Nutritional Value and Water Use of African Leafy Vegetables for Improved Livelihoods

A Oelofse & W van Averbeke

WATER RESEARCH COMMISSION

TT 535/12

NUTRITIONAL VALUE AND WATER USE OF AFRICAN LEAFY VEGETABLES FOR IMPROVED LIVELIHOODS

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by

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EXECUTIVE SUMMARY

In this report, the findings of an inter-disciplinary research project aimed at determining the drought- and heat tolerance, water use, agronomic requirements and potential contribution to human nutrition of a selection of eight African leafy vegetables (ALVs) are presented. Research work was done as part of five thrusts, each with its own specific objective. The objective of the first thrust was to review existing knowledge of the nutritional status of South Africans and the strategies used to address malnutrition in vulnerable communities. The objective of the second thrust was to review literature on the use and status of ALVs in contemporary South Africa. This included the identification of key plant species and their contribution to household food security. The objective of the third thrust was to characterise a selection of eight important ALV species in terms of their drought- and heat tolerance and water use. The fourth thrust was aimed at generating knowledge of the agronomic requirements of the eight selected ALV species. Empirical work focused on germination and emergence of their seed and on their plant nutrient requirements. In addition, existing knowledge was reviewed to provide an indication of their disease- and pest control requirements. The objective of the fifth thrust was to assess human nutritional aspects of the eight ALV species. Empirical work covered the determination of their micronutrient and antioxidant content, as well as patterns of availability and consumption of ALVs among households at selected sites in the KwaZulu-Natal and Limpopo Provinces. Vitamin A and iron were selected as the nutrients of primary interest due to their significant association with malnutrition in the developing world in general and South Africa in particular.

The research project was justified, because African leafy vegetables, which are defined as the collective of leafy vegetable species that form part of the culinary repertoire of particular contemporary African communities, are perceived to have important advantages over exotic leafy vegetable species, which include:

- Consumption of ALVs is not subject to acceptability constraints, because they from part of the traditional foods of local communities;
- ALVs are easier to produce and require less resources, such as fertilisers and water, than exotic vegetable species, because they are adapted to local conditions, such as high temperatures, water deficit and low soil fertility;
- ALVs are rich sources of micronutrients and their production and consumption could provide a host of health benefits to communities in which malnutrition and poor health are prevalent.

Research work conducted in the project enabled assessment of the validity of these perceived advantages. This was considered important, because if these perceptions were indeed valid, ALVs could contribute greatly to improved food security among food-insecure households, many of whom are living in water-stressed areas of the country.

The review of existing knowledge of the nutritional status of South Africans and the strategies used to address malnutrition in vulnerable communities showed that under- and over-nutrition co-existed in the same communities and often in the same households. Increased intake of fruit and vegetables was identified as one of the possible strategies to address under-nutrition and this was where home-production of ALVs could fit in. Important also was that increased intake of fruit and vegetables could be effective in addressing over-nutrition as well, by forming part of weight management programmes in line with the general health promotion messages contained in the South African food-based dietary guidelines. Since the rural poor in South Africa are disproportionately affected by the burden of malnutrition, investigating the option of home production of ALVs to improve their diet and nutritional status was considered to be a useful proposition.

The review of case studies documenting the use and status of ALVs in different parts of South Africa identified opposing trends. Most case studies indicated that the use of ALVs was in decline and that their status was low, being associated with poverty and lifestyles of the past. There were also studies that indicated

that ALVs were being mainstreamed in certain parts of South Africa, in line with the trend observed in many other African countries. The Vhembe District in Limpopo Province was one of the areas where large numbers of smallholders and home gardeners produced selected ALVs and where these vegetables held the status of fresh produce commodities that featured prominently on the shelves of local supermarkets. The review showed that a wide variety of plant species were being consumed by black people in one or other part of South Africa and, therefore, qualified as ALVs. Using three criteria, namely distribution of consumption, seasonality, and evidence of cultivation, eight ALV species were selected from the population of ALVs for empirical study in the project. These eight species were:

- Pigweed (amaranth) (*Amaranthus cruentus* L.);
- Jew's mallow (wild jute) (*Corchorus olitorius* L.);
- Non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*);
- Nightshade (*Solanum retroflexum* Dun.);
- Spider flower (*Cleome gynandra* L.);
- Pumpkin (*Cucurbita maxima* Duchesne);
- Tsamma melon (bitter water melon) (*Citrillus lanatus* Thunb.); and
- Cowpea (*Vigna unguiculata* (L.) Walp.).

Fieldwork investigating use and status of ALVs at selected sites in the KwaZulu-Natal and Limpopo Provinces demonstrated that ALVs were still consumed extensively by contemporary rural households. Amaranth species were particularly important. The potential to develop selected ALV species into fresh produce commodities was confirmed but most ALVs that were being consumed were harvested from the wild. Both review and empirical work showed that the transfer of knowledge of ALVs occurred from one generation to the next, with women holding most knowledge. Loss of this indigenous knowledge was identified as a threat in parts of the country where the use of ALVs was in decline and this confirmed the relevance and timeliness of the current research project.

Differences among the water requirements of the eight ALV species for optimum production were identified. The water requirement over the full growing season was 340 mm for pumpkin, tsamma melon and cowpeas, 360 mm for pigweed, 368 mm for Jew's mallow, 381 mm for nightshade, 382 mm for non-heading Chinese cabbage and 463 mm for spider flower. All eight ALVs were sensitive to water stress, because biomass production was affected by irrigation treatment. This indicated that production of these crops is dependent on the availability of adequate amounts of water. Full irrigation, whereby the rooting depth was regularly recharged to field capacity was necessary to achieve maximum biomass and high quality produce. Assays used to test the response of the eight vegetables to drought and heat stress revealed that all ALVs were more tolerant to drought and heat stress than Swiss chard (*Beta vulgaris* var. *cicla*), a widely grown exotic dark-green leafy vegetable that featured as the control in the assays.

Seven of the eight ALVs germinated best when it was warm but non-heading Chinese cabbage preferred relatively cooler conditions. The seed of several of the ALV species was subject to dormancy and ways of dealing with dormancy were developed for each of the species affected. All eight ALVs responded positively to the application of plant nutrients. Optimum fertiliser application rates were determined for all crops using pot studies and field experiments. Frequent harvesting delayed flowering and encouraged new shoot and root growth in all the crops but in some of the experiments this practice compromised leaf quality.

Analysis of the edible portions of the eight vegetables showed that these plants could contribute substantially to intake of vitamin A and iron in both young children and women, two of the groups most vulnerable to malnutrition. Some plants provided more than 50% of the RDA (Recommended Daily Allowance) for vitamin A and all eight ALVs provided at least 30% of the EAR (Estimated Average Requirement). The ALVs also provided varying amounts of other important nutrients, such as protein and fibre, which supported

the idea of using a variety of ALVs to address the nutritional health of the vulnerable. The antioxidant content and the associated ability to reduce the oxidative damage in erythrocytes, plasmid DNA (deoxyribonucleic acid) and cell cultures, was higher in ALVs than in Swiss chard. Considering the presence of both communicable and non-communicable diseases in vulnerable communities, this could be an important quality of these plants, which could assist in nutrient uptake and utilisation.

The household consumption study demonstrated that ALVs contributed significantly to the total dietary intake of calcium, iron and vitamin A of learners and their caregivers. Analysis for β-carotene content of both fried and boiled amaranth confirmed the potential of these leaves to contribute significantly to vitamin A requirements of nutritionally vulnerable communities. The study also confirmed the general belief that information on ALVs is passed down from the old to the young generation, mainly along female lines. Differences between provinces, as well as rural/urban differences within provinces in terms of type and source of ALVs, preparation methods, consumption patterns, and preference were observed. Therefore, it was recommended that data on the use of ALVs should be reported within the local context of the study area where the information was collected.

In conclusion, the results of this multi-disciplinary study have contributed substantially to knowledge of the water requirement and agronomy of a selection of important African leafy vegetables and the nutritional contribution these crops could make to human health and well-being. The eight ALVs tested showed differential responses to agronomic factors, had varying nutrient contents and differed in their popularity across study sites but the idea of introducing variety (production and consumption of different species) was considered important to optimise the nutritional contribution these plants could make in vulnerable communities. Apart from an important contribution to micronutrient malnutrition, consumption ALVs could also contribute to the prevention of both non-communicable and communicable diseases, such as certain types of cancer and HIV, because of their cell protective effects that arise from their relatively high antioxidants contents. Cultivation of these crops posed some challenges but none more than those experienced when growing exotic species, such as white cabbage and Swiss chard. The eight ALV species also tended to be more drought and heat tolerant than Swiss chard, which was the reference crop in this study. This could prove significant in the context of climate change. The study demonstrated that ALVs could be grown in home gardens using local resources. Increased consumption of ALVs, which could be brought about through cultivation, could assist in addressing malnutrition and food insecurity at the level of the household. Hence, the findings of this study support the promotion of the production and consumption of these crops.

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Dr AJ Sanewe	Water Research Commission (Chairman)
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ABBREVIATIONS AND ACRONYMS

°C	degree Celsius
AAPH	2,2′-azobis(2-amidinopropane) dihydrochloride
ABTS	2,2′-Azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt
ACC/SCN	Administrative Committee on Coordination Sub-Committee on Nutrition
AI	adequate intake
AICR	American Institute for Cancer Research
ALVs	African leafy vegetables
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
ARC – ISCW	Agricultural Research Council – Institute for Soil, Climate and Water
ARC – LBD:API	Agricultural Research Council – Livestock Business Division: Animal Products Institute
ARC – VOPI	Agricultural Research Council – Vegetable and Ornamental Plant Institute
AUC	area under the fluorescence curve
CAA	cellular antioxidant activity
Caco-2	human adenocarcinoma colon cancer
CDL	chronic diseases of lifestyle
CE	catechin equivalents
cm	centimetre
CMS	cell membrane stability
CV	coefficient of variance
DCF	dichlorofluorescein
DCFH-DA	dichlorofluorescein diacetate
dL	decilitre
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-2-picrylhydrazyl
dw	dry weight
EAR	estimated average requirement
EC	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
EER	estimated energy requirements
ET	evapotranspiration
ETc	crop evapotranspiration
ETo	potential evapotranspiration
FAO	Food and Agriculture Organization of the United Nations
F-C	Folin Ciocalteu
FC	field capacity
FGD	focus group discussion
fw	fresh weight
g	gram
GA_3	gibberellins
GAE	gallic acid equivalents
H	hydrogen
h	hour
ha	hectare
Hb	haemoglobin
HCF	high chemical fertilizer
HIV/AIDS	human immunovirus / acquired immune deficiency syndrome
HPLC	high performance liquid chromatography
i.e.	that is
IC	ion chromatograph
IFOAM	International Federation of Organic Agricultural Movements
ILSI	International Life Sciences Institute

IPGRI	International Plant Genetic Resources Institute
IPM	Integrated pest and disease management
ISRD	Integrated Sustainable Rural Development
K	potassium
Kc	crop coefficient
KCl	potassium chloride
Kg	kilogram
kJ	kilo Joules
KNO_3	potassium nitrate
L	litre
LA	leaf area
LAN	limestone ammonium nitrate
LSD	least significant difference
M	molar
m	metre
MCF	medium chemical fertilizer
mg	milli gram
ml	milli litre
mM	milli molar
mm	milli metre
mmol	milli moles
mo	months
MRC	Medical Research Council
N	nitrogen
Na_2HPO_4	di-sodium hydrogen orthophosphate dehydrate
NaH_2PO_4	sodium phosphate monobasic
NFCS-FB	National food consumption survey-fortification baseline
NIRU	Nutritional Intervention Research Unit
nm	nanomitre
O_2	oxygen
OH	hydroxyl
ORAC	oxygen radical absorption capacity
P	phosphorus
pBR	plasmid Boliver and Rodrigues
PBS	phosphate buffer solution
pH	potential hydrogen
ppm	parts per million
RAE	retinol activity equivalents
RDA	recommended daily allowance
RDA	recommended dietary allowance
RE	retinol equivalents
RE	retinol equivalents
RWC	relative water content
SADHS	South Africa demographic and health survey
SAFOODs	South African food data system
SANAS	South African National Accreditation Services
SD	standard deviation
SEM	standard error of means
TAE	Tris acetate ethylenediaminetetraacetic acid
TE	Trolox equivalents
TFC	total flavonoid content
THUSA	Transition and Health during Urbanisation in South Africa
TIFF	tagged image file format
TMV	Tobacco Mosaic Virus
TPC	total phenolic content
TTC	2,3,5-triphenyl-tetrazolium chloride reduction rate
TUT	Tshwane University of Technology

TW	turgid weight
UNICEF	United Nations Children's Fund
USDA	United States Department of Agriculture
v	volume
WCRF/AICR	World Cancer Research Fund/American Institute for Cancer Research
WRC	Water Research Commission
y	year
µg	micro gram
µl	micro litre
µM	micro molar
µmol	micro moles

CHAPTER 1: INTRODUCTION

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1.1 BACKGROUND

South Africa is largely food secure at national level (De Klerk *et al*., 2004; Laker, 2007), but large numbers of South Africans are food-insecure at household level, because they cannot afford to provide their households with adequate nutrition (Altman *et al*., 2009). Food insecurity in South Africa is predominantly experienced as micronutrient deficiencies, which are the result of diets that lack the necessary variation (Maunder and Meaker, 2007; Faber and Wenhold, 2007). Poverty contributes significantly to malnutrition in South Africa, because the diet of many poor people tends to be high in starch and low in nutrient-dense foods, such as fruit and vegetables (Maunder and Meaker, 2007). Ensuring that all South Africans are able to meet their recommended daily allowances (RDA) of the various nutrients for adequate nutrition and a healthy life is the principal food security challenge facing South Africa. Home gardening and other forms of small-scale agriculture could and should play a significant role in addressing this challenge but in many parts of the country the lack of water limits plant production and, therefore, the ability of households to grow enough food to provide key nutrients.

Four out of ten South African families live in poverty (UNICEF and The South African Human Rights Commission, 2011). Children raised in these families are at risk of infant mortality, low birth weight and subsequent stunting due to malnutrition (Black *et al*., 2003). Worldwide, iron deficiency is the most common micronutrient deficiency problem (Murray-Kolb & Beard, 2009). In South Africa, deficiencies in vitamin A, iodine and zinc are also important (Faber and Wenhold, 2007). It has been estimated that elimination of iron, iodine and vitamin A deficiency could lead to a population-wide increase of 10-15 IQ points, reduce maternal deaths by one third, decrease infant and childhood mortality by one third, and increase the capacity to work by almost half (Darton-Hill *et al*., 2005). Deficiencies, such as vitamin A and iron, have important negative consequences for children's growth and development. Local statistics on the severity of the problem indicate that one in three children under the age of six years are vitamin A deficient, one in four child deaths are suspected to be associated with vitamin A deficiency, and one out of two children aged between one and nine years old have less than 50% of the required intake of energy, vitamin A, iron, zinc and vitamin C (Labadarios *et al*., 2005).

Most of the 14.3 million people who are poor and vulnerable to food insecurity in South Africa live in rural communities, informal urban settlements and on farms, with women, children and the elderly being most affected (Department of Agriculture, 2006). Statistics South Africa reported that the causes of death among children were linked to poverty and that malnutrition was the seventh leading cause of death in children below the age of fifteen years (Stats SA, 2005). Despite the obvious need to improve household nutrition and the tremendous opportunity to address micronutrient deficiencies through farming, especially in community- and homestead gardens, there is evidence that gardening does not make a major contribution to nutrition in most households (Hebinck and Monde, 2007, Van Averbeke, 2007; Van Averbeke & Khosa, 2007). In many areas, gardening activities are hampered by the lack of sufficient water for irrigation (Wenhold *et al*., 2007; Laker, 2007). There is, therefore, a need to optimise the use of available water for irrigation in homestead crop production. Effective production of micronutrient-rich crops with the water that is available could contribute substantially to the reduction or elimination of micronutrient malnutrition in vulnerable

communities. Therefore, there is a need to investigate the relationship between water use, crop production and malnutrition, with specific reference to home gardening and small-scale farming.

South Africa is particularly vulnerable to climatic change (Schulze, 2011). It is a dry country in which plant production depends heavily on level of soil water availability (Laker, 2007). The threat of further drying of the climate, exacerbated by rising temperatures as a result of climate change, could have a serious impact on the availability of food. The impact of climate change could extend beyond food shortages. It could negatively affect the national economy by reducing the country's ability to export crops and generate foreign currency. South Africa is a water stressed country. Annandale *et al*. (2011) reported that the total renewable water available per inhabitant was a mere 1 106 m^3 $year^{-1}$ and in the next two to three decades, water availability is likely to drop below the benchmark of 1 000 m^3 person $year^{-1}$. The risk of land degradation and crop failure is likely to increase, especially in marginal areas. One way to combat inadequate availability of water is to select or develop crops that are particularly tolerant to water stress. It is in this context that indigenous and traditional African crops offer exciting opportunities for enhanced exploitation.

For centuries, indigenous edible plants and traditional crops have sustained rural populations in developing countries (Jansen van Rensburg *et al*., 2007). Several of these plants and crops are native to the places where they are grown. For this reason, they are expected to be particularly well adapted to the prevailing conditions. Anecdotal evidence suggests that these types of plants can be cultivated successfully without the use of expensive external resources, such as irrigation and agrochemicals. However, the utilisation, water use and agronomy of South African indigenous and traditional crops are not well documented (Jansen van Rensburg *et al*., 2004). Their nutritional value and the bioavailability of the nutrients they contain have not been determined systematically. At this stage, selection, propagation and knowledge of the agronomy of indigenous and traditional crops in South Africa is largely indigenous knowledge, whilst collection and consumption of these crops tends to be associated with poverty and lifestyles of the past.

Various organisations in Africa and abroad have done research on indigenous the leafy-, seed- and tuber plants that are used as vegetables and pot herbs but this research is fragmented and not always well documented. Research undertaken ranges from investigations into cultivation practices, nutrient contents (mostly of fresh material), situation analyses and eco-geographical surveys. The water and plant nutrient requirements of these crops and their response to variability in these factors, await systematic scientific enquiry.

Indigenous and traditional African vegetables are rich in micronutrients and increase the bioavailability and absorption of micronutrients from staple foods. Awareness of the nutritional benefits that can be derived from the consumption of these vegetables is part of indigenous knowledge. For example, after giving birth, women in Uganda consume *Cleome gynandra*, which is rich in iron and other micronutrients (Chweya and Mnzava, 1997). Rural people grow up eating these vegetables and most like their taste, which is not always the case with exotic vegetables. However, the contribution indigenous and traditional vegetables make to human nutrition depends heavily on the way they are prepared and on the quantity and frequency of consumption. Generally the leaves are a good source of calcium, magnesium, beta carotene, iron and vitamin C, whereas the roots and seeds are rich in proteins.

Many traditional food plants grow in the wild, or as weeds in cultivated areas, particularly leafy vegetable species. On the other hand, some traditional leafy vegetables have been domesticated and are actively reproduced. In some cases they have even reached the stage of being cultivated species. Over time, several exotic leafy vegetable species became part of the culinary heritage/diet of local people. For example blackjack, which is thought to have been brought to Africa with the horse fodder of the British army in the late nineteenth century, is now considered a traditional leafy vegetable (MacDonald *et al*., 2003). A large

number of different species of leafy vegetables are used in the Southern African region. Among these most, are consumed in specific parts of the country but some are consumed more extensively.

The lack of concerted research and development attention has contributed to the underutilisation of traditional food plants. Genetic diversity approaches involving studies of micronutrient composition and bioavailability could enhance the contribution of these plants to improved nutrition of vulnerable groups. Plant diversity research may also contribute towards identifying synergistic food combinations or preparation methods that mediate the absorption of micronutrients, such as beta carotene.

The current project is aimed at strengthening people's abilities to generate food for themselves, as opposed to programmes to enhance direct access to food, such as the social grants programme and the primary schools nutrition programme. It is aimed at empowering communities to help themselves to become food secure and to maintain a healthy balanced diet. It is aimed at raising the status of traditional food plants in South Africa by pointing out the valuable contribution these plants could make to the food security and hence, nutrition security of South African households.

1.2 AIM OF THE PROJECT

The aim of this project is to improve livelihoods through increased food security and well-being among groups that are vulnerable to malnutrition by means of increased water productivity using indigenous crop production in South Africa.

1.3 SPECIFIC OBJECTIVES

The specific objectives of the project were to:

1. Review the current nutritional status of the South African human population with specific reference to malnutrition.
2. Identify a list of potential crops from the perspectives of:
    - availability and accessibility of crops;
    - agricultural repertoire;
    - nutrient content of crops;
    - nutritional value of crops;
    - eating habits, food preferences and current consumption patterns (portion size).
3. Determine key nutrient contents of crops.
4. Identify crops to fill the nutritional gaps.
5. Draw up a short list of crops for further investigation.
6. Determine the water requirements of the selected crops through modelling and/or trials.
7. Determine the nutrient (fertilizer) requirements and pest/disease control for the selected crops.
8. Determine and test appropriate production practices for the selected crops.
9. Evaluate productivity/potential yields of the selected crops.
10. Determine the potential contribution of the selected crops to the nutrient requirements of the households.
11. Determine the potential effect of the selected crops on nutritional status.
12. Recommend crops and resource requirements/production system/technology for implementation.

1.4 CROP SELECTION

In line with the WRC terms of reference of WRC project K5/1579//4 (WRC, 2006), the research team responsible for the execution of the project initially proposed the adoption of a pyramid approach, starting out broadly when considering indigenous crops, and subsequently narrowing down the focus to two or three crops on which most of the research work would be done, both agronomically and in terms of their potential contribution to human nutrition, with particular reference to micro nutrient deficiencies. During 2006-07, the rationale of this approach was questioned, following lessons learnt from the Indigenous Crops Symposium, the visit report by Mr Schippers (Schippers and Van Averbeke, 2006), a situation analysis and team meetings. After careful consideration, the team reached consensus on a new approach to be followed for the remaining period of project implementation and made the following decisions:

1. The study would be limited to indigenous and traditional African leafy vegetables (ALVs).
2. Vitamin A and iron would be the focus of the nutritional contribution.
3. Selection of species for research would be broadened from two or three species to eight species with a view of providing dietary variety throughout the seasons and avoiding anti-nutritional effects associated with the sustained consumption of particular leafy vegetables.
4. Research on plant water relations involving the eight ALV species would assess their drought- and heat tolerance relative to an appropriate reference crop, and determine their water requirements and water productivity.
5. Agronomic research involving the eight ALV species would focus on conditions for germination and emergence and response to plant nutrient availability, whilst research dealing with the pests and diseases of the eight ALV species would largely be based on an extensive review of existing knowledge to be complemented with observations during field trials.

Collectively the eight ALVs represented a fairly comprehensive selection of the traditional leafy vegetable species that are consumed in South Africa. Perhaps with the exception of *Corchorus olitorius* they have a wide distribution in terms of consumption, which meant that the findings would have broad application. The eight selected vegetables were:

- *Amaranthus cruentus* L. (pigweed; amaranth),
- *Vigna unguiculata* L. Walp. (cowpea),
- *Corchorus olitorius* L. (Jew's mallow, wild jute),
- *Cleome gynandra* L. (spider flower, cat's whiskers, spiderplant, bastard mustard),
- *Citrillus lanatus* (Thunberg) (tsamma melon; bitter watermelon, *egusi* watermelon),
- *Cucurbita maxima* Duchesne (pumpkin),
- *Solanum retroflexum* Dun. (black nightshade), and
- *Brassica rapa* L. subsp. *chinensis* (non-heading Chinese cabbage).

1.5 CONTRIBUTION OF THE INDIVIDUAL CHAPTERS TO THE OBJECTIVES OF THE PROJECT

In this section, the content of the different chapters is briefly described and the contribution of each chapter to the achievement of the project objectives is pointed out.

1.5.1 Chapter 2

In Chapter 2 a summary of information contained in a more comprehensive report titled "Nutritional status of South Africans: Links to agriculture and water" (Wenhold and Faber, 2008) is presented. Several of the key issues raised in this chapter have also been published by Faber and Wenhold (2007). Information from two national studies, the National Food Consumption Survey – Fortification Baseline (NFCS-FB) of 2005

(Labadarios, 2007) and the South Africa Demographic and Health Survey (SADHS) of 2003 (Department of Health *et al.*, 2007), both of which had not been published by the time the above two publications were written, and the findings of these recent studies have been incorporated selectively in the chapter. The aim of the chapter was, therefore, to provide a concise overview of the current anthropometric and nutrient status and the food intake of South Africans reported on mainly in national studies but complemented with findings in local studies, for adults and children separately. Overall, this review presents the basis for implementing timely, needs-driven food-based approaches for combating malnutrition. The chapter is concluded with a summary of possible strategies to address the nutritional problems that are of importance to public health in South Africa. This chapter deals predominantly with objective 1.

1.5.2 Chapter 3

Chapter 3 provides an overview of the status of ALVs in contemporary South African society based on a review of literature. It presents information on the local nomenclature, botanical description, ecology, use and cultivation of eight groups of leafy vegetable species that were identified as most important in South Africa and explores the potential of ALVs to improve human nutrition, particularly among poor people. The chapter addresses objectives 2 and 5.

1.5.3 Chapter 4

Chapter 4 presents the findings of the assessment of the drought and heat tolerance of six ALVs relative to Swiss chard, which was selected as the reference crop, because it is an exotic leafy vegetable that is commonly grown by resource poor communities. Plants have developed a wide diversity of drought and heat tolerance mechanisms at the metabolic and physiological levels, and adaptation to abiotic stress has been identified at molecular, cellular and whole plant level. Drought and heat tolerance is the phenotypic expression of a number of morphological characteristics and physiological mechanisms. A combination of mechanically linked traits, such as drought and heat avoidance and tolerance, were investigated. The drought and heat tolerance traits were examined using various techniques, including relative water content, leaf area, cell membrane stability, cell viability and early drought tolerance. This chapter contributes towards objectives 6 and 9.

1.5.4 Chapter 5

Chapter 5 reports the water requirements of the eight selected ALVs. Water is an important plant growth factor which is of particular significance in water-stressed South Africa. Research was conducted at ARC – VOPI at Roodeplaat to determine the water use of the ALVs, as well as to evaluate the effect of different irrigation regimes on biomass production and water productivity of these crops. This chapter contributes towards objectives 6, 8 and 9.

1.5.5 Chapter 6

Chapter 6 deals with the processes of germination of the seed of the eight selected ALV species. Knowledge of these processes is a critical component of the agronomy of crop species, because it affects both yield and monetary value of crops. Modelling of these processes is important to assess the suitability of climatic conditions for the production of different crops and the scheduling of sequential planting in order to meet market demand and continuity of supply. It also enables prediction of the optimum planting date. Many factors affect the processes of germination and seedling emergence, including light, water, temperature, dormancy, soil physical conditions and oxygen availability. Among these factors, temperature, light and dormancy are arguably cardinal, explaining why they were selected for investigation. The work covered in this chapter contributes to objective 8.

1.5.6 Chapter 7

In Chapter 7, the biomass response of the ALVs to rate of application of nitrogen (N), phosphorus (P) and potassium (K) is characterised. The availability of these three plant nutrients has an important effect on biomass production and yield of crops in general. The extent to which the availability of these three nutrients affected biomass and yield of the eight ALV species was investigated using greenhouse pot studies that involved both chemical fertilisers and a selection of three types of animal manure. Field experiments involving chemical fertilisers were used to test the findings under field conditions. This chapter contributes to objectives 7, 8 and 9.

1.5.7 Chapter 8

Chapter 8 deals with pests and diseases that affect ALVs. Appropriate non-chemical control strategies for use in ALV production were identified. These were considered best suited for the communities for which the recommendations are intended. Components of pest and disease control strategies for ALVs were investigated for the following phases: selection of seed and plant material, field production, harvesting and storage. Because of a lack of literature available on pest and disease control for many of the traditional ALVs under investigation, comparisons were made with pest and diseases prevailing in conventional vegetable production of some of the major vegetable crops. Divided into two parts, the first part of the chapter deals with general organic control strategies for pests and disease in ALVs and other related genotypes, while the second part addresses specific organic control measures, where one pest and one disease was selected for each crop, and their specific organic control techniques and strategies were studied. This chapter specifically addresses objective 7.

1.5.8 Chapter 9

Chapter 9 focuses on the determination of the nutrient content of the eight selected ALV crops grown under conditions of adequate availability of growth factors in the field. Appropriate ALV crop sampling protocols were developed by the project team members of the Agricultural Research Council-Livestock Business Division: Animal Products Institute (ARC – LBD:API) and the Nutritional Intervention Research Unit (NIRU) of the Medical Research Council (MRC) responsible for the nutrient analysis, in collaboration with the horticulturists growing the ALVs. This ensured that a representative sample of the food material was taken for nutrient analysis. The decisions to grow the ALV crops under conditions of adequate availability of growth factors and to analyse the raw plant material were made in order to obtain good baseline values characterising each ALV species. The nutrient values provided a good indication of the most suitable ALV species to address micronutrient malnutrition. The content of this chapter contributes to objectives 2, 3 and 4.

1.5.9 Chapter 10

Assessment of the antioxidant properties of a selection of the ALVs is covered in Chapter 10. The ALVs selected for study were pigweed, Jew’s mallow, cowpea and pumpkin. The specific objectives of this chapter were fourfold. Firstly, to determine the total phenolic content, total flavonoid content and total antioxidant activity of the selected ALVs. Secondly, to identify and quantify selected flavonoids in extracts of ALVs, using high-performance liquid chromatography (HPLC). Thirdly, to determine the effects of boiling and extraction solvent on the total phenolic content, total flavonoid content and total antioxidant activity; and lastly to determine the ability of extracts of raw and boiled ALVs to protect against AAPH-induced oxidative damage in biological molecules (erythrocytes and plasmid DNA) and cell cultures (SC-1 mouse fibroblast and human adenocarcinoma colon cancer (Caco-2) cells). This chapter contributes to objectives 3, 10 and 11.

1.5.10 Chapter 11

Chapter 11 deals with the availability of ALVs, access to ALVs and nutrition-related uses of ALVs in two rural and one urban site in South Africa. In addition, empirical work looked at the contribution of ALVs to total nutrient intake of primary school children and their caregivers in one rural and one urban site and analysed a prominent ALV for β-carotene content. The study was done after the first summer rains, from October to December 2008 at three sites; a rural site in Limpopo Province, and a rural and an urban site in the KwaZulu-Natal Province. These two provinces have the highest prevalence of vitamin A deficiency. In the Limpopo Province (where the term *morogo* is used to refer to ALVs), the rural villages selected for this study were located in the Greater Sekhukhune District Municipality and within the local municipality of Makhudutamaga. Greater Sekhukhune forms part of one of the Integrated Sustainable Rural Development (ISRD) nodes. ISRD nodes consist of districts and local municipalities that have been prioritized by the South African government for development as they are among the poorest areas in the country and are characterised by poor infrastructure, limited resources and economic depression. In the KwaZulu-Natal Province (where the term *imifino* is used to refer to ALVs), data were collected at both a rural and an urban site. The rural site was a mountain village falling under the KwaXimba tribal authority bordering the Valley of a Thousand Hills just north of Pinetown. A home-garden project promoting, among other, dark-green leafy vegetables, was conducted in the area from 1998 to 2002 (Faber *et al*., 2002). The urban site was situated in the Mariannhill area, Pinetown, approximately 20 km south of the rural site and consisted of a fairly densely populated, poor settlement. The study consisted of four major components. The first was a qualitative explorative survey of the availability and use of ALVs at the two rural sites through observation, semi-structured interviews with key informants, and focus group discussions and was done at the two rural sites. The second was made up of a quantitative household-level survey of the procurement and consumption of *morogo/imifino* and was done at the two rural sites and the urban site. The third comprised a quantified dietary survey on the nutrient contribution of *imifino* to total nutrient intake for children and caregivers at the two sites in KwaZulu-Natal. The last component was concerned with the β-carotene content of fresh, boiled and fried amaranth, which was identified as the primary ALV at the various study sites. Chapter 11 contributes to objectives 10 and 11.

1.5.11 Chapter 12

Chapter 12 is the concluding chapter in this report. It brings together the main findings of the inter-disciplinary research project and uses the insights that have been developed to formulate recommendations for the production and consumption of ALVs in order to improve livelihoods through better nutrition.

1.6 CONCLUSIONS

The research chapters contained in this report make a considerable contribution to understanding the potential role African leafy vegetables play or could play in the livelihoods of people who are vulnerable to malnutrition. ALVs form part of indigenous and traditional crops, which are often referred to as ‘orphaned crops’, because they have long been ignored by research and development. The authors of this report hope that their work has placed these crops, particularly ALVs, a little closer to the limelight. They also hope that the research contained in this report helps to remove the persistent divide between agricultural and nutritional aspects of crops, which is of no use in the context of addressing malnutrition through production and consumption of crops.

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HUMAN NUTRITION IN SOUTH AFRICA

CHAPTER 2: NUTRITIONAL STATUS OF SOUTH AFRICANS AND STRATEGIES TO ADDRESS MALNUTRITION

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2.1 INTRODUCTION

This chapter is a selection of information from a more comprehensive report titled "Nutritional status of South Africans: Links to agriculture and water" (Wenhold and Faber, 2008). The main aspects thereof have already been published elsewhere (Faber and Wenhold, 2007). Information from two national studies, the National Food Consumption Survey – Fortification Baseline (NFCS-FB) of 2005 (Labadarios *et al.*, 2007) and the South Africa Demographic and Health Survey (SADHS) of 2003 (Department of Health *et al*., 2007), both of which had not been published by the time the above two publications were written, has selectively been incorporated. The aim of the chapter is therefore to provide a concise overview of the current anthropometric and nutrient status and the food intake of South Africans as mainly published in national studies, supplemented by referring to smaller scale studies, for adults and children separately. Overall, this should form the basis for implementing timely, needs-driven food-based approaches for combating malnutrition. The chapter is thus concluded by a summary of potential strategies to address the nutritional problems found to be of public health importance in South Africa.

Malnutrition is defined as both undernutrition and overnutrition. Overnutrition mainly refers to excessive intake of energy, whereas protein-energy malnutrition and micronutrient deficiencies can be distinguished in undernutrition. Globally, iron, vitamin A, iodine and zinc deficiency are the most prevalent forms of micronutrient deficiency, collectively sometimes called "hidden hunger". South Africa has a double burden of nutrition-related disease through the co-existence of under- and over-nutrition, with a rising incidence of overweight and obesity related to the nutrition transition. Dietary intake over time reflects a shift towards an increased dietary intake of fat in both urban and rural areas, and the intake of animal-source foods are increasing at the expense of plant-based foods (Bourne *et al*., 2002). Healthier food choices are generally more expensive than commonly consumed foods, and as a result, the large majority of the population cannot afford a healthy diet (Temple *et al*., 2011).

Nutritional status refers to the health status of people as affected by nutrition. It can be assessed by anthropometric, laboratory, dietary and clinical methods, ideally in combination and by putting these into a particular context and supplementing the findings with appropriate other information (e.g. risk factors) from the individual or group being assessed. Sometimes nutrient-specific assessments are made, e.g. iron or protein status.

2.2 ANTHROPOMETRIC STATUS

2.2.1 Adults

The body mass index (BMI) provides a good reflection of the general energy status of adults. In most cases it is positively correlated with total body fatness and refers to weight in kilograms divided by height in metres squared. The World Health Organization (WHO) defines underweight as BMI<18.5; normal weight as 18.5=<BMI<25; overweight as 25=<BM<30; and obesity as BMI>=30 (WHO, 1998).

Tables 2.1 and 2.2 show the percentage of adult males and females according to the BMI categories as determined by the SADHS of 1998 and 2003. These data clearly show that overnutrition is prevalent among South Africans, particularly women of all ages, with 52.3% being either overweight or obese at national level in 2003 (Department of Health *et al*., 2007). The NFCS-BF of 2005 focussed on women aged 16 to 35 years. Also in this age bracket the national prevalence of overweight and obesity combined was 51.5% (Kruger *et al*., 2007). KwaZulu-Natal province has the highest prevalence of female overnutrition, with figures of 62.8% and 57.5% reported in 1998 and 2003 respectively (SADHS, 1998; Department of Health *et al*., 2007). Smaller studies have shown pockets of severe female overweight/obesity. In a rural area in KwaZulu-Natal, for example, 76.9% of the females were either overweight or obese (Oelofse *et al*., 1999).

Determinants of overweight and obesity in South Africa include age, level of education, ethnicity, and area of residence (Puoane *et al*., 2002). Obesity is more common in urban than in rural areas (Department of Health *et al*., 2007). With the current rapid urbanisation as part of the demographic and nutritional transition of South Africa, the prevalence of obesity in adults is expected to increase further (Vorster *et al*., 2000). Bourne *et al*. (2002) summarised dietary data available from urban and rural studies from 1940 to 1992 and concluded that the diets of the black South African population shifted towards an atherogenic Western diet. According to Steyn *et al*. (2006a), the high prevalence of overweight and obesity in the adult population reflects the westernised eating pattern.

The black population constitutes 76.7% of the South African population (Statistics South Africa, 2000). The acceptance of overweight and obesity in the black population could make it difficult to prevent or treat obesity. Senekal *et al*. (2003) suggested that specific ethnic characteristics, such as obesity tolerant attitudes, should be taken into consideration when developing weight management programmes. Kruger and co-workers (2005) argued that obesity could not be managed solely at the individual level, but that a multi-sectoral approach is needed to modify the environment so that it is less conducive to weight gain.

In addition to BMI, waist circumference is often performed in adult anthropometric assessment. It provides an indication of excessive accumulation of fat in the abdominal region. This, in turn, is an independent risk factor for developing non-communicable diseases, such as type 2 diabetes mellitus, coronary heart disease and hypertension. For waist circumference the cut-off point for increased risk is gender-specific: above 102 cm and 88 cm for men and women respectively (Gibson, 2005). The SADHS of 2003 identified that nationally 5.0% of men and 33.7% of women had waist circumferences above the critical cut-off point. In the age groups 35 to 54 over 50% of all women were affected. Black women represented the group with the highest prevalence (39%) of abdominal obesity (Department of Health *et al*., 2007).

Table 2.1: Percentage of men aged 15 years and above by body mass index (BMI) categories to background characteristics, South Africa 1998 and 2003

Background characteristic	Underweight BMI<18.5		Normal weight BMI: 18.5-24.9		Overweight BMI: 25-29.9		Obese BMI: 30+		Number	
	1998	2003	1998	2003	1998	2003	1998	2003	1998	2003
Age										
15-24	21.3	20.3	67.5	68.2	8.4	9.7	2.7	1.8	1 796	1 086
25-34	8.5	8.4	62.9	60.9	20.7	20.9	7.8	9.8	1 103	702
35-44	8.5	8.3	52.8	52.2	24.9	26.6	12.8	12.9	990	569
45-55	9.2	8.4	45.2	45.9	28.1	31.9	17.3	13.8	678	418
55-64	9.1	9.9	47.5	48.0	28.3	29.0	14.4	13.1	510	282
65+	9.9	9.3	47.7	44.7	28.5	32.0	13.9	14.0	482	218
Residence										
Urban	10.8	11.8	55.5	57.3	22.2	20.3	11.1	10.6	3 486	2 189
Non-urban	16.4	14.0	61.4	58.5	15.6	22.4	6.3	5.1	2 072	1 086
Province										
Eastern Cape	11.5	10.9	57.6	63.8	20.5	16.5	10.1	8.8	750	355
Free State	18.8	18.0	56.7	60.0	16.3	13.5	8.1	8.6	439	217
Gauteng	9.7	14.3	58.5	57.9	21.1	20.2	10.2	9.7	1 060	865
KwaZulu-Natal	11.1	4.1	56.8	55.0	21.4	31.9	10.4	9.0	1 047	734
Limpopo	19.7	19.5	57.9	61.4	16.0	11.0	6.2	4.6	505	268
Mpumalanga	16.9	16.4	59.1	55.9	16.6	16.3	7.5	6.0	366	206
Northern Cape	23.1	25.7	54.3	55.1	14.4	13.8	7.6	5.4	132	58
North-West	17.5	19.8	61.4	55.0	15.4	17.5	5.5	4.8	544	243
Western Cape	5.8	9.2	55.3	52.7	25.3	23.6	13.1	14.5	706	331
Education										
None	12.2	13.9	58.3	51.2	21.3	24.1	8.2	10.8	549	276
Grade 1-5	14.6	14.4	58.3	51.2	18.4	24.5	8.2	10.0	760	329
Grade 6-7	15.9	16.1	58.5	58.6	17.6	16.3	7.4	9.0	745	371
Grade 8-11	15.0	14.1	59.8	60.2	17.2	19.3	7.6	6.3	2 256	1 300
Grade 12	5.6	9.7	58.7	60.5	22.2	19.9	13.4	10.0	785	669
Higher	7.1	5.4	41.6	51.5	33.5	30.3	17.8	12.9	430	300
Population group										
African	14.0	13.3	60.8	59.5	17.1	20.1	7.8	7.1	4 191	2 720
African urban	11.8	13.0	59.4	60.3	18.8	18.7	9.6	8.1	2 329	1 673
African non-urban	16.7	13.9	62.6	58.3	15.0	22.5	5.5	5.3	1 862	1 047
Coloured	11.4	11.5	56.6	52.4	22.1	21.3	9.2	14.9	628	256
White	4.7	4.9	38.1	47.0	36.1	25.1	20.8	23.0	536	203
Asian	16.6	10.1	50.7	45.2	23.7	33.7	9.0	10.9	189	82
Total	12.9	12.5	57.7	57.7	19.8	21.0	9.3	8.8	5 558	3 275

Source: SADHS, 1998:246; Department of Health, 2007:276 (SADHS, 2003)

Table 2.2: Percentage of women aged 15 years and above by body mass index (BMI) categories to background characteristics, South Africa 1998 and 2003

Background characteristic	Underweight BMI<18.5		Normal weight BMI: 18.5-24.9		Overweight BMI: 25-29.9		Obese BMI: 30+		Number	
	1998	2003	1998	2003	1998	2003	1998	2003	1998	2003
Age										
15-24	9.5	11.1	60.7	58.2	20.0	19.7	9.6	11.0	2 044	1 199
25-34	5.1	5.2	38.4	39.3	29.2	29.4	27.0	26.0	1 679	934
35-44	2.7	4.3	27.2	30.5	30.7	29.6	39.3	35.6	1 436	852
45-55	3.7	2.9	23.9	27.7	26.5	28.2	45.5	41.2	1 087	684
55-64	2.7	4.9	25.6	27.0	25.6	34.2	46.1	33.9	895	455
65+	7.4	5.2	32.5	29.1	26.5	33.9	33.3	31.8	829	357
Residence										
Urban	5.0	5.7	35.6	36.1	26.0	27.1	33.2	31.0	4 886	2 864
Non-urban	6.5	7.1	41.9	43.7	26.2	28.2	25.1	21.0	3 084	1 616
Province										
Eastern Cape	5.8	3.2	38.8	36.7	25.7	28.2	29.7	31.9	1 130	527
Free State	7.0	7.6	37.9	42.8	26.0	23.3	29.2	26.2	517	289
Gauteng	3.4	5.6	34.3	36.1	26.6	28.2	35.6	30.1	1 842	1 025
KwaZulu-Natal	5.4	3.2	31.2	39.3	27.4	33.0	35.4	24.5	1 554	831
Limpopo	7.2	9.1	48.7	45.0	24.0	24.2	20.1	21.8	831	496
Mpumalanga	4.9	6.0	43.8	40.1	24.9	25.9	25.8	28.0	500	294
Northern Cape	12.5	12.2	37.5	41.9	24.9	21.6	24.8	24.2	166	89
North-West	8.1	8.0	46.8	42.6	25.8	25.1	18.9	24.4	642	361
Western Cape	4.9	9.5	37.8	34.3	25.9	25.9	31.2	30.3	788	559
Education										
None	5.8	6.6	34.4	36.7	27.2	30.3	32.6	26.4	1 166	544
Grade 1-5	6.4	7.1	32.3	30.9	25.2	32.4	36.0	29.5	1 055	429
Grade 6-7	4.8	6.3	33.4	32.6	28.1	25.8	33.2	35.3	1 102	485
Grade 8-11	5.6	7.1	41.2	41.6	24.3	24.9	28.7	26.4	3 039	1 796
Grade 12	6.3	4.8	38.8	43.2	30.0	27.4	24.8	24.6	1 096	878
Higher	4.1	3.9	49.1	38.4	23.3	33.9	23.3	23.8	477	322
Population group										
African	4.9	5.6	37.7	38.2	25.9	27.7	31.2	28.5	6 143	3 687
African urban	3.9	5.1	34.0	34.0	25.5	27.1	36.3	33.8	3 293	2 161
African, non-urban	6.0	6.3	42.0	44.2	26.5	28.6	25.3	21.0	2 850	1 526
Coloured	9.9	12.1	36.1	35.8	25.3	25.7	28.5	26.5	800	438
White	2.9	4.9	44.2	57.0	27.4	24.3	25.5	13.7	731	236
Asian	15.6	5.7	35.8	35.1	27.3	34.4	21.3	24.8	284	110
Total	5.6	6.2	38.1	38.9	26.1	27.5	30.1	27.4	7 970	4481

Source: SADHS, 1998:247; Department of Health, 2007:277 (SADHS, 2003)

2.2.2 Children

In the context of anthropometric assessment of children, *underweight* is the term used when the weight for the age of a child is less than a specified cut-off value. This index is the cornerstone of growth monitoring on primary health care level, and is seen in South Africa as the first line of assessment of children on community level. It is a measure of both chronic and acute malnutrition, yet it cannot distinguish between the two. When weight-for-age is combined with the presence or absence of oedema (swelling) in a child, it aids in the diagnosis of kwashiorkor, marasmus or combinations thereof.

Stunting is a condition reflected by low height/length-for-age. It is a measure of chronic or long-term malnutrition. The onset of stunting is often associated with the age of the introduction of complementary feeding (Brown *et al*., 1995). Stunting does not change rapidly, and it may be irreversible in children older than 2 years (Cogill, 2003). Stunting is associated with a number of long-term factors such as chronic insufficient protein and energy intake, frequent sub-acute infections, sustained poor feeding practices, certain micronutrient deficiencies, particularly iron and zinc, poverty (Cogill, 2003) and poor socio-economic conditions (Gorstein *et al*., 1994). Because of its socio-economic dimension, stunting should be viewed in a broader societal context and not merely in a narrow nutritional sense (Zere and McIntyre, 2003). It has been suggested that integrated interventions include socio-economic variables, such as sanitation, housing, literacy and employment (Walsh *et al*., 2002) and the caring capacity of mothers be improved (De Villiers and Senekal, 2002) if the overall prevalence of stunting is to be decreased.

Wasting, on the other hand, refers to weight-for-height (or length) below a defined cut-off point. It is a measure of acute malnutrition. Causes include inadequate food intake, poor feeding practices, acute disease and infection, or mostly, a combination of these factors (Cogill, 2003). Wasting in individuals and populations can change rapidly with changes in the availability of food or disease prevalence.

Overweight and *obesity* are preferably expressed in relation to BMI. In the case of children interpretation of BMI is gender- and age-specific. Weight-for-height (or length) is also sometimes used to define overweight, particularly in older studies. Increasingly *low BMI for age* is also used to describe wasting; this implies that in future weight-for-height (or length) may become redundant.

The cut-offs are often expressed as z-scores (e.g. height-for-age z-score [HAZ] < -2 SD would mean that height-for-age is less than two standard deviations from the population mean), but other reporting systems and exact cut-off values are also used.

In the studies reviewed here, the WHO Growth Standards of 2006 for children from birth to five years old were not yet used. When comparing findings included in this review to more recent work which interpreted child anthropometry against the newer standards this should be kept in mind, as prevalence figures are not comparable (Norris *et al*., 2009; Duggan, 2010). Bosman and co-workers (2010) compared the anthropometric status of children aged from 12 to 60 months using different growth reference standards. They showed that the prevalence of stunting, overweight and obesity was significantly higher, and the prevalence and underweight and wasting lower when using the WHO 2006 growth standards. Algorithms are available to convert “old” prevalence estimates into “new” estimates (Yang and de Onis, 2008).

National anthropometric data are available for 6-71-mo-old children for 1994 (Labadarios *et al.*, 1995) and for 1-9-y-old children from 1999 (Labadarios *et al*., 2000) and from 2005 (Kruger *et al*., 2007). In 2003, the SADHS assessed children under the age of five years (Department of Health *et al*., 2007). Table 2.3 summarises the findings of these surveys. The mentioned studies showed a low prevalence of wasting (<5%), a low (<10%) to medium (10-20%) prevalence of underweight, and a medium (20-29%) prevalence of stunting at the national level. The problem therefore is chronic under-nutrition, rather than acute under-

nutrition. The South African Vitamin A Consultative Group study (SAVACG) of 1994 (Labadarios *et al*., 1995), the National Food Consumption Survey (NFCS) of 1999 (Labadarios *et al*., 2000) and the NFCS-FB of 2005 (Kruger *et al*., 2007) showed that prevalence figures differed between provinces, that children living on commercial farms were severely affected, and that the prevalence of malnutrition was higher in the rural areas compared with the urban areas. Secondary data analysis of the Living Standards and Development Survey of 1993 showed that children in the lowest socio-economic strata bear a greater burden of malnutrition (stunting and underweight, but not wasting), and that income-related inequalities in malnutrition were lowest in rural settings and highest in metropolitan areas (Zere and McIntyre, 2003).

Numerous smaller studies reported on the anthropometric status of children (Sickle *et al*., 1998; Faber and Benade, 1999; Oelofse *et al*., 1999; Van Stuijvenberg *et al*., 1999; Faber *et al*., 2001; Oelofse *et al*., 2002; Chopra, 2003; Mamabolo *et al*., 2004; Faber *et al*., 2005; Mamabolo *et al*., 2005; Smuts *et al*., 2008). As was the case in earlier studies (Vorster *et al.,* 1997), the data show that the prevalence and severity of malnutrition differ from area to area, and that pockets of malnourished children exist. The prevalence of malnutrition (in terms of underweight and stunting) tends to increase from the first to the second year of life. Thereafter the prevalence of malnutrition remains fairly constant. The period 6-12 mo, in particular, carries a great risk of growth faltering and malnutrition because of the inadequate nutritional quality of complementary foods and the increased risk of infections due to the decline in breastfeeding.

Secondary analysis of the NFCS data showed that child growth was associated with dietary diversity (Steyn *et al*., 2006c). Boys were shown to have a bigger chance of being stunted than girls (Lesiapeto *et al*., 2010). In Hlabisa, a rural area in KwaZulu-Natal, risk factors for stunting and underweight were reflective of maternal status and socio-economic status of the household (Chopra, 2003). Low maternal education was shown to be associated with child underweight (Lesiapeto *et al*., 2010). Stunting and underweight, but not wasting, are responsive to improvements in the socio-economic status of the household (Zere and McIntyre, 2003). A community-based nutrition education programme in combination with food-aid resulted in improvements in weight-for-age and weight-for-height of 2-5-y-old children in the Free State and Northern Cape, but the programme failed to facilitate catch-up growth in stunted children. The authors recommended that socio-economic variables such as sanitation, housing, literacy and employment, be included in integrated interventions if the overall prevalence of stunting is to be decreased (Walsh *et al*., 2002). It has also been suggested that initiatives to address the problem of growth failure should focus on improving the caring capacity of mothers (De Villiers and Senekal, 2002).

In the SADHS of 2003 weight, height, waist circumference and hip circumference of adolescents aged 15 to 19 were measured in a national sample. The interpretation thereof was problematic. The adult guidelines and the age-dependent cut-offs from Brazil that were used resulted in markedly different prevalence figures of anthropometric status (Department of Health *et al*., 2007). The WHO growth references of 2007 were not yet available for the interpretation at the time of data analysis.

Table 2.3: Anthropometric status of South African children nationally and by province in 1994 (SAVACG Study), 1999 (NFCS), 2003 (South Africa Demographic and Health Survey) and 2005 (NFCS-FB)

	Year	Age group	Eastern Cape	Free State	Gauteng	KwaZulu -Natal	Mpuma-langa	Northern Cape	Limpopo	North-West	Western Cape	RSA
	1994	6-71 mo	N=1 540	N=1 420	N=837	N=1 277	N=1 277	N=944	N=1 457	N=1 644	N=842	N=11 238
	1999	1-9 y	N=381	N=203	N=411	N=465	N=144	N=135	N=321	N=229	N=324	N=2 613
	2003	< 5 y	N=149	N=89	N=269	N=51	N=130	N=38	N=173	N=112	N=149	N=1 159
	2005	1-9 y	N=294	N=142	N=483	N=397	N=174	N=47	N=252	N=185	N=183	N=2 157
STUNTING												
% HAZ < -2	1994	6-71 mo	28.8	28.7	11.5	15.6	20.4	22.8	34.2	24.7	11.6	22.9
% HAZ < -2	1999	1-9 y	20.5	29.6	20.4	18.5	26.4	29.6	23.1	24.9	14.5	21.6
		1-3 y	23.2	39.8	26.2	25.1	29.1	30.0	19.9	31.9	14.2	25.5
		4-6 y	19.9	27.2	15.6	16.8	25.0	31.3	29.0	18.3	14.8	20.7
		7-9 y	16.9	3.4	6.0	4.8	21.4	23.5	16.7	17.6	14.8	13.0
% HAZ < -2	2003	< 5 y	28.5	32.9	26.5	13.3	22.2	37.1	26.6	24.0	34.7	27.4
% HAZ < -3			12.4	14.6	13.1	11.2	11.3	14.1	9.2	10.0	12.1	11.9
% HAZ < -2	2005	1-9 y	18.0 (12.9-23.2)	28.2 (19.0-37.3)	16.8 (13.7-19.8)	15.1 (11.1-19.1)	17.8 (12.5-23.1)	27.7 (10.3-45.0)	23.8 (17.6-30.0)	15.1 (11.3-18.9)	12.0 (6.4-17.7)	18.0 (16.3-19.6)
% HAZ < -3			6.5 (3.7-9.2)	7.0 (2.3-11.8)	5.2 (2.9-7.4)	3.0 (1.2-4.8)	5.7 (1.7-9.8)	8.5 (0.0-24.7)	8.3 (4.6-12.1)	4.9 (1.3-8.4)	0.5 (0.0-1.6	5.1 (4.2-6.1)
UNDERWEIGHT												
% WAZ < -2	1994	6-71 mo	11.4	13.6	5.6	4.2	7.3	15.6	12.6	13.2	7.0	9.3
% WAZ < -2	1999	1-9 y	7.1	14.3	8.8	6.0	4.2	23.7	15.0	15.3	8.3	10.3
		1-3 y	10.6	20.4	9.9	6.5	7.3	27.1	14.0	18.6	9.9	12.4
		4-6 y	3.8	9.9	9.4	6.6	3.3	20.8	16.0	12.2	4.9	8.8
		7-9 y	7.2	6.9	2.0	3.6	0.0	17.6	14.8	11.8	11.5	7.7
% WAZ < -2	2003	< 5 y	7.1	15.9	10.1	11.3	9.1	25.8	14.2	12.4	10.9	11.5
% WAZ < -3			0.8	8.2	0.8	10.9	2.2	5.4	1.6	2.4	4.6	2.9
% WAZ < -2	2005	1-9 y	7.8 (3.7-11.9)	14.1 (6.0-22.1)	6.4 (3.9-8.9)	5.0 (2.9-7.2)	10.9 (4.6-17.2)	38.3 (11.1-65.5)	12.3 (7.5-17.1)	12.4 (7.3-17.5)	8.2 (4.0-12.4)	9.3 (7.9-10.7)
% WAZ < -3			-	2.1 (0.0-4.4)	0.8 (0.0-1.6)	0.5 (0.0-1.2)	0.6 (0.0-1.8)	4.3 (0.0-17.3)	2.0 (0.3-3.7)	1.6 (0.0-3.6)	0.5 (0.0-1.7)	1.0 (0.6-1.4)
WASTING												
% WHZ < -2	1994	6-71 mo	3.2	4.5	1.2	0.7	1.7	2.5	3.8	4.5	1.3	2.6
% WHZ < -2	1999	1-9 y	1.8	3.4	1.2	4.3	2.8	9.6	7.5	5.7	0.9	3.7
		1-3 y	2.8	3.2	1.3	2.3	1.8	12.9	11.0	5.3	1.4	4.0
		4-6 y	1.9	1.2	1.6	5.4	3.3	4.2	5.3	7.3	0.8	3.4
		7-9 y	0.0	10.3	0.0	7.2	3.4	11.8	3.7	2.9	0.0	3.4

	Year	Age group	Eastern Cape	Free State	Gauteng	KwaZulu -Natal	Mpuma-langa	Northern Cape	Limpopo	North-West	Western Cape	RSA
% WHZ < -2	2003	< 5 y	0.8	8.3	4.2	7.5	6.0	10.0	5.3	6.0	6.2	5.2
% WHZ < -3			0.0	2.6	3.0	6.8	1.0	3.0	0.0	1.3	2.0	1.8
% WHZ < -2	2005	1-9 y	4.4 (1.3-6.8)	2.8 (0.0-6.2)	3.3 (1.7-4.9)	1.3 (0.2-2.4)	7.5 (2.9-12.1)	19.1 (2.3-36.0)	4.4 (1.5-7.3)	3.2 (0.5-6.0)	11.5 (6.1-16.8)	4.5 (3.6-5.4)
% WHZ < -3			1.4 (0.0-2.7)	-	1.0 (0.0-2.1)	-	2.3 (0.0-5.1)	-	0.4 (0.0-1.2)	-	4.4 (1.8-6.9)	1.0 (0.6-1.4)
OVERWEIGHT												
% WHZ > 2	1999	1-9 y	7.9	6.4	5.6	6.5	16.7	4.4	3.7	0.9	5.2	6.0
% WHZ > 2	2005	1-9 y	6.1 (3.2-9.1)	1.4 (0.0-3.5)	6.4 (4.3-8.5)	6.3 (4.0-8.6)	3.4 (0.0-7.0)	-	2.4 (0.7-4.1)	4.9 (1.4-8.3)	3.3 (0.4-6.2)	4.8 (3.9-5.7)

Sources: Labadarios *et al*., 1995; 2000; Kruger *et al*., 2007; Department of Health *et al*., 2007:150

It is important to evaluate anthropometric status in terms of both stunting and overweight. Mamabolo *et al* (2005) showed that 19% of 3-y-old children residing in the central region of Limpopo province were both stunted and overweight.

Studies in KwaZulu-Natal and the Eastern Cape showed that approximately 20% of infants (<12 mo) were overweight (Table 2.3). In the NFCS of 1999 six percent of 1-9-y-old South African children were overweight, and childhood obesity was higher in urban areas, particularly for children of well-educated mothers (Labadarios *et al*., 2000). The results from the NFCS-BF of 2005 suggest that on the national level the prevalence of overweight has slightly declined to 4.8% (Kruger *et al*., 2007).

In the early 1990s, Cameron *et* a*l*. (1994) reported that rural black girls showed a rapid weight gain in fatness after peak height velocity, and they hypothesized that the fat gain may be a physiological adaptation to an environment of suboptimal energy availability to buffer the energy cost of reproduction. Secondary analysis of the NFCS of 1999 data showed that stunting was associated with an increased risk of being overweight (Steyn *et al*., 2005). A study by Kruger *et al*. (2004) showed that stunted girls seem to be at risk of relatively greater fat deposition, especially in the abdominal area.

2.3 MICRONUTRIENT STATUS

Micronutrient malnutrition is also referred to as “hidden hunger”, as the consequences thereof often go unnoticed. Three micronutrient deficiencies have captured most of the world’s attention in the last decade, namely, vitamin A deficiency, iron deficiency anaemia and iodine deficiency disorders. Zinc deficiency has also recently come to the forefront. Poor and underprivileged children in developing countries are at particular risk of these nutritional deficiencies. In the following sections the main focus is on the results from laboratory (biochemical) assessments of micronutrient status.

2.3.1 Vitamin A

Vitamin A is an essential micronutrient needed for maintaining vision and eye health, integrity of epithelial cells, embryonic development, and maintenance of the immune system (Gibson, 2005). The term vitamin A deficiency includes both clinical and sub-clinical vitamin A deficiency. The prevalence of serum retinol <20 µg/dL (often used as cut-off for sub-clinical vitamin A deficiency) in 6-71-mo-old children as determined by the SAVACG study in 1994 and in 1-9y old children (NFCS-FB) in 2005 is given in Table 2.4.

Table 2.4: Prevalence of serum retinol <20 µg/dL in 6-71-mo-old children in South Africa in 1994 and 1-9 year old children in 2005; by province

	6-71 mo-old children, 1994		1-9 year old children, 2005	
Province	**N**	**%**	**N**	**%**
South Africa	4 283	33.3	1397	63.6
Rural	2 168	37.9	600	67.3
Urban	2 040	25.1	788	60.7
Northern Cape	497	18.5	26	23.0
Western Cape	403	21.0	177	43.5
Gauteng	321	23.5	276	65.2
Free State	626	26.8	133	61.7
Eastern Cape	734	31.1	207	64.2
North-West	442	32.0	121	49.6
Mpumalanga	460	33.0	96	52.1
KwaZulu-Natal	511	38.0	208	88.9
Limpopo	559	43.5	144	75.7

Source: Labadarios *et al.*, 1995; Labadarios *et al*., 2007b

According to these two national surveys, the prevalence of compromised vitamin A status increased (it almost doubled from 33.3% to 63.6%) in the ten-year period between the two assessments. Differences between provinces occurred at both time points. The 2005, data should be interpreted cautiously because of the relatively small sample size (the sample size for the Northern Cape was for example only 26). Nonetheless, KwaZulu-Natal and Limpopo remain the provinces with the highest prevalence of vitamin A deficiency. Equally, those living in rural areas had a higher prevalence of vitamin A deficiency than urban dwellers (Labadarios *et al*., 1995 and 2007b).

Numerous smaller studies reported on the vitamin A status of infants and children (Faber and Benadé, 1999; Oelofse *et al*., 1999; Van Stuijvenberg *et al*., 1999; Faber *et al*., 2001; Oelofse *et al*., 2002; Sibeko *et al*., 2004 ; Faber *et al*., 2005). The studies showed that there are pockets within the provinces where the prevalence of vitamin A deficiency is substantially higher than that reported at provincial level in the 1994 SAVACG study. A study in rural KwaZulu-Natal showed that home-deliveries, the attitude of the caregiver towards family life, and the health status of the infant were risk factors for vitamin A deficiency. All infants who were underweight and all infants of widowed caregivers were vitamin A deficient (Faber and Benadé, 2000). For adults, the only national data come from the NFCS-FB of 2005, where women of child-bearing age were assessed. The results per region are presented in Table 2.5.

Table 2.5: Prevalence of serum retinol <20 µg/dL in South African women (16-35 years) in 2005; by province

Province	**N**	**%**
South Africa	1834	27.2
Rural	791	31.3
Urban	1043	24.2
Northern Cape	-	-
Western Cape	200	9.0
Gauteng	428	20.4
Free State	140	24.3
Eastern Cape	268	22.0
North-West	154	16.2
Mpumalanga	104	11.5
KwaZulu-Natal	347	64.6
Limpopo	161	25.5

Source: Labadarios *et al*., 2007b

Several smaller studies have also reported on the prevalence of vitamin A deficiency in adults (Oelofse *et al*., 1999; Faber *et al*., 2001; Visser *et al*., 2003; Sibeko *et al*., 2004; Kruger *et al*., 2005). A high prevalence of vitamin A deficiency was observed in HIV+ patients. It was further shown that the number of patients with low levels of plasma retinol was significantly higher among those with stage III and IV disease, compared with patients with early disease (Visser *et al*., 2003).

2.3.2 Iron

Iron is an essential micronutrient needed for transferring oxygen from the lungs to tissues, and for electron and enzyme transport (Gibson, 2005). Iron deficiency is the most common and widespread nutritional disorder in the world (WHO, 2001) and is particularly prevalent in infants, children and pregnant women (Gibson, 2005). Iron deficiency is most common among groups of low socioeconomic status (Gibson, 2005). In the interpretation of the prevalence of iron deficiency the indicator (type and cut-off) used must be considered.

The prevalence of compromised iron status in 6-71-mo-old children as determined by the SAVACG study in 1994 and the NFCS-FB of 2005 is given in Table 2.6. Nationally the prevalence of low haemoglobin concentrations (indicative of anaemia) appears to have increased from about 21% to about 28%. Differences among provinces occurred. KwaZulu-Natal had the lowest prevalence of anaemia, while Western Cape and Limpopo had the highest prevalence in both time points. In the 2005 national data set poor iron status was most prevalent in the age group 1-3 years (Labadarios *et al*., 2007c).

Table 2.6: Prevalence of different types of iron status in 6-71-mo-old children in 1994, and 1-9 year old children in 2005 by province

	Children 6-71 mo-old in 1994				Children 1-9 year old in 2005			
Province	**N[a]**	**Hb < 11 g/dL**	**Ferritin < 12 µg/dL**	**Hb < 11 g/dL and ferritin < 12 µg/dL**	**N[a]**	**Hb[b]**	**Ferritin < 12 µg/dL**	**Hb < 11 g/dL and ferritin < 12 µg/dL**
South Africa	4 206-4 494	21.4	9.8	5.0	768-1730	27.9	13.7	11.3
Rural	2 107-2 264	21.1	8.3	4.6	328-706	24.6	11.4	7.9
Urban	2 032-2 169	20.7	12.1	5.4	440-1024	30.1	15.4	13.9
KwaZulu-Natal	474-516	10.4	13.4	3.5	115-256	21.7	12.1	11.3
Gauteng	332-390	16.3	9.2	3.8	154-410	26.6	12.6	10.4
Free State	601-646	17.1	6.8	3.9	62-132	22.0	30.2	16.1
Eastern Cape	457-498	20.6	5.0	2.4	83-238	30.3	6.4	8.4
Northern Cape	475-513	21.5	10.9	6.5	18-36	11.1	5.6	-
North-West	462-553	24.5	8.1	5.0	69-135	28.1	11.9	8.7
Mpumalanga	461-500	27.7	11.5	7.0	43-144	25.0	12.4	11.6
Western Cape	392-413	28.6	16.4	8.2	125-213	38.0	16.5	12.0
Limpopo	552-578	34.2	11.0	9.1	109-173	34.1	16.8	13.8

[a] Sample size varies for the different indicators
[b] Hb < 11 g/dL for <60mo; <15 g/dL for >=60mo;
[c] Ferritin < 12 µg/dL;
[d] Hb < 11 g/dL and ferritin < 12 µg/dL.
Sources: Labadarios *et al*., 1995; Labadarios *et al*., 2007c.

The prevalence of iron deficiency for children has also been reported in smaller studies (Sickle *et al.*, 1998; Faber and Benadé, 1999; Oelofse *et al*., 1999; Van Stuijvenberg *et al*., 1999; Faber *et al*., 2001; Oelofse *et al*., 2002; Sibeko *et al*., 2004 ; Faber *et al*., 2005). These studies reveal the presence of pockets within provinces where the prevalence of iron deficiency is substantially higher than that reported at provincial level. The iron status of adult women of South Africa was investigated in 2005 on a national level as part of the NFCS-FB (Labadarios *et al*., 2007c). The findings are presented in Table 2.7.

Table 2.7: Prevalence (%) of different types of iron status in South African women (16-35 years) in 2005; by province

Province	**N[a]**	**Hb < 12 g/dL**	**Ferritin < 12 µg/dL**	**Hb < 12 g/dL and ferritin < 12 µg/dL**
South Africa	1906-2126	29.4	14.5	10.5
Rural	809-898	31.4	14.1	11.2
Urban	1097-1228	27.9	14.9	10.1
KwaZulu-Natal	382-394	37.6	12.6	8.7
Gauteng	464-517	26.5	19.8	13.2
Free State	104-142	23.2	10.6	9.7
Eastern Cape	265-299	33.1	6.0	6.9
Northern Cape	32-44	6.8	9.4	-
North-West	156-166	17.5	10.9	4.5
Mpumalanga	113-133	33.1	24.8	15.7
Western Cape	204-237	24.9	5.4	3.9
Limpopo	186-194	37.6	27.4	24.7

[a] Sample size varies for the different indicators; Source: Labadarios *et al*., 2007c

From Table 2.7 it is evident that nationally about 29% of women aged 16 to 35 years have haemoglobin levels below 12 g/dL, which is a common cut-off for defining anaemia. Prevalence figures for the provinces range from about 7% in the Northern Cape to 38% in KwaZulu-Natal, but for the formers value the small sample size may be problematic.

Some smaller studies provide an indication of iron status in particular regions, e.g. Oelofse *et al. (*1999) and Faber *et al.* (2001) in KwaZulu-Natal, Kruger *et al.* (2005) in North-West, and Sibeko *et al.* (2004) amongst breastfeeding mothers in the Western Cape.

2.3.3 Iodine

Iodine is an essential micronutrient for thyroid hormone metabolism, and is required for optimal growth and development (Gibson, 2005). Women of reproductive age, pregnant women and young children are at highest risk of iodine deficiency. At school-going age girls tend to be at higher risk than boys (Gibson, 2005). Before 1995, dietary iodine deficiency was widespread in South Africa (Benadé *et al.*, 1997; Kalk *et al.,* 1998). The recommended method for preventing iodine deficiency globally is through the use of iodized salt (Gibson, 2005). In South Africa, mandatory iodization of household salt was introduced through revised legislation in December 1995. A series of studies showed that iodization of salt resulted in dramatic improvements in the short term, both for process and outcome indicators of iodine deficiency and endemic goitre (Witten *et al.*, 2001). However, people of the three northern provinces of the country, rural people, households using predominantly poorly iodized coarse salt, and low socio-economic households are still exposed to under- or non-iodized salt (Witten *et al.*, 2001). A study in the North-West Province showed that urban people had a higher median urinary iodine concentration than rural people. Many rural people used non-iodated salt (Kruger *et al.*, 2005).

In Table 2.8 the prevalence of suboptimal iodine status of women and children as determined in the NFCS-FB of 2005 is given. On the other side of the coin, in six provinces the urinary iodine levels are reportedly excessive (Jooste *et al.*, 2007). Thus nationally the iodine status of children is considered adequate and in some provinces excessive.

Table 2.8: Prevalence (%) of suboptimal iodine status (urinary iodine <100 µg/L) in South African women (N=2237) and children (N=1332) in 2005; by province

Province	**Women (16-35 y)**	**Children (1-9 y)**
South Africa	26.8	19.2
Northern Cape	-	-
Western Cape	25.6	17.7
Gauteng	33.3	21.3
Free State	18.6	10.8
Eastern Cape	24.3	28.8
North-West	33.6	25.2
Mpumalanga	27.9	20.3
KwaZulu-Natal	20.6	11.7
Limpopo	29.7	15.8

Source: Jooste *et al.* (2007)

2.3.4 Zinc

Zinc is an essential micronutrient needed for physical growth, immunocompetence, reproductive function, and neuro-behavioural development (Hotz and Brown, 2003). Epidemiological data on zinc deficiency remains scarce, predominantly due to a lack of reliable indicators for zinc status, but zinc deficiency is

considered as important as iron deficiency (ACC/SCN, 2000). Few South African studies have focused on zinc status. From the NFCS-FB of 2005, information is available for 400 children (Table 2.9).

Table 2.9: Prevalence of zinc deficiency (<65µg/dL) in 1-9 year old children in 2005 in South Africa; by province

Province	N	%
South Africa	400	45.3
Northern Cape	-	-
Western Cape	159	58.5
Gauteng	49	36.7
Free State	41	43.9
Eastern Cape	40	35.0
North-West	56	41.1
Mpumalanga	22	27.3
KwaZulu-Natal	-	-
Limpopo	33	27.3

Source: Dhansay *et al.* (2007)

Two smaller studies reported the prevalence of zinc deficiency in infants. The prevalence of zinc deficiency ranged from 32% in Kayamandi (Western Cape) to 45% in KwaZulu-Natal for infants 6-12 months of age (Oelofse *et al.,* 2002; Faber *et al.,* 2005). In addition, a study on HIV+ patients showed that the number of patients with low plasma zinc concentrations was significantly higher among those with stage III and IV disease (36-45%) compared with patients with early disease (20%) (Visser *et al*., 2003).

2.4 DIETARY INTAKE

The authoritative national studies, which performed a comprehensive dietary assessment focused on children, but in the SADHS of 2003 (Department of Health *et al*., 2007) a dietary screening of adults, resulting in a score/index, was also included. From the national studies only the former (i.e. the NFCS) is reviewed here.

2.4.1 Nutrient intakes

The NFCS of 1999 showed that a large number of children had an inadequate dietary intake of vitamin A, vitamin C, thiamine, riboflavin, niacin, vitamin B_6, vitamin B_{12}, folic acid, calcium, iron and zinc (see Table 2.10). Rural children were worse off than urban children, in line with the trend in 36 developing countries (Smith *et al*., 2004). Food insecurity, whether due to food accessibility or availability, was directly related to an inadequate dietary intake and increased levels of stunting and underweight. In the NFCS-FB of 2005 hunger on the national level was experienced by 51.6% of children aged 19 years, ranging from 29.3% in the Western Cape to 66.7% in the Eastern Cape (Gericke and Labadarios, 2007).

Inadequate intake of micronutrients starts during infancy. Oelofse *et al*. (2002) showed in two urban areas that particularly iron and zinc intakes were low during infancy. Faber (2005) reported that complementary foods consumed by rural infants were of low nutrient density, especially for iron, zinc and calcium.

2.4.2 Food intakes

Food consumption is one of the most important factors in the aetiology of malnutrition. Food items consumed most frequently by 1-9-y-old children as reported during the NFCS of 1999 are listed in Table 2.11.

Table 2.10: Percentage of 1-9-y-old children who consumed less than 67% of the RDA according to the NFCS

Nutrient	Age group	Eastern Cape	Free State	Gauteng	KwaZulu-Natal	Mpuma-langa	Northern Cape	Limpopo	North-West	Western Cape	RSA	Urban	Rural
N	1-3 y	168	96	241	237	60	82	147	115	162	1308	664	644
	4-6 y	170	83	136	217	65	55	144	81	132	1083	513	570
	7-9 y	86	29	50	101	37	16	61	34	63	477	239	238
Energy	1-3 y	37.5	61.5	49	32	63	72	58.5	45	23	45	41	49
	4-6 y	47	66	54	33	58.5	62	56	57	25	47	41	53
	7-9 y	48	72	64	37	59.5	56	64	65	29	50.5	45	56
Protein	1-3 y	12	13.5	7	4.5	10	20.5	11.5	5	2	8.5	8.5	8
	4-6 y	12.4	13.5	7.5	4	9	18	15.5	18.5	4.5	10	6.5	13.5
	7-9 y	20	17	16	7	8	31.5	13	6	0	11.5	7.5	15.5
Vitamin A	1-3 y	68	76	71	62	85	74	56	79	36	65	60	70
	4-6 y	75	78	65	76	82	80	49	81	50	69	64	73
	7-9 y	83	83	84	86	84	87	66	94	56	79	72	85
Vitamin C	1-3 y	74	80	70	65	82	77	83	80	38	69	62	78
	4-6 y	76	88	74	67	75	78	80	70	49	72	67	76
	7-9 y	78	72	72	67	70	100	80	74	44	70	64	77
Thiamine	1-3 y	20	32	23	12	20	50	23	22	14	22	22	21
	4-6 y	19	33	22	11	25	40	19	19	18	20	19	21
	7-9 y	21	24	28	8	14	44	11	9	17	17	16	17
Riboflavin	1-3 y	45	42	34	35	42	55	60	41	15	39	31	48
	4-6 y	66	67	55	55	68	64	71	64	27	58	46	69
	7-9 y	63	72	58	53	59	56	67	62	27	56	43	70
Niacin	1-3 y	64	72	46	39	53	54	50	44	20	47	39	54
	4-6 y	63	60	34	37	43	53	44	51	17	53	33	53
	7-9 y	57	45	42	34	35	50	33	41	14	38	30	46
Vitamin B_6	1-3 y	48	69	47	24	47	67	58	61	11	44	37	51
	4-6 y	45	64	37	22	41	60	53	59	15	40	31	48
	7-9 y	44	59	38	17	35	62	44	50	6	34	27	41
Vitamin B_{12}	1-3 y	53	47	44	41	40	56	73	43	16	45	37	53
	4-6 y	60	52	40	44	48	42	74	52	22	48	35	60
	7-9 y	58	55	50	50	54	37	66	44	14	49	33	64

Table 2.10: Percentage of 1-9-y-old children who consumed less than 67% of the RDA according to the NFCS *(CONTINUED)*

Nutrient	Age group	Eastern Cape	Free State	Gauteng	KwaZulu-Natal	Mpuma-langa	Northern Cape	Limpopo	North-West	Western Cape	RSA	Urban	Rural
Folate	1-3 y	63	85	69	57	83	84	75	80	47	68	62	74
	4-6 y	52	89	64	45	72	64	72	81	32	59	52	65
	7-9 y	45	72	56	46	59	75	69	68	27	53	46	60
Iron	1-3 y	86	92	85	77	78	93	62	81	64	79	78	80
	4-6 y	74	88	63	59	71	82	42	68	45	63	59	66
	7-9 y	70	72	64	53	62	75	36	73	44	58	53	63
Zinc	1-3 y	89	94	89	85	90	91	84	90	70	86	82	90
	4-6 y	85	83	78	66	82	75	76	76	58	74	66	81
	7-9 y	80	83	74	66	78	88	70	79	52	72	63	81
Magnesium	1-3 y	8	5	5	3	7	15	8	3	2	5	5	6
	4-6 y	9	18	7	2	11	29	6	9	10	9	9	9
	7-9 y	14	17	8	4	0	31	2	6	3	7	6	9
Phosphorus	1-3 y	30	28	22	16	25	43	31	18	9	23	21	24
	4-6 y	23	22	15	9	18	33	19	20	10	17	13	21
	7-9 y	28	17	14	8	11	37	11	12	2	14	9	19

Source: Labadarios *et al.*, 2000

Table 2.11: Most frequently consumed food items for 1-9-y-old children as reported in the 24-hr recall of the NFCS 1999

	National N=2868			Eastern Cape N=424			Free State N=208			Gauteng N=427			KwaZulu-Natal N=555			Limpopo N=352			Mpumalanga N=162			Northern Cape N=153			North-West N=230			Western Cape N=357		
	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d
Maize	1	78	442	2	79	439	1	95	541	2	80	369	1	80	420	1	95	524	1	81	463	2	78	400	1	91	483	10	31	278
White sugar	2	76	21	1	80	31	3	58	16	1	80	18	2	76	20	2	59	16	2	80	17	1	80	24	2	85	22	1	86	23
Tea	3	46	235	3	46	277	4	46	220	4	47	230	3	56	226	5	42	227	4	60	227	4	43	215	4	60	225	16	24	252
Whole milk	4	42	167	5	36	159	2	66	203	3	54	130	9	26	122	9	13	144	6	27	186	5	41	125	3	64	141	2	64	247
Brown bread	5	37	101	11	20	90	5	34	98	5	45	86	5	37	104	3	58	117	3	60	116	8	19	95	5	34	107	13	28	90
White rice	6	27	140	4	37	185	14	9	102	11	18	117	4	37	173	21	6	171	15	14	172	6	22	109	13	13	152	3	57	82
White bread	7	27	96	6	36	102				8	26	91	7	30	108				17	10	93	3	46	75	14	12	99	5	57	89
Hard margarine	8	26	13	7	24	13	10	11	14	6	32	12	6	32	13	12	11	10	12	18	10	12	14	10	15	11	9	4	57	17
Chicken	9	25	80	14	12	68	7	16	81	7	30	70	11	25	104	6	29	71	5	36	76	11	17	80	7	25	66	9	36	85
Potatoes	10	22	117	9	22	129	8	15	112	12	17	94	10	26	149	23	6	134	9	21	98	9	18	98	9	20	122	6	48	99
Beef	11	17	108				11	10	86	9	25	88	14	18	143	10	13	131	8	22	81	14	14	87	10	15	97	12	29	108
Fruit (other)	12	15	159	21	8	132	12	9	162	10	19	149	19	10	146	11	12	151	14	15	165	17	12	218	19	9	154	8	41	169
Cabbage group	13	14	82	12	15	87	6	18	84	16	15	72	16	14	103	24	6	67	11	20	86	19	10	73	6	27	75			
Cordial with water	14	14	295	13	12	335	23	4	228	20	10	261	17	14	303	16	9	244	23	7	263							7	43	307
Eggs	15	13	74	18	9	72	15	8	100	15	17	79	18	12	62	14	9	77	13	18	79	15	13	71	16	11	80	18	21	70
Green leaves	16	12	142				9	12	124	19	12	110	20	10	144	4	46	155	18	10	155				25	5	89			
Rooibos tea	17	12	241	24	6	268	18	7	201	14	17	245				8	16	218	10	20	222				11	14	256	19	20	250
Sour milk	18	11	310	8	22	369				23	9	172	8	28	334															
Vegetables (other)	19	11	81				16	8	94	13	17	84	15	14	71	15	9	105	16	13	94	20	9	76	12	13	62			
Non-dairy milk	20	10	7										13	21	7	7	17	7	7	26	6				23	6	8			
Salty snacks	21	9	30	23	6	21				22	10	31	25	6	31	18	8	29	20	8	25							14	27	32
Pumpkin	22	9	92	17	10	106	19	7	103				22	7	107	25	5	80				16	12	115				20	20	82
Peanut butter	23	8	14	22	7	16				17	13	11				13	9	15										24	18	16
Breakfast cereals	24	8	40							18	12	41							25	7	58							11	31	38
Legumes	25	8	166				22	5	127				12	24	199							22	6	63						
Samp & beans				10	21	350																								
Soup				15	12	181	24	4	105							20	6	145												
Samp & rice				16	11	327																								
Mageu				19	8	503																								
Coffee				20	8	219													24	7	237	10	17	299	18	9	265	23	19	214
Sweets				25	5	24																						15	24	28
Fruit (vitamin A-rich)							13	9	195																					
Mutton							17	7	120													7	20	136	21	6	91			
Mabella							20	5	313																8	23	343			
Pilchards/sardines							21	5	143				21	7	79							21	6	91	17	10	87			
Sugar (brown)							25	3	15							17	8	14												
Sweet spreads										21	10	17										13	14	23				22	19	24
Pork & ham										24	8	37	24	6	27													21	19	47

	National N=2868			Eastern Cape N=424			Free State N=208			Gauteng N=427			KwaZulu-Natal N=555			Limpopo N=352			Mpumalanga N=162			Northern Cape N=153			North-West N=230			Western Cape N=357		
	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d	N	%	g/d
Fruit juice										25	8	280	23	7	302	19	8	244				23	6	284	20	8	254	17	22	224
Gravy																			19	10	50									
Fat (Poli-unsaturated / Medium fat)																			21	8	7									
Salads																			22	8	73									
Animal fat																						18	11	14						
Vetkoek																						24	6	133	22	6	161			
Maize / samp rice																						25	6	188	18	9	265	23	19	214
Worms																22	6	63										15	24	28
Cookies / biscuits																									24	5	85			
Cheese																												25	15	24

Source: Labadarios *et al.*, 2000

In Table 2.11, the foods are ranked according to the number of children eating the food during the recall period. This means that the column with the heading "Rank" refers to the rank position of the food, based on the reported number of times the food was reported as being eaten. If a food does not have a rank number, it means that that food was not among the top 25. The percentage of children consuming these foods and the average amount of the food item eaten per day for consumers are also given in the table. Differences among provinces were observed. Maize was one of the most frequently and consistently consumed food items, followed by whole milk and brown bread. Cereals were consumed by 99% of all children. The average consumption of cereals was 493 g for 1-5-y-old children, 559 for 6-9-y-olds and 690-879 g for 10+ y olds when taking the groups of consumers into consideration (Nel and Steyn, 2002).

Overall, the contribution to all nutrients by fruit and vegetables was very low, as were the per capita portions. Consumption of fruit and vegetables was low because of low access and low availability (Steyn *et al.*, 2006b). Fruit and vegetable intake needs to be increased in South African children (Steyn *et al.*, 2006b). This could be achieved through food production in home gardens. In a study in rural KwaZulu-Natal, promotion of local production of β-carotene-rich crops lead to an increase in vitamin A and micro-nutrient intake and ultimately vitamin A status in 2-5-y-old children (Faber *et al.*, 2002a and 2002b).

Dietary diversity for South African children at national level was shown to be low (Steyn *et al.*, 2006c). In Sekhukhune in the Limpopo province, households with low dietary diversity were shown have fewer assets, experienced more food shortages, and were more food insecure when compared to households with a higher dietary diversity (Faber *et al*., 2009).

In the North-West province it was shown that urban adults ate a variety of food, whereas rural adults, especially those living on large commercial farms, ate mainly staple foods. The varied diets of the urban adults contributed to higher intakes of most vitamins and iron, and to higher serum retinol concentrations. Adults from urban areas had significantly higher dietary intakes of most micronutrients than rural adults. Urban adults had higher intakes of animal protein, fruit and vegetables than rural adults (Kruger *et al*., 2005).

2.5 CONCLUSIONS REGARDING NUTRITIONAL STATUS OF SOUTH AFRICANS

This summary of the more comprehensive review of literature on the current nutritional status of South Africans, which was published in a separate report, shows that anthropometrically over-and undernutrition are rife: amongst adult females overweight and obesity are of concern, whilst children suffer from various forms of undernutrition, mainly of a chronic nature. In respect of the micronutrient status vitamin A, iron and zinc undernutrition stand out. The dietary data confirm that inadequate intakes are major contributors to the current malnutrition of South African children.

It is thus concluded that:

- Amongst adult females there is a high prevalence of overweight and obesity (excessive energy status) of which the aetiology was not investigated (yet assumed to be related to the nutrition transition and being multifactorial), and which is evidenced by body mass index exceeding accepted cut-off values (25kg/m^2 and 30kg/m^2 for overweight and obesity respectively).

- Amongst young children there is a medium prevalence of stunting and low to medium prevalence of underweight primarily related to the inappropriate introduction of complementary feeding, but inter-related with other factors. This is evidenced by height (length)-for-age (for stunting) and weight-for-age (for underweight) below a specified cut-off value (usually <2SD from the reference).

- Overall there is a high prevalence of micronutrient malnutrition related to (in the case of children) inadequate nutrient intakes (mainly as a result of insufficient consumption of fruit, vegetables and animal-foods), and evidenced by (combinations of) biochemical indicators of vitamin A status (serum retinol), iron status (haemoglobin, ferritin or combinations thereof) and zinc status (serum zinc).

2.6 STRATEGIES TO ADDRESS MALNUTRITION

Malnutrition, be it over- or undernutrition, can be addressed by treating or preventing the condition. This overview focuses on the latter, with an attempt to highlight evidence-based current best practice.

In order to prevent malnutrition its causes must be known and addressed. This report did not explore the causes of all the forms of malnutrition in detail, yet it can be stated that the causes of the various nutrition-related public health problems of South Africa are not identical and consequently prevention strategies will differ.

2.6.1 Overweight and obesity

The evidence of effectiveness of interventions to prevent overweight and obesity in general has been systematically reviewed by the WHO (WHO, 2009). This adds to many previous original studies and reports, including the International Obesity Task Force (e.g. Kumanyika *et al*., 2002; Swinburn *et al*., 2005), the World Cancer Research Fund (WCRF/AICR, 2009) and recently the Lancet Series on Obesity (e.g. Gortmaker *et al*., 2011). Despite the fact that Lemmens *et al*. (2008), following their systematic review on the evidence of efficacy of obesity prevention specifically in adults, state that "the current evidence of efficacy studies is too small to draw firm conclusions about intervention types or approaches that are more effective than others" the following would represent generally acknowledged current state of knowledge:

Firstly, it is clear that there are many actors when it comes to the prevention of obesity. This ranges from multinational bodies, civil society organisations, government, industry/private sector, (mass) media, schools, workplaces/institutions to professions (WCRF/AICR, 2009; WHO, 2009). Secondly, many intervention strategies are available and the effectiveness of these has been summarised (WHO, 2009). Thirdly, Gortmaker *et al*. (2011) summarise the general implications of current knowledge regarding effective obesity prevention interventions as follows:

- Comprehensive strategies are needed
- Interventions should be integrated throughout society from the international to the individual context
- Coordination, networking and communication will maximise effect
- Life-course targeting of all demographic groups and long-term sustainability of interventions are important, where initial, limited impact work can form the base for subsequent long-term interventions
- Direct causes (related to energy imbalance, i.e. dietary energy intake versus energy expenditure in physical activity) and indirect influences (e.g. the physical, economic, social and personal dimensions / environment) should be addressed
- Governments should fund research on monitoring and evaluation of obesity prevention interventions
- Prevention of obesity should be considered alongside other major issues that confront societies

Many of the above but in particular the last point suggest that other nutrition problems of a society should be kept in mind when implementing obesity prevention strategies. In the case of South Africa child undernutrition and micronutrient deficiencies should thus inform decisions regarding the optimal strategy.

2.6.2 Undernutrition

Child stunting and underweight, as well as micronutrient deficiencies represent mainly undernutrition. The causes of undernutrition are universally summarised in terms of a framework, the so-called UNICEF conceptual framework, where three key areas, i.e. food insecurity, inadequate maternal and child care, and insufficient health services and unhealthy environment are considered the underlying causes of undernutriton. These are usually inter-related and each warrants attention, even though the relative importance can vary from one situation to another and may change.

Numerous publications and organizations have dealt with the (cost) effectiveness of interventions to address undernutrition (FAO/ILSI, 1997; Allen and Gillespie, 2001; Ismail *et al*., 2003; Lancet series, 2008; Bezanson and Isenman, 2010; Pearson and Ljungqvist, 2011). The “window of opportunity” for interventions is seen as from conception to 24 months of age for high impact in reducing death and disease and avoiding irreversible harm. Priority interventions (from the abovementioned publications) include those listed in Table 2.12.

In the current South African context under- and overnutrition often co-exist in the same communities, and even in the same household. Furthermore, there appears to be an overlap in the potential strategies, in the sense that increased intakes of fruit and vegetables will not only address micronutrient intakes per se, but would at the same time be advisable for weight management (this refers in particular to vegetables) and be in line with the general health promotion messages contained in the South African food-based dietary guidelines. Given that the rural poor in South Africa are disproportionately affected by the burden of malnutrition, investigating the options of home production of such crops and the potential contribution of wild growing plants, for example green leaves, known to be part of the traditional African cuisine, to the dietary intakes and nutritional status appears appropriate.

Table 2.12: Examples of strategies to prevent undernutrition

Prevent low birth weight (by improving maternal nutrition)	• Food supplementation during pregnancy • Micronutrient supplementation during pregnancy • Improve adolescent health and nutrition
Improve child growth	• Promote exclusive breastfeeding, including early initiation of breastfeeding and continued breastfeeding after introduction of complementary feeding • Improve complementary feeding for infants after age 6 months
Increase intake of vitamins and minerals (prevent micronutrient deficiency)	Non-food approaches • Periodic high-dose vitamin A supplements • Zinc supplements for diarrhoea management • Multiple micronutrient powders (e.g. sprinkles) • Deworming drugs (reduce losses of nutrients) Food-based approaches • Increase small-scale production of micronutrient-rich foods • Increase commercial production of micronutrient-rich foods • Maintain micronutrient levels in commonly eaten foods • Plant selection and breeding to increase micronutrient levels (biofortification) • Food fortification • Communication strategies to increase consumption of micronutrient-rich foods (nutrition education
Non-nutrition interventions (care and health related)	• Hygiene practices (hand washing with soap) • Household water treatment • Bed nets • Conditional cash transfers and safety nets

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AFRICAN LEAFY VEGETABLES IN SOUTH AFRICA

CHAPTER 3: AFRICAN LEAFY VEGETABLES

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3.1 INTRODUCTION

In South Africa, the use of green leafy vegetables as food is as old as modern man. The !Kung people, who have lived in Southern Africa for at least 120 000 years, relied heavily on the gathering of plants from the wild for their survival (Fox and Norwood Young, 1982; Parsons, 1993). The Bantu-speaking tribes, which started to settle in South Africa about two thousand years ago, also collected leafy vegetables from the wild (Bundy, 1988). Hunting and the collection of edible plants were particularly important in their food acquisition systems during times of emergency, when crops had failed or livestock herds had been decimated (Peires, 1981; Bundy, 1988).

Of all countries in Sub-Saharan Africa, western influences have been most intense in South Africa. This had a major influence on the food consumption patterns of local people. Yet, in contemporary South Africa, collecting green leafy vegetables for use as food continues to be widespread practice among rural African people (Bhat and Rubuluza, 2002; Jansen van Rensburg *et al*., 2004; Husselman and Sizane, 2006; Modi *et al*., 2006; Faber *et al*., 2010).

This chapter starts with an analysis of the concept ‘African leafy vegetables’ (ALVs). The apparent controversies that surround the meaning of ‘traditional’, ‘indigenous’, ‘indigenised’ and ‘exotic’, which are used to describe food plants in relation to particular areas, such as Africa, are dealt with and a proposal is made for conceptual clarity. The importance of these terms for research on food plants is pointed out. In the second part of the chapter, the use and current status of ALVs in South Africa is reviewed. In the third part, the potential role of ALVs in improving nutrition among vulnerable groups of people in South Africa is explained. In the last part, an overview of important South African leafy vegetable species is presented and the choice of eight ALV species that were selected for further research is justified.

3.2 ‘TRADITIONAL’, ‘INDIGENOUS’, ‘INDIGENISED’ AND ‘EXOTIC’ AFRICAN LEAFY VEGETABLES: CONCEPT ANALYSIS AND ELABORATION

The meaning of the term ‘vegetable’ is understood by most people. Yet, what constitutes a vegetable is difficult to define, because it is linked to culinary and cultural traditions, which vary spatially and temporally. Plant parts eaten as vegetables include leaves, petioles, bulbs, stems, tubers, roots, flower, fruits and seeds and these can be consumed raw or cooked (Splittstoesser, 1990). Leafy vegetables are plant

species of which the leafy parts, which may include young succulent stems, flowers and fruits, are used as vegetables (Jansen van Rensburg *et al*., 2007). In other parts of this report, reference is made to 'green' or 'dark green' leafy vegetables. These adjectives are significant, because they distinguish between leaves that were exposed to light, which produces the green colour, and leaves that were not, as in the case of white cabbage (*Brassica oleraceae* L. var. *capitata*) and Belgian endive (*Cichorium endivia* L.). This distinction is important for human nutrition, because dark green leaves tend to be denser in nutrients than pale-green, yellow or white leaves (Faber *et al*., 2007).

As indicated in the title, this chapter deals with ALVs. Before this concept is defined, it was deemed useful to deal with some of the controversies that surround the labelling of plants that are used as leafy vegetable species on the African continent. There are a host of terms describing the leafy vegetables that are consumed by African people. Among others, these include African traditional leafy vegetables (Gockowski *et al*., 2003), indigenous leafy vegetables (Mooketsi, 2011), traditional leafy vegetables (Orech *et al*., 2005; Etèka *et al*., 2010) and ALVs (Abukutsa-Onyango, 2007; Jansen van Rensburg *et al*., 2007). Schippers (2002) and Shackleton *et al*. (2009) argued that the meaning of all these terms is subject to dispute and identified the need for the clarification of these concepts.

Within specified spatial boundaries, which can be defined locally, nationally or continentally, two main categories of food plants, in this case leafy vegetables, can be identified, namely a category that is made up of 'traditional' species and another that is comprised of 'exotic' species (Shackleton *et al*., 2009). In the context of the African continent, the label 'exotic' is assigned to food plants that have been recently introduced in Africa, whilst the label 'traditional' is given to food plants that have been used over a long period of time. Whereas the distinction between traditional and exotic is often made in relation to space, i.e. Africa, the term 'traditional' is located in the social domain. Food plants and more specifically crops, which can be defined as food plants that are cultivated, are called 'traditional' in relation to a group of people. Broadly speaking, groups of people occupy fairly well delineated physical spaces[1] and this explains why food plants are called 'traditional' in relation to a particular area. However, it is the people living in that area that have learnt where and how to find and harvest these plants, or how to produce them under local growing conditions, and how to prepare them for consumption. The accumulated knowledge covering harvesting or production, storage, consumption and reproduction of particular food plants, which is passed on from generation to generation, and is continuously added to through observation and experimentation, constitutes an indigenous knowledge system. Therefore, typical of a 'traditional' food plant is the rich indigenous knowledge system that surrounds it.

Knowledge systems develop over time and this means that food plants 'become' traditional through a process of indigenisation. Phillips-Howard (1999) defined indigenisation as the incorporation by a people of externally derived technology and other resources (such as new crops) into their culture. Accordingly, a food plant can be labelled 'traditional' when it has been indigenised. Phillip-Howard suggested that once the process of indigenisation has proceeded sufficiently for people to consider externally derived ideas, technology, techniques or materials, such as new food plants, as legitimate elements of their own culture and knowledge system, these incorporations can be regarded as 'indigenised'. Even though distinction between 'traditional' and 'exotic' food plants can never be clear-cut, various indicators can be used to assist differentiation. One of these is language. Traditional crops typically have local names (Van Averbeke *et al*., 2007). Another is reproduction. Typically, traditional crops are reproduced by local farmers, through the

[1] People who migrate tend to transfer their food preferences to their new places of residence and work, creating new markets for crops and foods previously unknown in these places, enriching the local food culture and opening opportunities for related enterprise development in agriculture and the food industry at large.

collection and storage of seed (Van Averbeke *et al*., 2007). The presence of 'land races' each with specific characteristics, often captured by labels (descriptive names in the local language), is another indication that the food plant has the status of a traditional crop in a particular area, because land races are evidence of farmer selection over many years (Schneider, 1999; Van Averbeke *et al*., 2007). A fourth indicator is the extent of local knowledge that surrounds the cultivation, consumption and reproduction of the crop. Limited or borrowed knowledge is an indication that the crop is exotic. Deep, local knowledge is characteristic of traditional crops (Van Averbeke *et al*., 2007).

From a botanical perspective, the category of traditional food plants can be subdivided into two sub-categories, namely indigenous and indigenised food plants. Here the distinction between indigenous and indigenised is not cultural but geographic. A traditional food plant is called indigenous to an area, when it was not deliberately or accidentally introduced to that area and when its occurrence in that area is the result of its natural dispersal from where it originated or was native (Bean, 2007). Indigenised food plants can then be defined as 'traditional' food plants that were deliberately or accidentally introduced to an area. For this reason, it is proposed that the label 'traditional' be used with specific reference to a group of people who occupy a specific area and that this label be assigned only to those food plants that are surrounded by rich indigenous knowledge systems. Furthermore, it is proposed that the label 'indigenous' be used to refer to food plants that are indigenous to the area in a botanical sense and that the label 'indigenised' be used to refer to all other traditional food plants. Differentiation between indigenous, indigenised and exotic food plants is important from the perspective of adaptation. It can be argued that indigenous food plants are particularly well adapted to the local ecology as a result of natural processes over long periods of time. Indigenised food plants have also been subjected to a process of adaptation, either through natural adaptation, when they grow in the wild or through human intervention in the form of farmer selection when they are grown as crops. The process of crop adaptation through farmer selection was aptly illustrated by Schneider (1999) for sweet potatoes in Papua New Guinea, where following the introduction of this crop about four centuries ago, a total of about 1000 land-races had been developed, some of which were adapted to specific micro-environmental conditions or natural stress factors, such as flooding. The potential for high levels of adaptation to local conditions of the different plant species used by different groups of African people in South Africa, particularly with regard to drought tolerance, was central in the thinking that yielded the research proposal for WRC Project K5/1579//5.

African people in South Africa refer to plant species of which the green leaves are used as food by means of a collective label such as *merogo* (Sesotho) and *imifino* (isiZulu, isiXhosa), which freely translated means green leafy vegetables. This label is particularly useful when approaching green leafy vegetables from the perspective of contemporary indigenous knowledge. What exactly constitutes *merogo* or *imifino* is subject to spatial and temporal variation. The plant species that are included under the label depend on the local ecology and culinary traditions (Levy *et al*., 1993; Gericke, 2000; Jansen van Rensburg *et al*., 2007; Van Wyk and Vorster *et al*., 2009). Focusing on the use rather than the origin of plant species, these terms allow for the addition of new species to the collective, including fairly recently introduced exotic species. One of these species is Swiss chard. White cabbage, on the other hand, is not regarded as *morogo* or *imifino*, probably because the leaves of white cabbage are not green. The distinction between use and origin is important, because the terms *morogo* and *imifino* are used to refer to all the different green leafy vegetables that are consumed, irrespective of whether they are harvested from indigenous plants, indigenised plants or from recently introduced plants, such as Swiss chard, beetroot or sweet potatoes (Faber *et al*., 2007). Accordingly, in this chapter, ALVs are defined as 'the collective of green leafy vegetable species that form part of the culinary repertoire of particular contemporary African communities'. However, from an agronomic perspective it remains important to distinguish between traditional and exotic species and

between indigenous and indigenised among the traditional plant species. This distinction can easily be indicated by referring to indigenous, indigenised and exotic ALVs.

3.3 USE AND STATUS OF AFRICAN LEAFY VEGETABLES IN CONTEMPORARY SOUTH AFRICA

Traditionally, the collecting of leafy vegetables and the knowledge associated with this practice has been a female domain among both the !Kung (Fox and Norwood Young, 1982; Parsons, 1993) and the Bantu-speaking tribes (Jansen van Rensburg *et al.*, 2004). In contemporary South Africa, collection continues to be associated with women but once a particular plant species becomes domesticated and is grown as a crop, men readily become involved, especially when its production is commercialised (Van Averbeke *et al*., 2007). Leafy vegetables also tend to be regarded as a female food but gender distinctions in their consumption are much less universal than in their collection (Whitbread, 1986; Hart and Vorster, 2006).

In South Africa, Wehmeyer and Rose (1983) identified more than 100 different species of plants that were being used as leafy vegetables. A shortlist of important species and their utilisation is provided in Table 3.1. ALVs are harvested from the wild or from fallowed and cultivated fields. Many of the plant species from which the leaves are collected, grow as weeds but there are also selected species that are cultivated for use as leafy vegetables. For most species the young growing points and tender leaves are the plant parts that are used in the preparation of vegetable dishes. Petioles and in some cases young tender stems are also included, but old, hard stems are discarded (Vorster *et al*., 2009). The leaves and other selected plant parts are prepared as pot herbs or as relishes, primarily to accompany maize and sorghum porridge. The leafy vegetable dishes may be prepared from a single species or from a combination of different species (Faber *et al*., 2007). Other ingredients, such as tomatoes, onions, peanut flour and spices may be added to the leafy vegetables to enhance their taste (Tshikalange, 2006). Cooking methods vary from thorough boiling, which may include the replacement of the first cooking water with fresh water in the case of bitter-tasting species, such as *S. retroflexum* (Van Averbeke *et al*., 2007), to steaming involving the use of very small quantities of water and short cooking times, as in the case of the leaves and flowers of pumpkin. According to Vorster *et al*. (2005), the recipes used to prepare the different leafy vegetables tend to be fairly homogeneous among people belonging to a particular cultural group, limiting culinary diversity.

ALVs are seasonal and highly perishable. To extend the period during which they are available, different ways of preserving these vegetables have been developed. The two main methods are the sun-drying of fresh leaves and the sun-drying of blanched or cooked leaves. Both these methods transform the leafy vegetables into dry products that have long shelf lives (Vorster *et al*., 2005). Invariably the dry vegetables are re-hydrated by cooking in water. Electrification of the rural areas has introduced new preservation technologies, including the blanching and freezing of leaves (Van Averbeke *et al*., 2007).

The role of ALVs in the food consumption patterns of South African households is highly variable and depends on factors such as poverty status, degree of urbanisation, distance to fresh produce markets and time of year (Vorster *et al*., 2002). Quantitatively, collection and consumption of ALVs tend to be inversely proportional to household income (Vorster *et al*., 2002). Poor households tend to use ALVs more than their wealthier counterparts, because they lack the financial means to purchase vegetables and the means to produce their own (Vorster *et al*., 2002). The use of wild food, including ALVs, forms part of the safety net that rural people use to cope with poverty, disaster or livelihood stress (Rose and Guillarmod, 1974; Rubaihayo, 1997; Shackleton *et al*., 2000).

Table 3.1: African leafy vegetables commonly harvested from the wild or obtained through semi-cultivation or full cultivation in South Africa

Crop Species	Limpopo	Mpumalanga	Gauteng	Free State	North West	KwaZulu-Natal	Northern Cape	Eastern Cape	Western Cape
Abelmoschus esculentus Moench.	⭘					○			
Amaranthus spp. (*A. cruentus* L., *A. Graecizans* L., *A. retroflexus* L., *A. spinosus* L., *A. thunbergii* Moq., *A. tricolor* L. and others)	◎	○	○	○	○	◎		◎	
Bidens spinosa L.	○				○	◎		◎	
Brassica rapa L. subsp. *chinensis*	●		●						
Chenopodium album L.				○	○	○		○	
Citrillus lanatus Thunb.	⭘	◎			◎	◎			
Cleome gynandra L.	◎	○			○	○		◎	
Corchorus olitorius L.	◎	○							
Cucumis melo L.	○								
Cucurbita spp. (*C. maxima* Duchesne, *C. pepo* L. and *C. moschata* Duchesne ex Poir.)	●	●	●	●	●	●	●	●	●
Galinsoga parviflora Cav.								○	
Momordica balsamina L.	⭘	○							
Portulaca oleracea L.							○	○	
Solanum retroflexum Dun.	●	○				○		○	
Vigna unguiculata (L.) Walp	⭘	●	◎	◎	⭘	●		●	

○ = consumed ◎ = rarely cultivated (<20%) ⭘ = often cultivated (20-75%) ●= mostly cultivated (>75%)

During periods of drought or when the breadwinner in the household becomes unemployed, affected rural households intensify their collection and consumption of wild food (Shackleton *et al.*, 1999; Dovie, *et al.*, 2002; Shackleton, 2003). Social disturbances can also lead to increased use of leafy vegetables from the wild. For example, during the pre-1994 political struggle in the Transkei region of the Eastern Cape, people fleeing their houses to escape violence relied heavily on food collected from veld and forest for survival (Jansen van Rensburg and Vorster, 2005). In poor rural communities consumption of wild food is particularly important for women and children (Shackleton *et al*. 2002a; Vorster and Jansen van Rensburg, 2005). In rural areas the use of ALVs is enhanced by remoteness, because households in remote rural areas have limited access to fresh produce markets (Jansen van Rensburg and Vorster, 2005; Hart and Vorster, 2006). Urban households use ALVs less than rural households, because they lack access to localities where these vegetables grow naturally. It is for this reason that urban areas have great potential for the marketing of the marketing of ALVs.

The monetary value of the products rural people harvest from the wild, including leafy vegetables, has been determined using case studies in the Limpopo, KwaZulu-Natal and Eastern Cape Provinces of South Africa (Shackleton *et al*., 1999; Magasela *et al*., 2001; Twine *et al*., 2001; Shackleton *et al*., 2002a; Shackleton *et al*., 2007). Across the case studies, the most widely used leafy vegetable species were *Amaranthus hybridus*, *Bidens pilosa*, *Bidens biternata*, *Cleome gynandra*, *Chorcorus tridens*, *Chenopodium album* and *Tribulus terrestris*. Use of these leafy vegetables was largely restricted to home consumption. Generally, harvesting leafy vegetables from the wild was most important at the study sites in Limpopo Province and least at the study sites in the Eastern Cape.

On the African continent as a whole, production, trade and consumption of ALVs is expanding (Schippers, 2000, 2002, 2006). In South Africa, the trend is less clear. South African researchers and policy makers have ignored ALVs for a long time but this has changed during the past two decades. ALVs have increasingly received research attention and at the policy level the value of these plants has been recognised (Department of Agriculture, 2004). The growing interest in ALVs in both research and policy circles contrasts sharply with the negative image these plants have developed among important groups of potential consumers in South African society, particularly the youth and the urbanised, who tend to associate their consumption with poverty and the past (Vorster *et al*., 2002 and 2009; Hart and Vorster, 2006). Even in selected rural areas of the country a decline of the consumption ALVs in favour of exotic vegetables has been observed. This was evident from the case study conducted by Jansen van Rensburg and Vorster (2005) in three parts of the former Transkei in the Eastern Cape Province. This study showed that the use of ALVs had been declining at all three study sites, particularly at Qunu, where access to western vegetables was the easiest, because of its proximity to Mthatha, the largest urban centre in the Transkei region. At Dimfi, which was the most remote of the three rural study sites, the decline in use was least. By contrast, during the past 50 years in the Vhembe District of the Limpopo Province, the status of nightshade (*Solanum retroflexum* Dun.) has been elevated from that of a wild to that of a fresh produce commodity that is being cultivated extensively under irrigation by local smallholders and retailed by both petty traders and large supermarket outlets in the District (Van Averbeke *et al*., 2007).

Marketing of ALVs in South Africa is still limited and mostly restricted to dried products (Vorster *et al*., 2002; Vorster and Jansen van Rensburg, 2005; Hart and Vorster, 2006). Whitbread (1986) reported the marketing of different species of amaranth, which were collected as weeds from fields and gardens in the KwaZulu-Natal Midlands. Marketing of amaranth was mainly by petty traders, but the leaves were available at the municipal fresh produce market of Pietermaritzburg and were also found on the shelves of selected green grocers in that city. According to Weinberger and Pichop (2009), 88 traders at informal markets in Durban and Soshanguve (Plate 3.1) sold 27 tons of ALVs in one year with a retail value of R67 000.

Plate 3.1: An informal trader selling amaranth and 'modern' vegetables at the Soshanguve train station

A case study of the street trade in ALVs that were produced at the 136 ha Dzindi smallholder canal irrigation scheme in the Vhembe District of the Limpopo Province in 2009 showed that the population of 101 street traders who purchased fresh produce from farmers at the scheme derived a total of R345 304 from the sale of Chinese cabbage, nightshade and pumpkin leaves (Manyelo, 2011). Even though this income contributed only 4.3% to the total trade income of the 101 fresh produce street traders, the findings do underline the potential of ALVs production to contribute to the livelihoods of smallholder farmers and people involved in economic activities that are linked to the production of these crops.

3.4 THE POTENTIAL CONTRIBUTION OF AFRICAN LEAFY VEGETABLES TO HOUSEHOLD FOOD SECURITY

In Chapter 2 of this report, it was pointed out that micronutrient malnutrition is widespread in contemporary South Africa. Supplementation, food fortification, bio-fortification and dietary diversification are the main strategies that exist to address micronutrient deficiencies (Faber and Wenhold, 2007). Steyn *et al.* (2001) argued that dietary diversification be favoured, because it represented a sustainable solution to malnutrition. Consumption of green leafy vegetables is one of the measures that can be used to enhance dietary diversification. The dark green leaves of plants, such as amaranth and nightshade are known to be denser in micronutrients, such as beta-carotene, vitamin C, protein, calcium and iron, than the pale green leaves of vegetables, such as cabbage and lettuce (Latham, 1997).

It is imperative to consider factors affecting the bio-availability of micronutrients in plant foods to ensure long-term effectiveness of programmes designed to improve the nutritional status. Data on the nutrient content of the ALVs that are commonly consumed in South Africa are limited. Green leaves are biological matter and show natural variability in nutrient composition. Factors affecting natural variability include variety, seasonality, climatic conditions, edaphic factors, growth stage at harvest and method of harvesting (Hornick, 1992). Post-harvest factors affecting nutrient content include storage conditions, preparation methods and processing.

Leafy vegetables are high in bulk and low in protein, fat and energy, mainly because of their high water content. Carbohydrates are the main source of energy they contain (Uusiku *et al.*, 2010). Micronutrient content of leafy vegetables is highly species dependent, but in general leafy vegetables contain substantial amounts of carotenoids (lutein and β-carotene), iron, folate, riboflavin and calcium (Uusiku *et al.*, 2010). However, dark green leafy vegetables are known to contain oxalates, phytates and nitrates, compounds that reduce absorption of certain micronutrients (Latham, 1977; Steyn *et al.* 2001). Nesamvuni *et al.* (2001)

collected and analysed the ten most commonly consumed wild green leaves eaten in Venda and showed that a portion of 180 g cooked leafy vegetables would make a significant contribution to the daily requirement of women for iron, vitamin C, folate and beta-carotene. Although some leafy vegetables contain substantial amounts of vitamin C in the unprocessed form, processing greatly affects the vitamin C content (Uusiku *et al*., 2010).

3.4.1 Vitamin A

Dark green leafy vegetables in general are considered to be good sources of β-carotene, but there is some controversy around the potential of dark green leafy vegetables to effectively address vitamin A deficiency (De Pee *et al*., 1995). This uncertainty is based largely on questions regarding the conversion factor that has been used for the bioconversion from β-carotene from food to retinol in the body. It is generally accepted that 6 μg of β-carotene equals 1 μg retinol (FAO/WHO, 2001). However, De Pee *et al*. (1998) proposed that 1 μg retinol is equivalent to 26 μg β-carotene for leafy vegetables. FAO/WHO (2001) acknowledge that recent data in general suggest the need for a revision towards lower bioavailability, but until definitive data are available, their recommendation remains to use a conversion factor of 6 μg.

Despite the uncertainty about the bio-availability of β-carotene in dark-green leafy vegetables, controlled studies have demonstrated that consumption of cooked and pureed green leafy vegetables improved the vitamin A status of children, men and pregnant women (Takyi, 1999; Haskell *et al*., 2004; Haskell *et al*., 2005). A South African study showed that consumption of dark-green leafy vegetables and other vegetables rich in β-carotene that were produced in home-gardens improved children's vitamin A status (Faber *et al*., 2002).

Preparation of green leafy vegetables could improve the bioavailability of β-carotene. For example, the bioavailability of β-carotene in processed spinach was found to be higher than in the raw spinach (Rock *et al*., 1998). Adding fat during preparation of green leafy vegetables is expected to enhance vitamin A status of consumers because fat enhances carotenoid absorption (Jayarajan *et al*., 1980). The addition of fat (Jalal *et al*., 1998) or foods with a high fat content, for example avocado fruit (Unlu *et al*. 2005), to a meal that contains β-carotene sources enhances carotenoid absorption. It appears that between 3 g (Jalal *et al*., 1998) and 5 g (Jayarajan *et al*., 1980) fat per meal is required to ensure maximum carotenoid absorption. Interventions aimed at improving the vitamin A status of groups of people that are at-risk must, therefore, include recommendations to add the minimum amount of fat to the meal. Conversely, microwave cooking, steaming, boiling, and sautéing (or stir-frying) result in provitamin A losses. Substantial losses of provitamin A occur with deep-frying, prolonged cooking, combinations of several preparation/processing methods, baking, and pickling (Rodriguez-Amaya, 1997).

To optimise vitamin A absorption, it is imperative to prevent intestinal helminthes. Controlled feeding trials with dark green leafy vegetables showed that de-worming had a beneficial effect on the bio-availability of β-carotene and consequently vitamin A status of children (Jalal *et al*., 1998; Takyi 1999).

3.4.2 Iron

Dark-green leafy vegetables contain relatively large amounts of iron, but they also contain oxalates and phytates, which inhibit the absorption of non-heme iron (Latham, 1977; Steyn *et al*. 2001). The bioavailability of non-heme iron in plant foods is therefore low and the potential contribution of plant foods towards controlling iron deficiency in developing countries has been questioned (De Pee *et al*., 1996). Agricultural interventions to increase the supply and intake of iron from plant foods are not popular. Instead, the production and consumption of animal foods are usually encouraged, because of the high bioavailability of heme iron from animal foods (Ruel, 2001).

Removing the antinutrients or inhibiting compounds from green leafy vegetables, preferably using traditional preparation methods, could increase the bioavailability of iron. This has been extensively studied in cereal-based diets (Makokha 2002; Lestienne *et al*., 2005).

From this overview, it can be concluded that ALVs offer potential nutritional benefits for nutritionally vulnerable populations. Effective nutrition education programmes promoting these vegetables will enhance the impact on the nutritional status of communities at risk.

3.5 IMPORTANT AFRICAN LEAFY VEGETABLES IN SOUTH AFRICA

In South Africa, ALVs are mostly gathered and not obtained by means of cultivation. Limited seed broadcasting in fields does occur (Vorster *et al*., 2002; Hart and Vorster, 2006). Selected species, particularly pumpkins, melons, cowpeas and amadumbe (taro) are being cultivated fairly widely (Kirsten, 1977; Fox and Norwood Young, 1982; Hart and Vorster, 2006). Several of the most popular ALV species, such as amaranth and spider flower, are pioneer plants, which emerge naturally when soils are disturbed as a result of cultivation. They are regarded as weeds in commercial farming systems (Grabandt, 1985), but not in African smallholder cropping systems. Women, who do most of the weeding in smallholder cropping systems, often distinguish between undesirable weed species, which are hoed or pulled out, and species that belong to the local collective of ALV species, which are harvested or left undisturbed for subsequent use (selective weeding) (Hart and Vorster, 2006). Most of the species that are consumed as leafy vegetables grow in summer. Exceptions are the local *Brassica* species and *Chenopodium album*, which grow during winter (Levy *et al*., 1936; Whitbread, 1986). The distribution and cultivation status of an abridged list of African vegetables is presented in Table 3.1.

The popularity of specific species is function of many factors, including availability, ease of preparation, taste, consistency and appearance. Ubiquitous availability of amaranth species explains why these plants are used as a leafy vegetable in most parts of South Africa. The soft, fast-cooking leaves of pumpkin and nightshade species are preferred to the fibery leaves of cowpeas and old amaranth plants, which require long cooking times (Fox and Norwood Young, 1982). Taste, another very important factor, is subject to regional and gender diversity. In the north of South Africa the bitter taste of nightshade and cleome are highly appreciated, particularly by males, whereas in the south the sweet taste of amaranth leaves is preferred (Vorster *et al*., 2002; Vorster *et al*., 2009). Similarly, many people in the north enjoy the mucilaginous texture of Jew's mallow (wild jute), whilst people in the south find the sliminess offensive (Vorster *et al*., 2002).

Ranking ALVs in terms popularity at a national level is necessarily subject to a degree of bias, because of the spatial variability in the plants that are used. The available evidence from different parts of the country indicates that seven groups of species are most important. These are amaranth (*Amaranthus* spp.), spider flower (*Cleome gynandra*), non-heading Chinese cabbage (*Brassica rapa* subsp. *chinensis*), nightshade (*Solanum nigrum* complex*)*, Jew's mallow (*Corchorus olitorius* and *C. tridens*), cowpeas (*Vigna unguiculata*) and pumpkins (*Cucurbita pepo, C. maxima* and *C. moschata*), melons (*Cucumis melo*) and selected other indigenous and traditional cucurbits, such as balsam pear (*Momordica balsamina*) and tsamma melon (*Citrillus lanatus*).

3.5.1 Amaranth (*Amaranthus* spp.)

Amaranth is known as *misbredie, hanekam, varkbossie* in Afrikaans, pigweed, cockscomb and hell's curse in English, *unomdlomboyi, imbuya, umifino umtyuthu* in isiXhosa, *imbuya, isheke, indwabaza* in isiZulu, *thepe, theepe* in IsiPedi, Sesotho and Setswana, *umbuya, isheke* in siSwati, *vowa, theebe* in Tshivenda and *theyke, cheke* in Xitsonga (Fox and Norwood Young, 1982; Bromilow, 1995; Van Wyk and Gericke, 2000, Vorster *et al*., 2002).

Amaranth belongs to the Amaranthaceae family and is an extremely variable, erect to spreading, herbaceous herb. The height of mature plants varies between 0.3 m and 2 m, depending on the species, growth habit and environment. Some species have distinct markings on their leaves. Terminal and auxiliary inflorescences occur. The small seeds of the leafy amaranths are usually very shiny and dark brown to black, contrary to the grain types, which usually have seeds that are cream coloured. Different species of amaranth are utilized all over South Africa, except in the arid south-western areas (Schippers, 2000; Van Wyk and Gericke, 2000; Hart and Vorster, 2006; Vorster *et al*., 2009).

Amaranth is a C4 plant that grows optimally under warm conditions (day temperatures above 25ºC and night temperatures not lower than 15ºC, bright light and adequate availability of plant nutrients (Van den Heever and Coertze, 1996a; Maboko, 1999; Schippers, 2000). The various amaranth species are tolerant to adverse climatic conditions (Grubben 2004; Maundu and Grubben, 2004). They are quite drought-tolerant, but prolonged dry spells induce flowering and decrease leaf yield (Schippers, 2000; Palada and Chang, 2003). Amaranth is photoperiod sensitive and starts to flower as soon as the day length shortens. Under cultivated conditions amaranth produces fresh leaf yields of up to 40 t ha^{-1} (Van den Heever and Coertze, 1996a; Maboko, 1999; Schippers, 2000; Mhlonthlo *et al*., 2006).

Amaranthus thunbergii (L.), *A. greazicans* (L.) (Plate 3.2), *A. spinosus* (L.), *A. deflexus* (L.), *A. hypochondriacus* (L.), *A. viridus* (L.) and *A. hybridus* (L.) are among the most widely used amaranth species in South Africa (Norwood and Fox Young, 1982; Schippers, 2000; Van Wyk and Gericke, 2000; Vorster *et al*., 2002; Hart and Vorster, 2006). Plate 3.3 illustrates the variation in morphology of different amaranth species. According to Germishuizen *et al*. (2006) there are 16 indigenous and indigenized *Amaranthus* species in South Africa. The young leaves, growing points and whole seedlings of amaranth are harvested and cooked for use as a vegetable. Amaranth also has other uses. In the Tzaneen area, the leaves and stems of *A. spinosus* are dried and ground for use as snuff (Hart and Vorster, 2006). In areas where in the past access to salt was limited, such as in parts of the Limpopo Province, the whole dried plants of different amaranth species were burnt to produce ash, which was dissolved in water and the precipitate of the filtrate of the ash was used as salt (Fox and Norwood Young, 1982).

Amaranth is rarely cultivated in South Africa because, as with many other ALVs, people believe the plants will grow naturally. In the Bushbuckridge area of the Limpopo and Mpumalanga Provinces women do harvest and store seed of amaranth, which they broadcast in their fields when they observe a decline in the natural population. Women also practise selective weeding to replenish natural seed reserves (Vorster *et al*., 2002; Hart and Vorster, 2006). Selective weeding refers to the control of weeds with due regard to the weed species concerned. When practising selective weeding, ALV species, such as amaranth, are treated as crops and allowed to grow without being disturbed, whilst other weed species that are not used as food are controlled. When selective weeding is used with the intention of raising the natural population of a particular ALV species, the plants are left in the field to complete their full life cycle, including the release of seed. Of all the weeds that feature as ALVs in South Africa, amaranth is part of the group of species that have the best potential to be developed as crops.

Plate 3.2: The spoon shaped leaves of *Amaranthus greazicans*, an indigenous amaranth specie with good taste but low yield potential

Plate 3.3: Variation in morphology of different amaranth species in a field trial

3.5.2 Spider flower (*Cleome gynandra* L.)

Spider flower is known as *oorpeultjie, palmbossie, vingerblaartee* in Afrikaans; spider flower or plant, cats whiskers and African cabbage in English; *lude, ulude, ulube* in isiNdebele; *amazonde* in IsiZulu; *lerotho* in IsiPedi and Sesotho; *murudi* in Tshivenda and *rirhudzu, bangala* in Xitsonga (Fox and Norwood Young, 1982; Bromilow, 1995; Van Wyk and Gericke, 2000, Vorster *et al*., 2002).

Spider flower belongs to the Capparaceae family and is an herbaceous, erect, mainly branched plant. The height of the plant varies between 0.5 m and 1.5 m, depending on the environment. Leaves are compound and palmate with three to seven leaflets. Stems and leaves are covered with glandular hair. Pigmentation on the stems varies from green to pink and purple. The terminal inflorescences have very distinct small white flowers, but pink and lilac coloured flowers also occur. The fruit consists of small siliques (Van Wyk and Gericke, 2000).

According to Germishuizen *et al*. (2006), there are 15 cleome species in South Africa. Among the different spider flower species that occur, *C. gynandra* (Plate 3.4) is the most widely used as a leafy vegetable but *C. monophylla* (Plate 3.5) and *C. hirta*, which are close relatives, are also occasionally used. *C. hirta* has bright pink, purple and white flowers and is a popular ornamental plant in South African gardens (Fox and

Norwood Young, 1982; Van den Heever and Coertze, 1996b; Chweya and Mnzava, 1997; Schippers, 2000; Van Wyk and Gericke, 2000; Vorster *et al*, 2002; Hart and Vorster, 2006).

Spider flower grows best during summer and is sensitive to cold. It does not grow well when the temperature drops below 15ºC. Cleome prefers well-drained, medium-textured soils and does not grow well in poorly drained soils or heavy clay soils. It requires full exposure to sunlight and performs poorly when shaded. Cleome grows best when adequately supplied with water. It does tolerate a degree of water stress, but prolonged water stress hastens flowering and senescence. Application of fertilisers containing appreciable amounts of nitrogen delays flowering and increases the number and size of leaves. Spider flower is harvested by uprooting and ratoon harvesting (Van den Heever and Coertze, 1996b; Chweya and Mnzava, 1997; Schippers, 2000; Schippers *et al*., 2002b; AVRDC, 2003; Mnzava and Chigumira Ngwerume, 2004).

In the hot northern parts of South Africa, where spider flower grows naturally, it is generally preferred to amaranth. The plant parts used include the leaves and the growth tips. People in the south find cleome too bitter but bitterness can be reduced by changing the cooking water or by cooking it in milk. When preparing spider flower as a vegetable, amaranth leaves are often added to increase bulk (Fox and Norwood Young, 1982; Chweya and Mnzava, 1997; Van den Heever and Coertze, 1996b; Van Wyk and Gericke, 2000; Vorster *et al*., 2002; Hart and Vorster, 2006).

Spider flower is not fully cultivated in South Africa but as with amaranth, women occasionally raise the natural population of plants that grow as weeds in fields by broadcasting seed or by practicing selective weeding (Vorster *et al*., 2002; Hart and Vorster, 2006; Vorster *et al*., 2009). Spider flower is also among the group of ALVs that has good potential for development as a crop, particularly in the north of South Africa (Fox and Norwood Young, 1982).

Plate 3.4: The very distinct flower of C*leome gynandra* with its extremely long stamens gave rise to its common name of cats whiskers or spider flower

Plate 3.5: *Cleome monophylla* with simple leaves and pink flowers is also used as a leafy vegetable but is not as popular as *C. gynandra*

3.5.3 Chinese cabbage (*Brassica rapa* L. subsp. *chinensis* and subsp. *pekinensis*)

Chinese cabbage is known as rape in English, *Sjinese kool* in Afrikaans and *mutshaina* in Tshivenda and other local African languages.

Chinese cabbage is a member of the Cruciferae family. *Brassica campestris*, the progenitor form of Chinese cabbage, is believed to have evolved in the Mediterranean area. It was introduced to China more than 2000 years ago, where farmers developed two main types, namely heading Chinese cabbage (*B. rapa* L. subsp. *pekinensis*) and non-heading types (*B. rapa* L. subsp. *chinensis*). Heading Chinese cabbage forms a compact to elongated head with green crinkled leaves and white midribs whilst in non-heading Chinese cabbage, dark green leaves supported by light green to white petioles form a rosette (Opeňa *et al*., 1988; Hill, 1990; Rubatzky and Yamaguchi, 1997) as seen in Plate 3.6. Chinese cabbage is an annual, flowering vegetable which takes 6 to 11 weeks from sowing to the end of the vegetative stage when the plants reach a height of 15 to 30 cm (Hong-Fu, 1988; Manrique, 1993; Rubatzky and Yamaguchi, 1997). It has a stout taproot, which is sometimes partly swollen. The inflorescence is a terminal umbel-like raceme, which can be up to 60 cm long. (Toxopeus and Baas, 2004).

Chinese cabbage is a cool season crop, which requires adequate availability of soil water and plant nutrients for optimum growth. The crop does not tolerate poorly drained conditions. Chinese cabbage is usually sweet in flavour when cooked (Opeňa *et al*., 1988). In Eastern Asia, it is used in soups, egg rolls, stir-fry and for pickling (Hill, 1990), while in Korea, it is prepared as a fermented side dish called kimchi (Chun, 1981; Opeňa *et al*., 1988). In South Africa, *B. rapa* subsp. *chinensis* is the most popular type of Chinese cabbage among black people, who use it as a relish to accompany maize porridge (Van Averbeke *et al*., 2007). Ethiopian Kale and Ethiopian Mustard (*B. carinata* and *B. juncea*), which are both closely related to Chinese cabbage, are popular in central and east Africa and are indigenous to Africa. *Brassica napus* or rape kale is very popular in the Limpopo and Mpumalanga provinces of South Africa and Zimbabwe, where it is known as *murhodisia* (Schippers, 2000; Toxopeus and Mvere, 2004). A common characteristic of all *Brassica* species is the presence of glucosinolate compounds, which are converted by the enzyme myrosinase to give bitter-tasting and gitrogenic substances, such as isothiocyanates, nitriles and goitrin (Peirce, 1987; Rubatzky

and Yamaguchi, 1997). These compounds contribute to flavour and odour, but they also inhibit thyroxin production and cause thyroid enlargement, known as goiter, when consumed in large quantities (Schippers, 2000).

Vhembe District in the north of Limpopo Province is the centre of origin of the cultivation of non-heading Chinese cabbage in South Africa. In Vhembe an informal seed multiplication and distribution system is being maintained by selected producers. Despite the absence of any extension efforts to promote the production of the crop, smallholder cultivation of Chinese cabbage has been spreading rapidly from Vhembe to other parts in the Limpopo, Mpumalanga and Gauteng Provinces. Locally, the crop is either sown directly or raised from seedlings and harvested as single leaves (Van Averbeke *et al*., 2007; Van Averbeke and Netshithuthuni, 2010). Elsewhere in the world, the crop is also harvested as complete plants (Matsumura, 1981; Peirce, 1987). Smallholders in Vhembe consider the fifth true leave to be the first marketable leaf and they continue picking the leaves on a regular basis until the peduncle starts to elongate (Van Averbeke *et al*., 2007; Van Averbeke and Netshithuthuni, 2010). The fresh leaf yield of Chinese cabbage typically ranges between 5 t ha^{-1} and 30 t ha^{-1} (Tindall, 1983). In Vhembe District leaf yield of Chinese cabbage was found to be heavily dependent on planting date (Van Averbeke, 2008).
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Plate 3.6: The loose leafy rosette of non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*)

3.5.4 Nightshade (*Solanum nigrum* complex)

Nightshade (English) is known as *nastergal, galbessie* and *nagskade* in Afrikaans; *ixabaxaba* in isiNdebele; *umsobo, sheshoa-bohloko* and *umsobo-sobo* in isiXhosa; *umsobo, isihlalakuhe, udoye, umagqa, umgwaba, umsobo-sobo* (fruit) and *umqunbane* in isiZulu; *lethotho* in IsiPedi; *seshoa-bohloko, sehloabohloko* and *momoli* in Sesotho; *msobo* and *umsobo* in SiSwati; *muxe* in Tshivenda; and *kophe* in Xitsonga (Fox and Norwood Young, 1982; Bromilow, 1995; Van Wyk and Gericke, 2000, Vorster *et al*., 2002; Van Averbeke *et al*., 2007).

Nightshades (*S. nigrum* complex) are erect, branched annual or biannual herbaceous plants that can reach a height of 75 cm. The leaves are alternate and bright green in colour but purple pigmentation may be present. The small flowers are about 4 mm to 10 mm long with white petals and conspicuous yellow anthers that are arranged in a drooping umbel-like inflorescence (Plate 3.7). Nightshade is also well known for its small, shiny, black to purple-black fruit (Norwood and Fox Young, 1982) as seen in Plate 3.8.

Plate 3.7: The infloresence of nightshade (*Solanum retroflexum*) showing the recurved petals

Plate 3.8: Ripe berries of nightshade are black in colour

In nature, *S. nigrum* species are mainly found in fairly humid environments with at least 500 mm of rain per annum (Edmonds and Chweya, 1997). They prefer fertile soils that are high in nitrogen and phosphorus (Van Averbeke *et al*., 2007). The optimal temperature for growth ranges between 20ºC and 30ºC, but most species tolerate a temperature range of 15ºC to 35ºC. When grown during winter, maximum growth and biomass production are obtained when the plants are exposed to full sunlight, whilst during summer shading up to 60% can be beneficial (Edmonds and Chweya, 1997). The yield potential of *S. nigrum* species depends on a number of factors, including type of species, length of the growing season, number of harvests and agro-ecological conditions, but under favourable conditions cumulative leaf yields of 20 t ha^{-1} can be achieved. Plant spacing, nutrient and water supply and plant protection are the important management practices that determine to what extent the yield potential of the crop will be realised (Edmonds and Chweya, 1997).

The nightshade complex contains many species and its taxonomy is complicated. According to Germishuizen *et al*. (2006), *S. nigrum* is indigenised in South Africa but *S. retroflexum* is indigenous (Van Averbeke *et al*., 2007). In South Africa, *S. americanum*, *S. nigrum* and *S. retroflexum* are the most commonly used species (Schippers, 2000; Manoko and Van den Weerden, 2004). When used as a leafy vegetable, the leaves and tender shoots of nightshade are harvested and cooked (Van Averbeke *et al*., 2007) but Norwood and Fox Young (1982) mentioned reports of leaves being eaten raw. The ripe fruit is also consumed extensively, either fresh or as a preserve, but the green fruit is poisonous (Norwood and Fox Young, 1982).

In South Africa, nightshade is mostly harvested from the wild except in the Vhembe District where it is being cultivated, with local farmers producing their own seed (Van Averbeke *et al*., 2007). *S. nigrum* species are commonly propagated from seed but the use of shoot cuttings as propagules, especially during the rainy

season, has also been reported, though plants propagated in this way yield less than those propagated by seed (Edmonds and Chweya, 1997). Poor germination is a problem commonly encountered when growing nightshade, Poor germination has been ascribed to inadequate removal of sugars and germination inhibitors present in the fruit during extraction of the seed (Mwai and Schippers, 2004). Typically, *S. nigrum* species are ready for harvest when the plants are about 15 cm high (Edmonds and Chweya, 1997), which they reach about four to six weeks after transplanting (Chweya, 1997; Schippers, 2000; Mwai and Schippers, 2004). The preferred harvesting method is to cut the entire shoot, because this encourages re-growth of the side shoots. Regular harvesting of the young shoots and de-budding encourages the production of lateral shoots and extends the harvesting period. Leaves are harvested until the fruit starts to develop and the leaves become narrow, thin and leathery (Manoko and Van den Weerden, 2004; Van Averbeke *et al*., 2007). Although *S. retroflexum* is being produced commercially by smallholders in Vhembe District, *S. scabrum* probably has better potential for development as a crop, because it has more and larger leaves.

3.5.5 Jew's mallow (*Corchorus olitorius* and *C. tridens)*

Jew's mallow or wild jute (English) is known as *jute, wilde jute* in Afrikaans; *thelele* and *ligusha* in IsiPedi, Sesotho and Setswana; *delele* in Tshivenda; and g*uxe, ligushe in* Xitsonga and Shangaan (Fox and Norwood Young, 1982; Bromilow, 1995; Van Wyk and Gericke, 2000, Vorster *et al*., 2002).

Jew's mallow belongs to the Tiliaceae family and is an erect annual herb that varies from 20 cm to approximately 1.5 m in height. The stems are angular with simple oblong to lanceolate leaves that have serrated margins and distinct hair-like teeth at the base. The bright yellow flowers are usually very small and the fruit is a straight, angular capsule. The capsule of *C. tridens* ends in three small "horns" (Norwood and Fox Young, 1982). According to Schippers (2000), the seeds of *Corchorus* spp. show a high degree of dormancy, which can be broken by means of hot water treatment (Schippers *et al*., 2002a).

Jew's mallow prefers warm, humid conditions and performs well in areas with high rainfall (600 to 2000 mm) and high temperature (30ºC during the day and 25ºC at night). As a result, it is known only in the northern and eastern regions of South Africa. Growth of Jew's mallow is seriously depressed when the temperature drops below 15ºC or when the plants are subjected to a prolonged period of water deficit. Corchorus prefers rich, well-drained, medium-textured soils but will also grow in coarse and fine textured soils.

According to Germishuizen *et al*. (2006), there is 13 indigenous and indigenized *Chorchorus* species in South Africa. These include *C. asplenifolius* (Plate 3.9), *C. tridens, C. trilocularis and C. olitorius* amongst others (Schippers *et al*., 2002a; Van Wyk and Gericke, 2000). Cooked Jew's mallow has a mucilaginous texture, similar to okra, which is highly appreciated by some and offensive to others. When preparing coarse-textured leaves, such as those of cowpeas, inclusion of Corchorus makes it easier for older people to swallow the vegetables. To reduce the sliminess bicarbonate of soda or even cow urine is added to the cooking water (Norwood and Fox Young, 1982; Van Wyk and Gericke, 2000; Schippers *et al*., 2002a).

In South Africa, Jew's mallow is only harvested from the wild, but it has potential for development as a crop, particularly in the north and east of the country.

Plate 3.9: *Corchorus asplenifolius* is one of the indigenous wild jute species in South Africa

3.5.6 Pumpkin, melons and indigenous cucurbits

The leaves of "ordinary" pumpkin (*Cucurbita pepo, C. Moschata* and *C. maxima*) are known as *pampoenblare* in Afrikaans; *ibobola* in siNdebele; *cetshana* in isiXhosa; *intanga* and *umliba* in isiZulu; *mphodi, monyaku, motshatsha* and *mophotse* in IsiPedi; *mekopu* and *maphutse/lephotse* in Sesotho and Setswana, *phuri* and *thanga* in Tshivenda; and *tinwembe* in Xitsonga.

The leaves of the tsamma melon, also known as bitter water melon (*Citrillus lanatus*), are called *bitterwaatlemoenblare*; *karkoerblare* and *maketaanblare* in Afrikaans; *ibotola* in isiNdebele; umxoxozi, *ujodo* and *ityabontyi* in isiXhosa; *ibece* and *ikhabe* in isiZulu; *mogapu, habu, lethikithi, mathikithi, lerotse, matyathya, motshatsha* and *mochacha* in IsiPedi; *lehapu, makakabane, tjoto* and *thoomo* in Sesotho; *makataan, kgengwe, lekatane* and *makopuntji* in Setswana; *brani* and *gwadi* Tshivenda, and *t'samma* in Khoisan (Fox and Norwood Young, 1982; Bromilow, 1995; Van Wyk and Gericke, 2000, Vorster *et al*., 2002).

Members of the Cucurbitaceae all almost all vine like annual, herbaceous plants. The leaves and stems of some species are covered in sharp, stiff translucent hairs that can irritate the human skin. The leaves vary considerably in shape and size from almost entire leaves to deeply lobed leaves that vary from dark to light green in colour. Flowers are monoecious, yellow or white and vary in size. Fruit also vary in size and colour (Norwood and Fox Young, 1982). Some wild cucurbits are also harvested in the wild. The most popular cucurbit species are the tsamma melon (*Citrullus lanatus* Thunb.) (Plate 3.10), the melon (*Cucumis melo*), the bottle gourd or calabash (*Lagenaria siceraria*) and the pumpkins and squashes (*C. pepo, C. maxima* and *C. moschata*, illustrated in Plate 3.11) (Bosch, 1998; Coertze, 1996; Schippers, 2000). The leaves of *nkaka*, balsam pear or laloentjie (*Momordica balsamina*), a local climber (Plate 3.12), are a popular vegetable in the eastern parts of South Africa (Norwood and Fox Young, 1982; Van Wyk and Gericke, 2000; Vorster *et al*., 2002; Hart and Vorster, 2006).

C. maxima and *C. pepo* are considered to be drought-tolerant and said to require relatively little water for growth but they are also known to respond positively to irrigation when conditions are very dry (Chigumira Ngwerume and Grubben, 2004; Messiaen, and Fagbayide, 2004). *C. moschata* is the most heat-tolerant type of pumpkin and it is also fairly drought tolerant (Grubben and Chigumira Ngwerume 2004). Cucurbits are said to respond well to fertilisers, particularly animal manure (Philip *et al*., 2009).

Plate 3.10: Tsamma melon (*Citrillus lanatus*) intercropped with maize

Plate 3.11: Pumpkins (*Cucurbita maxima*) growing in a maize field

Plate 3.12: *Momordica balsamina* is a wild indigenous cucurbit that is said to have medicinal properties but its consumption as leafy vegetable has been declining due to its extremely bitter taste

Members of the Cucurbitaceaea (pumpkin and relatives) family are very popular leafy vegetable in South Africa and one of the few ALVs that are cultivated. When harvested as a leafy vegetable, the leaves, flowers and young fruit are picked and cooked. The roasted seed is also a very popular snack (Norwood and Fox Young, 1982; Van Wyk and Gericke, 2000; Vorster *et al.*, 2002, Hart and Vorster, 2006). The fruit of the

bitter melon and other indigenous cucurbits are also well known sources of water in desert areas (Fox and Norwood Young, 1982; Van Wyk and Gericke, 2000).

In South Africa, pumpkins and melons are often grown as a minor crop in a mixed cropping arrangement with maize as the major crop. In these mixed crop arrangements, the cucurbits act as live mulch, because of their creeping growth habit and they help to control weeds (Silwana, 2000; Schippers, 2000; Vorster *et al*., 2002). Cucurbits are directly sown in spring after the period with frost has ended. The creeping plants occupy a lot of space and spacing of the plants is usually wide, ranging from 0.75 m x 1 m to 2 m x 2 m (Schippers, 2000).

3.5.7 Cowpeas (*Vigna unguiculata* L.)

Cowpeas are known as *akkerboontjie, koertjie* in Afrikaans; *dinawa* in IsiNdebele, *iimbotyi* in IsiXhosa; *imbumba, indumba, isihlumanya* in IsiZulu; *monawa* in IsiPedi; *monawa, dinawa, nawa* in Sesotho; *dinawa, nawa-ea-setswana* in Setswana; *munawa* (plant*), nawa* (beans) in Tshivenda; *dinaba, munaoa, tinyawa* in Xitsonga; and *murowi we nyemba* in Shona (Fox and Norwood Young, 1982; Bromilow, 1995; Van Wyk and Gericke, 2000, Vorster *et al*., 2002).

Cowpeas are a leaf and pulse crop that belongs to the Leguminosae family. They are annual or perennial herbaceous plants with tri-foliate leaves. Different varieties exist, varying from prostate indeterminate types to erect, determinate, low-branching types. The varieties mainly used as a leafy vegetable are the spreading, prostrate types (Plate 3.13).

Plate 3.13: Cowpeas (*Vigna unguiculata*) growing in a homegarden

The seed of cowpeas is reniform to oblong and varies in colour from white to dark red and black. The seed is often mottled or shows a black “eye” at the hilum. Cowpeas are indigenous to Africa and have been cultivated for a long time on the continent (Norwood and Fox Young, 1982; Schippers, 2000; Vorster *et al*., 2002; Hart and Vorster, 2006). Various subspecies of cowpeas are found in the wild in the eastern parts of the KwaZulu-Natal, Mpumalanga and Limpopo Provinces. According to Germishuizen *et al*. (2006), there are indigenous and indigenized *V. unguiculata* subspecies in South Africa. These subspecies include *Vigna unguiculata* subsp. *dekindtiana* var. *dekindtiana, V. unguiculata* subsp. *dekindtiana* var. *huillensis, V. unguiculata* subsp. *rotracta, V. unguiculata* subsp. *stenophylla, V. unguiculata* subsp. *tenuis* var. *ovata, V. unguiculata* subsp. *unguiculata*, with *V. unguicalata* subsp. *unguiculata* the most commonly found (Vorster *et al*., 2002).

Cowpea is a heat-loving crop, which is thought to have lower soil fertility requirements than many other crops and which is also considered to be drought-tolerant (Schippers 2000). Cowpea derives an important part of its nitrogen requirements from the atmosphere (Schippers, 2000) and is resistant to major bacterial, fungal and viral diseases and to root knot nematodes and important parasitic weeds (Singh, 2006). All of these attributes make the crop well suited for inclusion in South African smallholder systems.

Cowpeas are primarily grown for the seed, but young leaves and growth points are used as a leafy vegetable. The leaves are no longer harvested after the flower initiation because when the plant has reached this growth stage the leaves become very fibrous (Schippers, 2000; Vorster *et al*., 2002; Hart and Vorster, 2006).

Cowpeas are often grown in a mixed cropping system with taller crops, such as maize. Cowpeas are especially important in dry regions. Harvesting of the leaves commences as soon as plants are well established (Schippers, 2000; Vorster *et al*., 2002; Hart and Vorster, 2006).

3.6 SELECTION OF AFRICAN LEAFY VEGETABLES FOR FURTHER RESEARCH

The terms of reference of WRC Project K5/1579/5 required that research be done on two or three 'indigenous crops' with particular potential to address nutritional deficiencies in the diet of vulnerable groups, particularly the rural poor. The choice to focus on ALVs was justified by their potential to improve the vitamin A and iron status of people. As indicated, these two micronutrients are particularly deficient in the diets of vulnerable groups. The review of the various leafy vegetable species that are consumed in South Africa indicated that there was substantial diversity, necessitating the selection of a few important ALV species for the conduct of research aimed at determining their water use, agronomic requirements and nutrient composition. Three criteria were used during selection, namely:

- the extent of consumption of the different leafy vegetable species in South Africa;
- the extent of their cultivation; and
- their primary growing season.

Both the extent of consumption and production were considered to be important indicators of the potential of leafy vegetable species for incorporation in the farming or home-garden systems of rural and urban poor, whilst their primary growing season – winter versus summer – was considered important for year-round production of a variety of greens. Using these criteria, eight ALVs, listed in Table 3.2, were selected for further research.

Table 3.2: Leafy vegetable species selected for agronomic research

Scientific name	English names
Amaranthus cruentus L.	Pigweed (amaranth)
Vigna unguiculata (L.) Walp.	Cowpeas
Corchorus olitorius L.	Jew's mallow, wild jute
Cleome gynandra L.	Spiderplant, Cat's whiskers, Spider flower, Bastard mustard
Citrillus lanatus (Thunb.	Tsamma melon, bitter watermelon, Egusi watermelon
Cucurbita maxima (Duchesne)	Pumpkin
Solanum retroflexum Dun.	Black nightshade
Brassica rapa L. subsp. *chinensis*	Chinese cabbage (non-heading)

Amaranth, represented by pigweed, was selected, because it appeared to be the most widely consumed green leafy vegetable in South Africa. Spider flower and wild jute were included in the selection, because they are very popular in the northern parts of South Africa and offer potential for development into cultivated crops. All other five species in the list are already being cultivated. Collectively the eight plant species listed in Table 3.2 represent a fairly comprehensive selection of the leafy vegetable species that are consumed (and in

some cases produced) in the northern part of South Africa but many have a wider distribution in terms of consumption (Table 3.1), perhaps with the exception of jute. This means that the findings of the research that was done on these eight plant species should have wide application.

3.7 CONCLUSIONS

The well-known Pedi proverb, 'Meat is a visitor but morogo a daily food', captures the important role that ALVs have played in the food systems of black people in South Africa. Indications are that these vegetables are still used extensively by contemporary rural households in this country and the potential to develop selected species into commodities has been recognised. Considering their potential nutritional value, they could contribute significantly to food security and balance in the diets of vulnerable rural and urban households. There are questions pertaining the bio-availability of the nutrients contained in ALVs and these need research attention. Generally, the use of ALVs in South Africa has diminished. At community and household level, knowledge associated with ALVs is essentially passed on from one generation to the next and in certain parts of the country there is a threat that this knowledge will be lost. Further research on the different aspects of ALVs species, including their ecology, use, cultivation and nutritional contribution is, therefore, warranted.

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Drought Tolerance, Heat Tolerance and Water Requirement of Selected African Leafy Vegetables

CHAPTER 4: THE RESPONSE OF SIX AFRICAN LEAFY VEGETABLES TO DROUGHT AND HEAT STRESS

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4.1 INTRODUCTION

In many parts of South Africa, the production of crops is subject to the environmental limitation of water deficit (Slabbert and Van den Heever, 2007). Water deficit reduces crop productivity, causing economic losses (Fuglie, 2007; Hyman *et al*., 2008). Water deficit often causes plant water stress, which has a negative effect on growth and quality of plants and can cause substantial reductions in yield (Wang *et al.*, 2003). Water stress affects plant growth by altering metabolism and gene expression (Ludlow, 1993; Jensen *et al.*, 1996). Drought inhibits cell elongation, reduces photosynthesis, impedes nutrient uptake and alters plant hormone levels (Pennypacker *et al*., 1990), because water is a fundamental constituent in the maintenance of normal physiological processes and membrane transport activities in plants (Jones and Tardieu, 1998).

Water stress studies conducted on a range of different plants have shown that plants develop mechanisms to survive and grow under conditions of extremely low or frequently changing water supply and extreme heat. Plant response to abiotic stress includes various mechanisms of escape, tolerance or avoidance (Monneveux and Belhassen, 1996; Slabbert, 2007). Knowledge of the response of African leafy vegetable (ALV) species to water and heat stress is limited (Van Vuuren, 2006; Slabbert, 2007).

Plants have developed a wide diversity of drought and heat tolerance mechanisms at the metabolic and physiological levels, and adaptation to abiotic stress has been listed at molecular, cellular and whole plant level (Ashral and Foolad, 2005; Yin *et al.*, 2005). Drought and heat tolerance is the phenotypic expression of a number of morphological characteristics and physiological mechanisms, and a combination of mechanically linked traits is such as drought and heat avoidance and tolerance were investigated in the current study. The drought and heat tolerance traits examined include the following techniques: relative water content, leaf area, cell membrane stability, cell viability and early drought tolerance.

In this study the response of six selected ALVs to drought and heat stress is measured and compared with that of a reference crop. Swiss chard (*Beta vulgaris* var. *cicla*), an exotic leafy vegetable commonly cultivated in rural and urban home gardens, was selected as the reference crop. The results should contribute to choosing particular ALV species for production in specific agro-ecological environments.

4.2 OBJECTIVES

The objectives of the study were to measure the morphological and physiological response of selected ALV species to drought and heat stress; to characterize the selected species according to their level of drought and heat tolerance/sensitivity, and to rank the eight ALVs and Swiss chard in terms of their drought and heat tolerance/sensitivity.

4.3 APPROACH TO THE STUDY

Different screening techniques were used to test the drought and heat tolerance of a selection of ALV species. The screening techniques included relative water content (RWC), cell membrane stability (CMS), leaf area (LA), cell viability (2,3,5-triphenyl-tetrazolium chloride reduction rate (TTC)) and early drought

screening (wooden box technique). Test crops included five summer ALVs, namely, pigweed (*Amaranthus cruentus*), tsamma melon (*Citrillus lanatus*), pumpkin (*Cucurbita maxima*), nightshade (*Solanum retroflexum*) and cowpea (*Vigna unguiculata*) and one winter ALV, namely non-heading Chinese cabbage (*Brassica rapa* subsp. *chinensis*). For all the screening techniques, the responses to drought and heat stress of the five summer ALVs and the one winter ALV were compared with those of Swiss chard (*Beta vulgaris* var. *cicla*), which served as the reference crop.

All crops were grown in a greenhouse at TUT. For all screening tests, except the wooden box technique, the crops were grown in pots with a diameter of 25 cm using a potting-soil/vermiculite/soil (2:1:4) mixture as the growing medium. Osmocote^{TM}(5-6) was added at the rate of 10 g per pot. Details on the materials and method used when applying the wooden box technique appear in section 4.6. The five summer ALVs and the reference crop were grown during September to November 2007, 2008 and 2009. The winter ALV and the reference crop Swiss chard were grown during April to June 2007 and 2008. During summer, the temperature in the greenhouse ranged between 25 and 27˚C during the day and between 10 and 15˚C at night. During winter, temperatures of 20-24˚C (day) and 8-12˚C (night) prevailed.

Plants were well watered until they reached the eight-leaf stage, which occurred seven to eight weeks after planting. Then the pots were allocated randomly to two treatments, namely a well-watered control and a water stress treatment in which water was completely withheld. Watering of the control plantlets occurred every second day. A different set of five plants (not stressed) was used for the 2,3,5-TTC (cell viability) evaluation. Measurements were made from the 3rd-4th leaf from the bottom, sampling towards the top every 2-3 days, for both control and stress plants. Ten control and ten stress plants were tested for each genotype, one sample taken from each plant was used for all the different screening techniques.

4.4 MECHANISMS OF TURGOR MAINTENANCE AND LEAF EXPANSION: MEASUREMENT OF RELATIVE WATER CONTENT AND LEAF AREA OF AFRICAN LEAFY VEGETABLES IN RESPONSE TO DROUGHT

4.4.1 Background

The importance of water for normal plant functioning is well recognised. Depletion of soil water reserve causes a variety of symptoms in plants, ranging from few minutes (wilting, stomatal closure) to weeks (change in leaf growth, senescence) or months (decrease in total biomass or yield). Water acts as a hydraulic agent in maintaining turgor and powering expansion growth, a biochemical reactant in photosynthesis and other important metabolic reactions, a solvent and transport agent for all substances moving into, throughout, and out of the plant, and a protoplasmic structural agent and filler (Spomer, 1984). Plant growth is also influenced indirectly through the functions of water as a thermal buffer against rapid and extreme temperature changes and as an evaporative cooling agent. Water stress is defined by an imbalance between supply, linked to soil water potential, and demand, linked to climate (Fisher and Turner, 1978; Tardieu, 1996).

There are sets of integrated processes in the plant, which avoid water stress. Exposure of plants to extreme conditions such as drought causes a diverse set of physiological, morphological and developmental changes (Jensen *et al.*, 1996; Gulen and Eris, 2004). The plant will try to maintain some form of regulation to allow the crop to avoid events that would cause early plant death (Tardieu, 1996). According to Tardieu (1996) water loss through vapour can be controlled on the short term by stomatal closure. This leads to a decreased difference between root and leaf water potentials. Because this short term regulation is usually not so effective, plants change their architecture by reducing leaf growth, increasing root/shoot ratio and reducing leaf area by early senescence (Tardieu, 1996).

Plant responses to water deficit are dependent on the amount of water loss, the rate of loss and the duration of the stressed condition (Bray, 1997). According to Bray (1997), it is difficult to separate the function of drought-induced genes from other stress-induced changes. Therefore, it is important to integrate information about cellular and whole plant responses in order to understand the behaviour of the plant during water deficit.

The importance of determining plant water status in research for water requirement of horticultural crops is well recognised. The use of some recognised mechanisms in the breeding of drought tolerance is a major issue in order to compare tolerance/sensitivity at a certain plant water status. In this study, two techniques, namely, relative water content (RWC) and leaf area (LA), were applied to measure whole plant response and plant water status.

Relative water content (RWC)

Relative water content (RWC) is an old method that has been used since 1962 (Barrs and Weatherley, 1962). This method is a very reliable way for measuring leaf water status and plant stress (Blum, 1998). RWC estimates the percentage of water present in the leaf as a fraction of the total volumetric water that the leaf can hold at full turgor (Blum, 1998; Jones and Tardieu, 1998). The effect of osmotic adjustment is represented by the relationship between RWC and LWP (Ψ_L) during moisture stress (Blum, 1998). RWC measurement is a direct method to determine leaf water status in plants during water deficit periods, indicating the ability of the plant to maintain a high water content and possible drought tolerance during severe drought conditions (Abdalla and El-Khoshiban, 2007).

Leaf area

Phenology and leaf area have a very important role in whole plant response and adaptation to water deficit. This is an important factor to take into consideration before more intricate physiology is tested for crop response to drought stress (Blum, 1996). Blum (1996) states further that, whereas leaf area is determined by phenology, stem morphology, rates of leaf emergence and potential leaf size, any effect of drought on these factors would alter the leaf area. Plants modulate their leaf areas and thereby adjust water loss from the canopy to the size that can be effectively supplied by the existing soil water (Passioura, 1996). It is important for a crop to be able to adjust the leaf expansion, because it enables the crop to control water-use and reduce water losses through transpiration (Blum, 1996).

The degree of leaf expansion serves as an indicator of the plant's growth response to drought, as many plants use this for drought avoidance (Blum, 1996). The effective live light-intercepting leaf area on a plant is reduced by drought by means of reduced cell expansion, reduced cell division, leaf rolling, paraheliotropism, death of apical parts and leaves, and death of whole leaves (first basal and then apical) (Blum, 1996.). Using a leaf area meter, the leaf surface can be measured, possibly indicating rate of dessication and thus a mechanism that could distinguish between drought sensitive and drought tolerant plants.

4.4.2 Material and methods

Relative water content (RWC)

The leaf samples were taken predawn. Using a no. 6 cork borer, leaf disks were cut and weighed immediately after harvest (within 30 minutes) to obtain the fresh weight (FW). Five disks were used for each replicate. The samples were re-hydrated by placing the disks in small glass bottles and adding approximately 3 ml of distilled water to allow the disks to float at room temperature of approximately 20˚C. After 4 hours the leaf disks were pat dry thoroughly with towelling paper, and weighed again to obtain the turgid weight (TW). The samples were then oven dried overnight at 70 ˚C, cooled in a desiccator, and

weighed again to obtain the dry weight (DW). The RWC was measured by calculating the following parameters:

RWC = [(W-DW)/(TW-DW)] x 100

Plants were water stressed until a RWC decreased below 50%, where after the plants were rewatered, and RWC for recovery was measured.

Leaf area

Leaf area was measured for both stress and control leaves. One sample was taken early in the morning from each plant, using ten replicate plants for each treatment. The samples were put into a plastic bag directly after sampling and leaf area was measured using a leaf area meter (LI-3100^{TM}). Leaves were placed one at a time, with their apical side down, on the conveyer belt passing an interrupted light source and sensor. A digital measurement was noted.

Statistical analysis

Results were subjected to statistical analysis, and standard deviations in values were calculated at a 95% probability ($p \leq 0.05$). Standard deviations are noted in figures.

4.4.3 Results

Relative water content (RWC)

Summer crops

After ten days of drought stress, the RWC dropped from 87 to 35% in *A. cruentus*, and recovery after rewatering was fairly high at 67% (Figure 4.1a). The RWC of *C. lanatus* decreased from 88 to 47% after 12 days of drought stress. Recovery was good and 2 days after rewatering the RWC recovered to 88% (Figure 4.1b). The RWC of *C. maxima* decreased from 74 to 45% after 12 days water stress, but recovery was very good and RWC was 84% two day after rewatering (Figure 4.1c). *S. retroflexum* was the crop that stressed the first upon water deficit, and RWC decreased from 79 to 45% within 8 days of drought stress only. Recovery was good however, and RWC was 85% after rewatering (Figure 4.1d). *V. unguiculata* could endure 21 days of water stress before the RWC dropped from 86 to 46%. RWC after rewatering and recovery was 74% (Figure 4.1e). *B. vulgaris* var. *cicla* had the most significant drop in RWC, and reached 33% after 10 days water stress (Figure 4.1f).

When the overall reduction in RWC is considered, taking into account the number of days taken to reach a RWC below 50%, it is clear that *V. unguiculata* was able to maintain the highest RWC during prolonged water stress, followed by *C. maxima, C. lanatus, S. retroflexum, A. cruentus* and lastly *B. vulgaris* var. *cicla* (Table 4.1).

Winter crops

The RWC of *B. rapa* subsp. *chinensis* decreased from 93 to 47% within 12 days of water stress, and recovery after rewatering was 89% within 2 days (Figure 4.2a). The RWC of *B. vulgaris* var. *cicla* decreased from 85 to 45% within 12 days of water stress, and recovery after rewatering was 79% within 2 days (Figure 4.2b). *B. vulgaris* var. *cicla* can only be ranked slightly more drought tolerant than *B. rapa* subsp. *chinensis* (Table 4.1).

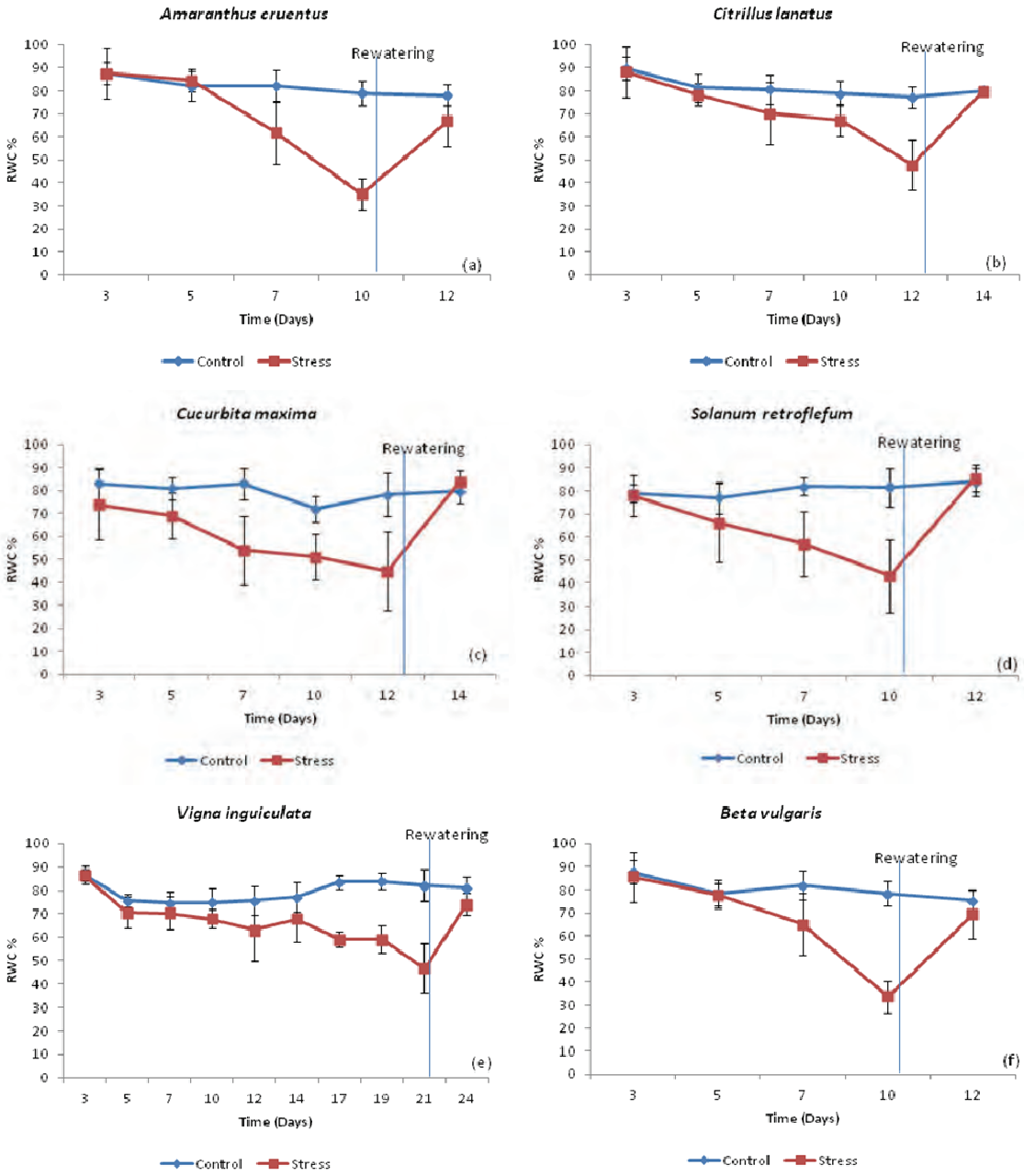


Figure 4.1: Effect of drought stress on relative water content (RWC) of (a) *Amaranthus cruentus*, (b) *Citrillus lanatus*, (c) *Cucurbita maxima*, (d) *Solanum retroflexum*, (e) *Vigna unguiculata* and (f) *Beta vulgaris* var. *cicla*

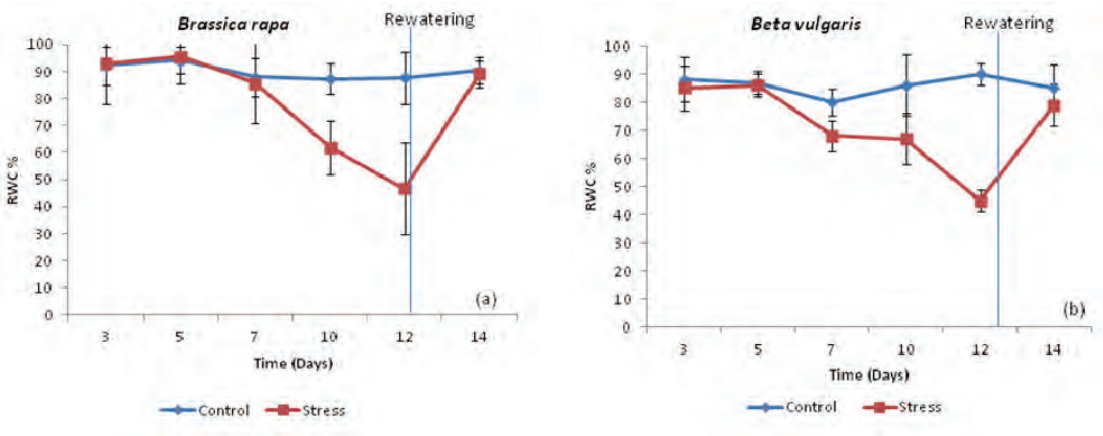


Figure 4.2: Effect of drought stress on relative water content (RWC) of (a) *Brassica rapa* subsp. *chinensis* and (b) *Beta vulgaris* var. *cicla*

Table 4.1: Summary of the relative water content (RWC) of *Amaranthus cruentus*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata*, *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla* after severe drought stress

Summer crops	**RWC**	**Days taken**	**Ranking***
A. cruentus	35	10	5
C. lanatus	47	12	2
C. maxima	45	12	2
S. retroflexum	43	10	4
V. unguiculata	46	21	1
B. vulgaris var. *cicla*	33	10	6
Winter crops	**RWC**	**Days taken**	**Ranking**
B. rapa subsp. *chinensis*	47	12	2
B. vulgaris var. *cicla*	45	12	1

* 1 = most drought tolerant and 6 = most drought sensitive

Leaf area (LA)

Summer crops

Figures 4.3 and 4.4 clearly indicates the changes in leaf area for control and stress leaves of the seven ALVs tested over the period of 12 to 21 days of drought stress. Leaf size was not influenced markedly in *A. cruentus* due to the influence of water withholding and rewatering, for both control and stress plants, and leave size ranged between 100 mm^2 and 200 mm^2 in size (Figure 4.3a).

Both control and stress leaves showed a decrease in leaf area over time for *C. lanatus*, but reduction in size was more evident in the stressed leaves after 12 days water withholding, from 315mm^2 on day three, to 154 mm^2 after 12 days of water stress. After rewatering, LA recovered and increased to 207 mm^2 within two days (Figure 4.3b).

Both control and stress leaves showed a decrease in leaf area over time for *C. maxima*, being an indication of the decrease in leaf size as sampling continue towards the top of the plant where the leaves are generally smaller (Figure 4.3c). Decrease in leaf size was however more evident in the stressed leaves after 10 days water withholding from 429 mm^2 on day three, to 190 mm^2 after 10 days, and recovery of the stressed leaves was high (228 $mm^{2)}$ within two days of rewatering.

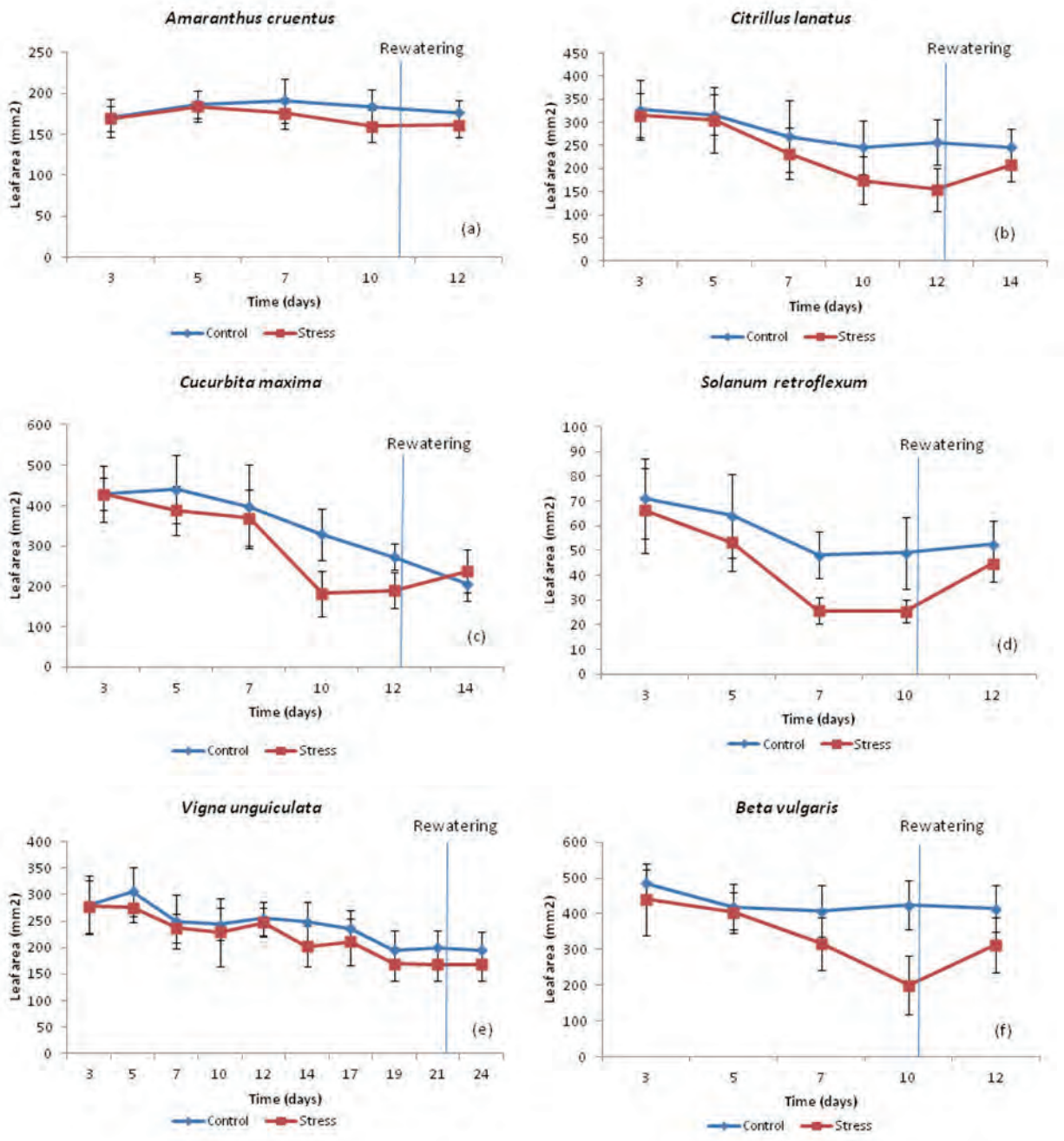


Figure 4.3: Change in leaf area with increasing drought stress and rewatering in (a) *Amaranthus cruentus*, (b) *Citrillus lanatus*, (c) *Cucurbita maxima*, (d) *Solanum retroflexum*, (e) *Vigna unguiculata* and (f) *Beta vulgaris* var. *cicla*

A decrease in leaf area was already evident in *S. retroflexum* on day three of water stress from, where the stressed leaves had an average leaf area of 66 mm^2 compared to 71 mm^2 for the control. On day 10 the stressed leaves had an average leaf area of 25 mm^2, and recovery was slow where the leaves reached 43 mm^2 two days after rewatering, compared to 52 mm^2 for the control leaves (Figure 4.3d).

Leaf area was slightly reduced in both control and stressed plants of *V. unguiculata* stressed plants after 21 days of water stress, form 261 mm^2 to 235 mm^2 for the control and 278 mm^2 to 211 mm^2 for the stress plants (Figure 4.3e), but it was not statistically different.

Both control and stress plants of *B. vulgaris* var. *cicla* showed a leaf area range of between 400 mm^2 and 450 mm^2 after 3 days water stress, and the stress plants showed a steady decline in LA over time, where the average leaf size decreased from 402 mm^2 to 313 mm^2 after 10 days drought stress (Figure 4.3f). The mean leaf area for drought stress can be seen in Figure 4.4.

When the overall decrease in LA considered, taking into account the number of days taken to reach a RWC below 50%, it is clear that the greatest reduction in leaf area occurred after 10 days in *B. vulgaris* var. *cicla* (-95 mm^2), 12 days for *C. maxima* (-46 mm^2) and *C. lanatus* (-45 mm^2), followed by 12 days for *S. retroflexum* (-14 mm^2) and *A. cruentus* (-12 mm^2), and 19 days for *V. unguiculata* (-22 mm^2) (Table 4.2).

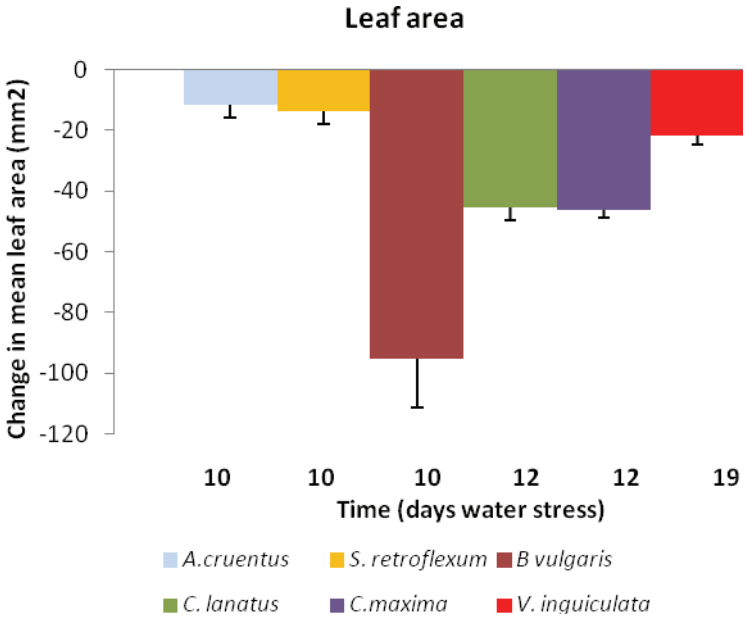


Figure 4.4: Mean leaf area with increasing drought stress and rewatering in *Amaranthus cruentus*, *Brassica rapa* subsp. *chinensis*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata* and *Beta vulgaris* var. *cicla*

Table 4.2: Summary of the reduction in leaf area of *Amaranthus cruentus*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata*, *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla* after severe drought stress at a RWC below 50%

Summer crops	**Mean reduction in LA**	**Days taken**	**Ranking***
A. cruentus	-12	10	2
C. lanatus	-45	12	4
C. maxima	-46	12	4
S. retroflexum	-14	10	2
V. unguiculata	-22	21	1
B. vulgaris var. *cicla*	-95	10	6
Winter crops	**Mean reduction in LA**	**Days taken**	**Ranking**
B. rapa subsp. *chinensis*	-51	12	2
B. vulgaris var. *cicla*	-4	12	1

*1 = most drought tolerant and 6 = most drought sensitive

Winter crops

Both control and stress plants of *B. rapa* subsp. *chinensis* showed a steady decline in LA over time (Figure 4.5). Water stress had a significant impact on the average leaf size, and the average leaf size decreased from 190 mm^2 to 59 mm^2 after 14 days increasing drought stress (Figure 4.5a). Recovery of the stressed plants

after rewatering was slow, and leaves reach a leaf area of only 74 mm^2 compared to 102 mm^2 for the control plants.

Leaf area in *B. vulgaris* var. *cicla* decreased from 177 mm^2 on day 5 to 91 mm^2 after 14 days water stress, compared to the control where leaf area decreased from 180mm^2 to 119 mm^2 for the same period of time (Figure 4.5b). Two days after rewatering recovery was good and the leaf area for the stressed plants was 103 mm^2 compared with 92 mm^2 for the control.

When the overall decrease in LA considered, taking into account the number of days taken to reach a RWC below 50%, it is clear that the greatest reduction in leaf area occurred in *B. rapa* subsp. *chinensis* than the reference crop *B. vulgaris* var. *cicla* (Table 4.2).

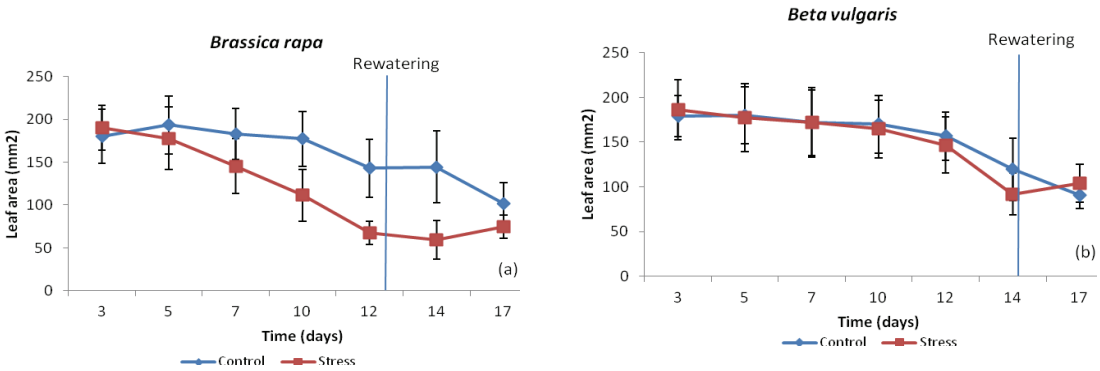


Figure 4.5: Change in leaf area with increasing drought stress and rewatering in (a) *Brassica rapa* subsp. *chinensis* and (b) *Beta vulgaris* var. *cicla*

When the mean leaf area of *B. vulgaris* var. *cicla* and *Brassica rapa* subsp. *chinensis* were calculated, it is clear that the most prominent reduction in leaf area occurred in *B. rapa* subsp. *chinensis* (-51 mm^2), followed by *B. vulgaris* var. *cicla* (-4 mm^2) after 10 days water stress (Figure 4.6).

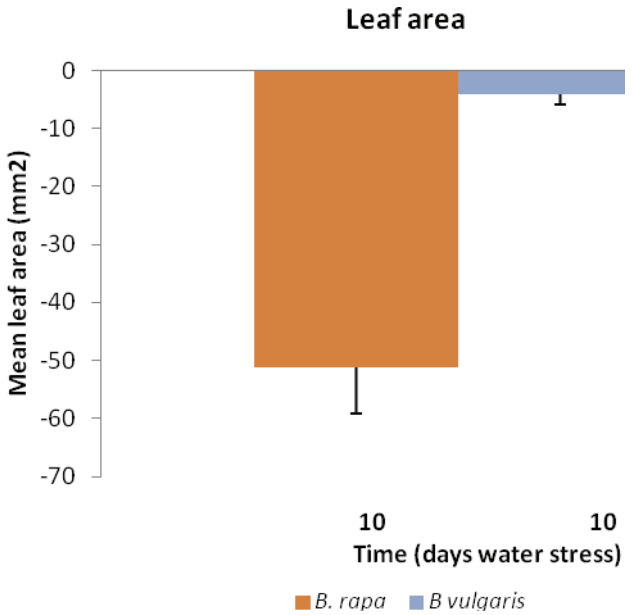


Figure 4.6: Mean leaf area with increasing drought stress and rewatering in (a) *Brassica rapa* subsp. *chinensis* and (b) *Beta vulgaris* var. *cicla*

4.4.4 Discussion and conclusion

Relative water content

It is clear from Table 4.1 that the summer ALVs with the highest reduction in RWC were *A. cruentus, S. retroflexum* and *B. vulgaris* var. *cicla*, where the crops reached a RWC below 50% within 10 days of water stress. *C. lanatus* and *C. maxima* were able to maintain a RWC above 50% for 12 days, while *V. unguiculata* was able to maintain the highest RWC during drought stress, and only reached a RWC of 46% after 21 days of drought stress.

The ability of seed propagated plants to adapt to water stress involves, at its most basic level, either tolerance to cellular dehydration or minimisation of water loss and maintenance of turgor pressure (Morgan, 1992). The ability to maintain turgor pressure during a change in plant water status ensures that metabolic processes in the plant continue and normal growth and productivity proceed (Begg and Turner, 1976). This ability is an indicator of drought tolerance (Abdulla andEl-Khishiban, 2007; Hassanzadeh *et al.*, 2009). RWC can be maintained in cells and tissues to allow for metabolic activity. This is as a consequence of osmotic adjustment and other traits of adaptation to drought, such as root development and reduced transpiration (Monneveux and Belthassen, 1996). Generally, the RWC in green tissues of plants (expressed in percent of fresh weight) ranges between 70 and 80% and genetic variability of this attribute is rather limited (Monneveux and Belthassen, 1996). Higher plants cannot support great variations in RWC but are able to regulate this component. In evolutionary terms, this ability seems to be related to the presence of vascular organs and roots and to the existence of regulatory mechanisms, such as stomatal regulation and osmotic adjustment (Monneveux and Belthassen, 1996). Among the summer crops evaluated in the current study, *B. vulgaris* var. *cicla* was the first plant to stress. The RWC dropped below 50% ten days after water was withheld. Swiss chard was, therefore, the most drought sensitive of all species tested, followed by *A. cruentus, S. retroflexum C. lanatus* and *C. maxima*, with *V. unguiculata* being the most drought tolerant species, because its RWC only dropped below 50% after 21 days of withholding water. The winter crop *B. rapa* subsp. *chinensis* only performed slightly better than the reference crop *B. vulgaris* var. *cicla* reaching a RWC below 50% after 12 days.

Recovery in RWC upon rewatering was very high (100%) in *C. lanatus, C. maxima* and *S. retroflexum*, and slightly lower (approx. 95%) in *V. unguiculata* and *B. vulgaris* var. *cicla,* with *A. cruentus* showing the lowest recovery (88%) in RWC, which was still high. Restoration and repair upon rehydration are critical indicators of desiccation tolerance (Blum, 1996). These processes involve the return of the ‘dormant’ or even the severely disrupted system to full function, indicating drought stress tolerance or sensitivity. Drought response indexes have strong associations with water loss during water deficit conditions, indicating cultivars showing low water loss to be more drought resistant (Dhanda *et al.*, 1999).

Leaf area

Leaf area can be controlled by either reducing individual leaf growth, or by reducing the number of leaves (Tardieu, 1996). Decrease in leaf size is usually a sign that the evaporation demand is higher than rate of water replacement in the cells, which is usually the first symptom of mild water deficits. The plant cannot maintain a high leaf water turgor and the leaves start to wilt. The more drought sensitive the species, the more wilting will occur.

Calculation of the mean leaf area of the summer ALVs (Table 4.2) showed that the most prominent reduction in leaf area occurred after 10 days in *B. vulgaris* var. *cicla* (-95 mm^2); after 12 days in *C. maxima* (-46 mm^2) and *C. lanatus* (-45 mm^2), after 19 days in *V. unguiculata* (-22 mm^2); and after 12 days in *S retroflexum* (-14 mm^2) and *A. cruentus* (-12 mm^2). Among the winter crops, LA was higher in *B. rapa* subsp. *chinensis* than in the reference crop *B. vulgaris* var. *cicla.* The reduction in LA could be directly attributed to a reduction in RWC. Cell expansion is one of processes affected by water deficit that is most sensitive (Hsiao, 1973). When

cell expansion is restricted, leaf extension and growth are reduced. Plant growth has been correlated directly with cell turgor (Boyer, 1987).

RWC can be maintained in cells and tissues to allow metabolic activity. This is a consequence of osmotic adjustment and other traits of adaptation to drought, such as root development and reduced transpiration. Higher plants cannot support great variations in RWC but are able to regulate this component. In evolutionary terms, this ability seems to be related to the presence of vascular organs and roots and to the existence of regulatory mechanisms such as stomatal regulation and osmotic adjustment.

Plants can modulate leaf area and thereby adjust water loss from the canopy to the size that can be effectively supplied by the existing soil water reserve. It is important for a crop to be able to adjust leaf size, as this is a means by which a water-stressed crop can maintain control over water-use, avoid desiccation under severe drought conditions, and enhance its chance of survival. Leaf dessication and a decrease in leaf area is an indication of water stress experienced by the plant. *B. vulgaris* var. *cicla* experienced the greatest decrease in leaf area, and this correlated with the RWC results. Conversely, among the summer crops, *V. unguiculata* shown to be able to maintain a relative high RWC for the longest period of time and also demonstrated the smallest decrease in leaf area. Similar relationships between RWC and LA were observed for the other species. This could point at the importance of turgor pressure in the maintenance of leaf function, such as stomatal conductance, maintenance of leaf area, and all other biochemical functions linked to these attributes, such as enzyme function, photosynthesis and respiration. Drought tolerant plants have the ability to respond to water deficit by rapid stomatal closure to reduce transpiration rate and stem water flow and in this way maintain a high leaf RWC. All six ALVs were all able to maintain a higher RWC and leaf area than the reference crop. This supports the hypothesis that these crops are well adapted to dry climatic conditions.

4.5 MEASUREMENT OF CELL MEMBRANE STABILITY AND CELL VIABILITY IN RESPONSE TO DROUGHT AND HEAT STRESS IN AFRICAN LEAFY VEGETABLES

4.5.1 Background

According to Blum and Ebercon (1981) drought tolerance of tissue may be exhibited and measured in any of the tissue's physiological or metabolic functions. Drought tolerance is process-specific since different physiological processes may show tolerance within a genotype (Blum, 1979). A valid and functional drought tolerance test should therefore relate to integrated plant responses at low plant organisational level (i.e. tissue growth), or a single attribute that is related to the basic dynamics of cellular or tissue responses to stress (Blum and Ebercon, 1981).

There are a few methods that could be used for assessing plant cellular or tissue viability following a stress treatment, i.e. regrowth (Ishikawa *et al.*, 1995; Wu and Wallner, 1983), vital staining (Chen and Gusta, 1982) and plasmolysis (Palta *et al.*, 1977). There are, however, two methods that are most often used in determining cell viability, namely, cell membrane stability *via* electrolyte leakage, and 2,3-5-triphenyl tertazolium chloride (TTC) reduction assays.

Cell membrane stability (CMS)

The rate of injury to cell membranes is commonly used as a measure of tolerance to plant stresses, such as cold/freezing (Sulc *et al.*, 1991), heat (Reynolds *et al.*, 1994; Srinivasan *et al.*, 1996), drought (Bewley, 1979; Farooz and Azam, 2006), as well as salinity and mineral deficiency or toxicity (Blum, 1988).

The rate of injury to cell membranes by drought may be estimated through measurement of electrolyte leakage from cells (Farooz and Azam, 2006; Gomes *et al.*, 2010). The ability of the membrane to retain and selectively transport cellular solutes gives an indication of the cellular membrane function. Excessive

leakage of cellular electrolytes is an indication of cellular membrane injury (Blum, 1998; Kosheva *et al.*, 2004; Labuschagne *et al.*, 2008). CMS in terms of electron leakage is a way of measuring the plant's resistance to stress conditions in terms of conserved cellular membrane function. This technique for screening plant material for potential anti-oxidant activity (= stress tolerance) is based on indirect monitoring of cell membrane intactness after a stress treatment, where K^+ leakage is measured in a solution with an atomic absorption spectrophotometer or a conductivity meter (Malan, 1989). Measuring CMS gives an indirect indication of membrane damage, reflecting oxidative stress tolerance or sensitivity, and will be tested as a possible drought tolerance mechanism in amaranth.

Reduction assays of 2,3-5-Triphenyl tetrazolium chloride (TTC)

Cell viability is assessed by TTC assays. In the past, TTC reduction has been primarily used as a qualitative response to determine seed viability, but subsequently it has been refined successfully for use as a screening method of various abiotic stresses. TTC assays are used as a measure of cell viability for heat (de Ronde *et al.*, 1995; Porter *et al.*, 1995); salt (Ishikawa, 1995), drought (de Ronde and van der Mescht, 1997) and cold/freezing (Ishikawa *et al.*, 1995; Manley and Hummel, 1996) tolerance. The results of these tests were shown to be correlated strongly with field performances for several crops, including soya beans (Martineau *et al.*, 1979) and sorghum (Sullivan and Ross, 1979). Schaff *et al.* (1987), on the other hand, found no correlation between TTC reduction and yield performances for 26 cultivars of kidney beans (*Phaseolus vulgaris*).

TTC assay measures cell viability and is based on the principles of tetrazolium salt reduction to formazan salts by dehydrogenase respiratory enzymes (Berridge *et al.*, 1996; Blum, 1998). According to Fokar *et al.* (1998), the TTC assay evaluates the mitochondrial electron transport chain and is therefore representative of respiratory activity. Leaf disks are given a mild mannitol shock and this shock triggers mechanisms in the cell leading to the reduction of TTC to formazan salts. The amount of formazan formed dehydrogenase activity serves as an indicator of the tolerance of the plant (De Ronde and Van der Mescht, 1997). Formazan formation is measured spectrophotometrically and a drought or heat index can be calculated using these data.

4.5.2 Material and methods

Greenhouse plants were cultivated and maintained as described above in section 4.3. When the plants reached an age of 5-6 weeks, the cell viability tests were carried out as follow:

Cell membrane stability (CMS)

Leakage of electrolytes from tissue to an external solution was measured by the electron conductivity of the solution. Cell membrane stability was measured according to the methods of Slabbert and Krüger (2000). Heat stress was induced by placing the leaf disks in a temperature controlled growth chamber. The drought stress was induced by incubating the leaf discs in a sodium hydrogen maleate buffer containing 0.5 M mannitol (-1.24MPa) as an osmoticum. The CMS was calculated as the reciprocal of the cell membrane injury after the method of Blum and Ebercon (1981): CMS (%) = [1-(T1/T2)/1-(C1/C2)] x 100

Reduction assays of 2,3,5-Triphenyltetrazolium chloride (TTC)

Leaf disks were obtained using a no. 6 cork bore from healthy leaves. The drought stress was induced by incubating the leaf discs in a sodium hydrogen maleate buffer containing 0.5 M mannitol (-1.24MPa) as an osmoticum. The TTC reduction assay was carried out as described by De Ronde *et al*. (1998).

4.5.3 Results

Cell membrane stability (CMS)

Summer crops

A clear difference between the results of CMS for the drought and heat treatments of all six crops was observed, and it was evident that the crops were all more heat sensitive than drought sensitive (Figure 4.7).

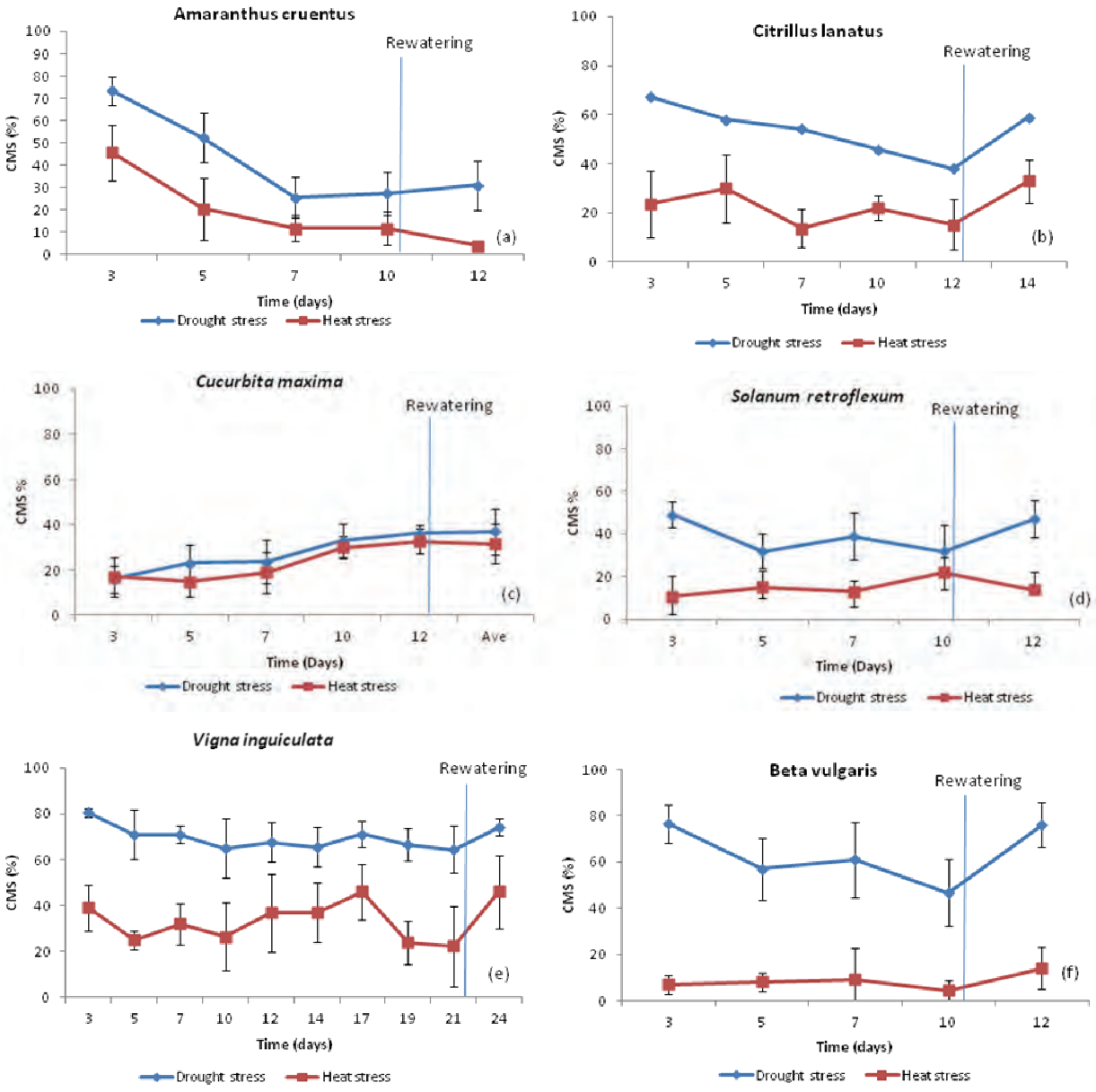


Figure 4.7: Effect of drought and heat stress on cell membrane stability (CMS) of (a) *Amaranthus cruentus*, (b) *Citrillus lanatus*, (c) *Cucurbita maxima*, (d) *Solanum retroflexum*, (e) *Vigna unguiculata* and (f) *Beta vulgaris* var. *cicla*

Drought: Data for 10 days drought and heat stress are summarised in Table 4.3. *A. cruentus* had the most significant decrease in CMS after 10 days drought stress from 73% to 27%, and recovery after rewatering was poor and reached only 31% (Figure 4.7a). The CMS of *C. lanatus* decreased from 67% after 3 days water stress, to 46% after 10 days water stress, and CMS after rewatering was 59% (Figure 4.7b). The CMS

for *C. maxima* was overall the lowest for all the crops tested and increased from 16% after three days waters stress, to 36% after 10 days water stress. The CMS after rewatering reached 37% (Figure 4.7c). CMS for *S. retroflexum* decreased from 67% to 46% after 10 days drought stress, but recovery was good and reached 59% (Figure 4.7d). *V. unguiculata* was able to maintain the highest CMS after 10 days water stress, where CMS decreased from 81% after three days water stress, to 65%, and after 21 days recovery was 64% after rewatering (Figure 4.7e). There was a slight significant decrease in CMS for *B. vulgaris* var. *cicla* from 76% to 47% over a period of 10 days water stress, but recovery was good and CMS was 76% two days after rewatering (Figure 4.7f). The mean CMS for drought stress can be seen in Figure 4.8. The summary and ranking of the cell membrane stability can be seen in Table 4.3, which shows that *V. unguiculata* had the highest CMS during the drought stress period, followed by *B. vulgaris* var. *cicla, C. lanatus, S. retroflexum* and *A. cruentus*, with *C. maxima* having the lowest CMS for drought stress.

Heat: All the ALVs tested had a lower CMS for heat tolerance, than drought tolerance (Figure 4.7). All ALVs were able to maintain a higher CMS during heat stress, than the reference crop *B. vulgaris* var. *cicla*. CMS for *A cruentus* decreased from 45% after 3 days heat stress treatment, to 12% after 10 days, and reached 4% after 12 days heat stress treatment (Figure 4.7a). CMS ranged between 22 to 24% for the first 10 days of heat stress treatment in *C. lanatus*, and reached 33% after 14 days (Figure 4.7b). CMS for *C. maxima* had a slight increase from 17 to 33% during the first ten days heat stress treatment, and maintained at 32% after 14 days (Figure 4.7c). *S. retroflexum* was also not able to maintain a high CMS, which ranged between 11-22% within the first 10 days of heat stress treatment, and the CMS decreased after 12 days to 14% (Figure 4.7d). *V. unguiculata* was able to maintain the highest CMS during the heat stress treatment, and had a CMS of 27 to 39% for the first 10 days of heat stress treatment, with a CMS of 22% after 21 days of heat stress treatment (Figure 4.7e). The CMS for heat tolerance was the lowest for the reference crop *B. vulgaris* var. *cicla* and ranged from 5% to 7% during the first 10 days of heat stress treatment, with a CMS of 14% after 12 days (Figure 4.7f). The mean CMS for heat stress can be seen in Figure 4.9.

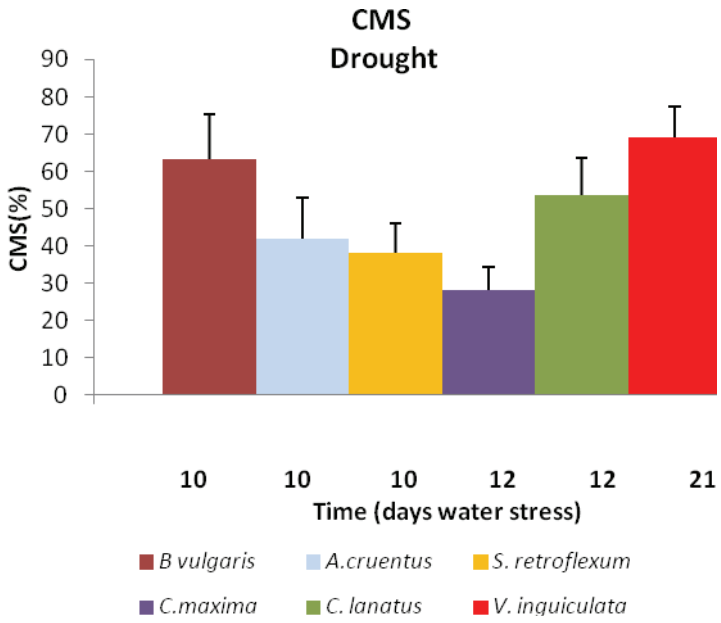


Figure 4.8: Mean cell membrane stability for drought stress in *Amaranthus cruentus*, *Brassica rapa* subsp. *chinensis*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata* and *Beta vulgaris* var. *cicla*

The summary and ranking of the cell membrane stability, presented in Table 4.3, indicates that *V. unguiculata* had the highest CMS during the heat stress period, followed by *A. cruentus*, *C. lanatus* and *C. maxima*, with *B. vulgaris* var. *cicla*, whilst *S. retroflexum* had the lowest CMS for heat stress.

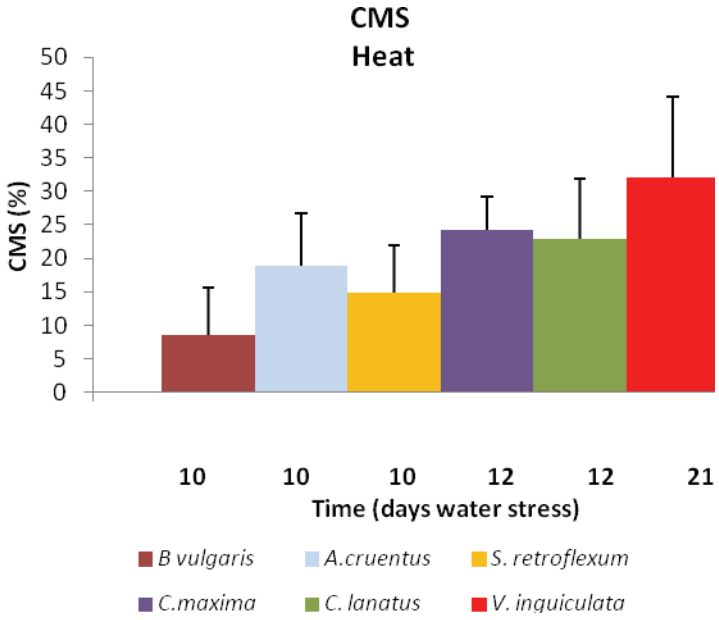


Figure 4.9: Mean cell membrane stability for heat stress in *Amaranthus cruentus*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata* and *Beta vulgaris* var. *cicla*

Winter crops

CMS for drought tolerance of *B. rapa* subsp. *chinensis* was only slightly higher than that of the reference crop *B. vulgaris* var. *cicla* (Figure 4.10). An average of 76 to 77% CMS was maintained during the first 10 days of drought stress for *B. rapa* subsp. *chinensis* (Figure 4.10a), compared to CMS of 60 to 75% for *B. vulgaris* var. *cicla* (Figure 4.10b) during the same period of time. CMS recovery upon rewatering was also good, and *B. rapa* subsp. *chinensis* reached 65% compared with 77% for *B. vulgaris* var. *cicla.* The mean CMS for drought stress can be seen in Figure 4.11. The summary and ranking of the cell membrane stability in Table 4.3 show that there were no statistical differences in drought tolerance for the two species tested.

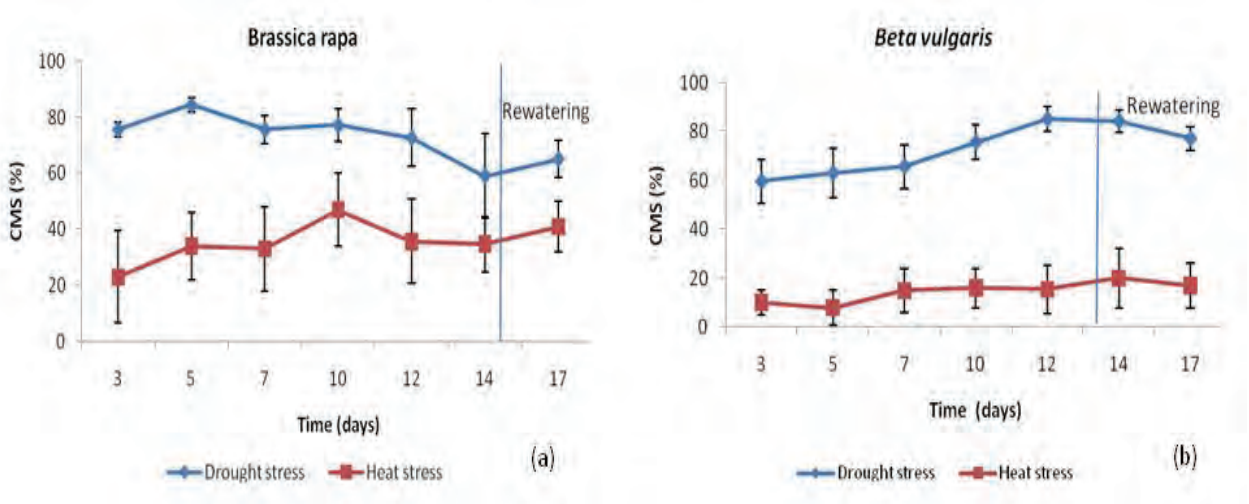


Figure 4.10: Effect of drought and heat stress on cell membrane stability (CMS) of (a) *Brassica rapa* subsp. *chinensis* and (b) the winter reference crop *Beta vulgaris* var. *cicla*

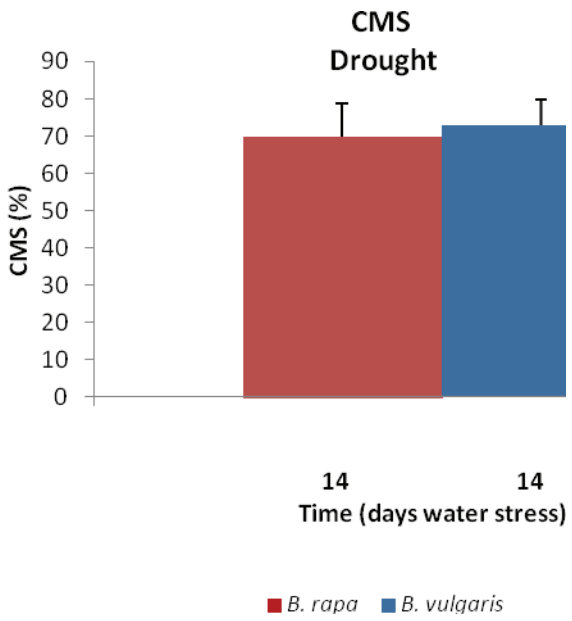


Figure 4.11: Mean cell membrane stability for drought stress in *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

B. vulgaris var. *cicla* had a lower heat tolerance than *B. rapa* subsp. *chinensis* (Figure 4.10) and CMS ranged from 10 to 16% during the first 10 days heat stress treatment for the former, while it maintained 23 to 47% CMS for the same stress period for *B. rapa* subsp. *chinensis.* The mean CMS for heat stress can be seen in Figure 4.12. The summary and ranking of the cell membrane stability in Table 4.3 indicates that *B. rapa* subsp. *chinensis* was statistically more heat tolerant than *B. vulgaris* var. *cicla.*

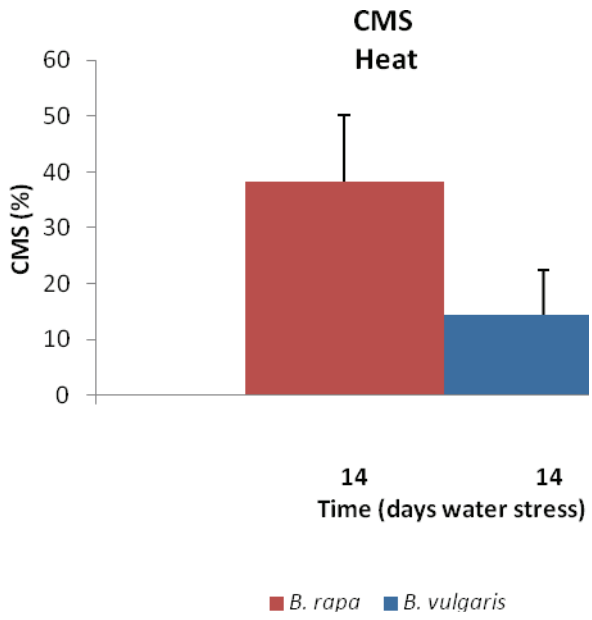


Figure 4.12: Mean cell membrane stability for heat stress in *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

Table 4.3: Summary and ranking of the cell membrane stability of *Amaranthus cruentus*, *Brassica rapa* subsp. *chinensis*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata* and *Beta vulgaris* var. *cicla* for drought and heat stress

Cell membrane stability (%)										
Summer crops	**Drought**					**Heat**				
	After 3 days	**After 10 days**	**RWC below 50%**	**Ave**	**Rank**	**After 3 days**	**After 10 days**	**RWC below 50%**	**Ave**	**Rank**
A. cruentus	73	27	31	42	4	45	12	4	19	2
C. lanatus	67	46	59	54	2	24	22	33	23	3
C. maxima	16	36	37	28	6	17	33	32	24	3
S. retroflexum	49	27	47	38	4	11	22	14	15	5
V. unguiculata	81	65	64	69	1	39	27	22	32	1
B. vulgaris var. *cicla*	76	47	76	63	2	7	5	14	9	5
Winter crops	**Drought**					**Heat**				
	After 3 days	**After 10 days**	**RWC below 50%**	**Ave**	**Rank**	**After 3 days**	**After 10 days**	**RWC below 50%**	**Ave**	**Rank**
B. rapa	76	77	65	70	1	23	47	41	38	1
B. vulgaris var. *cicla*	60	75	77	73	1	10	16	17	14	2

Cell viability (TTC)

Summer crops

Drought

The cell viability (TTC) results for drought stress over time are shown in Figure 4.13. Very little decrease in formazan absorbancy can be observed in *C. lanatus* (Figure 4.13b). *B. vulgaris* (Figure 4.13f) also had a decrease in formazan absorbancy, while an increase in formazan absorbancy can be seen for *C. maxima* (Figure 4.13c), *S. retroflexum* (Figure 4.13d), *A. cruentus* (Figure 4.13a) and *V. unguiculata* (Figure 4.13e). According to the results presented in Figure 4.14, *C. maxima*, *S. retroflexum* and *V. unguiculata* were drought tolerant, and *C. lanatus*, *A. cruentus* and *B. vulgaris* var. *cicla* were drought sensitive.

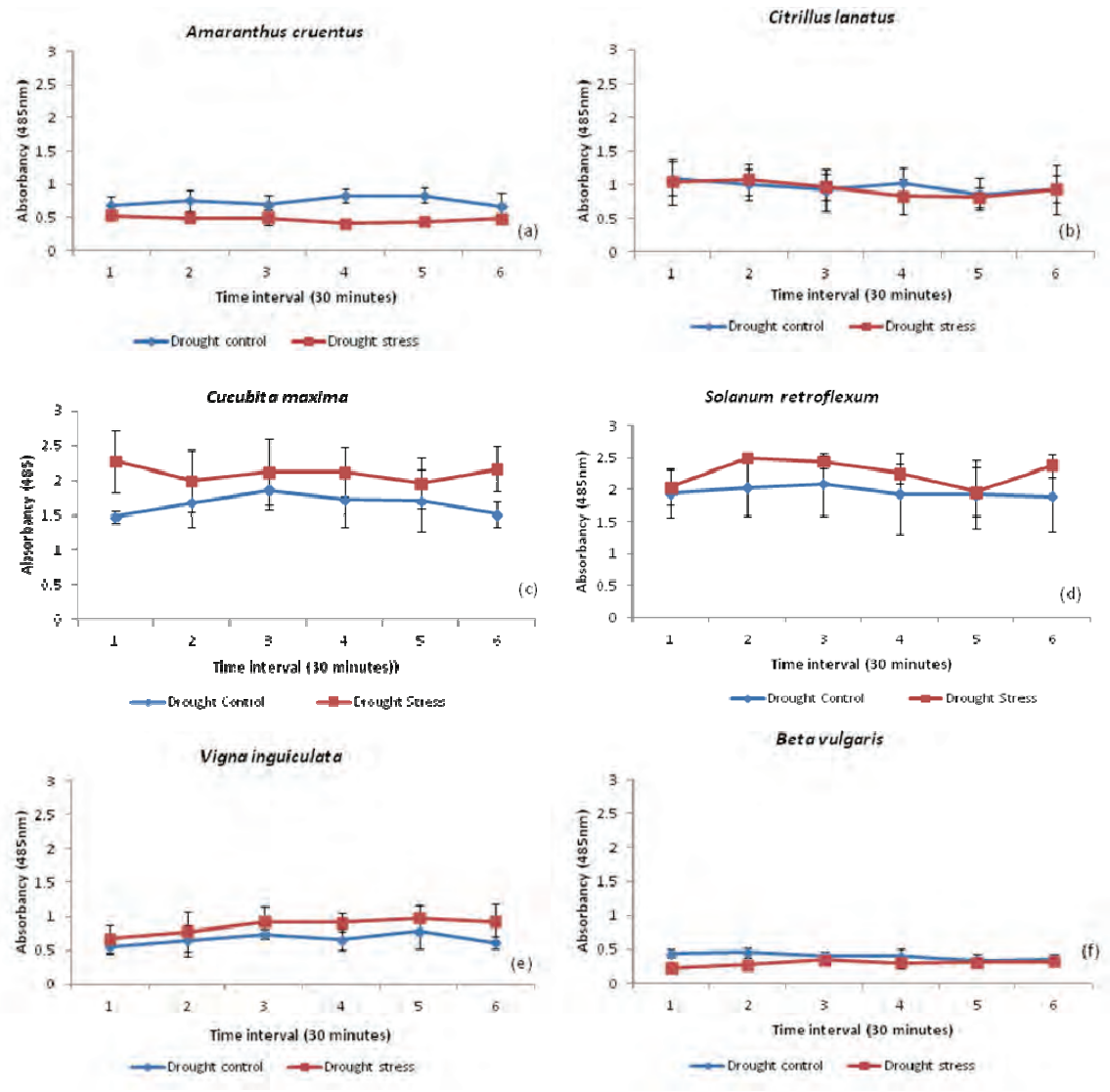


Figure 4.13: Changes in formazan absorbancy with time upon drought stress using the 2,3,5-triphenyltetrazolium chloride assay for (a) *Amaranthus cruentus*, (b) *Brassica rapa* subsp. *chinensis*, (c) *Citrillus lanatus*, (d) *Cucurbita maxima*, (e) *Solanum retroflexum*, (f) *Vigna unguiculata*, and (g) *Beta vulgaris* var. *cicla*

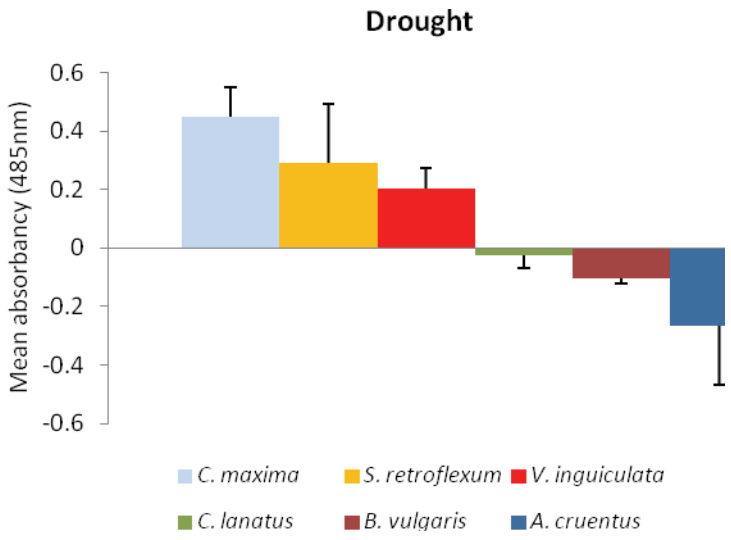


Figure 4.14: Mean changes in formazan absorbancy with time upon drought stress using the 2,3,5-triphenyltetrazolium chloride assay for *Amaranthus cruentus*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata* and *Beta vulgaris* var. *cicla*

Heat

The results shown in Figure 4.15 clearly demonstrate that the formazan absorbancy was relatively unchanged for heat stressed *A. cruentus* (Figure 4.15a), *C. maxima* (Figure 4.15c) and *B. vulgaris* var. *cicla* (Figure 4.15f). Formazan absorbancy decreased as a result of heat stress for *C. lanatus* (Figure 4.15b), *S. retroflexum* (Figure 4.15d) and *V. unguiculata* (Figure 4.15f), indicating heat sensitivity. It is clear from Figure 4.16 that the mean formazan absorbancy indicated greatest heat tolerance in *A. cruentus* and *C. maxima*, followed by *B. vulgaris* var. *cicla*, *V. unguiculata*, *S. retroflexum* with *C. lanatus* being most heat sensitive (Figure 4.16).

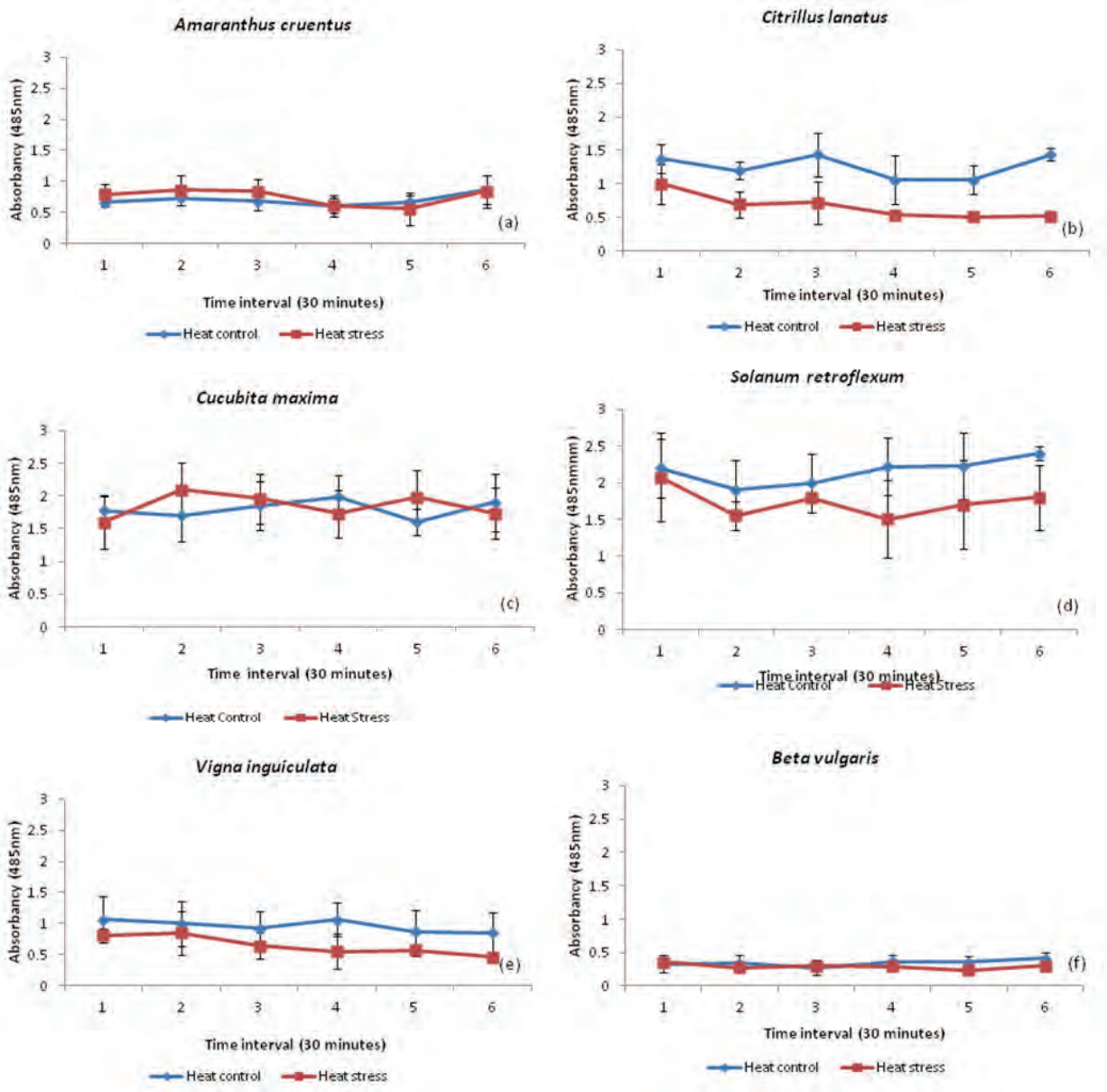


Figure 4.15: Changes in formazan absorbancy with time upon heat stress using the 2,3,5-triphenyltetrazolium chloride assay for (a) *Amaranthus cruentus*, (b) *Brassica rapa subsp. chinensis*, (c) *Citrillus lanatus*, (d) *Cucurbita maxima*, (e) *Solanum retroflexum*, (f) *Vigna unguiculata* and (g) *Beta vulgaris* var. *cicla*

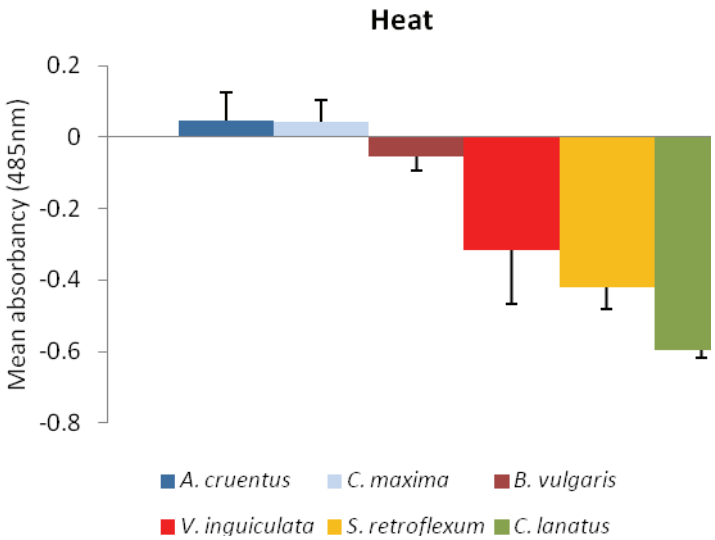


Figure 4.16: Mean changes in formazan absorbancy with time upon heat stress using the 2,3,5-triphenyltetrazolium chloride assay for *Amaranthus cruentus*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum*, *Vigna unguiculata* and *Beta vulgaris* var. *cicla*

Winter crops

Drought

Both *B. rapa* subsp. *chinensis* (Figure 4.17a) and *B. vulgaris* var. *cicla* (Figure 4.17b) formazan absorbancy was low for the stressed plants, and both were drought sensitive. The mean absorbancy results indicate that the formazan absorbancy for the two species did not differ statistically for drought tolerance (Figure 4.18).

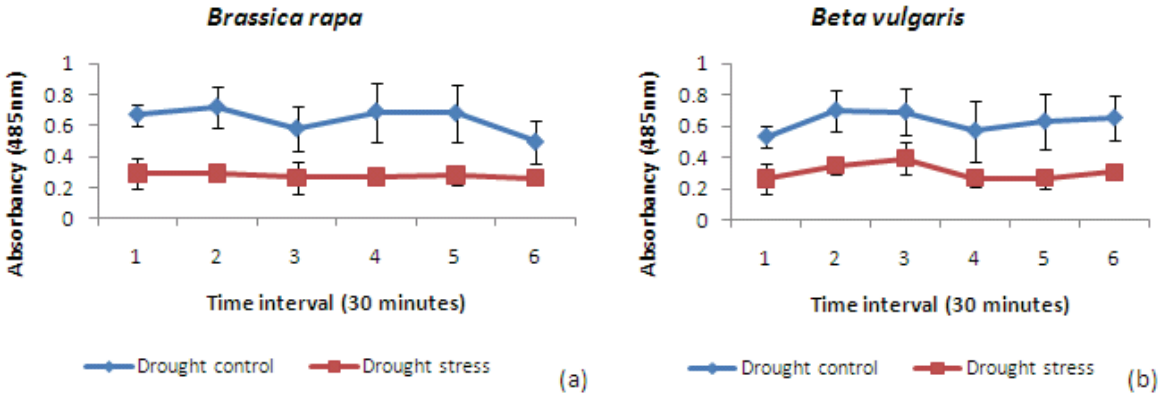


Figure 4.17: Changes in formazan absorbancy with time upon drought stress using the 2,3,5-triphenyltetrazolium chloride assay for *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

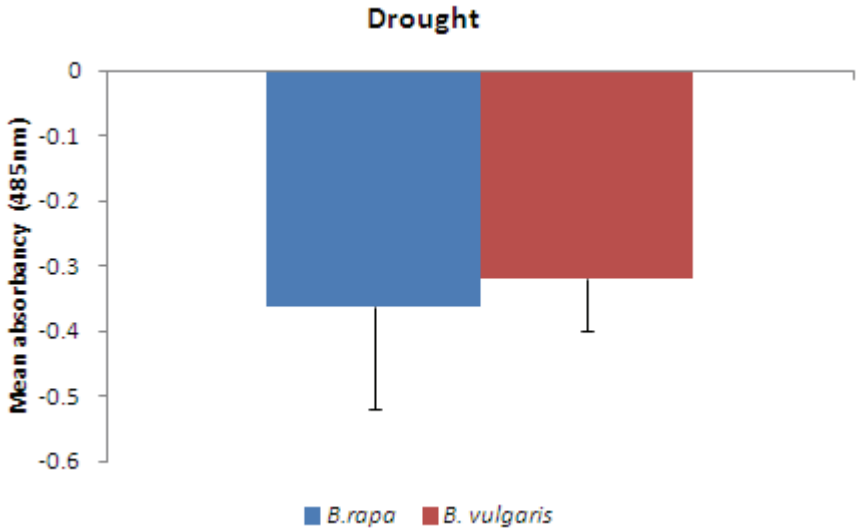


Figure 4.18: Mean changes in formazan absorbancy with time upon drought stress using the 2,3,5-triphenyltetrazolium chloride assay for *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

Heat stress

Both *B. rapa* subsp. *chinensis* (Figure 4.19a) and *B. vulgaris* var. *cicla* (Figure 4.19b) formazan absorbancy was lower for the stressed plants, and both were heat sensitive. The mean absorbancy results indicate that the formazan absorbancy for the two species did not differ statistically for heat tolerance (Figure 4.20).

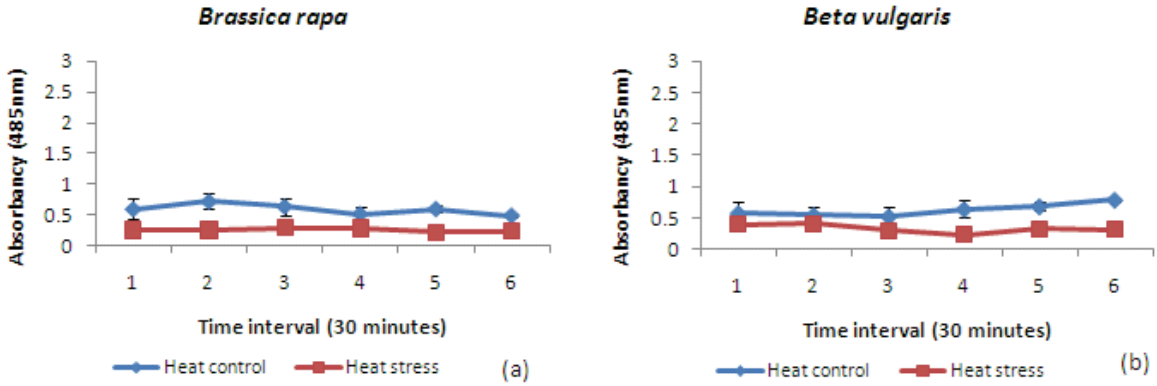


Figure 4.19: Changes in formazan absorbancy with time upon heat stress using the 2,3,5-triphenyltetrazolium chloride assay for *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

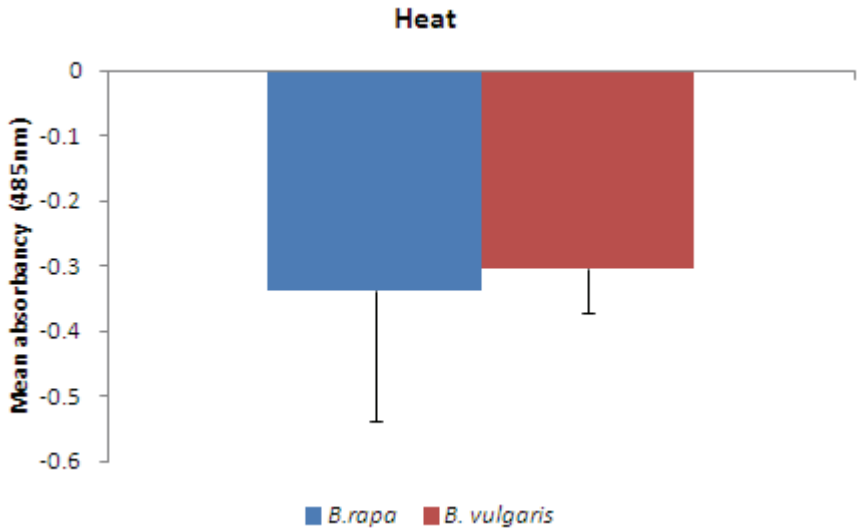


Figure 4.20: Mean changes in formazan absorbancy with time upon heat stress using the 2,3,5-triphenyltetrazolium chloride assay for *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

A summary of the findings obtained by means of the cell membrane stability *via* electrolyte leakage and the 2,3-5-triphenyl tertazolium chloride (TTC) reduction assays is presented in Table 4.4.

Table 4.4: Mean changes in formazan absorbancy with time upon drought stress using the 2,3,5-triphenyltetrazolium chloride assay for (a) *Amaranthus cruentus*, (b) *Brassica rapa* subsp. *chinensis*, (c) *Citrillus lanatus*, (d) *Cucurbita maxima*, (e) *Solanum retroflexum*, (f) *Vigna unguiculata* and (g) *Beta vulgaris* var. *cicla*

Cell viability Mean formazan absorbancy (485nm)				
Summer crops	**Drought**	**Ranking**	**Heat**	**Ranking**
A. cruentus	-0.27	6	0.05	1
C. lanatus	-0.03	4	-0.60	6
C. maxima	0.45	1	0.04	2
S. retroflexum	0.30	2	-0.42	4
V. unguiculata	0.20	3	-0.32	5
B. vulgaris var. *cicla*	-0.10	5	-0.05	3
Winter crops	**Drought**	**Ranking**	**Heat**	**Ranking**
B. rapa subsp. *chinensis*	-0.364	2	-0.340	2
B. vulgaris var. *cicla*	-0.320	1	-0.303	1

4.5.4 Discussion and conclusion

Cell membrane stability

Cellular membrane function is generally assessed by its ability to retain and selectively transport cellular solutes (Blum, 1998). Generally, excessive leakage of cellular electrolytes can is seen as a sign of membrane injury. *V. unguiculata* was both the most heat and drought tolerant of all the species tested. Electrolyte leakage in *B. vulgaris* var. *cicla* indicated low leakage during drought, suggesting drought tolerance, but heat excessive leakage of electrolytes occurred during heat stress, possible indicating heat sensitivity. Consequently, wilting of *B. vulgaris* var. *cicla* during hot days could probably be due to heat sensitivity, rather than drought sensitivity. CMS measures a plants ability to resist stress in terms of conserved cellular

membrane function. There are various reports on the use of CMS in the assessment of heat tolerance (Blum and Ebercon, 1981; Shanahan *et al*., 1990) as well as drought tolerance (Blum and Ebercon, 1981; Gomes *et al*., 2010). Saadalla *et al*. (1994) reported that CMS assays for heat tolerance in wheat seedlings could be used as a predictor of the thermotolerance of mature plants. Blum (1998) compared CMS for measuring thermotolerance at different stages of wheat development after imposing controlled heat hardening of either seedlings or plants. Blum (1998) found that thermotolerance tended to increase from seedling to flowering stage and thermotolerance for CMS was well correlated between growth stages of the eight wheat cultivars tested.

Cell viability (TTC)

The principle of the TTC assay is that formazan production is relatively lower in stressed leaves of sensitive cultivars compared to the leaves of the unstressed control treatment. However, for tolerant cultivars the opposite reaction was observed where the formazan levels were higher in the stress treatment compared to the unstressed control treatment. Similar tendencies were found in cotton for drought as well as heat stress (Nachlas *et al*., 1960). When exposed to water stress, cell viability was low in *A. cruentus* and *B. vulgaris* var. *cicla*, and highest in *C. maxima* and *S. retroflexum*. For heat stress, cell viability was low in *C. lanatus* and *V. unguiculata* and highest in *C. maxima* and *A. cruentu*s. Low cell viability was due to inefficient tolerance mechanisms to survive a moderate drought or heat stress during the TTC analysis and the plant could not adapt to the stress (indicated by the reduction of cell viability and production of formazan salts, compared to the control). According to De Ronde and Van der Mescht (1997), TTC analyses of potato plants showed that heat and drought tolerance is organ and cultivar specific.

It is difficult to select species tolerant to either drought, as plants respond to these two environmental stresses simultaneously, because in many places dry conditions are usually accompanied by warm temperatures. Physiological screening techniques are used to distinguish between different stress conditions, and to limit the selection to only one parameter. Drought simulation in the laboratory eliminates heat stress, and *vice versa.* This allows for the distinction between species which are drought and/or heat sensitive. Cell viability by means of the TTC assay could assist plant breeders to establish stress indices to distinguish between plant responses to heat and drought (de Ronde *et al.*, 1995). Similarly drought and heat tolerance can be measured *in vivo* through cell membrane stability. Differences in plant growth and production during stress are due to the differences in stress tolerance between species. By selecting tolerant species, management of single parameters hampering plant production, such as drought, could be accomplished. This knowledge could assist breeders in the selection of the best performing (highest production) species for specific climatic environments.

4.6 ESTIMATING EARLY DROUGHT TOLERANCE OF AFRICAN LEAFY VEGETABLES USING THE WOODEN BOX TECHNIQUE

4.6.1 Background

Numerous constitutive traits affect crop performance under drought stress. The most notable constitutive traits affecting plant productivity under drought stress are plant phenology, early plant vigour, canopy architecture, plant surface characteristics, root size and depth, root penetration capacity, stem reserve utilization for grain filling and potential yield (Blum, 1996). During its lifetime a plant can encounter several dry spells, which can affect it adversely. These dry spells can occur at any stage of the development of the plant (Singh *et al.*, 1999).

Singh *et al.* (1999) developed the wooden box technique for screening plants for early drought tolerance according to root development at seedling stage. The advantage of this technique is that it is relatively easy to use whilst still providing a good indication of the drought tolerance of plants. For breeding purposes,

plants that avoid drought can be detected and the drought tolerant plants that survived this procedure can be mass propagated afterwards.

4.6.2 Material and methods

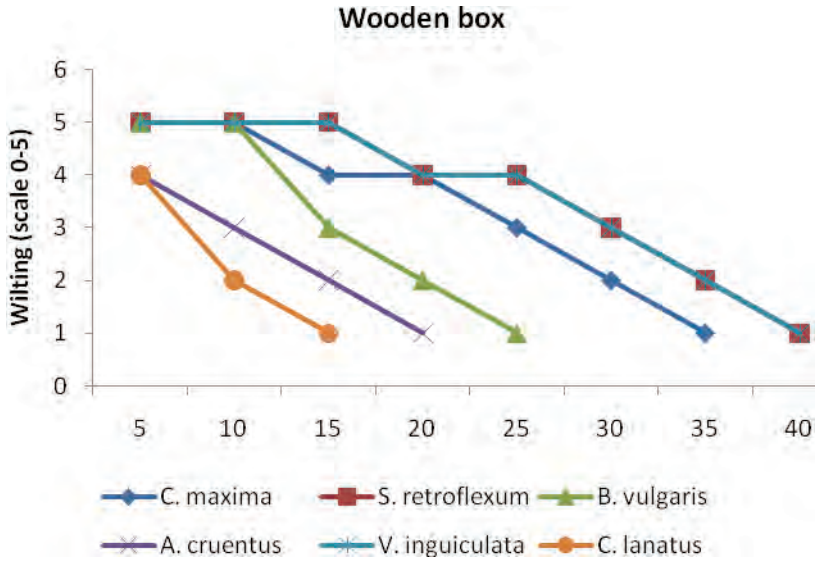
Wooden boxes were built according to the methods described by Slabbert and Krüger (2000). Seed were sown in shallow furrows and thinned out to 12 seedlings per row and 16 rows per box. Liquid fertilizer was applied every week until drought stress commenced. Irrigation continued every second day using watering can (measured equal volumes of water for each box). Watering was stopped when seedlings reached the 2nd-3rd leaf stage.

Drought stress was measured daily over a period of 2-3 weeks, using wilting on a 0-5 scale as criterion: 0 = 100% wilted and dry; 1 = 100% wilted; 2 = 75% wilted; 3 = 50% wilted; 4 = 25% wilted/started wilting; 5 = no wilting. Physiological changes such as chlorosis, leaf drop, and anthocianin formation was also noted. Rewatering was done when seedlings reached the 100% wilting ratio and recovery was noted.

4.6.3 Results

Summer crops

It is clear from Figure 4.21 that early drought tolerance was very diverse for the different species trested. *A. cruentus* and *C. lanatus* seedlings started to wilt (25% wilting) after 5 days of water stress. *C. lanatus* reached 50% wilting between 5-10 days water stress, and after 15 days water stress 100 wilting was reached already.



5= upright, healthy; 4= starts wilting/25% wilted; 3= 50% wilted; 2= 75% wilted; 1= 100% wilted; 0= dry/dead

Figure 4.21: Effect of increasing water stress (days withholding water) on early drought tolerance by measuring wilting in *Amaranthus cruentus*, *Beta vulgaris* var. *cicla*, *Citrillus lanatus*, *Cucurbita maxima*, *Solanum retroflexum* and *Vigna unguiculata*

A. cruentus reached 50% wilting after 10 days water stress. The first two bottom leaves turned chlorotic and dropped off in *A. cruentus* after reaching 75% wilting within 15 days water stress. *A. cruentus* reached 100% wilting after 20 days of water stress. *B. vulgaris* var. *cicla* showed symptoms of chlorosis after three days of water stress and started wilting (25% wilting) after 10 to 15 days of water stress, with 50% of the plants

wilted after 15 days of stress, when the two bottom leaves dropped off. The seedlings were 75% wilted after 20 days of water stress and 100% wilted after 25 days of water stress. *C. maxima* only started wilting (25%) after 15 days water stress, and reached 50% wilting after 25 days of water stress. The seedlings reached 75% wilting after 30 days, and 100% wilting after 35 days. The seedlings of both *V unguiculata* and *S. retroflexum* started (25%) to wilt after 20 days, and reached 50% wilting after 30 days water stress. The seedlings were 75% wilted after 35 days and 100% after 40 days. Anthocyanin formation in was noticed in the bottom three leaves of *V. unguiculata* after 20 days of water stress.

Winter crops

It is clear from Figure 4.22 that both *B. vulgaris* var. *cicla* and *B. rapa* subsp. *chinensis* seedlings started to wilt (25% wilting) after 20 days of water stress.

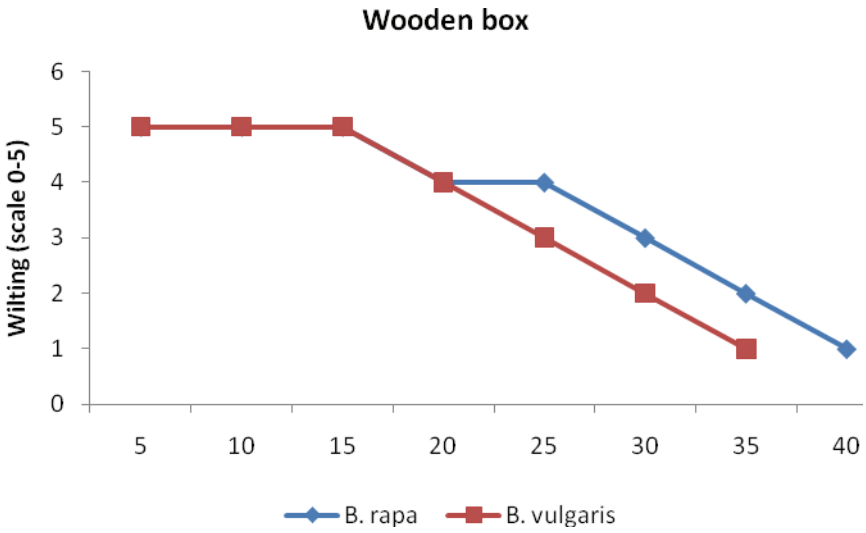


Figure 4.22: Effect of increasing water stress (days withholding water) on early drought tolerance by measuring of wilting in *Brassica rapa* subsp. *chinensis* and *Beta vulgaris* var. *cicla*

B. vulgaris var. *cicla* reached 50% wilting between 25 days water stress, and after 30 days water stress 75% wilting was reached, followed by 100% wilting after 35 days water stress. *Brassica rapa* subsp. *chinensis* seedlings reached 50% wilting after 30 days water stress, followed by 75% wilting and 100% wilting after 35 and 40 days, respectively. A summary of the findings obtained by means of the wooden box technique are presented in Table 4.5

Table 4.5: Ranking of African leafy vegetables according to early drought tolerance as measured by wilting index

Summer crops	**Ranking***
A. cruentus	5
C. lanatus	6
C. maxima	3
S. retroflexum	1
V. unguiculata	1
B. vulgaris var. *cicla*	4
Winter crops	**Ranking**
B. rapa subsp. *chinensis*	1
B. vulgaris var. *cicla*	2

*1 = most drought tolerant and 6 = most drought sensitive

4.6.4 Discussion and conclusion

Early drought screening gives us an indication of the ability of seedlings to withstand water stress conditions at an early stage of development. Seedlings of *V. unguiculata, S. retroflexum* and *C. maxima* were more drought tolerant at an early developmental stage than those of *B. vulgaris* var. *cicla, A. cruentus* and *C. lanatus. V. unguiculata* took 40 days to reach 100% wilting, while *C. lanatus* was 100% wilted after only 15 days. Leaf drop during drought stress is an indication of drought avoidance, as was observed among the *B. vulgaris* var. *cicla* seedlings. Anthocyanin formation, which was noticed after three weeks of drought stress in *V. unguiculata* seedlings, is a known phenomenon in juveniles (Gitz *et al.*, 1998) and can be linked to abiotic stresses such as drought (Balakumar *et al.*, 1993). Numerous reports suggest that plant cells containing anthocyanin are often tolerant to abiotic stresses, such as cold and drought. It has been suggested that anthocyanin formation can be used as a visual tool to identify possible drought tolerance in certain crops. In some species, leaf abscission is an indicator of drought avoidance, achieved by reducing the surface area for transpiration. This mechanism enhances the likelihood of survival of the plant.

4.7 GENERAL CONCLUSION

The development of a wider choice of crops, including crops adapted to dry areas is critical if the growing human population of South Africa will continue to obtain its food from local production. If global warming persists, areas currently under irrigation could in future be without water supply, which means that cultivation will require the use of drought tolerant crops. To complicate matters, drought seldom occurs in isolation, and mostly interacts with a variety of other abiotic and biotic stresses. Usually drought is accompanied by high temperatures and stressed plants are then again more susceptible to biotic stresses, such as disease. This complex challenge requires improved yield and productivity during abiotic stress conditions, which could involve the use of new crop species.

Drought affects almost all aspects of plant growth and, most importantly, productivity and product quality. The majority of people from resource poor areas do not have irrigation equipment and are dependent on rainfall for their crop harvests. No rain means no harvest for many resource poor people, leading to famine and malnutrition. Knowledge of the drought/heat tolerance status of ALVs is expected to provide resource-poor farmers and extension officers with information that will enable them to make better crop choices for specific agricultural regions. It opens the possibility to exploit many marginal areas where arid and semi-arid conditions prevail, and drought occurs periodically during the growth season. Under such conditions, it is important to understand the plant responses to water deficit. Plant responses to water stress depend on many factors, of which the amount of water loss, the rate of loss and the duration of the stressed condition play important parts.

The main objective of this study was to investigate the response of ALVs when exposed to drought and heat stress conditions and to suggest possible strategies for the selection of specific ALVs for use in areas of South Africa where soil water availability is limiting, a condition that is usually associated with high temperatures. In the prevailing context of human population growth, persistent poverty and associated malnutrition, broadening knowledge of alternative vegetables for use dry areas is an important element of a national strategy aimed at maintaining and improving nutritional security at household level.

The ALVs and the exotic vegetable differed in their response to drought stress and heat stress, which corresponded with existing theory and was a confirmation of the idea that plant response to drought and heat stress depends on plant species and stress severity. Drought and heat tolerance are multidimensional stress factors and for this reason, studies of drought and heat tolerance mechanisms are of a multidisciplinary nature. A complementary approach to improve plant performance in water limited environments involves the identification and selection of traits that contribute to drought avoidance, drought tolerance or high water use

efficiency. A partial list of potential important drought and heat traits was examined in this study, i.e. relative water content, leaf area, cell membrane stability, cell viability and early drought tolerance. The reactions of all eight ALVs and reference crop in response to drought and heat stress are summarised in Table 4.6. The variation in reaction among the eight ALVs tested supported existing theory that plant species display different adaptations/avoidance to water and heat stress.

Table 4.6: Summary of ranking for drought and heat tolerance of African leafy vegetables

	Drought*							Heat*			
	RWC	**LA**	**CMS**	**TTC**	**WB**	**Average**	**Rank**	**CMS**	**TTC**	**Average**	**Rank**
Summer crops											
A. cruentus	5	2	4	6	5	(4.4)	5	2	1	(1.5)	1
C. lanatus	2	4	2	4	6	(3.6)	4	3	6	(4.5)	4
C. maxima	2	4	6	1	3	(3.2)	3	3	2	(2.5)	2
S. retroflexum	4	2	4	2	1	(2.6)	2	5	4	(4.5)	4
V. unguiculata	1	1	1	3	1	(1.4)	1	1	5	(3.0)	3
B. vulgaris var. *cicla*	6	6	2	5	4	(4.6)	6	5	3	(4.0)	6
Winter crops											
B. rapa subsp. chinensis	2	2	1	2	1	(1.6)	2	1	2	(1.5)	1
B. vulgaris var. *cicla*	1	1	1	1	2	(1.2)	1	2	1	(1.5)	1

*1 = most drought- or heat tolerant and 6 = most drought- or heat sensitive

RWC = relative water content; LA = leaf area; CMS = cell membrame stability; TTC = 2,3-5-triphenyl tetrazolium chloride reduction; WB = wooden box (early drought tolerance)

Overall, the results of this study indicated that the ALVs had a higher degree of drought and heat tolerance than the reference crop *B. vulgaris* var. *cicla* but also that this general conclusion needed to be qualified. The findings show interesting differences and tendencies, emphasizing that drought tolerance is a multi-genetic trait. In practical terms this means that among different plant species and even among plants within a particular genotype, different mechanisms of drought tolerance are switched on at different stages of plant development. While some species show a higher drought or heat tolerance at an early stage of development, others become more tolerant over time. For this reason it was important is to use a variety of screening techniques (cellular level and whole plant level) to eventually arrive at a balanced conclusion concerning the general drought and/or heat tolerance of a given plant. The findings on the drought and heat tolerance of the ALVs that were tested could assist farmers in optimising crop choice when combined with knowledge of temperature and rainfall of their local environments.

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CHAPTER 5: WATER REQUIREMENT OF SELECTED AFRICAN LEAFY VEGETABLES

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5.1 INTRODUCTION

Crop water requirement is the quantity of water required by a crop or mixed population of crops during a given period of its normal growth under field conditions (Doorenbos and Pruitt, 1977). The water requirement of a crop varies during its growing period, mainly due to variation in crop canopy, agronomic practices, irrigation methods and weather conditions (Doorenbos and Kassam, 1979). About 99% of the water taken up by a crop from the soil is lost as evapotranspiration (ET) (Smith, 1992, 1993). Measurement of the actual crop evapotranspiration (ETc) on a daily basis during the entire growing period of a crop provides the total water requirement of a given crop (Doorenbos and Pruitt, 1977). The most commonly used technique to estimate ETc makes use of the crop coefficient (Kc) approach, whereby the ETc is calculated using potential evapotranspiration ETo and the Kc, which takes into account the relationship between atmosphere, crop physiology and agronomic practices (Allen *et al*., 1998). The ETo is the amount of water a healthy, actively growing cool-season grass subjected to the weather condition where the crops grew over a specified time (Allen *et al*., 1998). The importance of this value is that crops at a particular site, such as Roodeplaat, will use water in direct proportion to the evapotranspiration of this reference grass. Therefore, accurate estimates of crop water use (crop evapotranspiration, ETc) depend to a large extent on the accuracy of the coefficients used to relate ETo to ETc. The objective of this study was to determine crop water requirement, biomass production and crop water productivity of eight ALVs.

5.2 MATERIALS AND METHODS

Two experiments were conducted in 2009/10 and 2010/11 to achieve the objectives. The first experiment was conducted to establish the water requirement of the ALVs and mini-lysimeters were used to determine the Kc values. The second experiment was conducted under a rain shelter to evaluate the effect of water stress on biomass production and crop water productivity.

5.2.1 Water requirement

The experiment was carried out at Agricultural Research Council-Roodeplaat 30 km North East of Pretoria (Latitude 25°35' N, Longitude 28°21' E and Altitude 1164 m), in Gauteng Province, Republic of South Africa, using plastic mini-lysimeter pots. The actual ETc was estimated using a water balance method of weighable lysimeters. The lysimeters had a size of 0.5 m diameter and a height of 0.5 m. A total of 15 lysimeters were prepared for each crop. The lysimeters were filled with local soils collected from the site. Eight ALVs of known cultivar were used for the experiment as described in Table 5.1. The ALVs were sown based on traditional knowledge and after thinning the containers contained three plants per pot at each harvest. According to Allen *et al*. (1998) well watered condition should be considered when ETc is measured for crop coefficient development. The lysimeters were irrigated to field capacity and were irrigated every three to four days. Catch cans were used to capture water that drained out of the lysimeters after each irrigation. The water was then reapplied to the lysimeter to keep even water application in all the lysimeters. The plants received adequate radiation and fertilizer solution during their growing period. Fresh weight of the above ground biomass was taken four to five times in the growing season and dry weight was determined by drying the fresh biomass in a 60^{o}C oven for 48 hours. Climate influences duration of the total growing

period and the various growth stages of crops. The total growing period (in days) from transplanting to the last day of the harvest and the harvest days were recorded. At least four harvests were made for each crop and ETo was determined for each period from the automatic weather station connected to the internet through radio network. The lysimeters were weighed at a 48 hours interval to calculate the ETc. ETc were measured by determining the mass loss of the weighing lysimeters. The daily ETc measurements obtained from the weighing mini-lysimeters were related to reference ETo computed from the Penman-Monteith equations (Allen *et al.*, 1998). Crop coefficients (Kc) values were calculated by dividing the actual ETc derived from the lysimeters by the cumulative ETo for that period (Kc = ETc/ETo) determined using the automatic weather station.

5.2.2 Biomass and water productivity

Field experiments were also carried out under rain shelters at ARC – Roodeplaat experimental site during the growing period 2009-2011. The average daily maximum and minimum temperature for Roodeplaat is 34 and 8°C in summer (November-April), 23 and 4°C in winter (May-August) and 29 and 11°C in autumn (September-October) (Figure 5.1). The hottest month is December, which an average maximum temperature over 29 year period (1980-2009) of 32°C. Based on the mean monthly record, July is the coldest month with the mean maximum and minimum temperatures being 21 and 2°C. December, January and February are hot months, whereas May, June and July are cold and dry months.

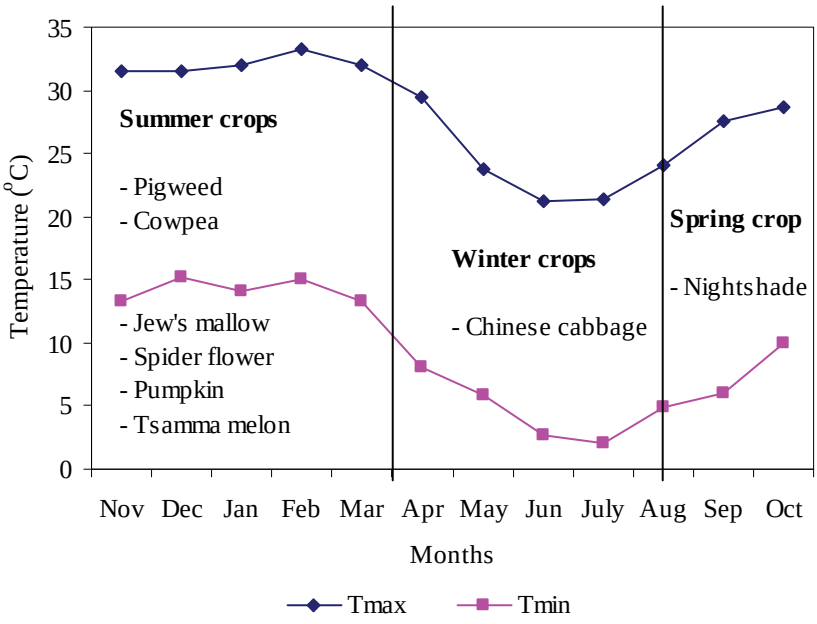

Figure 5.1: Average maximum and minimum temperature of Roodeplaat from 1980 to 2009 (ARC – ISCW, March 2010)

The daily potential evapotranspiration of summer and winter seasons of Roodeplaat varies and the atmospheric demand in the winter is lower than the summer season (Figure 5.2).

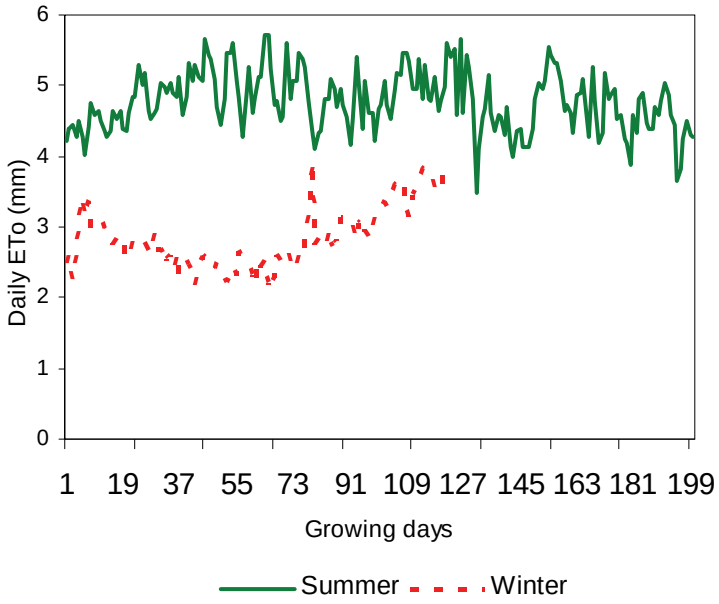

Figure 5.2: Daily potential evapotranspiration (ETo) at Roodeplaat (1998-2011)

The soil type under the rain shelters is sandy loam with 15-20% clay and 70-80% of sand. The bulk density of the soil is 1.51 g cm^{-3}. At field capacity the volumetric soil water content was on average 23%, at permanent wilting point 14%, and at saturation 43%. Soil samples were also taken at 30 cm depth and 60 cm depth to provide a baseline to manage the irrigation water and make fertilizer recommendations.

Seeds of the eight ALVs were planted in seedling and transplanted to the experimental site after four weeks of establishment in the seedling trays. Depending on the soil analysis the appropriate rate of N: P: K fertilizer (2:3:4 (30) was thoroughly mixed into the soil at planting (Table 6.1). The remaining amount of fertilizer was added two weeks after transplanting. The crop also received nitrogen topdressing in the form of limestone ammonium nitrate comprising 28% nitrogen (LAN (28)). Full irrigation was applied during the first two weeks to have a good stand, avoid flowering and to keep the vegetable quality marketable, where after the irrigation treatments were started.

A drip irrigation system was designed for a field block of 12 m x 24 m for the rain shelter. The block was divided into 40 plots to include different treatment replications. A plastic of 2 mm thickness and a width of 1 m were buried to a depth of 1.2 m in the ground to divide the irrigation field into forty plots to avoid water redistribution between the irrigation treatments. Treatments were then completely randomized and replicated five times. The envisaged irrigation levels were irrigation to field capacity (FC) when the Plant Available Water depleted to 40% is considered to be 100% -100FC. An amount of water that applies 75% of the water applied to refill to FC is 75FC, an amount of water that applies 50% of the water applied to refill to FC is 50FC and amount of water that applies 25% of the water applied to refill to FC is 25FC. The Neutron probe calibrated for the field was used to take readings per week, before each irrigation cycle. The drip irrigation system comprised an electric powered pump, control unit (Solenoid valves and Controller), filter, water meters and polyethylene drip tape. The amount of water applied to each treatment was recorded from the water meters installed inline to the main pipe.

Measurement of meteorological data is important for calculation of evaporative atmospheric demand of a crop and was collected from an automatic weather station which was set up at 50 m distance from the irrigated field. These weather stations measures every fifteen minutes meteorological data such as temperature and RH using CS-500 Vaisala temperature and relative humidity probe, solar radiation using LI -200 Pyranometer, rainfall and

amount intensity using a tipping bucket Texas instrument inc. rain gauge (Campbell scientific Logan UT.USA), and wind speed and direction, using R.M. Young cup anemometer. These measurements are converted to hourly values, ETo is calculated using the FAO-Penman Monteith equation and stored through the radio network in a databank which is accessed using the Internet. Table 5.1 shows the agronomic practices, herbicides and insecticides of (May-August and September-April) of the monitored vegetables.

Table 5.1: Agronomic practices used in the experiments with different African leafy vegetables

African leafy vegetable specie	Planting month	Plant population (plants ha^{-1})	Fertilizer application rate	Herbicides /insecticides
Non-heading Chinese cabbage *(Brassica rapa* subsp. *chinensis)*	June	111 111	N:190 kg ha^{-1}; P:50 kg ha^{-1}; K:50 kg ha^{-1}	Ridomil, for the control of white rust
Nightshade *(Solanum retroflexum)*	September	111 111	N:150 kg ha^{-1} P:20 kg ha^{-1} K: 200 kg ha^{-1}	-
Pumpkin*(Cucurbita maxima)*	December	33 333	N:150 kg ha^{-1} P:20 kg ha^{-1} K: 200 kg ha^{-1}	Malasol and Decis to control aphids and red spider mites
Tsamma melon *(Citrillus lanatus)*	December	33 333	N:150 kg ha^{-1} P:20 kg ha^{-1} K: 200 kg ha^{-1}	Malasol and Decis to control aphids and red spider mites
Jew's mallow (wild jute) *(Corchorus olitorius)*	December	95 238	N:150 kg ha^{-1} P:36 kg ha^{-1} K: 284 kg ha^{-1}	-
Cowpea *(Vigna unguiculata)*	December	95 238	N:150 kg ha^{-1} P:36 kg ha^{-1} K: 284 kg ha^{-1}	Malasol to control aphids
Pigweed (amaranth) *(Amaranthus cruentus)*	December	166 667	N:150 kg ha^{-1} P:20 kg ha^{-1} K: 200 kg ha^{-1}	Malasol to control aphids
Spider flower (*Cleome gynandra*)	December	166 667	N:150 kg ha^{-1} P:20 kg ha^{-1} K: 200 kg ha^{-1}	Malasol to control aphids

In the initial irrigation trial, carried out in the 2007 and 2008 growing seasons, a number of challenges prevented success. These included bolting at an early growth stage, flowering before irrigation treatment commenced, flowering of seedlings in the seedling trays, uncertainty in cultivation practices and serious infestations of insect pests and diseases. These challenges resulted in poor data sets that could not be used to build up reasonable conclusions and recommendations. Another challenge was the absence of registered chemicals for use against insect pests and diseases that affected the ALVs. The data collection procedures and methodologies were also not good enough to get a complete data set as there were uncertainties on harvesting protocols, planting time and plant population. In winter 2009 and summer 2009/10, the trial was carried out with a better understanding of the crops. For example, the insect damage was prevented using chemical sprays identified to be effective on these crops. Water-stressed plants are prone to bolt (flower and go to seed prematurely), and both stalks and leaves could turn fibrous and bitter in terms of leaf quality and when a flower stalk appeared. Flowers were, therefore, clipped off to prolong the harvest. Chinese cabbage is one of the eight vegetables that were starting to flower about 20 days after transplanting. Harvesting was carried out every 14 to 21 days, so that the plants continue to produce new growth at the central stem. Depending on the crop, different vegetative growth

components such as leaf fresh mass, leaf dry mass, number of edible leaves, number of flowers, number of buds, and number of fruits were sampled and measured during different growth stages. Leaf area was determined using a ceptometer and dry matter was determined after four to five days of oven drying at 60^{o}C in the laboratory. Sampled fresh leaves were also counted to obtain the total number per plant. The stem length was determined by using a measuring tape while the stem diameter was determined by using a calliper or a ruler.

5.3 RESULTS

5.3.1 Crop water requirement

Results indicated that the crop coefficient (Kc), which related the actual evapotranspiration to the reference evapotranspiration, varied for different growth stages. The ALVs were harvested four or more times and each cutting was essentially considered as a harvesting cycle within the season. The length of the harvesting cycle was based on observations and the first harvest was longer in duration than the other cycles due to slow crop growth rates after transplanting. Seasonal actual crop water use (ETc) for each crop is indicated in Table 5.2. The Kc values of pumpkin and watermelon were similar to the values reported in the FAO manuals. Kc values for the different growth stage of eight African leafy vegetables determined from data collected in 2009/10 and 2010/11 are shown in Table 5.2.

Table 5.2: Crop coefficient (Kc) estimates for different growth stages and growing period for eight African leafy vegetables

African leafy vegetable species	Initial growth stage		Development growth stage		Middle growth stage		Late growth stage		Seasonal ETc (mm)
	Days	Kc	days	Kc	days	Kc	days	Kc	
Chinese cabbage (*Brassica rapa* subsp. *chinensis*)	25	0.83	20	0.98	20	1.00	15	1.00	382
Nightshade (*Solanum retroflexum*)	20	0.78	40	0.53	35	0.91	30	1.00	381
Pumpkin *(Cucurbita maxima)*	40	0.80	35	1.00	25	0.53	20	0.46	340
Tsamma melon *(Citrillus lanatus)*	40	0.80	35	0.90	25	0.53	20	0.46	340
Jew's mallow *(Corchorus olitorius)*	35	0.78	30	0.92	25	0.90	20	1.06	368
Cowpea *(Vigna unguiculata)*	30	0.81	25	0.85	25	0.80	30	0.80	340
Pigweed (amaranth) *(Amaranthus cruentus)*	35	0.70	30	0.90	25	0.90	20	0.80	360
Spider flower (*Cleome gynandra*)	35	0.90	30	1.10	25	1.10	20	0.80	463

The Kc values developed from the mini-lysimeter studies can be used to determine the water requirements of African leafy vegetables for maximum biomass production. The Kc values enable precise water applications, which are needed to achieve high irrigation efficiency. When using these Kc values it is important to consider

agronomic practices, such as spacing and canopy architecture. Environmental conditions should also be considered, because high air temperatures and water vapour pressure deficits are known to cause temporal and transient leaf stomatal closure, which impede plants from transpiring water at their full potential.

5.3.2 Biomass production

In this section, the effects of four irrigation treatments on the growth or yield components of the ALVs are presented and discussed. There are different plant parts that can be used to determine the extent of water stress on crop growth. In this study, shoot (leaf, stem, flower, buds, branches) fresh and dry weights, and root fresh and dry weights were considered for each crop.

Pigweed (amaranth) (*Amaranthus cruentus* L.)

Biomass production of pigweed in the four irrigation treatments was determined over two seasons (2009/10 and 2010/11). Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield components of pigweed (Table 5.3). In the 2009/10 experiment, yield components of pigweed were not affected by the amount of water applied except for the leaf fresh weight, on which irrigation amount applied had a significant ($p<0.01$) effect. In the 2010/11 experiment, leaf and stem dry weight, number of leaves, root length, root fresh and dry mass were all affected significantly by the amount of irrigation applied.

Table 5.3: Analysis of variance for the different plant parts of pigweed (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	6.66	0.004 **	22	1.809	0.1762 ^{ns}	35
Leaf DW	0.61	0.62 ^{ns}	46	50	0.001**	23
Stem FW	2.64	0.0847 ^{ns}	35	1	0.3788 ^{ns}	26
Stem DW	1.46	0.2616 ^{ns}	41	15	0.001**	15
No of leaves	1.26	0.3215 ^{ns}	43	8.9	0.0011**	15
No of flowers	1.09	0.3803 ^{ns}	38	-	-	
No of stems	0.75	0.5346 ^{ns}	57	-	-	
No of branches	0.49	0.6950 ^{ns}	26	-	-	
Root length	-	-	-	4	0.0206*	24
Root fresh mass	-	-	-	17.98	0.0001**	34
Root dry mass	-	-	-	14.06	0.001**	28

** highly significant at $p \leq 0.01$; * significant at $p \leq 0.05$; ns = not significant at $p>0.05$

Treatment means were separated using Fisher's LSD test ($p=0.05$) and the results are shown in Tables 5.4 and 5.5.

Table 5.4: Effect of irrigation treatment on leaf and stem fresh (FW) and dry weight (DW), number of leaves, flowers, branches and buds of pigweed obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10)

Plant parts	Irrigation treatment (mm)				$LSD_{0.05}$
	100FC (384)	75FC (289)	50FC (193)	25FC (96)	
Leaf FW (g)	3510 [a]	2328 [b]	2293 [b]	2099 [b]	747
Leaf DW (g)	560 [a]	402 [a]	419 [a]	513 [a]	Ns
Stem FW (g)	2519[a]	1576[ab]	1959[b]	1472[b]	874
Stem DW(g)	265[a]	231[a]	176[a]	166[a]	Ns
No of leaves	5001 [a]	4040 [a]	4255 [a]	2893 [a]	Ns
No of flowers	289 [a]	289 [a]	328 [a]	210 [a]	Ns
No of stems	112 [a]	100 [a]	129 [a]	75 [a]	Ns
No of branches	539 [a]	484 [a]	488 [a]	441 [a]	Ns
Number of buds	36 [a]	34 [a]	33 [a]	30 [a]	Ns

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

In the 2009/10 experiment, the highest leaf fresh, dry and stem weight and number of leaves and branches were obtained from the 100FC irrigation treatment plots (Table 5.4). This indicated that pigweed could be grown at 75FC or deficit irrigation but that better growth could be expected when the crop was irrigated at 100FC. Higher numbers of branches were also observed in the 100FC irrigation treatment. This indicated the capacity of this crop to fill lateral space above ground when irrigated to field capacity and is also an indicator of competitive capacity of the crop for radiation interception.

Table 5.5: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves and below ground biomass of pigweed obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2010/11)

Plant parts	Irrigation treatment (mm)				$LSD_{0.05}$
	100FC (443)	75FC (381)	50FC (342)	25FC (259)	
Leaf FW (g)	7315[a]	6972[a]	5170[a]	4688[a]	2841
Leaf DW (g)	2551[a]	726[b]	824[b]	739[b]	379
Stem FW (g)	7356[a]	6206[a]	6296[a]	5467[a]	2225
Stem DW (g)	484[b]	319[b]	611[ab]	516[a]	96
No of leaves	12996[a]	12361[a]	10029[b]	8269[b]	2193
Root length (cm)	29[a]	29[a]	27[a]	17[a]	8
Root FW (g)	45[a]	43[a]	26[b]	4[c]	13
Root DW (g)	8.6[a]	8.6[a]	4.9[b]	2.7[b]	2

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

In the 2010/11 experiment, the amount of irrigation water applied was higher than in the 2009/10 experiment. The 100FC treatment produced the highest leaf dry weight but stem dry weight was higher in the driest treatments. Number of leaves, and leaf fresh and dry mass were highest in the 100FC treatment (Table 5.5). The results indicated that pigweed needed optimum application (100FC) of water for optimum biomass production. The below ground components of pigweed (root length, fresh and dry mass) were also affected by the irrigation treatments. High root fresh and dry mass were obtained in the 100FC, 75FC and 50FC treatments, which indicated the competitive capacity of this plant to draw water from deeper parts of the soil profile. In the 25FC irrigation treatment, root development was confined to the upper layer of the soil (17 cm).

Jew's mallow or wild jute (*Corchorus olitorius* L.)

Jew's mallow responses to four irrigation treatments were determined for in the 2009/10 and 2010/11 experiments. Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield components of Jew's mallow. In 2009/10 experiment, most yield components of Jew's mallow were not affected by the amount of water applied, except for leaf fresh weight. In the 2010/11 experiment, the above ground yield components of Jew's mallow were affected by the irrigation treatments (Table 5.6).

Table 5.6: Analysis of variance for the different plant parts of Jew's mallow (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	4.58	0.0169 *	79	21.22	0.0001 **	11
Leaf DW	2.65	0.0842 ^{ns}	30	11.21	0.0003 **	15
Stem FW	0.4470	0.93 ^{ns}	52	19.10	0.0001 **	16
Stem DW	0.42	0.7438 ^{ns}	36	20.80	0.0001 **	18
No of leaves	2.13	0.1427 ^{ns}	47	13.92	0.0001 **	17
No of branches	0.11	0.9505 ^{ns}	58	-	-	-
Number of buds	2.86	0.0854 ^{ns}	65	-	-	-
Root length	-	-	-	2.57	0.0903 ^{ns}	26
Root fresh mass	-	-	-	0.28	0.8377 ^{ns}	35
Root dry mass	-	-	-	0.47	0.7099 ^{ns}	45

** highly significant at $p \leq 0.01$; * significant at $p \leq 0.05$; ns = not significant at $p > 0.05$

Treatment means were separated using Fisher's LSD test (p=0.05) (Tables 5.7 and 5.8). In the 2009/10 experiment, irrigation amounts applied had a significant ($p<0.01$) effect on the leaf fresh weight. The highest leaf fresh weight was obtained from the 100FC treatment, whilst all other above-ground biomass indicators also tended to peak in this treatment (Table 5.7). This indicated that Jew's mallow favoured generous application of water for optimum growth and development.

Table 5.7: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves, flowers, branches and buds of Jew's mallow obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10)

Plant parts	Irrigation treatment (mm)				$LSD_{0.05}$
	100FC (462)	75FC (274)	50FC (176)	25FC (117)	
Leaf FW (g)	2834 ^{a}	1130 ^{b}	734 ^{b}	659 ^{b}	1425
Leaf DW (g)	367 ^{a}	326 ^{a}	288 ^{a}	212 ^{a}	121
Stem FW (g)	605 ^{a}	573 ^{a}	387 ^{a}	409 ^{a}	345
Stem DW (g)	54 ^{a}	56 ^{a}	59 ^{a}	44 ^{a}	31
No of leaves	3370 ^{a}	2686 ^{a}	1992 ^{a}	1593 ^{a}	1620
No of branches	236 ^{a}	290 ^{a}	235 ^{a}	260 ^{a}	214
Number of buds	2678 ^{a}	2192 ^{a}	1786 ^{a}	143 ^{a}	1996

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

In the 2010/11 experiment, the above ground biomass indicators of Jew's mallow were also affected by irrigation treatment (Table 5.8). The highest of values were again observed in the 100FC treatment followed by the 75FC treatment ($p<0.01$). The below-ground components of Jew's mallow (root length, fresh and dry mass) were not affected by irrigation treatment.

Table 5.8: Effect of irrigation treatment on leaf and stem fresh (FW) and dry weight (DW), number of leaves, flowers, branches and buds of Jew's mallow obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2010/11)

Plant parts	Irrigation treatment (mm)				$LSD_{0.05}$
	100FC (434)	75FC (237)	50FC (202)	25FC (123)	
Leaf FW (g)	2958 [a]	2548 [b]	2040 [c]	1693 [c]	362
Leaf DW (g)	772 [a]	779 [a]	590 [b]	478 [b]	131
Stem FW (g)	4434 [a]	3274 [b]	2400 [c]	2293 [c]	680
Stem DW (g)	660 [a]	532 [b]	316 [c]	329 [c]	109
No of leaves	16525 [a]	15338 [ab]	12520 [b]	8112 [c]	3005
Root length (cm)	29 [a]	23 [a]	20 [a]	19 [a]	8
Root FW (g)	7 [a]	6 [a]	7 [a]	8 [a]	3
Root DW (g)	5 [a]	4 [a]	4 [a]	6 [a]	3

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

Cowpea (*Vigna unguiculata* (L.) Walp.)

Cowpea responses to four irrigation treatments were determined for two seasons (2009/10 and 2010/11). Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield components of cowpea. In the 2009/10 experiment, leaf fresh and dry weight cowpea were affected by the amount of water applied. However, there was no significant difference in the number of leaves among treatments. In the 2010/11 experiment, more measurements were done to verify the results obtained in 2009/10. The effect of irrigation treatment was significant on leaf area and on bean dry and fresh mass. No significant effect was observed on the below-ground dry matter of cowpea (Table 5.9).

Table 5.9: Analysis of variance for the different plant parts of cowpea (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	14.38	0.0006 **	36	7.8	0.002 **	20
Leaf DW	15.26	0.0005 **	33	1.07	0.3897 [ns]	32
No of leaves	2.05	0.1508 [ns]	54	2.48	0.0982 [ns]	30
Leaf area	-	-	-	4.11	0.0276 *	25
Root length	-	-	-	0.57	0.6410 [ns]	15
Root fresh mass	-	-	-	1.3	0.3074 [ns]	20
Root dry matter	-	-	-	1.69	0.2087 [ns]	24
Seed fresh mass	-	-	-	14.51	0.0001 **	37
Seed dry mass	-	-	-	6.97	0.0042 **	46

** highly significant at $p \leq 0.01$; * significant at $p \leq 0.05$; ns = not significant at $p > 0.05$

Treatment means were separated using Fisher's LSD test (p=0.05) (Table 5.10). The highest number of leaves and the highest fresh and dry mass of leaves were obtained in the 75FC treatment of the 2009/10 experiment. This indicated the competitive capacity of cowpea for water and its potential for production under deficit irrigation. In the 2010/11 experiment, values for most of the plant parts were highest in the 100FC irrigation treatment except for root length and root fresh mass. The effect of irrigation amount on the number of leaves of cowpea was clearly evident but generally, cowpea grew well under deficit irrigation (75FC) without losing quality of the leaves. However, the results indicated that when the crop was grown for seed, irrigation to 100FC was expected to produce higher yields.

Table 5.10: Effect of irrigation treatment on leaf and stem fresh (FW) and dry weight (DW), number of leaves, flowers, branches and buds of cowpea obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10 and 2010/11)

Year	Plant parts	Irrigation treatments				$LSD_{0.05}$
		100FC (462)	75FC (274)	50FC (176)	25FC (117)	
2009/10	Leaf FW (g)	1160 [a]	1256 [a]	282 [b]	260 [b]	452
	Leaf DW (g)	225 [b]	322 [a]	71 [c]	75 [c]	96
	No of leaves	499 [ab]	502 [a]	331 [ab]	204 [b]	296
2010/11	Leaf FW (g)	5094 [a]	4835 [a]	3262 [b]	3089 [b]	1117
	Leaf DW (g)	788 [a]	786 [a]	641 [a]	580 [a]	Ns
	No of leaves	2675 [a]	2008 [a]	1938 [a]	1904 [a]	Ns
	Leaf area (m^2)	69214 [a]	68113 [a]	48633 [ab]	41124 [b]	21014
	Root length (cm)	18 [a]	20 [a]	20 [a]	19 [a]	Ns
	Root FW(g)	13 [a]	13 [a]	11 [a]	10 [a]	Ns
	Root DW(g)	4 [a]	5 [a]	3 [a]	5 [a]	Ns
	Beans FW(g)	835 [a]	450 [b]	315 [bc]	143 [c]	217
	Beans DW (g)	196 [a]	116 [b]	76 [b]	45 [b]	72

Means followed by the same letter/s within rows are not significantly different (p=0.05)
(Ns); LSD=Least Significant Difference

Chinese cabbage (*Brassica rapa L* subsp. *chinensis*)

Chinese cabbage responses to four irrigation treatments were determined over two seasons (2009/10 and 2010/11). Analyses of variance were performed to determine the effects of the irrigation amounts applied on leaf fresh and dry weight, number of flowers and leaf area. In the 2009/10 experiment, leaf fresh weight was significantly affected by irrigation treatment and the same applied to the results obtained in the 2010/11 experiment, with many of the differences being highly significant (Table 5.11).

Table 5.11: Analysis of variance for the different plant parts of non-heading Chinese cabbage (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	7.70	0.0096 **	12	11.22	0.0001**	21
Leaf DW	1.78	0.2290 [ns]	16	18.75	0.0001**	29
Number of flowers	1.37	0.3186 [ns]	30	-	-	-
Leaf area	-	-	-	7.67	0.0004**	54

** highly significant at $p \leq 0.01$; * significant at $p \leq 0.05$; ns = not significant at $p > 0.05$

Treatment means were separated using Fisher's LSD test (p=0.05) (Table 5.12). In the 2009/10 experiment, irrigation amounts applied had a significant ($p<0.01$) effect on leaf fresh weight. Highest leaf fresh weight was obtained from the 100FC treatment, which indicated that Chinese cabbage favoured high levels of soil water availability for optimum growth and development and confirmed the findings of Van Averbeke and Netshithuthuni (2010). Number of flowers was not affected significantly by treatment, which suggested that flower initiation and formation in Chinese cabbage was probably dependent on factors other than water (Table 5.12). In the 2010/11 experiment, highly significant differences in leaf fresh and dry weight and leaf area among

treatments were observed. The highest fresh leaf weight and leaf area was observed in the 100FC treatment but dry matter was highest in the 75FC treatment.

Table 5.12: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves, flowers, branches and buds of non-heading Chinese cabbage obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10)

Years	Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
		100FC (462)	75FC (274)	50FC (176)	25FC (117)	
2009/10	Leaf FW (kg)	7.95 [a]	7.31 [a]	7.35 [a]	4.90 [b]	2
	Leaf DW (kg)	0.67 [a]	0.62 [a]	0.60 [a]	0.49 [a]	Ns
	Number of flowers	245 [a]	253 [a]	279 [a]	242 [a]	Ns
2010/11	Leaf FW (g)	6305 [a]	5381 [ab]	4688 [b]	3674 [c]	952
	Leaf DW (g)	836 [a]	925 [a]	922 [a]	315 [b]	193
	Leaf area (cm^2)	54108 [a]	28308 [b]	29448 [b]	18035 [b]	15857

Means followed by the same letter/s within rows are not significantly different (p=0.05)
(Ns); LSD=Least Significant Difference

Nightshade (*Solanum retroflexum*)

The responses of nightshade to four irrigation treatments were determined over two seasons (2009/10 and 2010/11). Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield components of nightshade (Table 5.13).

Table 5.13: Analysis of variance for the different plant parts of nightshade (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Shoot DW	13.72	0.0001 **	29	-	-	-
Root FW	8.83	0.0006 **	25	-	-	-
Stem FW	20.8	0.0001 **	32	19.17	0.0001 **	31
Leaf area	-	-	-	16.55	0.0001 **	22
Leaf FW	2.73	0.0711 [ns]	65	16.54	0.0001 **	24
Stem FW	-	-	-	6.0	0.0061 **	55
Root DW	2.24	0.1146 [ns]	54	-	-	-
Leaf DW	9.15	0.0005 **	58	12.92	0.0002 **	23
Fruit DW	10.22	0.0003 **	50	-	-	-
Number of flowers	6.79	0.0024 **	40	-	-	-
Number of buds	4.48	0.0147 *	45	-	-	-
No of leaves	1.78	0.1830 [ns]	69	8.22	0.0015 **	29
Stem length	25.1	0.0001 **	9	-	-	-
Root length	1.25	0.3190 [ns]	36	-	-	-
Number of branches	2.2	0.1196 [ns]	18	-	-	-

** highly significant at $p \leq 0.01$; * significant at $p \leq 0.05$; ns = not significant at $p > 0.05$

In the 2009/10 experiment, most yield components of nightshade were affected significantly by the amount of water applied except for the leaf fresh weight, root dry mass, number of leaves, root length and number of branches (Table 5.13). In the 2010/11 experiment, there were highly significant differences among all the yield components (Table 5.13).

Treatment means were separated using Fisher's LSD test (p=0.05) (Table 5.14).

Table 5.14: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves, flowers, branches and buds of nightshade obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10)

Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
	100FC (158)	75FC (119)	50FC (80)	25FC (37)	
Shoot DW (g)	359 [a]	299 [a]	195 [b]	119 [b]	85
Root FW (g)	13 [a]	12	10	6 [b]	3
Stem FW(g)	24 [a]	9 [b]	9 [b]	9 [b]	5
Leaf area (g)	64 [a]	55 [ab]	27 [b]	27 [b]	33
Leaf FW (g)	24 [a]	9 [b]	9 [b]	9 [b]	5
Stem FW (g)	3 [a]	2 [ab]	2 [ab]	1 [b]	1
Root DW (g)	12 [a]	5 [bc]	1 [c]	7 [b]	4
Leaf DW (g)	40 [a]	15 [b]	13 [b]	12 [b]	12
Fruit DW (g)	141 [a]	123 [ab]	86 [bc]	47 [c]	47
Number of flowers	251 [a]	224 [a]	161 [ab]	92 [b]	99
Number of buds	370 [a]	321 [a]	164 [a]	193 [a]	219
No of leaves	61 [a]	52 [b]	48 [b]	37 [c]	6
Stem length (cm)	34 [a]	34 [a]	26 [a]	24 [a]	13
Root length (cm)	10 [a]	9 [ab]	9 [ab]	7 [b]	2

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

Table 5.15: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves, flowers, branches and buds of nightshade obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2010/11)

Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
	100FC (322)	75FC (242)	50FC (162)	25FC (81)	
Leaf FW (g)	2275 [a]	1787 [a]	1392 [b]	687 [c]	494
Leaf DW (g)	447 [a]	331 [b]	296 [b]	169 [c]	96
Stem FW (g)	2410 [a]	1604 [b]	985 [c]	480 [c]	569
Stem DW (g)	785 [a]	499 [ab]	389 [bc]	127 [c]	333
No of leaves	10461 [a]	7947 [ab]	6793 [bc]	3990 [c]	2807
Leaf area (m^2)	33481 [a]	32876 [a]	19569 [b]	13047 [b]	7443

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

In the 2009/10 experiment, irrigation amounts applied had a significant ($p<0.01$) effect on all growth components of nightshade that were measured. The highest values were obtained in the 100FC treatment, which indicated that nightshade grew best when irrigated to field capacity (100FC). Similarly in 2010/11 experiment, yield component values tended to peak in the 100FC irrigation treatment (Table 5.15), confirming the 2009/10 conclusion that nightshade favoured high levels of soil water availability for optimum biomass production.

Spider flower (*Cleome gynandra* L.)

The response of spider flower to four irrigation treatments was determined during two seasons (2009/10 and 2010/11). Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield component of the crop (Table 5.16). In the 2009/10 experiment, most yield components of spider flower were not affected by the amount of water applied, except for the leaf and stem fresh weight. In the 2010/11 experiment, the analysis indicated that above-ground yield components of spider flower were not affected by irrigation treatment (Table 5.16).

Table 5.16: Analysis of variance for the different plant parts of spider flower (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	4.31	0.0208 *	27	0.57	0.6451 ^{ns}	25
Leaf DW	1.26	0.3210 ^{ns}	65	0.16	0.9188 ^{ns}	24
Stem FW	5.3	0.0099**	30	0.84	0.4920 ^{ns}	33
Stem DW	0.64	0.6021 ^{ns}	47	2.65	0.0841 ^{ns}	27
No of leaves	1.03	0.4053 ^{ns}	63	0.69	0.5713 ^{ns}	43
No of flowers	0.97	0.4330 ^{ns}	38	0.09	0.9639 ^{ns}	28
No of stems	0.39	0.7627 ^{ns}	58	-	-	-
No of branches	0.31	0.8159 ^{ns}	37	-	-	-
No of buds	0.66	0.5878 ^{ns}	85	-	-	-
No of fruits	0.45	0.7182 ^{ns}	55	-	-	-

** highly significant at p< 0.01; * significant at p<0.05; ns = not significant at p>0.05

In the 2009/10 experiment, irrigation amounts applied had a significant (p<0.01) effect on the leaf and stem fresh weight (Table 5.17). The trend was for the values of the yield components to be highest in the 100FC treatment followed by the 75FC irrigation treatment. This indicated that spider flower tended to favour well-watered conditions for optimum growth and yield.

Table 5.17: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves, flowers, branches and buds of spider flower obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10)

Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
	100FC (384)	75FC (289)	50FC (193)	25FC (96)	
Leaf FW (g)	3798^{a}	3066^{ab}	2763^{cb}	2179^{c}	752
Leaf DW (g)	568^{a}	462^{a}	439^{a}	253^{b}	145
Stem FW (g)	5104^{a}	4074^{ab}	3862^{b}	2848^{c}	1051
Stem DW (g)	627^{b}	453^{a}	430^{b}	240^{c}	153
No of leaves	7032^{a}	6923^{a}	5141^{a}	3547^{a}	2170
No of flowers	263^{a}	258^{a}	273^{a}	394^{a}	194
No of stems	998^{a}	972^{a}	888^{a}	789^{a}	384
No of branches	405^{a}	339^{a}	283^{a}	266^{a}	133
No of buds	83^{ab}	58^{b}	60^{b}	101^{a}	40
No of fruits	462^{a}	258^{a}	233^{a}	140^{a}	92

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

Table 5.18: Effect of irrigation treatment on leaf and stem fresh and dry weight, number of leaves, flowers, branches and buds of spider flower obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2010/11)

Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
	100FC (443)	75FC (381)	50FC (342)	25FC (259)	
Leaf FW (g)	1999[a]	1919[a]	1712[a]	1553[a]	Ns
Leaf DW (g)	384[a]	292[a]	358[a]	351[a]	Ns
Stem FW(g)	5043[a]	4953[a]	2881[a]	2612[a]	Ns
Stem DW(g)	479[a]	504[a]	496[a]	345[a]	Ns
No of leaves	8147[a]	5613[a]	5552[a]	4682[a]	Ns
No of flowers	178[a]	166[a]	172[a]	157[a]	Ns

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

In the 2010/11 experiment, irrigation amounts applied had no significant effect on yield components (Table 5.18) but again the trend was for the values of the yield components to be highest in the 100FC treatment and to progressively decline as less irrigation water was applied. This indicated that spider flower tended to favour well-watered conditions for optimum growth and yield.

Pumpkin (*Cucurbita maxima*)

The response of pumpkin to the irrigation treatments was determined in the 2009/10 and 2010/11 experiments. Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield components of pumpkin. In 2009/10 experiment, most yield components of pumpkin were not affected by the amount of water applied. In the 2010/11 experiment, leaf dry weight showed significant differences among treatments (Table 5.19).

Table 5.19: Analysis of variance for the different plant parts of pumpkin (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	0.66	0.5876 [ns]	38	2.48	0.0986 [ns]	20
Leaf DW	0.18	0.9097 [ns]	41	5.5	0.0086 *	16
No of leaves	0.54	0.6588 [ns]	46	2.32	0.1141 [ns]	19
No of flowers	0.14	0.9317 [ns]	48	1.96	0.1600 [ns]	34
No of buds	1.25	0.3255 [ns]	33	0.33	0.8056 [ns]	40
No of fruits	1.06	0.3931 [ns]	67	2.0	0.1546 [ns]	15

** highly significant at $p \leq 0.01$; * significant at $p \leq 0.05$; ns = not significant at $p > 0.05$

Table 5.20: Effect of irrigation treatment on leaf and stem fresh (FW) and dry weight (DW), number of leaves, flowers, branches and buds of pumpkin obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10)

Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
	100FC (388)	75FC (256)	50FC (176)	25FC (86)	
Leaf FW (g)	2026[a]	2122[a]	1933[a]	1527[a]	965
Leaf DW (g)	327[a]	303[a]	296[a]	270[a]	165
No of leaves	449[a]	397[a]	340[a]	321[a]	235
No of flowers	107[a]	120[a]	118[a]	101[a]	72
No of buds	468[a]	699[a]	566[a]	591[a]	255
No of fruits	10[a]	9[a]	7[a]	5[a]	7

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

Treatment means were separated using Fisher's LSD test (p=0.05) for the 2010/11 experiment (Table 5.21). The highest leaf dry mass was obtained from the 100FC treatment and overall the various biomass indicators tended to peak in this treatment.

Table 5.21: Effect of irrigation treatment on leaf and stem fresh (FW) and dry weight (DW), number of leaves, flowers, branches and buds of pumpkin obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2010/11)

Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
	100FC (388)	75FC (300)	50FC (200)	25FC (100)	
Leaf FW (g)	1356[ab]	1356[ab]	1125[b]	994[b]	314
Leaf DW (g)	158[a]	106[b]	144[a]	133[ab]	28
No of leaves	256[a]	251[a]	235[a]	191[b]	58
No of flowers	48[a]	40[ab]	43[ab]	28[a]	18
No of buds	15[a]	32[a]	36[a]	32[a]	50
No of fruits	1.4[a]	0.6[ab]	0.8[ab]	0.2[b]	1

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

Tsamma melon (*Citrillus lanatus* (Thunberg))

Tsamma melon response to four irrigation treatments was determined during the 2009/10 and 2010/11 seasons. Analyses of variance were performed to determine the effects of the irrigation amounts applied on the yield components of tsamma melon. In the 2009/10 experiment, above-ground biomass of tsamma melon was not affected by the amount of water applied (Table 5.22).

Table 5.22 : Analysis of variance for the different plant parts of tsamma melon (2009/10 and 2010/11)

Plant parts	2009/10			2010/11		
	F value	P value	CV (%)	F value	P value	CV (%)
Leaf FW	0.08	0.9705[ns]	53	1.56	0.2379[ns]	25
Leaf DW	1.53	0.2889[ns]	16	2.55	0.0291 *	22
No of flowers	0.53	0.6747[ns]	50	5.81	0.0069 **	15
No of leaves	0.5371	0.8[ns]	27	3.11	0.0559[ns]	21
No of fruits	-	-	-	1.28	0.3160[ns]	49

** highly significant at $p \leq 0.01$; * significant $p \leq 0.05$; ns = not significant $p > 0.05$

In the 2010/11 experiment, the leaf dry mass and number of flowers were significantly affected by the irrigation treatments. In the 2009/10 experiment, leaf fresh weight tended to increase with increasing water deficit but the other biomass indicators did not support this trend.

Table 5.23: Effect of irrigation treatment on leaf and stem fresh (FW) and dry weight (DW), number of leaves, flowers, branches and buds of tsamma melon obtained from 5.2 m^2 plots (ARC – Roodeplaat, 2009/10 and 2010/11)

Years	Plant parts	Irrigation treatments (mm)				$LSD_{0.05}$
		100FC (388)	**75FC (293)**	**50FC (195)**	**25FC (96)**	
2009/10	Leaf FW (g)	2221[a]	2322[a]	2372[a]	2697[a]	2624
	Leaf DW (g)	320[a]	379[a]	377[a]	329[a]	174
	No of flowers	189[a]	219[a]	167[a]	278[a]	224
	No of leaves	1324[a]	1146[a]	1650[a]	1094[a]	833
		100FC (388)	**75FC (300)**	**50FC (200)**	**25FC (100)**	$LSD_{0.05}$
2010/11	Leaf FW (g)	1332[a]	1262[a]	1085[a]	971[a]	395
	Leaf DW (g)	148[a]	136[ab]	126[ab]	100[b]	38
	No of flowers	62[b]	83[a]	60[b]	65[b]	13
	No of leaves	349[a]	262[b]	261[b]	245[b]	80
	No of fruits	5[a]	5[a]	9[a]	7[a]	5

Means followed by the same letter/s within rows are not significantly different (p=0.05) (Ns); LSD=Least Significant Difference

In the 2010/11 experiment, fresh and dry mass of leaves tended to increase as water was more available and numerically the highest values were obtained in the 100FC treatment but in the absence of significant differences and the opposed trends in the results of the two experiments it was not possible to draw conclusions about the effects of water on growth and yield of tsamma melon.

5.3.3 Crop water productivity

From the previous section (5.3.2) it was evident that the eight ALVs responded differently to the four irrigation treatments. In this section the response of the ALVs to different water stress level from the point of view of yield and water productivity is discussed. Crop water productivity is the amount of water required per unit total biomass or specified biomass (yield) (Steduto *et al*., 2007). In tables 5.24 through to 5.31, the data on total FM (fresh matter) and DM (dry matter) yields refer to the consumable or marketable portions, primarily leaves. Total dry matter yield of the consumable portion was used in the calculation of the crop water productivities listed in these tables.

Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*)

The total yield of Chinese cabbage obtained in the 2009 and 2010 experiments ranged between 15 t ha^{-1} and 8 t ha^{-1} on fresh weight (FW) basis at a seasonal water application of 195 mm and 45 mm, respectively. The fresh mass yield obtained from the 25FC treatment was not of marketable quality. Therefore, irrigating Chinese cabbage at this level of water stress is not recommended, because both yield and quality were compromised. Maximum crop water productivity was obtained in the 25FC and 50FC treatments, where deficit irrigation was applied. Water productivity generally decreased as applied irrigation water increased but maximum marketable fresh mass yield was obtained in the 100FC treatment. The water use of Chinese cabbage for the 100FC, 75FC, 50FC and 25FC was found to be 1124, 769, 497 and 520 litres of water for each kg of marketable fresh mass

yield produced. Results of the study showed that irrigating to field capacity (100FC) gave higher above-ground yield and better quality leaves compared to other treatments.

Table 5.24: Total fresh and dry matter, irrigation water use and crop water productivity of non-heading Chinese cabbage (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	2009	2010	2009	2010	2009	2010	2009/10
100FC	15.2±1.4	13.7±1.7	1.2±0.8	2.1±0.5	195	180	0.9
75FC	14.0±0.6	10.1±1.4	1.2±0.0	2.4±0.3	146	135	1.3
50FC	14.1±1.1	11.2±0.4	1.3±0.1	2.4±0.1	97	90	2.0
25FC	9.2±0.7	8±0.5	1.0±0.5	0.8±0.1	49	45	1.9

[1]Consumable or marketable portion; [2]dry matter basis

Nightshade (*Solanum retroflexum*)

Nightshade was harvested four times in the 2009 and three times in the 2010 growing season. The total fresh marketable yield of nightshade obtained in 2009 was higher than in 2010 on fresh and dry weight basis as the number of harvests was greater in 2009 than in 2010 (Table 5.25).

Table 5.25: Total fresh and dry matter, irrigation water use and crop water productivity of nightshade (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	2009	2010	2009	2010	2009	2010	2009/10
100FC	8.6±1.8	4.5±0.4	2.1±0.5	0.9±0.1	158	322	0.81
75FC	9.7±1.2	3.6±0.3	2.4±0.5	0.6±0.1	119	242	1.12
50FC	8.4±0.8	2.8±0.4	2.1±0.4	0.6±0.1	80	162	1.47
25FC	7.2±0.8	1.4±0.2	2.0±0.4	0.4±0.2	37	81	2.88

[1]Consumable or marketable portion; [2]dry matter basis

On average the yield obtained did not correspond to the irrigation water applied in 2009 growing season. Higher yield was obtained in the 75FC treatment than in the 100FC irrigation treatment (9.7 t ha^{-1}). The reduced yield obtained in the 100FC treatment could be attributed to the high frequency of irrigation applied to replenish the soil water deficit, which may have caused nutrient leaching from the root zone. Maximum crop water productivity, however, was obtained in the driest treatment (25FC). Water productivity decreased when applied irrigation water increased.

Pigweed (amaranth) (*Amaranthus cruentus*)

Pigweed grown in the 25FC irrigation treatment produced the least average biomass yield on fresh and dry weight basis. In the 100FC, the fresh biomass yield averaged 17 t.ha^{-1} in 2009/10 and 13 t.ha^{-1} for the 2010/11 season, while the dry weight was 1.7 t.ha^{-1} and 1.2 t.ha^{-1} (Table 5.26). The large decrease in biomass yield in the 75FC irrigation treatment suggested that dry matter in this treatment was partitioned preferentially to the root system rather than the shoot system. Results also showed that the 25FC irrigation treatment was more water-productive than all other treatments. Average crop water productivities for the two seasons increased from

0.5 kg.m^{-3} to 1.1 kg.m^{-3} when irrigation water application was decreased. The amount of irrigation water applied indicated that pigweed production could be maintained with relatively little water.

Table 5.26: Total fresh (FM) and dry matter (DM), irrigation water use and crop water productivity of pigweed (*Amaranthus cruentus*) (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	09/10	10/11	09/10	10/11	09/10	10/11	2009-2011
100FC	16.9±1.6	13.3±4.5	2.8±0.5	1.6±0.2	384	443	0.5
75FC	11.2±0.5	14.0±2.5	2.6±0.4	1.6±0.2	289	381	0.7
50FC	11.1±1.6	9.9±0.1	1.9±0.4	1.3±0.2	193	342	0.7
25FC	10.0±0.8	9.2±1.0	1.7±0.2	1.2±0.2	96	259	1.1

[1]Consumable or marketable portion; [2]dry matter basis

Cowpea (*Vigna inguiculata* (L.) Walp.)

Cowpea planted directly to the seed bed emerged 75% at one week after planting in the 2009/10 and more than 90% in the 2010/11 season. Water use over the four month period ranged from about 462 mm for the most frequently irrigated treatment (100FC) down to 117 mm for the least irrigated treatment (25FC) in the 2009/10 experiment, whereas in the 2010/11 experiment, the application of water was between 429 mm and 135 mm (Table 5.27).

Table 5.27: Total fresh and dry matter, irrigation water use and crop water productivity of cowpea (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	09/10	10/11	09/10	10/11	09/10	10/11	2009-2011
100FC	9.5±0.8	10.6±1.6	0.4±0.2	1.6±0.2	462	429	0.23
75FC	11.7±0.8	9.65±0.9	0.5±0.0	1.5±0.2	274	229	0.42
50FC	7.4±0.2	6.49±1.6	0.3±0.4	1.2±0.2	146	195	0.41
25FC	4.8±0.6	6.11±1.6	0.2±0.4	1.2±0.2	117	135	0.53

[1]Consumable or marketable portion; [2]dry matter basis

Highest yields of cowpea leaves were obtained in the 75FC and 100FC irrigation treatments, both on fresh and dry weight basis. Maximum yield was attained in the 100FC, but maximum water productivity was obtained where deficit irrigation (25FC) was applied.

Jew's mallow (C*orchorus olitorius* L.)

Jew's mallow yield obtained from the irrigation treatments were in the range of 7.2 and 1.7 t ha^{-1} on a fresh weight basis (Table 5.28). The highest yield was obtained in the well irrigated treatment (100FC) in both growing seasons, showing a positive effect on increased water application. A tendency for yield to decrease was observed as irrigation was reduced from 100FC to 25FC. The highest water productivity was obtained in the driest irrigation treatment (25FC). The difference in water productivity between the 50FC and 75FC irrigation treatments indicated that Jew's mallow could be produced under relatively dry conditions.

Table 5.28: Total fresh and dry matter, irrigation water use and crop water productivity of Jew's mallow (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	09/10	10/11	09/10	10/11	09/10	10/11	2009-2011
100FC	7.2±2.5	5.9±0.8	0.7±0.1	1.5±0.2	462	434	0.26
75FC	2.9±0.5	4.9±0.6	0.7±0.0	1.5±0.2	274	237	0.44
50FC	1.9±0.2	4.0±0.5	0.6±0.1	1.1±0.1	146	202	0.48
25FC	1.7±0.3	3.3±0.3	0.4±0.1	0.9±0.1	117	123	0.56

[1]Consumable or marketable portion; [2]dry matter basis

Spider flower (*Cleome gynandra* L.)

Water use of spider flower over the three month period ranged from about 443 mm for the most frequently irrigated treatment (100FC) down to 96 mm for the least irrigated treatment (25FC) in the 2009/10 (Table 5.29). In the 2010/11 experiment, the seasonal water application was between 443 mm to 259 mm. In the 2009/10 experiment, the highest yields of spider flower leaves were obtained in the 75FC and 50FC irrigation treatments both on fresh and dry weight basis. This could be due to the number of plants survived after transplanting. The yield obtained in 2010/11 was lower than the 2009/10 experiment as more harvests were done in 2009/10 than in 2010/11. In 2010/11, the re-growth of the crop after harvesting was slow and this reduced the number of harvests per season. Maximum yield was attained in the 100FC in 2010/11 but maximum water productivity was measured where deficit irrigation (25FC) was applied.

Table 5.29: Total fresh and dry matter, irrigation water use and crop water productivity of spider flower (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	09/10	10/11	09/10	10/11	09/10	10/11	2009-2011
100FC	9.6±1.2	4.0±0.7	0.9±0.3	0.8±0.2	384	443	0.20
75FC	11.1±1.8	3.5±0.6	0.7±0.1	0.8±0.2	289	381	0.23
50FC	13.2±1.5	3.3±0.6	1.1±0.1	0.8±0.2	193	342	0.41
25FC	7.3±1.0	3.2±0.7	0.6±0.1	0.8±0.2	96	259	0.48

[1]Consumable or marketable portion; [2]dry matter basis

Pumpkin (*Cucurbita maxima*)

Water use of pumpkin over its growing period ranged from about 388 mm in the 100FC irrigation treatment down to 86 mm for the least irrigated treatments (25FC) in the 2009/10 experiment and 389 mm to 100 mm in the 2010/11 experiment (Table 5.30). The yield obtained in 2010/11 was lower than the 2009/10 experiment as the crop was harvested three times in the 2010/11 experiment and four times in the 2009/10 experiment. An additional harvest could have been made in the 2010/11 experiment but the young leaves were too small for marketing. Fresh and dry mass yield of pumpkin did not differ significantly among the treatments. Crop water productivity differed due to treatment effect and maximum productivity was obtained where deficit irrigation (75FC) was applied. The main challenge with pumpkin leaf production was the unavailability of a harvesting protocol. In this study, the growing tips were harvested, including the smallest

and softest leaves, together with approximately 20 cm length of the stem. This could be the main reason that the effect of water in all the treatments was insignificant, particularly in the 25FC treatments, where young leaves appeared but were too small for marketing purposes.

Table 5.30: Total fresh and dry matter, irrigation water use and crop water productivity of pumpkin leaves (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	09/10	10/11	09/10	10/11	09/10	10/11	2009-2011
100FC	4.0±0.6	1.3±0.6	0.7±0.9	0.3±0.0	388	389	0.13
75FC	3.7±0.8	1.4±0.8	0.6±0.1	0.2±0.2	256	300	0.92
50FC	4.1±1.1	1.1±1.0	0.5±0.1	0.3±0.2	176	200	0.23
25FC	3.3±0.5	0.9±1.3	0.6±0.1	0.3±0.3	86	100	0.46

[1]Consumable or marketable portion; [2]dry matter basis

Tsamma melon (*Citrillus lanatus* Thunberg)

Tsamma melon and pumpkin both received the same amount of water in 2009/10 and 2010/11. Yield (young leaves) of tsamma melon obtained in 2009/10 and 2010/11 for fresh and dry weight did not differ. The yield obtained in 2010/11, however, was lower than the 2009/10 experiment as three harvests was done in 2010/11 and four in 2009/10 (Table 5.31).

Table 5.31: Total fresh and dry matter, irrigation water use and crop water productivity of tsamma melon (2009 and 2010 growing season, ARC Roodeplaat)

Irrigation treatment	Total FM yield (t ha^{-1})[1]		Total DM yield (t ha^{-1})[1]		Irrigation water use (mm)		Average crop water productivity[1; 2] (kg m^{-3})
	09/10	10/11	09/10	10/11	09/10	10/11	2009-2011
100FC	5.2±0.9	1.3±1.1	0.6±1.0	1.1±0.1	388	389	0.14
75FC	5.1±0.8	1.0±0.9	0.8±0.1	0.3±0.1	293	300	0.15
50FC	5.1±0.9	1.3±0.8	1.5±0.1	0.2±0.1	195	200	0.43
25FC	4.8±1.7	1.1±1.2	0.9±1.5	0.1±0.1	96	100	0.41

[1]Consumable or marketable portion; [2]dry matter basis

The reason for the fewer harvests in 2010/11 was the small size of the young leaves. Watermelon vines grew continuously during the experimental period in the 100FC irrigation treatments. The leaves sprouted along the vine almost every 10 cm apart and both the vines and leaves were covered with hairs. Similar to pumpkins, there is no information on harvesting methods of tsamma melon that can be referred to. Therefore harvesting was done based on experience from senior technicians at ARC. The yield obtained from different treatments did not differ on weight basis but quality decreased as water application was reduced. Tsamma melon should, therefore, be watered regularly for best results.

5.4 DISCUSSION

The experiments conducted at ARC Roodeplaat to estimate crop water requirement of eight ALVs using mini-lysimeters and to evaluate the effect of different irrigation regimes on biomass production and water productivities were successful. Two year averaged Kc values were developed for each crop and were observed to

vary for different growth stages. The Kc values reported could greatly benefit farmers, in South Africa, where high irrigation efficiencies are required. It is, however, important to consider the different environmental conditions when using these Kc values and also the agronomic practices being applied. Crop water requirements of Chinese cabbage and spider flower over their full growing season were 382 mm and 463 mm, which were higher than those of pigweed (amaranth), Jew's mallow and nightshade (Table 5.2). Cowpea, pumpkin and tsamma melon (340 mm) required the least amount of water during their growing period (Table 5.2).

Pigweed can be grown using deficit irrigation (75FC) but better growth can be obtained when it is irrigated at 100FC. Highest numbers of branches were observed in the 100FC irrigation treatments. This confirms the capacity of a plant species to fill lateral space above ground, which is an indicator of competitive capacity of the crop for radiation interception when it is well irrigated.

In the Jew's mallow irrigation experiment, the highest leaf fresh weight and yield components were obtained by irrigating the crops to field capacity (100FC). This indicates that *Jew's mallow* favours good application of water for its growth and development. The below ground components (root length, fresh and dry mass) were found not to be affected by the irrigation treatments.

Cowpea seems to grow at deficit irrigation (75FC) without losing marketable quality of the leaves. However, if the crop is grown for seed or bean production it has to be irrigated to 100FC to get optimum yield.

In the Chinese cabbage irrigation experiment, highest leaf fresh weight was obtained from the 100FC treatment and this indicates that Chinese cabbage favours regular application of water for optimum growth and development, confirming the findings reported by Van Averbeke and Netshithuthuni (2010). Water-stressed plants are prone to premature bolting (flowering and going to seed prematurely), and both stalks and leaves are known to turn fibrous and bitter once the flower stalk elongates (Van Averbeke *et al*., 2007). The number of flowers recorded in the different irrigation treatments did not differ significantly among treatments, which could mean that flower initiation in Chinese cabbage is prompted by climatic factors other than water stress. Flowers need to be clipped off to prolong the leaf harvests.

For nightshade, the highest growth component indicator values were obtained in the 100FC treatment. This indicates that nightshade requires good application of water for optimum growth and development.

Spider flower yield components tended to peak in the100FC treatment followed by the 75FC irrigation treatment. This could indicate that spider flower requires sufficient amount of water for good marketable quality biomass production.

Yield obtained from pumpkins and bitter watermelon did not differ significantly among the irrigation treatments but quality decreased as water application was reduced. Harvesting of young leaves was done based on experience from senior technicians at ARC as there was no published information on harvesting methods. Both crops tended to give the highest leaf dry mass in the 100FC treatment. This indicated that pumpkin should preferably be irrigated frequently to field capacity to get acceptable quality of leaves.

The results showed that ALVs responded differently to different irrigation treatments. Less irrigated treatments (25FC) resulted in lower yields compared to the well irrigated treatment (100FC), implying that increased water application could lead to increased yields. Higher water productivity was obtained with the driest irrigation treatment (25FC), indicating that production of these crops is possible in areas where water is a limiting factor. However the yields obtained under water-stressed conditions may lack the quality needed to market the produce. Water stress caused by high evapotranspirative demand, and high air and soil temperatures, appeared to be the main causes of poor performance of the ALVs in the low water treatments. Using proper irrigation water management, farmers can increase their ALV production and gross income when they irrigate to field capacity,

but that will be at the cost of decreased water productivity. The water productivity concept is useful to evaluate the performance of these crops in terms of water use but future studies could benefit by including monetary value of the ALVs in order to estimate whether ALVs produced from the different levels of irrigations could be acceptable for marketing and be profitable. Average irrigation water use, average total dry matter and crop water productivity of eight ALVs are indicated in Table 5.32. Pumpkin and watermelon needed higher amounts of water per kilogramme of green leafy vegetable compared to the other ALVs. Chinese cabbage produced the highest amount of biomass per cubic metre of water followed by nightshade and pigweed (amaranth). Although, the water productivity results presented in this study came out of a study follow intensive monitoring, water productivity can vary with agronomic practices such as irrigation and nutrient management, and climatic condition of the growing area. Therefore, the results presented here must be seen as a first approximation to the understanding of the water use and water foot print of eight ALVs in South Africa.

Table 5.32: Average total above-ground dry matter yield, irrigation water use and crop water productivity of the eight ALVs obtained in the well-watered irrigation treatment (100FC) over two growing seasons (2009/10 and 2010/11, ARC Roodeplaat)

African Leafy Vegetables	Average total above-ground dry matter yield[1] (t ha^{-1})	Average irrigation water use (mm)	Crop water productivity[1; 2] (kg m^{-3})
Chinese cabbage (*Brassica rapa* subsp. *chinensis*)	1.6	190	0.90
Nightshade (*Solanum retroflexum*)	1.5	240	0.80
Pigweed (amaranth) *(Amaranthus cruentus)*	2.2	413	0.50
Jew's mallow *(Corchorus olitorius)*	1.1	448	0.26
Spider flower (*Cleome gynandra*)	0.9	414	0.20
Cowpea (*Vigna unguiculata)*	1.0	446	0.23
Pumpkin (*Cucurbita maxima)*	0.5	389	0.13
Tsamma melon (*Citrillus lanatus)*	0.4	389	0.14

[1]Consumable or marketable portion; [2]dry matter basis

5.5 CONCLUSIONS AND RECOMMENDATIONS

The response of ALVs to different water regimes varied strongly depending on the crop. All eight ALVs that were studied were sensitive to water stress (50FC and 25FC irrigation treatments) and the biomass production of the crops was influenced by the irrigation treatments. This indicates that the crops can only be produced with adequate amount of water, particularly if the yield is intended for human consumption. Frequent harvesting, every seven to ten days, was found to delay flowering and encourage new shoot and root growth in all the crops.

Highest water productivity was obtained by deficit irrigation (50FC and 25FC irrigation treatments) but deficit irrigation compromised leaf quality of the crops. Pumpkin and watermelon needed more water to produce a kg of green leafy vegetable. Chinese cabbage produced the highest biomass per metre cube of water compared to the other ALVs. Using proper irrigation water management, farmers can increase their harvest and gross income when they irrigate to field capacity, but that will be at the cost of decreased water productivity. The water productivity of crops presented in this study is the first attempt at estimating ALVs water requirement and effect of water stress on their biomass production but can serve in various ways such estimating the water foot print of ALVs in South Africa.

The water requirement of the ALVs determined in these studies can be used as a basis to schedule irrigation in different areas of South Africa. However, it is recommended that further studies be done on the agronomic practices of these crops to see if their nutritional water productivity is directly interlinked to soil fertility. Research that integrates water productivity of ALVs with different levels of irrigation and with monetary value of the produce is also required in order to determine whether ALVs grown at deficit irrigation will be profitable to farmers. Additional research studies on agronomic practices that investigate and adapt indigenous or traditional knowledge in South Africa are recommended.

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Agronomic Characterisation of Selected African Leafy Vegetables

CHAPTER 6: GERMINATION OF AFRICAN LEAFY VEGETABLES

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6.1 INTRODUCTION

Leafy vegetables that grow in the wild, or as weeds on cultivated land, are highly seasonal (Jansen van Rensburg *et al*., 2007). Cultivation of a diverse range of leafy vegetable species in home gardens, could enhance their availability at household level, both quantitatively and temporary. Moreover, evidence from elsewhere in Africa (Schippers, 2002) and also from South Africa (Van Averbeke *et al*., 2007) indicates that cultivation of traditional leafy vegetables often leads to their commercialisation. This, in turn, creates opportunities for rural households to derive income from the cultivation of these vegetables.

Knowledge of the agronomy of many traditional South African leafy vegetables is limited or totally lacking. The dearth of information applies particularly to plants that are not cultivated species. To assist the incorporation of these wild plants into the cropping- or gardening systems of people, information on how to grow these plants is needed. To develop such information for a selection of important South African leafy vegetables was one of the aims of WRC Project K5/1579//4. The process of germination of seeds of the eight leafy vegetables that were selected for research in this Project is the focus of this chapter. Seed germination was defined as the protrusion of the radical through the surrounding seed covering (Mpati, 2006).

The process of seed germination is the first stage of plant growth. It is particularly important in annual crops, because of its substantial effects on both yield and monetary value of crops (Wang, 2005; Ghaderi *et al*., 2008). Knowledge of seed germination is important to assess the suitability of climatic conditions for crop growth, particularly with regard to suitable and optimum planting dates, which largely depend on the local temperature regime and the growing period of the crop (Böhringer *et al*., 1999). Scheduling sequential plantings, in order to match supply with demand for the crop, which has a major effect on the monetary value of a crop, requires knowledge of seed germination (Wang, 2005).

Germination in non-dormant seed is governed by environmental factors, including temperature, light, moisture and oxygen and obviously the type of seed (Hemmat *et al*., 2003; Ghaderi *et al*., 2008). In the presence of adequate soil moisture and the absence of water-logged conditions, temperature, light and dormancy are the three factors that affect germination of seed in major ways (Bewley and Black, 1994). For this reason these three factors were selected for study. The eight African leafy vegetables that were included in the study were pigweed (*Amaranthus cruentus* L.), spider plant or cat's whiskers (*Cleome gynandra* L.), tsamma melon (*Citrillus lanatus* Thunb.), pumpkin (*Cucurbita maxima* Duchesne), jute or Jew's mallow (*Corchorus olitorius* L.), cowpea (*Vigna unguiculata* L.), non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*), and nightshade (*Solamum. retroflexum* Dun.). Swiss chard (*Beta vulgaris* L. var. cicla) was included in the study as the reference crop.

The objectives of the study were:

- To determine the cardinal temperatures for germination of the seed of eight ALVs at different controlled temperatures;
- To determine which of these ALV seeds displayed dormancy and to identify pre-sowing treatments that could break the dormancy in these seeds; and
- To determine the influence of light on the germination of the seed of the eight ALVs.

6.2 EFFECT OF TEMPERATURE

6.2.1 Background

Temperature is one of the prominent environmental factors regulating growth and development of plants (Garcia-Huidobro *et al*., 1982; Bruin, 1994; Craufurd *et al*., 1996; Koger *et al*., 2004; Mpati, 2006). The rate at which germination occurs usually increases linearly with temperature, within a well-defined range and then declines sharply at higher temperatures. The range of temperatures at which germination occurs is characterised by cardinal temperatures, which comprise the minimum, maximum and optimum temperature (Ghaderi *et al*., 2008). The minimum temperature is the lowest temperature at which germination occurs, regardless of how long the seeds are incubated. Germination proceeds at the minimum temperature but at a very slow rate. The optimum temperature is the temperature range that provides the greatest germination percentage within the shortest time. The maximum temperature is the highest temperature at which germination occurs (Bradford and Nonogaki, 2007).

Knowing the germination response of seeds of a plant to temperature and the cardinal temperatures that characterise this response is essential for sustainable crop production. This knowledge is necessary to identify the temperature conditions necessary for the plant to successfully germinate, emerge and grow and to model plant growth with a view of predicting harvest readiness. In South Africa, ambient temperature and soil temperature vary greatly, not only seasonally but also spatially, because of differences in latitude, altitude and proximity to the ocean among other factors. Knowing the cardinal germination temperatures of a plant enables identification of the window period during which the crop can be planted and reach harvest readiness at a particular locality (Craufurd *et al*., 1996; Roché *et al*., 1997; Ghaderi *et al*., 2008). Consequently, the objectives of this part of the study were to investigate the effect of temperature on germination and to determine the cardinal temperatures for 50% germination of the seed of the eight African leafy vegetables at constant temperatures.

6.2.2 Materials and methods

Seed source

Seeds of *A. cruentus*, *C. gynandra*, *C. lanatus* (tsamma melon), *C. maxima* (VOPI reference ‘ex Bushbuckridge”), *C. olitorius* and *V. unguiculata* (VOPI reference “Fahari”) were supplied by the Vegetable and Ornamental Plant Institute (VOPI) of the Agricultural Research Council (ARC) at Roodeplaat. The seed of *B. rapa* subsp. *chinensis* (*dabadaba* land race) and *S. retroflexum* were obtained from farmers in the Vhembe District, where these two species are cultivated. Details are provided in Chapter 7 of this report. The seed of *B. vulgaris* (variety Ford Hook Giant) was obtained from Hygrotech (Pyramid, Pretoria North).

Seed pre-treatment and sanitation

A hot water treatment was used to reduce a range of fungal and bacterial contamination during germination tests (Miller and Ivey, S.a; Department of Agriculture and Food, 2005). Thereafter, seeds were used for the standard germination tests (Miller and Ivey, S.a). In order to prevent secondary contamination, the temperature incubators, containers used for germination, as well as the bench surfaces in the laboratory were surface sterilized by wiping with 90% alcohol and 1% sodium hypochlorite before use.

Seed germination

The experiments were conducted in a laboratory using environmental controlled low-temperature incubators (Labcon™ 220v, 50 Hz) fitted with Lasec thermometer (GLAA504.110IMTJ, -10/110ºC) provided by Tshwane University of Technology (TUT). Incubators were allowed to run at desired temperatures for 48 h before samples were incubated.

Each treatment consisted of 50 seeds per vegetable species and was replicated four times, totalling 200 seeds per vegetable. Germination experiments were conducted by sowing small seeds on top of four layers (115 mm x 125 mm) of pre-cut brown anchor germination paper (top paper) moistened with 10 ml distilled water in a plastic container or into 160 mm x 100 mm rectangular transparent plastic containers, moistened with 20 ml distilled water. Large seeds were sown on four layers of rolled germination paper method (260 mm x 380 mm) moistened with 50 ml distilled water (ISTA, 2008). Treatments were arranged in a completely randomized design in controlled incubators for all experiments.

The effect of temperature on seed germination was investigated by monitoring germination under specified constant temperatures (treatment) of 4ºC to 44ºC with 4ºC increments under continuous darkness for 14 days (336 h). Seeds were only exposed to normal light during data collection. Seeds were sampled using tail sampling procedure (Hygrotech, 2008) under a magnifier lamp (model no.:8066D, AC 230v, 50 Hz, 22w, bulb type 22w G10Q). Contaminated, broken or alien seeds were removed and discarded.

Seeds were considered to have germinated once the radicle has protruded from the testa by at least 2 mm. Seed germination was recorded every 6 h over a period of 336 h (14 days), and expressed as a percentage of the total number of tested seeds counted and removed. After 10 days (240 h), recordings were taken every 12 h since a majority of seeds had already germinated. A Nikon microscope (model, C-LEDS, 100-240v, 0.2A, 50/60 Hz) was used to determine the length of the radicle if not visible to the naked eye.

Data transformation

The following parameters were determined:

i. The trend in germination over temperature and time (Jami Al-Ahmadi and Kafi, 2007)
ii. Time to 50% germination (Jami Al-Ahmadi and Kafi, 2007)
iii. Cardinal temperatures (Bruin, 1994)

Statistical analysis

Analysis of variance (ANOVA) was used to test for differences between the treatments. Treatment means were separated using Fishers' Protected t-test Least Significant Difference (LSD) at the 5% level of probability (Snedecor and Cochran, 1980). Data was analysed using the statistical programme GenStat® (Payne *et al.,* 2007). Time to 50% germination was analysed with SAS statistical software version 9.2 (SAS, 1999).

6.2.3 Results

Trend in germination over time

***Amaranthus cruentus* L.**

Germination percentage of *Amaranthus cruentus* L.is shown in Figure 6.1.

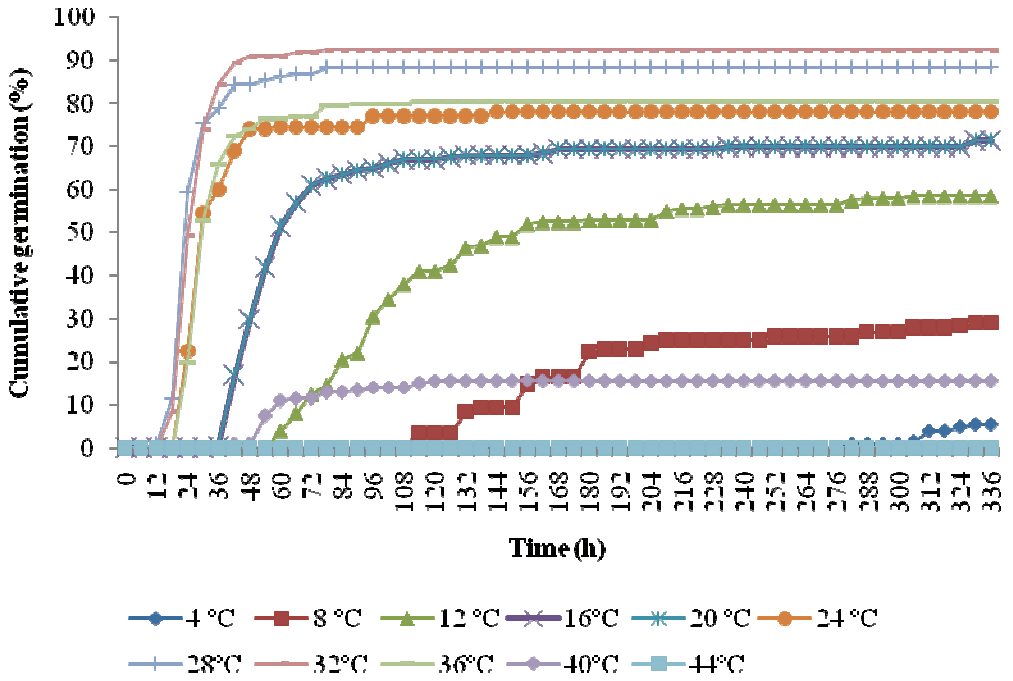

Figure 6.1: Cumulative percentage of germinated *Amaranthus cruentus* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

It is clear from the cumulative germination of *A. cruentus* that seeds germinated in nearly all temperature treatments although the germination percentage varied with time to germination. Commencement of germination of the seed of *A. cruentus* occurred after 12 h of incubation in the 28ºC, 32ºC, and 36ºC temperature treatments, after 18 h in the 40ºC treatment, after 24 h in the 24ºC treatment and after 30 h in the 20ºC treatment. All other temperature treatments substantially delayed commencement of germination.

Within the first 36 h at 20 to 40ºC, approximately 60% of seeds germinated, and differences between these treatments were statistically not significant. Thereafter, germination percentage remained unchanged at 20 and 24ºC, but at 28 to 40ºC, germination percentage significantly increased ($p \leq 0.01$) up to more than 90% after about 96 h. Below 20ºC and above 40ºC, germination was slow and germination percentage remained below 50%. At 16ºC, germination only started after 48 h and reached a level of only 30% after 96 h, while at 12ºC and 44ºC, germination started after 120 h reaching a level of 3% and 1.5%, respectively. No germination was observed at 4ºC. At 8ºC, germination started after 66 h and was very low (0.5%).

Time to final germination percentage was shortest at 40ºC (120 h) followed by 126 h in the 28ºC and 36ºC treatments. At 24ºC, time to final germination percentage was 156 h and at 20ºC it was 174 h. Time to final germination percentage at 32ºC was 192 h, while at 12ºC and 44ºC it was substantially delayed to 204 h and 264 h, respectively. Final germination percentage was significantly higher ($p \leq 0.01$) in the 28ºC to 40ºC temperature treatments than in the other temperature treatments. At 28ºC, final germination was 95%, which was not significantly different from the 99% attained in the 32ºC to 40ºC treatments. Final germination at 20ºC and 24ºC was 64.5% and 62.5%, respectively. It was 12% at 12ºC and 10.5% at 44ºC.

Beta vulgaris* L. var. *cicla

Germination percentage of *B. vulgaris* var *cicla* is shown in Figure 6.2. Cumulative germination of *B. vulgaris* var. *cicla* was affected by temperature. Germination of *B. vulgaris* var. *cicla* seed commenced after 24 h in the 28ºC to 40ºC treatments, after 30 h in the 24ºC treatment and after 36 h in the 20ºC treatment. Commencement of germination in all the other temperature treatments was delayed substantially. Seeds incubated at 20ºC to 40ºC germinated rapidly and reached maximum germination after 24 h to 48 h.

Significantly fewer ($p \leq 0.01$) seeds germinated during the same period at lower (4ºC to 16ºC) and higher temperatures (44ºC). Although onset of germination was delayed to 60 h at 16ºC and 108 h at 12ºC, final germination percentage in these two treatments was similar to that at 20ºC to 36ºC. Germination commenced only after 216 h at 8ºC and final germination was 53.0%, whilst at 40ºC it was 52.0% after 288 h. At 44ºC, final germination percentage (4%) was reached after 288 h and was significantly lower ($p \leq 0.01$) than that obtained at 40ºC (52.0%). No germination of *B. vulgaris* var. *cicla* seed was observed at 4ºC.

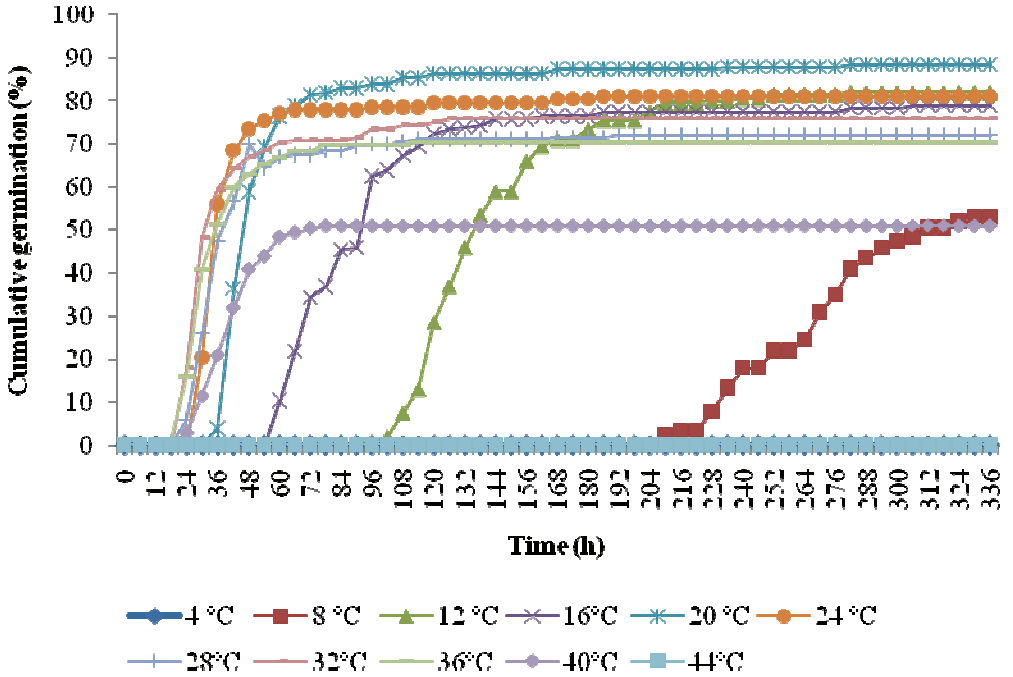

Figure 6.2: Cumulative percentage of germinated *Beta vulgaris* var. *cicla* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Final germination percentage was attained earliest in the 36ºC and 32ºC treatments, being after 114 h and 126 h, respectively. At 24ºC and 28ºC, time to final germination percentage was extended to 186 h and 192 h, respectively. Final germination percentage was reached after 264 h in the 40ºC treatment, after 276 h in the 20ºC treatment and after 282 h in the 12ºC and 16ºC treatments. Final germination percentage was highest (87.5%) in the 20ºC, but not statistically different from the 85% achieved at 12ºC, the 80% at 16ºC, the 81% at 24ºC and the 76% at 32ºC.

Brassica rapa* L. subsp. *chinensis

Germination percentage of *B. rapa* subsp. *chinensis* is shown in Figure 6.3. Commencement of germination of the seed of *B. rapa* subsp. *chinensis* occurred after 18 h in the 28ºC and 32ºC temperature treatments, after 24 h in the 24ºC and 36ºC treatments and after 30 h in the 20ºC treatment. Commencement of gemination was delayed to 36 h in the 16ºC treatment, 42 h in the 40ºC treatment, 60 h in the 12ºC treatment and 114 h in the 8ºC treatment. No germination of *B. rapa* subsp. *chinensis* seed was observed at 44ºC, while at 4ºC commencement of germination was delayed to 288 h. During the first 48 h, germination percentage was highest in the 20ºC to 36ºC constant temperature treatments.

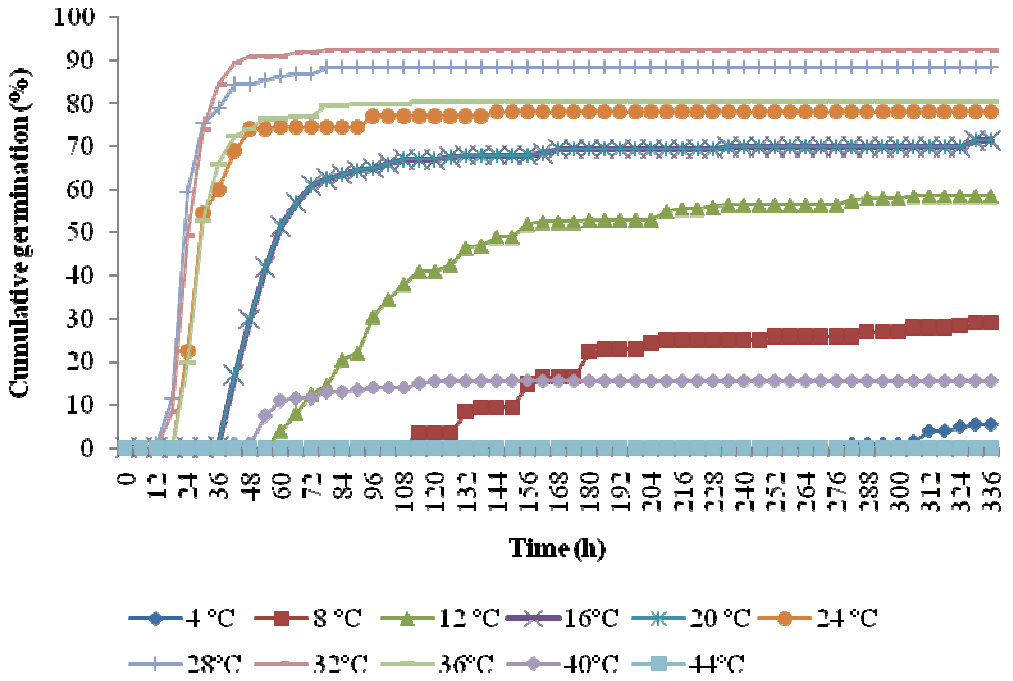

Figure 6.3: Cumulative percentage of germinated *Brassica rapa* subsp. *chinensis* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Germination after 18 h was similar in the 28ºC and 32ºC treatments and remained similar throughout the incubation period. After 144 h and 156 h, germination percentage at 36ºC and at 20ºC was statistically not different from the germination percentage in the 28ºC to 32ºC treatment. Germination percentage at 24ºC, though relatively high, was significantly lower ($p \leq 0.01$) than that attained at 32ºC but statistically not different from that at 20ºC, 28ºC and 36ºC. *B. rapa* subsp. *chinensis* seed germination in the 4ºC and 44ºC proceeded slowly. The onset of germination at 16ºC was delayed to after 48 h, but germination proceeded rapidly thereafter, reaching levels in excess of 60% after 60 h.

Final germination percentage was reached earliest (after 78 h) at 28ºC and 32ºC. In all other treatments, time to final germination percentage was delayed. Final germination percentage was reached after 114 h in the 36ºC treatment, 144 h in the 24ºC treatment, 276 h in the 20ºC treatment and after more than 300 h in all treatments below 20ºC. Final germination percentage was 92.5% at 32ºC, 88.5% at 28ºC, 81.5% at 36ºC, 78% at 24ºC and 82.5% at 20ºC. Relative to the final germination percentage in the 20ºC to 36ºC treatments, final germination percentage of *B.rapa* subsp. *chinensis* seeds was significantly lower ($p \leq 0.01$) at temperatures below 20ºC and above 36ºC. Final germination percentage was 71.5% at 16ºC, 58.5% at 12ºC, 29% at 8ºC, 15% at 40ºC and 5.5% at 4ºC.

***Citrillus lanatus* L.**

Germination percentage of *C. lanatus* is shown in Figure 6.4. Generally, germination of *C. lanatus* seed occurred at temperatures ranging between 20ºC and 40ºC. At the other temperatures, germination was either completely inhibited or extremely poor. Germination commenced after 36 h in the 32ºC to 40ºC treatments, after 42 h in the 24ºC treatment, after 48 h in the 28ºC treatment and after 54 h in the 20ºC treatment. Germination at 16ºC commenced after 312 h. No germination of *C. lanatus* seed was observed below 16ºC and above 44ºC.

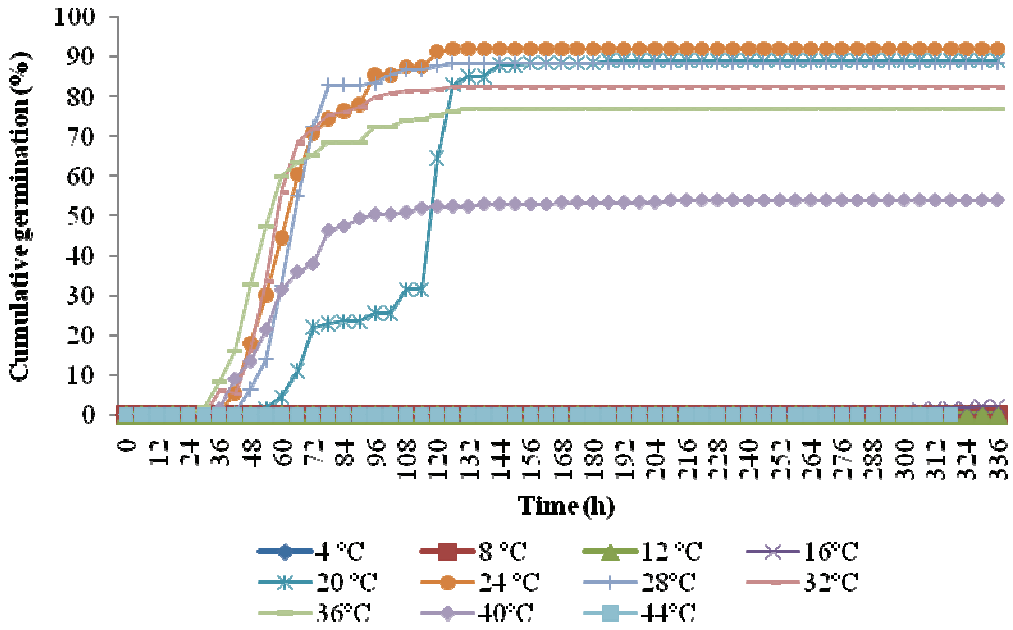

Figure 6.4: Cumulative percentage of germinated *Citrillus lanatus* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Although germination started after 36 h in the 32ºC to 40ºC treatments, germination percentage after 36 h was significantly higher ($p \leq 0.01$) at 36ºC (8.5%) than at 32ºC (2.5%) and 40ºC (1.5%). During the first 72 h, germination percentage varied among the 24ºC to 36ºC treatments, but after 72 h differences in germination percentage among these temperature treatments were no longer statistically significant. After 78 h to 144 h, germination percentage varied significantly ($p \leq 0.01$) among the 24ºC to 36ºC treatments. After 150 h, germination percentage in the 20ºC to 28ºC treatments was significanlty higher than that at 32ºC and 36ºC. However, the germination percentage of 82.5% at 32ºC was not significantly different from that at 20ºC (88%), 24ºC (88.5%) and 36ºC (77.5%). This trend in germination percentage remained the same until final germination was recorded after 336 h.

Final germination percentage was reached after 126 h in the 24ºC to 32ºC treatments. Final germination percentage was attained after 132 h at 36ºC, after 186 h in the 20ºC treatment and after 210 h in the 40ºC treatment. Final germination percentage of *C. lanatus* was highest (93%) in the 24ºC treatment, but statistically, this final germination percentage did not differ from that at 20ºC (89%) and 28ºC (88.5%). The final germination percentage of 82.5% attained at 32ºC was significantly lower than that at 24ºC. A final germination percentage of 77.5% was attained in the 36ºC treatment, while only 53.5% was attained in the 40ºC treatment.

***Cleome gynandra* L.**

Germination percentage for *Cleome gynandra* seeds is shown in Figure 6.5.

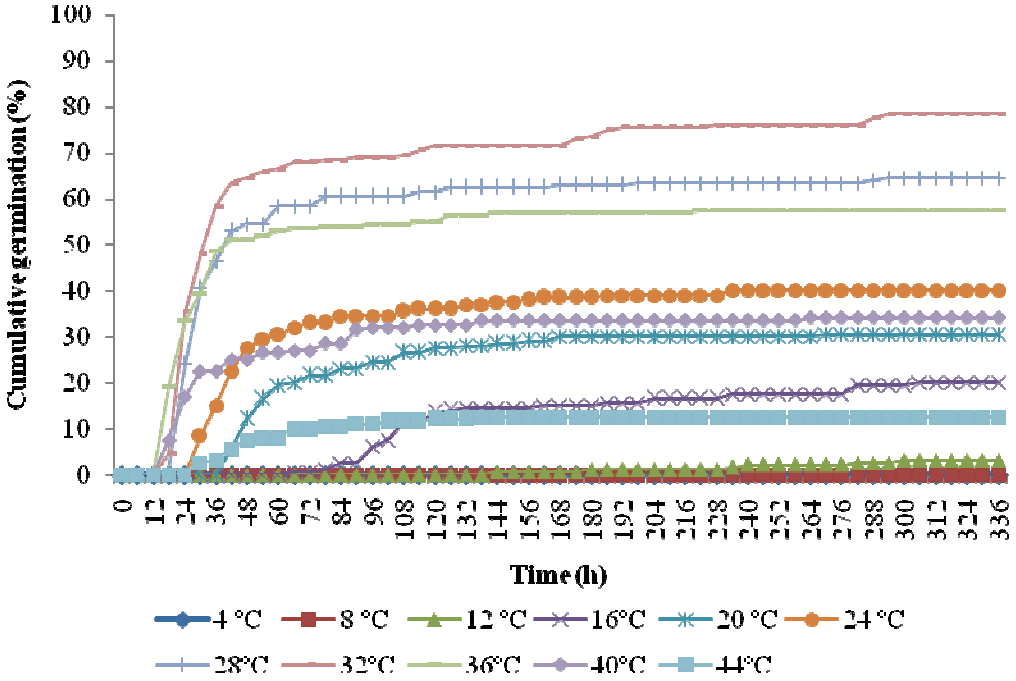

Figure 6.5: Cumulative percentage of germinated *Cleome gynandra* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Onset of germination of *C. gynandra* was recorded after 18 h in the 28ºC to 40ºC temperature treatments, after 24 h in the 24ºC treatment, after 30 h in the 44ºC treatment and after 42 h in the 20ºC treatment. Below 20ºC, commencement of germination was substantially delayed, and no germination was observed in the 4ºC and 8ºC temperature treatments. After 18 h of incubation, the germination percentage of 19% at 36ºC was significantly higher ($p \leq 0.01$) than that at 28ºC (1%), 32ºC (4.5%) and 40ºC (7.5%). After 24 h, germination percentage in the 28ºC to 36ºC treatments still differed significantly ($p \leq 0.01$) from that in the 40ºC treatment. Thereafter, germination percentage was significantly higher ($p \leq 0.01$) in the 28ºC and 32ºC treatments than in the other temperature treatments, and this trend was maintained for the remainder of the incubation period.

Final germination percentage was attained after 192 h at 40ºC but was very low (34.5%). At 36ºC, final germination percentage (56.5%) was attained after 216 h, while at 24ºC, final germination percentage (40%) was reached after 228 h. Time to final germination in the 28ºC and 32ºC treatments was delayed to 294 h. Final germination percentage (30.5%) was attained after 270 h at 20ºC, after 300 h (3.5%) at 12ºC and after 306 h (20%) at 16ºC. The final germination percentage in the 28ºC (66%) and 32ºC (78%) treatments was significantly higher ($p \leq 0.01$) than in all other treatments.

***Corchorus olitorius* L.**

Germination percentage of *Corchorus olitorius* is shown in Figure 6.6.

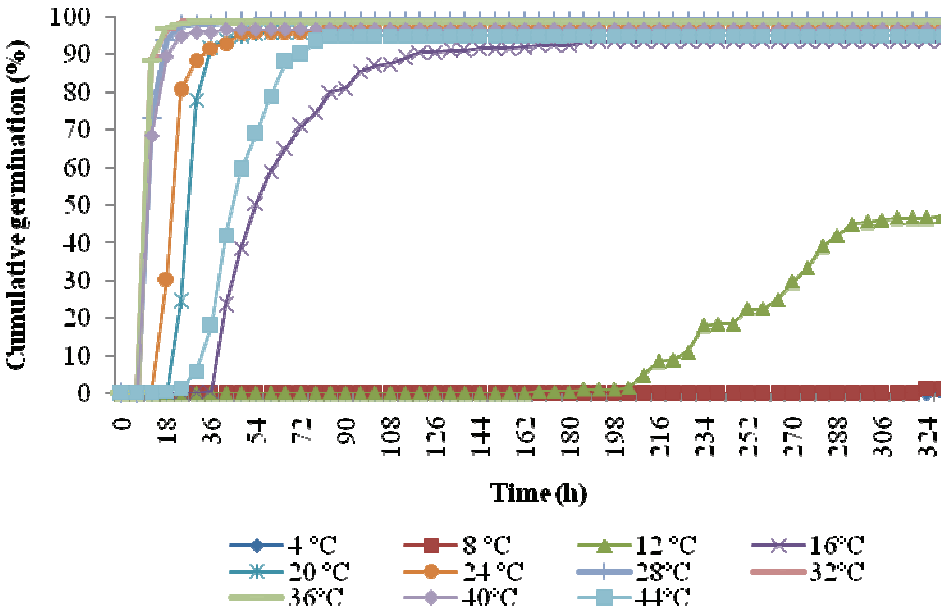

Figure 6.6: Cumulative percentage of germinated *Corchorus olitorius* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Germination commenced after 12 h in the 28ºC to 40ºC temperature treatments, after 18 h in the 24ºC and 44ºC treatments, after 24 h at 20ºC and after 36 h in the 16ºC treatment. Germination of *C. olitorius* seeds was observed after 168 h in the 12ºC treatment and after 324 h in the 8ºC treatment. No germination was observed in the 4ºC treatment.

After 12 h, germination percentage was significantly higher ($p \leq 0.01$) in the 28ºC, 32ºC and 36ºC treatments than in all other temperature treatments. After 24 h, germination percentage rose to above 90% in the 28ºC to 40ºC treatments. After 72 h of incubation differences in germination percentage among the 28ºC to 44ºC treatments were no longer statistically different ($p > 0.01$). After 234 h, the germination percentage of seed incubated at 16ºC was not statistically different from that in the 20ºC to 44ºC treatment.

Final germination percentage was attained after 24 h in the 32ºC treatment, after 36 h in the 36ºC treatment, after 42 h in the 40ºC treatment and after 54 h in the 28ºC treatment. Final germination percentage was attained after 84 h in the 20ºC , 24ºC and 44ºC treatments and after 186 h in the 16ºC treatment. Below 16ºC, attainment of final germination percentage was substantially delayed. Statistically, final germination percentage did not differ significantly among the 16ºC to 44ºC temperature treatments. In these treatments, final germination percentage ranged between 93% and 99%.

***Cucurbita maxima* Duchesne**

Germination percentage of *Cucurbita maxima* is shown in Figure 6.7.

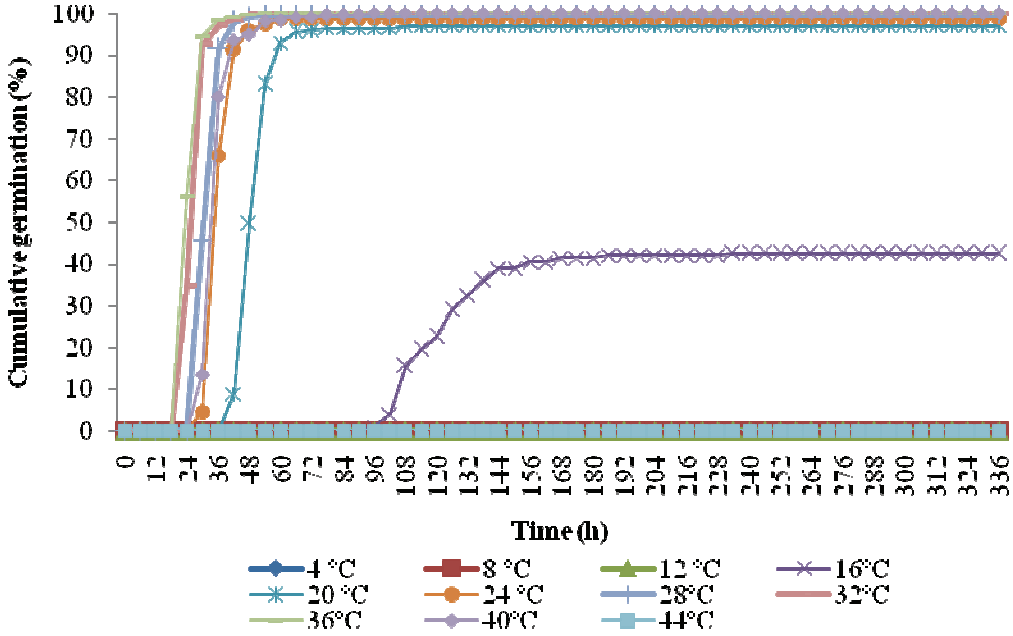

Figure 6.7: Cumulative percentage of germinated *Cucurbita maxima* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Seed germination of *C. maxima* seed started after 24 h at 32ºC and 36ºC, after 30 h at 24ºC and 40ºC, after 42 h at 20ºC and after 96 h in the 16ºC treatment. No seeds of *C. maxima* germinated below 16ºC or above 40ºC during the 336 h of incubation. Although germination was observed earliest in both the 32ºC and 36ºC treatments, after 24 h, the germination percentage of 56% in the 36ºC was significantly higher ($p \leq 0.01$) than the 34.5% in the 32ºC treatment. During the 36 h to 72 h period, germination percentage varied significantly ($p \leq 0.01$) among seed incubated in the 20ºC to 40ºC range of treatments but after 78 h, there were no longer significant differences in germination percentage among these treatments.

Final germination percentage was attained after 48 h in the 32ºC treatment, after 54 h in the 28ºC and 36ºC treatments, after 60 h in the 24ºC treatment and after 96 h in the 40ºC treatment. Temperature substantially delayed time to final germination percentage in the 20ºC (108 h) and 16ºC (189 h) treatments. Final germination percentage of more than 95% attained in the 20 to 40ºC treatments was significantly higher ($p \leq 0.01$) than the 45.5% in the 16ºC treatment.

***Solanum retroflexum* Dun.**

Germination percentage of *S. retroflexum* is shown in Figure 6.8.

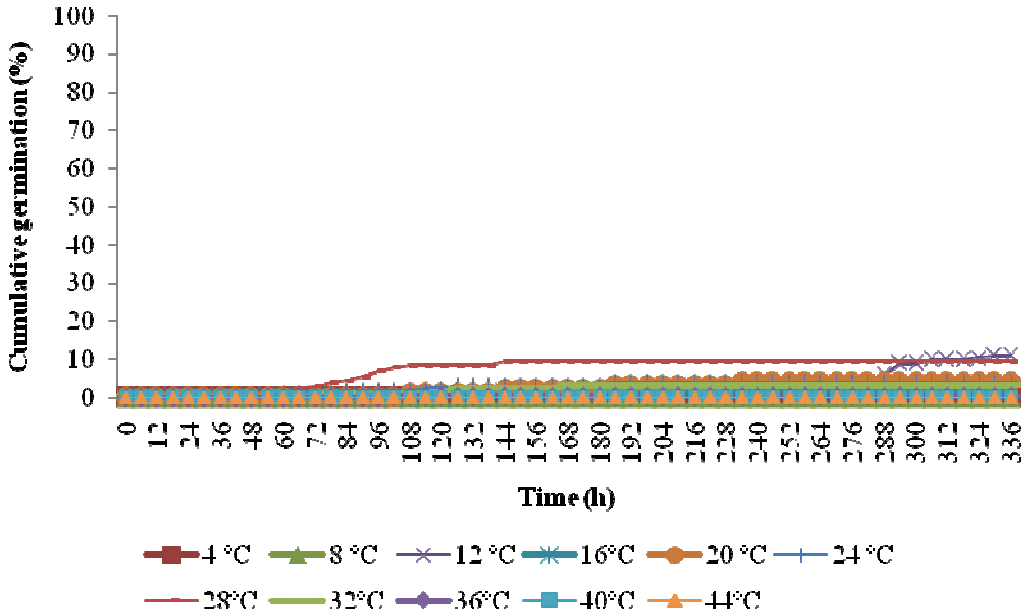

Figure 6.8: Cumulative percentage of germinated *Solanum retroflexum* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Germination of *S. retroflexum* seed was characterised by very low germination percentage across all temperature treatments over the entire incubation period. Germination was only recorded in the 12ºC to 36ºC temperature treatments. Germination of *S. retroflexum* commenced after 54 h in the 28ºC, after 60 h in the 24ºC treatment and after 78 h in the 32ºC treatement. All other temperature treatments substantially delayed commencement of germination. No germination of *S. retroflexum* was observed below 12ºC and above 36ºC.

After 78 h, the germination percentage of 3.5% in the 28ºC treatment was significantly higher ($p \leq 0.01$) than that in all other temperature treatments. Thereafter, germination percentage remained significantly higher in the 28ºC treatment than in the other treatments until 180 h, when differences between the 24ºC and 28ºC treatments were no longer significant. After 204 h, differences among the 20ºC to 28ºC temperature treatments were no longer significant. Although the onset of germination at 12ºC was delayed to 282 h, final germination percentage was significantly higher than that in the 16ºC, 20ºC, 24ºC and 32ºC treatments.

Time to final germination percentage was attained after 144 h in the 28ºC treatment, after 168 h in the 32ºC treatment, after 186 h in the 24ºC treatment, after 204 h in the 16ºC treatment, after 234 h in the 20ºC treatment and after 330 h in the 12ºC treatment. Final germination percentage was highest in the 12ºC (11%), 20ºC (4%), 24ºC (4%) and 28ºC (9.5%) temperature treatments. Final germination percentage was 3% in the 32ºC treatment and 2% in the 16ºC treatment.

***Vigna unguiculata* (L.) Walp.**

Germination percentage of *V. unguiculata* is shown in Figure 6.9.

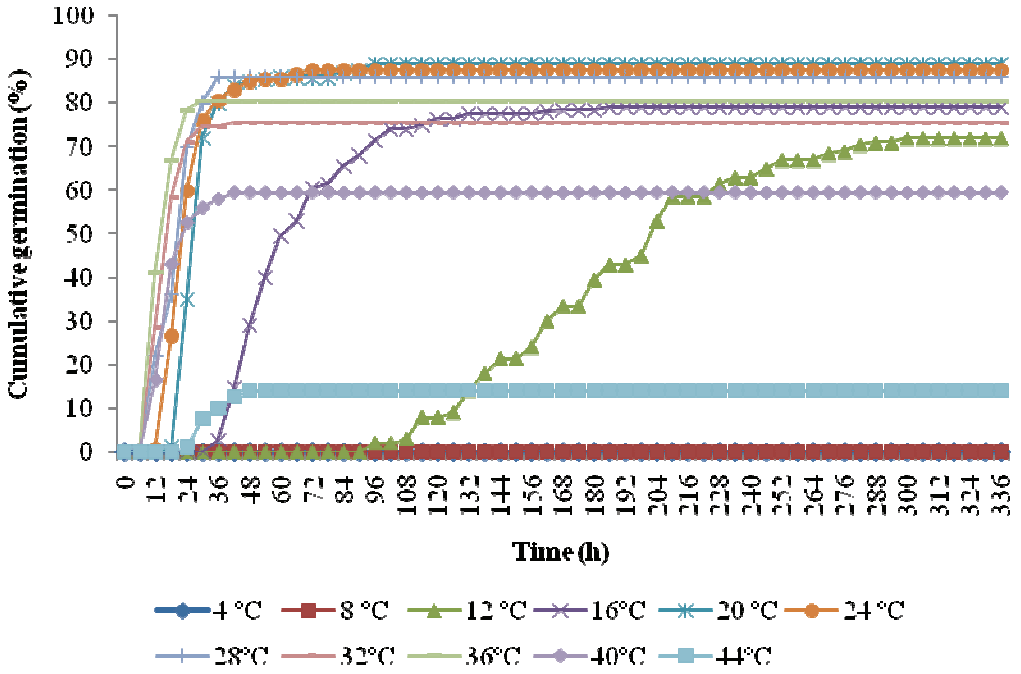

Figure 6.9: Cumulative percentage of germinated *Vigna unguiculata* seed over 336 h at different constant temperatures within the range of 4ºC and 44ºC

Germination of *V. unguiculata* seed occurred after 12 h in the 24ºC to 40ºC temperature treatments, after 18 h in the 20ºC treatment and after 24 h in the 44ºC treatment. Germination commenced after 36 h in the 16ºC treatment and after 102 h in the 12ºC treatment. No germination of *V. unguiculata* seed was obserbed below 12ºC.

The cumulative germination curve in the 20ºC to 44ºC temperature range showed a sharp increase in germination percentage during the first 36 h. After 12 h, germination percentage was 28.5% at 32ºC and 41% at 36ºC. These values were significantly higher ($p \leq 0.01$) than the 20.5% and 16.5% observed in the 28ºC and 40ºC treatments, respectively. After 30 h, differences in germination percentage among the 20ºC to 36ºC temperature treatments were no longer significant. After 102 h, germination percentage in the 16ºC treatment also no longer differed significantly from those in the 20ºC to 36ºC temperature treatments, and after 282 h, the germination percentage attained in the 12ºC treatment was also no longer different statistically from that in the 16ºC to 36ºC treatments.

Final germination percentage was reached after 30 h in the 36ºC treatment but took longer in all other treatments, being attained after 42 h in the 32ºC and 40ºC treatments, after 48 h in the 44ºC treatment, after 72 h in the 24ºC treatment and after 96 h in the 20ºC treatment. Final germination percentage was substantially delayed to 186 h and 288 h in the 16ºC and 12ºC temperature treatments, respectively. Final germination percentage (72.5% to 89%) was significantly higher ($p \leq 0.01$) in the 12ºC to 36ºC treatments than in the 40ºC (59.5%) and 44ºC (14%) treatments.

Time to 50% germination

***Amaranthus cruentus* L.**

The effect of temperature on time to 50% germination of *Amaranthus cruentus* seed is shown in Figure 6.10.

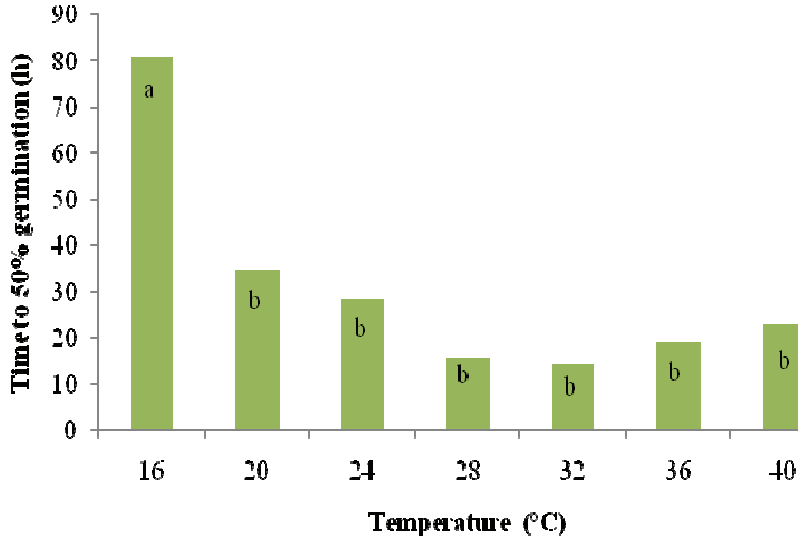

Figure 6.10: Effect of temperature on time taken to achieve 50% germination of *Amaranthus cruentus* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [p>0.01])

The minimum time required for 50% germination of *A. cruentus* was 14 h at 32ºC. Higher and lower temperatures tended to increase time to 50% germination, but statistically time to 50% germination was only increased significantly (p≤0.01) at 16ºC.

Beta vulgaris* L. var. *cicla

The effect of temperature on time to 50% germination of *B. vulgaris* var. *cicla* is shown in Figure 6.11.

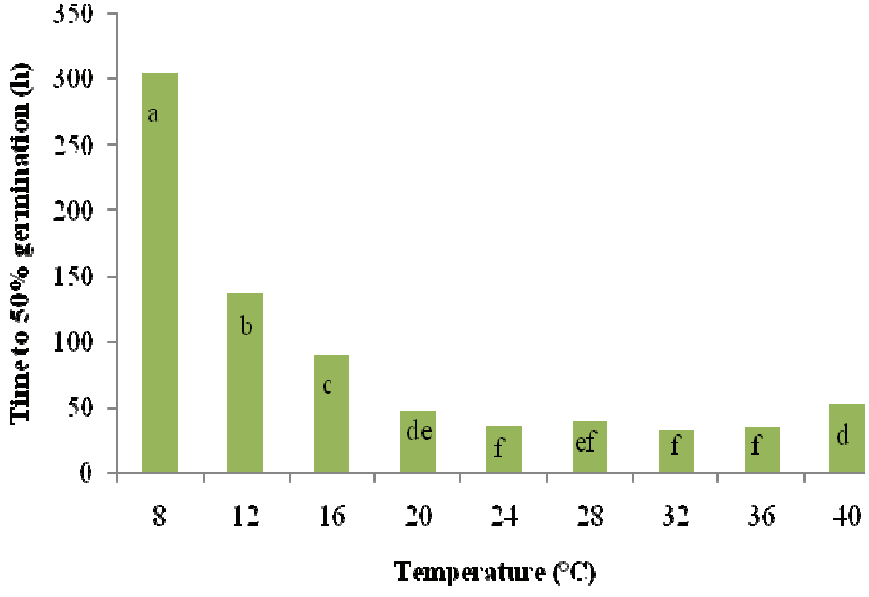

Figure 6.11: Effect of temperature on time taken to achieve 50% germination of *Beta vulgaris* var. *cicla* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [p>0.01])

Time to 50% germination of *B. vulgaris* var. *cicla* was shortest ($p \leq 0.01$) in the 24ºC to 36ºC treatments. At temperatures below 24ºC and above 36ºC, time to 50% germination was significantly increased ($p \leq 0.01$).

Brassica rapa* L. subsp. *chinensis

Figure 6.12 shows the effect of temperature on time to 50% germination of *B. rapa* subsp. *chinensis* seed. The shortest time to 50% germination of *B. rapa* subsp. *chinensis* was 23 h at 28ºC, but this did not differ significantly from the 31 h at 24ºC, 25 h at 32ºC and 31 h at 36ºC. At 16ºC and 12ºC, time to 50% germination was increased to 61 h and 128 h, respectively.

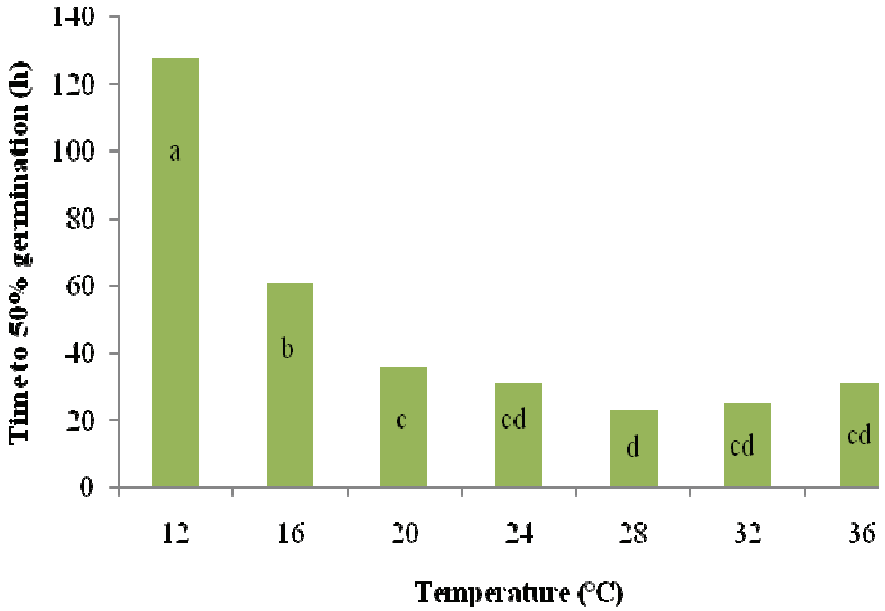

Figure 6.12: Effect of temperature on time taken to achieve 50% germination of *Brassica rapa* subsp. *chinensis* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [$p > 0.01$])

***Citrillus lanatus* L.**

The effect of temperature on time to 50% germination of *C. lanatus* seed is presented in Figure 6.13. The time to 50% germination of *C. lanatus* was shorter in the 24ºC to 36ºC treatment than in the 20ºC and 40ºC treatments. Time to 50% germination was 63 h at 24ºC, 65 h at 28ºC, 60 h at 32ºC and 58 h at 36ºC. At 20ºC, time to 50% germination was increased to 112 h and at 40ºC temperature; it was increased to 80 h.

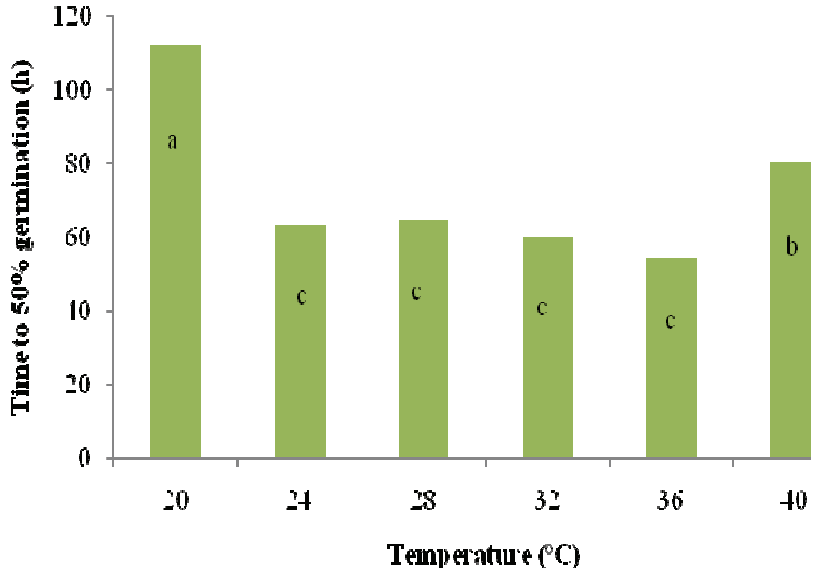

Figure 6.13: Effect of temperature on time taken to achieve 50% germination of *Citrillus lanatus* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [p>0.01])

***Cleome gynandra* L.**

The effect of temperature on time to 50% germination of *C. gynandra* is presented in Figure 6.14. Seeds incubated at temperatures higher than 36ºC or lower than 28ºC did not reach 50% germination. Only in the 28ºC, 32ºC and 36ºC temperature treatments was 50% germination attained. Statistically ($p \leq 0.01$), differences in incubation time needed to obtain 50% germination among these three treatments were not significant. Time required to reach 50% germination was 38 h at 28ºC, 30 h at 32ºC and 33 h at 36ºC.

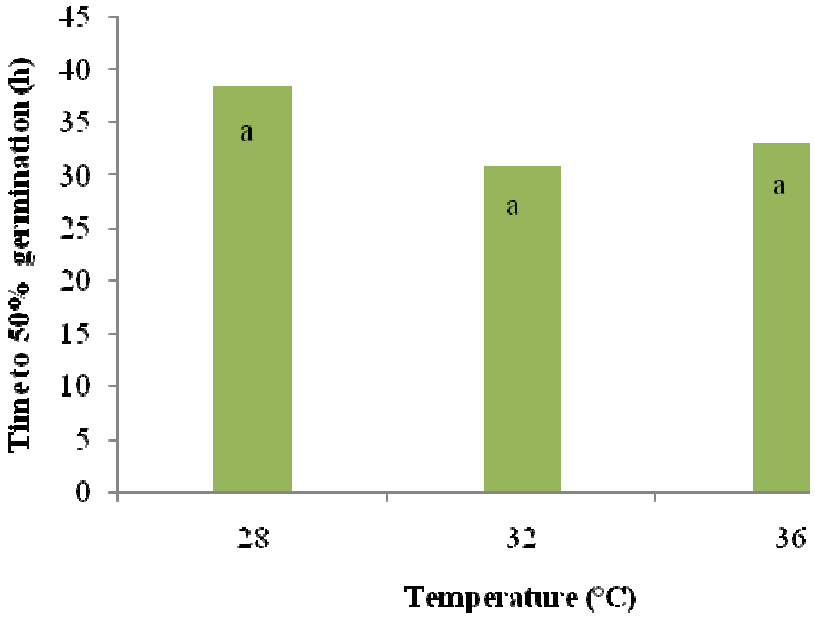

Figure 6.14: Effect of temperature on time taken to achieve 50% germination of *Cleome gynandra* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [p>0.01])

***Cucurbita maxima* Duchesne**

The effect of temperature on time to 50% germination of *C. maxima* seed is shown in Figure 6.15. Time to 50% germination was 25 h in the 32ºC treatment and 30 h in the 28ºC treatment, but the difference in time between these two treatments and the 36ºC treatment was statistically not significant ($p>0.01$). Reducing the incubation temperature to 24ºC or lower, significantly ($p\leq0.01$) increased incubation time to 50% germination when compared with the 32ºC and 36ºC treatments. Increasing the incubation temperature to 40ºC increased time to 50% germination from 24 h in the 36ºC to 33 h in the 40ºC treatment, an increase that was statistically significant ($p\leq0.01$).

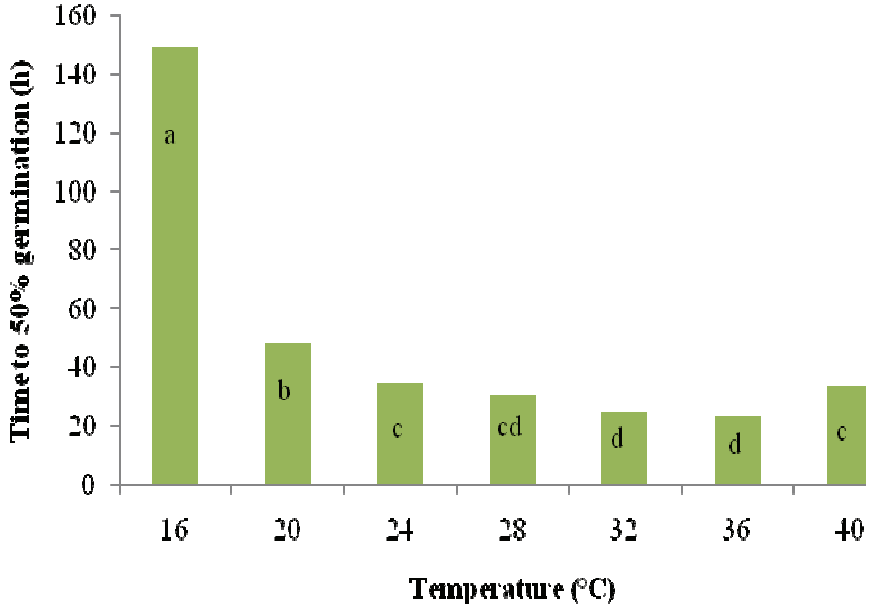

Figure 6.15: Effect of temperature on time taken to achieve 50% germination of *Cucurbita maxima* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [$p>0.01$])

***Corchorus olitorius* L.**

The effect of temperature on time to 50% germination of *C. olitorius* seed is shown in Figure 6.16. Minimum time to 50% germination of *C. olitorius* was 12 h in the 28ºC to 40ºC treatments. Below and above this temperature range, time to 50% germination was significantly increased ($p\leq0.01$). Time to 50% germination was 299 h at 12ºC, 54 h at 16ºC, 27 h at 20ºC and 50 h at 44ºC.

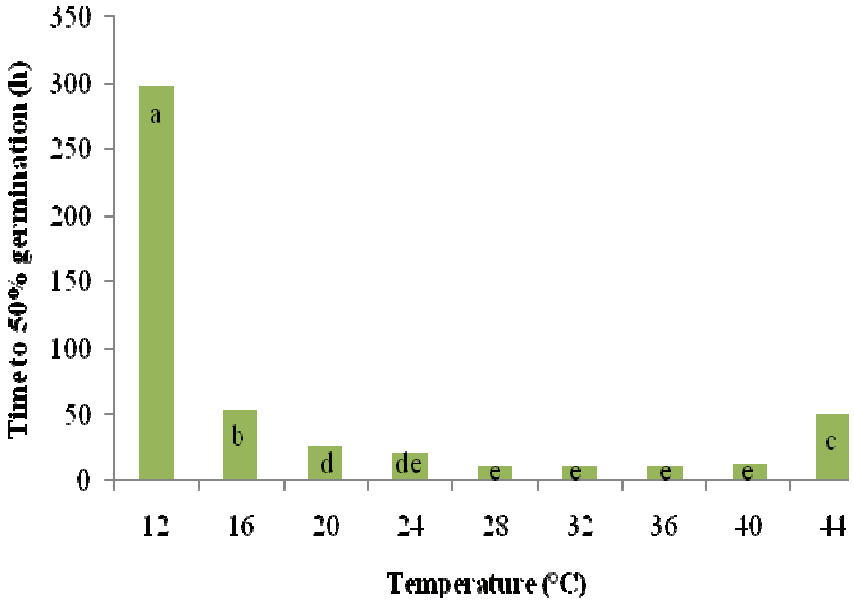

Figure 6.16: Effect of temperature on time taken to achieve 50% germination of *Corchorus olitorius* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [p>0.01])

***Vigna unguiculata* (L.) Walp.**

The effect of temperature on time to 50% germination of *V. unguiculata* seed is presented in Figure 6.17. The minimum time to 50% germination of *V. unguiculata* was 14 h at 36ºC, although this did not differ significantly (p≤0.01) from the time to 50% germination recorded in the 20ºC to 40ºC treatments, which ranged from 14 h to 27 h. At temperatures of 12ºC and 16ºC, time to 50% germination was increased significantly to 192 h and 66 h, respectively.

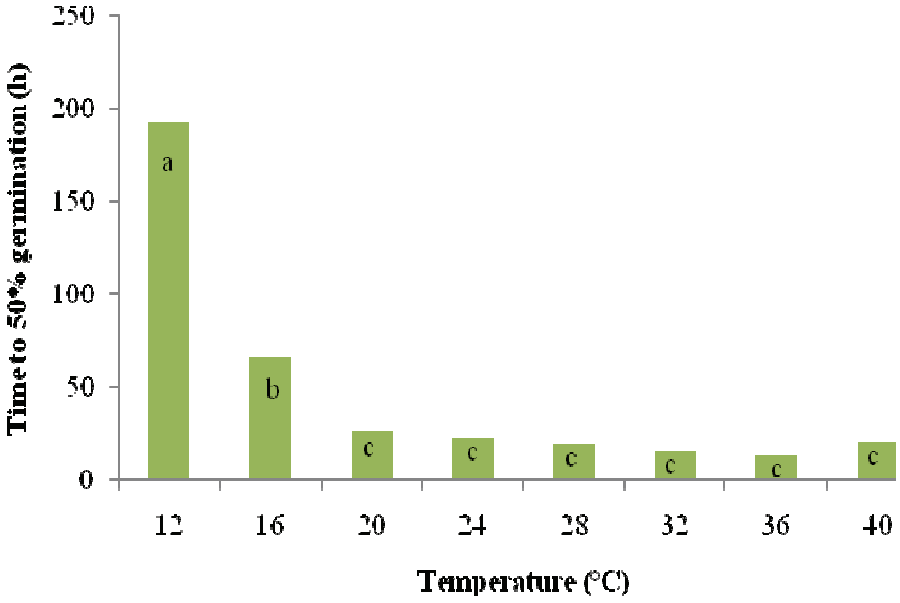

Figure 6.17: Effect of temperature on time taken to achieve 50% germination of *Vigna unguiculata* seed incubated at different constant temperatures within the range of 4ºC and 44ºC over 336 h (values with the same letter are not significantly different [p>0.01])

Cardinal temperatures

The cardinal temperatures for the selected ALVs are summarized in Table 6.1.

Table 6.1: Cardinal temperatures for the germination of selected African leafy vegetables

African leafy vegetables	Minimum temperature	Optimum temperature	Maximum temperature
		(°C)	
Amaranthus cruentus	16	31	40
Beta vulgaris var. *cicla*	8	31	40
Brassica rapa subsp. *chinensis*	12	29	36
Cleome gynandra	12	31	36
Citrillus lanatus	20	30	40
Corchorus olitorius	12	35	44
Cucurbita maxima	16	32	40
Vigna unguiculata	12	36	40
Solanum retroflexum	-----Did not reach 50% germination----		

6.2.4 Discussion

The results showed that germination of the seed of the selected African leafy vegetables was affected by temperature. The general trend was for germination percentage to increase from low temperatures towards a maximum achieved at an optimum temperature range, followed by a rapid decline at temperatures higher than the optimum, which is in line with the pattern that applies for most plants. High temperatures had a negative effect on final germination percentage and germination decreased for all eight plant species tested or failed completely at temperatures of 40ºC to 44ºC. These high temperatures are known to cause denaturing of proteins, membrane dysfunction and termination of metabolic activity (Kurtar, 2010), and this probably explains why the majority of seeds failed to germinate. Low temperatures delayed germination, which is known to increase the probability of fungal growth on seed (Singh and Dhaliwal, 1972).

In *A. cruentus*, germination in excess of 90% was obtained at temperatures ranging from 28ºC to 40ºC. Time to 50% germination was shortest in the 20ºC to 40ºC temperature range and final germination percentage of *A. cruentus* decreased at temperatures below 28ºC and above 40ºC. This is in agreement with the results of Gutterman, Corbineau and Côme (1992), who recorded 65% germination at temperatures ranging between 12ºC and 24ºC and 10% germination at temperatures ranging between 40ºC and 44ºC. The relationship between temperature and germination in *A. cruentus* underlines the importance of temperatures above 16ºC for rapid germination and high germination percentage (Aufhammer *et al*., 1998).

According to Van den Heever and Coertze (1996), amaranth species are known to be tolerant to relatively high temperatures and to generally thrive well within a temperature range of 22ºC to 30ºC. Cristaudu *et al*. (2007) stated that amaranth was adapted to a wide climatic range and grew optimally in the dry and hot summers of the Mediterranean environment, which could explain the germination percentage of 95 to 99% attained in the 28ºC to 40ºC observed in this study. The optimum temperature for maximum germination of *A. cruentus* observed in this study was 31ºC.

According to Drost (2005), *B. vulgaris* var. *cicla* seed germinated best when the temperature ranged between 12ºC and 23ºC, with temperatures above 26ºC reducing germination. Similar results were obtained in this

study. The highest germination percentage of more than 80% was attained at 12ºC to 24ºC. When temperature exceeded 24ºC, germination was reduced to below 80%. Time to 50% germination was, however, shorter when temperature ranged between 24ºC and 36ºC.

Generally, *Brassica* species, achieve high germination percentages at temperatures ranging between 15ºC and 35ºC, low germination percentage at 5ºC and very low germination percentage at 45ºC (Tokumasu *et al*., 1985). Toxopeus and Baas (2004) stated that optimum temperature for germination for *B. rapa* subsp. *chinensis* was between 20ºC and 25ºC, but that *B. rapa* subsp. *chinensis* seed was known to germinate even at temperatures as low as 5ºC. In this study the highest germination percentages were attained at temperatures ranging between 20ºC and 36ºC, lower germination percentages at 8ºC to 16ºC and very low at 4ºC and 40ºC. Time to 50% germination was shortest at 24ºC to 36ºC and the optimum temperature was 29ºC.

At 28ºC and 32ºC, maximum germination attained by *C. gynandra* was significantly higher than at other temperatures. Germination percentage at 44ºC was still noticeable (13%), suggesting that *C. gynandra* is adapted to hot environments as indicated by Fletcher (1999), Mnzava and Chigumira Ngwerume (2004) and Silué (2009). Poor and delayed seed germination of *Cleome* is believed to be due to the hard seed coat, immature embryos or induced secondary dormancy (Ochuodho and Modi, 2005; Ekpong, 2009). In this study the germination of *C. gynandra* was higher than reported by Böhringer *et al*. (1999). This could be due to the seed lots used in the current study having been in storage for a longer period than those used by Böhringer *et al*. (1999), which were only six months old. According to Chweya and Mnzava (1997), highest germination of *C. gynandra* seed occurs after 12 months of storage. However, this requires further investigation since it has been observed that seeds from dry capsules might germinate immediately after harvest. Long storage periods probably allow immature embryos to reach maturity (Ochuodho and Modi, 2005), since *C. gynandra* seeds have a rest period that extends to the fifth months after collection (Chweya and Mnzava, 1997; Fletcher, 1999).

According to Kurtar (2010), minimum and maximum temperatures for Cucurbits were 15ºC and 45ºC, respectively, with large differences amongst cultivars, whilst the reported optimum germination temperatures ranged from 20ºC to 32ºC. The germination of the seed of both Cucurbits (*C. lanatus* and *C. maxima*) used in this study was in line with the findings of Kurtar (2010). The minimum temperature for germination was 20ºC for *C. lanatus* and 16ºC for *C. maxima*, resulting in a germination percentage of approximately 3% and 42%, respectively. Time to 50% germination was shortest at 24ºC to 36ºC for *C. lanatus* and 28ºC to 36ºC for *C. maxima*. Maximum temperature for germination was 40ºC for both species. From this study it was evident that both cucurbits were warm climate crops that were sensitive to cold, explaining why both required relatively high temperatures for germination. These findings support those of Schippers (2002) and Chingumira Ngwerume and Grubben (2004).

In this study, germination of *C. olitorius* occurred over a wide temperature range, from 8ºC to 44ºC, with the highest germination percentage of 93% to 99% being obtained at 16ºC to 44ºC. Optimum temperature was 35ºC and maximum temperature 44ºC. At 12ºC, germination was reduced significantly. Time to 50% germination of *C. olitorius* was shortest (less than 20 h) at temperatures ranging between 24ºC and 40ºC. These findings are in line with those reported by Nkomo and Kambizi (2009) who stated that *C. olitorius* grew well when day temperatures averaged 30ºC and above.

Final germination percentage of *S. retroflexum* seed was very low, with a final germination of 11% at 12ºC being the highest that was recorded. According to Abukutsa-Onyango (2007) nightshades have inherent dormancy problems, especially if seeds are not properly harvested and processed. It was difficult to extrapolate the cardinal temperatures for *S. retroflexum* germination due to low germination, which might be

the result of inadequate removal of sugars and germination inhibitors present in the fruit during extraction of the seed (Abukutsa-Onyango, 2007; Jansen van Rensburg *et al*., 2007).

According to Vural *et al*. as quoted by Balkaya (2004), minimum and optimum temperatures for germination of *V. unguiculata* seeds were between 8 to 10ºC and 20 to 25ºC, respectively. In this study, minimum and optimum temperature for *V. unguiculata* germination were found to be 12ºC and 36ºC, respectively, with the maximum temperature being 40ºC. Time to 50% germination was shortest over the wide 20ºC to 40ºC range of temperatures. Cool temperatures (below 12ºC) and high temperatures (above 36ºC) significantly reduced germination of *V. unguiculata* seed. Balkaya (2004) reported that germination among vegetable legume species showed variability in terms of temperature response with seeds of some legumes, including *V. unguiculata*, germinating poorly at low temperatures. The trend in the response of germination to temperature and the cardinal temperatures of *V. unguiculata* recorded in this study were similar to those reported by Craufurd *et al*. (1996), who stated that the optimum temperature for germination of *V. unguiculata* seed ranged from 30ºC to 36ºC.

6.2.5 Conclusion

The temperature during seed imbibition was found to affect germination rate and final germination percentage of all eight African leafy vegetables tested. Every species tested had a temperature range at which germination was possible and for each specie temperature controlled the germination rate in a characteristic, unique pattern. The minimum, optimum and maximum temperature for seed germination varied among the species tested. A wide range of temperatures supported germination of *A. cruentus*, *C. gynandra*, *C. lanatus*, *C. maxima*, *C. olitorius*, *V. inguiculata*, *B. rapa* subsp. *chinensis*, and *B. vulgaris* var. *cicla*, and depending on the species, germination was successful at temperatures of 8ºC to 44ºC. In general, however, low temperatures of 8ºC to 20ºC delayed germination and raising the temperature increased germination rate until a temperature of about 30ºC to 35ºC was reached. When increasing temperature above 35 ºC, the rate of germination for all species decreased significantly and the extent of this reduction was species dependent. Very poor germination rates were observed for *S. retroflexum* over the whole spectrum of temperatures tested. *C. gynandra* also displayed a poor germination, and only reached 50% germination at temperatures between 28ºC and 36ºC.

The results of this study indicated that seed germination of the eight species was optimal in the temperature range of 29ºC to 36ºC. This result confirmed that these species were adapted to warm climates, and that they might grow optimally during summer when temperatures are warm. It is reported that most ‘weedy/wild’ species germinate better at high temperatures than exotic species. Therefore, simulation of germination at these high temperatures in this study probable was an important pre-adaptation for the weedy or wild habit of these species.

The minimum temperature range at which germination occurred was 8ºC to 20ºC. This might imply that below some critical temperature within this range, the ALVs may exhibit substantial reduction in rate of germination and subsequent growth, referred to as chilling injury. In addition, early sowing can be beneficial because it enables crops to have a long growing season and be ready for harvest prior to cool weather or rain at the end of the season. For the eight species tested, the maximum temperature range for seed germination ranged between 36ºC and 44ºC.

The ability to predict germination rate and the cardinal temperatures for seed germination is important for understanding seedling establishment in cropping ecosystems. Knowledge of seed germination response to temperature can help to optimise yield and monetary value of crops. For the majority of ALVs, the effect of temperature on seed germination has not been reported before. As was shown, the cardinal temperatures had a pronounced effect on rate and germination percentage of these crops. Considering that there has been

marked interest among many workers in modelling plant growth and development, the results of this study could contribute to these efforts. Models such as those predicting days to germination and those predicting optimum temperatures could be used to determine the optimum time for sowing of these crops in different regions and to utilize the growing season of these regions as productively as possible.

The cardinal temperatures derived for seed germination of the different ALVs could be used for the prediction of subsequent stages of growth. Germination is a function of environmental conditions and it can be expected that factors other than temperature might contribute to the field performance of these crop species. One implication of this study is that care should be taken to interpret germination responses to temperature for these species as adaptations to climatic factors. Future studies should aim to identify the selective forces acting on the ability to germinate under these temperature ranges, with a focus on post germination traits. It is also important to keep in mind that these experiments were conducted at constant temperatures, whereas under natural conditions temperature is subject to diurnal fluctuations. Consequently, the cardinal temperatures estimated in this study might differ from those that apply under natural conditions.

6.3 EFFECT OF DORMANCY ON GERMINATION

6.3.1 Background

In the majority of plant species, seed germination and development are separated by a period of low metabolic activity known as dormancy (Ochuodho and Modi, 2005). Seed dormancy can be defined as failure of seeds to germinate under optimal environmental conditions favouring germination (Bradford and Nonogaki, 2007). It is an important survival mechanism of many seeds in delaying germination, which allows time for dispersal and prevents germination of all the seeds at one time when conditions appear favourable (Bewley, 1997; Fenner and Thompson 2005). According to Bewley (1997), extensive domestication and breeding of crop species have seemingly removed dormancy mechanisms in wild seeds. However, under adverse conditions, dormancy may re-appear.

Dormancy may be caused by an impermeability of the seed coat which strongly resists water and gaseous exchange. Hard seed coat acts as barriers to germination by preventing embryo expansion or radical growth (Materechera and Materechera, 2001). Immature embryos, light and temperature requirements, or the presence of germination inhibitors are other factors causing dormancy (Stidham *et al*., 1980). Physiological dormancy in seeds may be related to the proportion between inhibitors (especially abscisic acid) and growth regulators (such as gibberellins) (Fenner and Thompson, 2005).

Several methods have been suggested to break seed dormancy. These include stratification, scarification and leaching and soaking of seeds amongst others. Stratification also known as cold treatment or pre-chilling according to Çetinbaş and Koyuncu (2006) stimulate structural GA synthesis. Stratification is believed to limit the effects of inhibitors in turn promoting growth stimulators (Stidham *et al*., 1980). Scarification is a treatment procedure that damages the seed coat of seeds to improve permeability to water and gaseous exchange (Stidham *et al*., 1980). These treatments may include rubbing seed against an abrasive surface, nicking of seeds and warm water treatment. Chemicals such as gibberellins and potassium nitrate have been known to stimulate germination (Stidham *et al.*, 1980; Hilton, 1984). Gibberellins may be used to eliminate the chilling requirement of peach and apple seeds and increase their germination (Çetinbaş and Koyuncu, 2006).

In agricultural crops, where rapid seed germination and growth are required, dormancy normally is an undesirable characteristic, since it leads to poor and delayed germination (Bewley and Black, 1997). Furthermore, dormancy may cause irregular emergence which may cause poor establishment of crops. One of the main problems that may prevent sustainable use of indigenous plants native to arid lands is that they

readily germinate within the native environment, but fail to show good germination under laboratory conditions or when cultivation is attempted (Nadjafi *et al*., 2006).

Therefore, the objective of this study was to determine whether the selected ALVs had any form seed of dormancy and how pre-sowing treatments can break seed dormancy. The ALVs investigated were: *A. cruentus, S. retroflexum, B. rapa* subsp. *chinensis, C. gynandra, C. lanatus, C. olitorius, C. maxima, V. unguiculata* and *B. vulgaris* (control).

6.3.2 Materials and methods

Seed Germination

A similar methodology for seed treatment and diagnosis of germination was followed as explained in 4.2.2: Materials and Methods. Pre-sowing test for seed dormancy included three treatments and a control:

i) Scarification: seeds were scarified by rubbing them between two sheets of cabinet abrasive paper (P100 GRIT)
    - Small seeds were gently rubbed 5 times between two abrasive papers without damaging the seed extensively
    - Large seed were rubbed at the seed tip (attachment of the funiculus) with abrasive paper (Travlos *et al*., 2007).
ii) Potassium nitrate (KNO_3): 0.2% KNO_3 was used instead of distilled water to moisten the germination substratum on which seeds were sown (Ochuodho and Modi, 2005; ISTA, 2008:14).
iii) Pre-chilling: imbibed seeds were incubated for 7 days at 5ºC under continuous darkness in a controlled Labcon™ low-temperature incubator (Ochuodho and Modi, 2005).
iv) Control: seeds were left untreated.

After the pre-sowing treatments were completed, seed were germinated as described in 4.3.1.

Statistical analysis

Analysis of variance (ANOVA) was used to test for differences between the treatments. Treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor and Cochran, 1980). Data was analysed using the statistical program GenStat® (Payne *et al.,* 2007).

6.3.3 Results

***Amaranthus cruentus* L.**

Effects of the dormancy pre-sowing treatments on the germination of *A. cruentus* are shown in Figure 6.18. Commencement of germination of the seed of *A. cruentus* occurred after 24 h of incubation in all the dormancy treatments, including the control. Scarifying and pre-chilling *Amaranthus* seed significantly ($p \leq 0.05$) improved the onset of germination after 24 h from 18.5% in the control to 92.5% for scarifying and 55.0% for pre-chilling. Furthermore, these two treatments also significantly ($p \leq 0.05$) increased final germination percentage, which was reached after 48 h and 96 h, respectively.

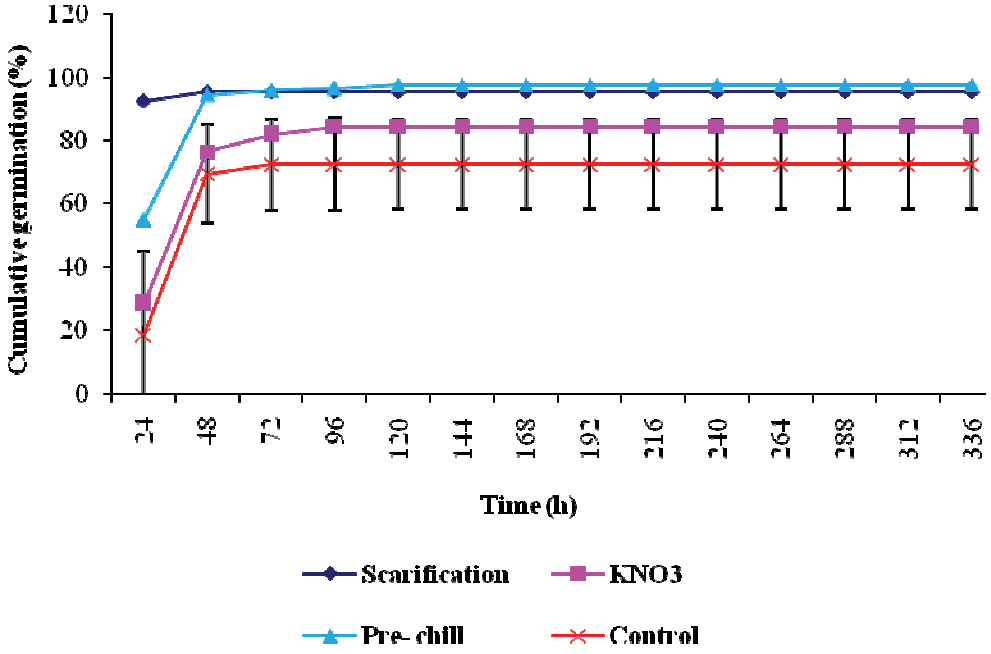

Figure 6.18: Cumulative germination percentage of *Amaranthus cruentus* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Soaking seeds in potassium nitrate (KNO_3) did not significantly ($p>0.05$) improve germination of *Amaranthus* relative to the control. However, the germination percentage of seeds imbibed with KNO_3 tended to be higher than in the control treatment over the full incubation period. Final germination of 97.5%, 95.5%, 84.5% and 72.5% attained for seed that were pre-chilled, scarified, soaked in KNO_3 and the control, respectively, was reached after 120 h, 48 h, 84.5 h and 72 h, respectively.

Beta vulgaris* L. var. *cicla

Effects of the dormancy pre-sowing treatments on the germination of *B. vulgaris* var. *cicla* seed are shown in Figure 6.19. The onset of germination started after 48 h in all treatments. After 72 h, germination percentage of scarified *B. vulgaris* var. *cicla* seed was significantly lower ($p\leq0.05$) (67.5%) than in the control (77.5%). After 96 h, there were no significant differences ($p>0.05$) among treatments. Seeds imbibed with KNO_3 tended to have a higher final germination percentage (87.0%), than seeds in the other treatments but final germination percentage in the KNO_3 treatment occurred later (after 264 h) than in the other treatments. Final germination percentages were 77.0% for pre-chilled seed, 73.5% for scarified seed and 79.0% in the control. Final germination was achieved after 216 h (scarified), 240 h (pre-chilled) and 264 h (KNO_3), compared to 120 h in the control.

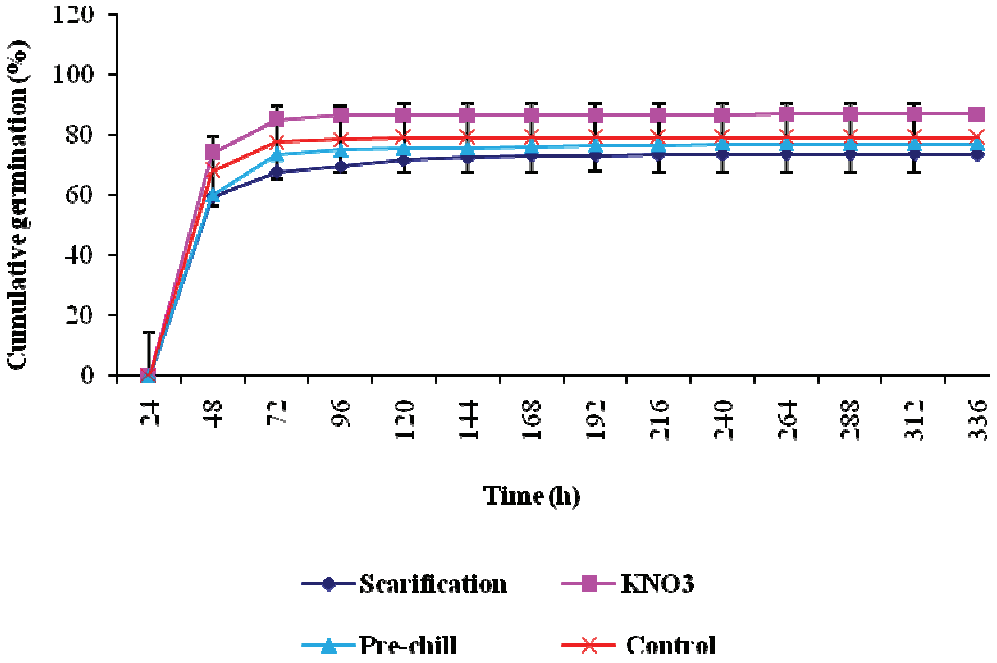

Figure 6.19: Cumulative germination percentage of *Beta vulgaris* var. *cicla* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Brassica rapa* L. subsp. *chinensis

The effects of the dormancy pre-sowing treatments on the germination of *Brassica rapa* subsp. *chinensis* are shown Figure 6.20.

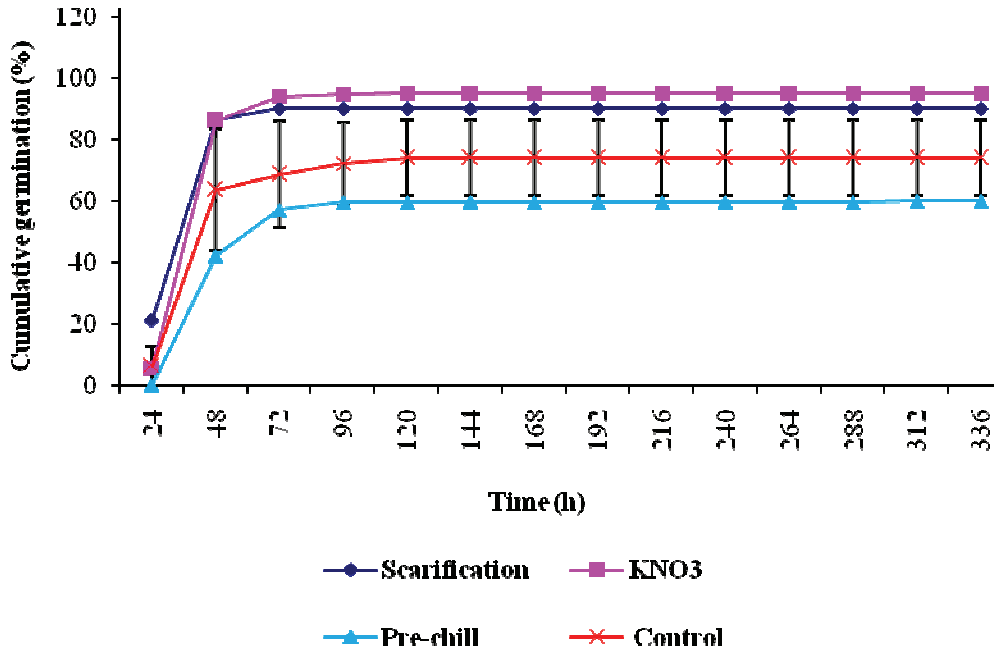

Figure 6.20: Cumulative germination percentage of *Brassica rapa* subsp. *chinensis* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Germination of *B. rapa* subsp. *chinensis* seed started after 24 h in the scarification, KNO_3 and control treatments. Commencement of germination in the pre-chill treatment was observed after 48 h. Germination percentage after 24 h was significantly higher ($p \leq 0.05$) when seed was scarified (21%) compared to the control (6%). After 48 h, the scarification and KNO_3 treatments significantly increased ($p \leq 0.05$) germination percentage of *B. rapa* subsp. *chinensis* from 63.5% in the control to 86.5% and 86.0%, respectively. Subjecting *B. rapa* subsp. *chinensis* seed to pre-chilling conditions significantly reduced ($p \leq 0.05$) germination percentage throughout the entire incubation period. The highest germination percentages of 90% and 95% were reached after 72 h and 120 h when seed was scarified and imbibed with KNO_3, respectively. Final germination percentages in these two treatments were significantly higher ($p \leq 0.05$) than those in the control (74%) and pre-chill (60%) treatments, which attained final germination percentage after 120 h and 312 h, respectively.

***Citrillus lanatus* L.**

Effects of the dormancy pre-sowing treatments on the germination of *Citrillus lanatus* are shown in Figure 6.21.

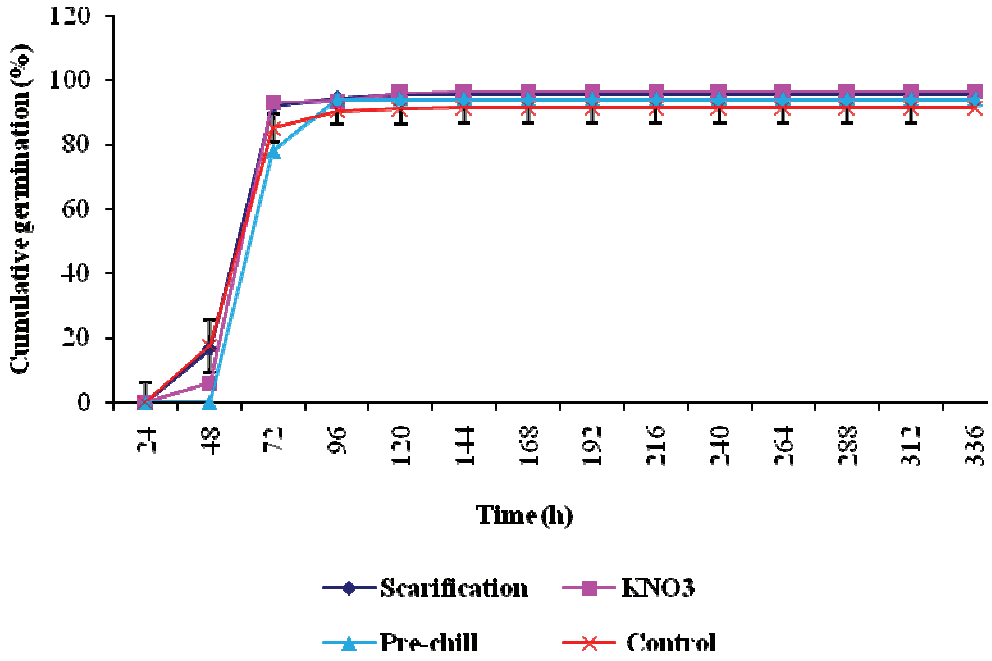

Figure 6.21: Cumulative germination percentage of *Citrillus lanatus* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Germination commenced after 48 h in the scarification, KNO_3 and control treatments and after 72 h in the pre-chill treatment. Onset of germination of seed of *C. lanatus* was delayed when seed of this specie was pre-chilled (0%) or imbibed with KNO_3 (6%), compared to when seed was scarified (16.5%). After 72 h, the germination percentage (78.0%) of pre-chilled seed, although statistically not different from that in the control (85.0%), was significantly lower ($p \leq 0.05$) than scarified seed (92%) or KNO_3 treated seed (93%). However, after 96 h, differences in germination percentage among treatments were no longer statistically significant and this persisted until the end. Final germination percentage in all dormancy pre-treatments tended to be higher than in the control, but statistically differences were not significant. . Final germination percentage of 96.5% (KNO_3), 95.5% (scarified), 94.5% (pre-chilled) and 91.5% (control) was reached after 144 h, 120 h, 96 h and 144 h, respectively.

***Cleome gynandra* L.**

Effects of the dormancy pre-sowing treatments on the germination of *Cleome gynandra* seed are shown in Figure 6.22.

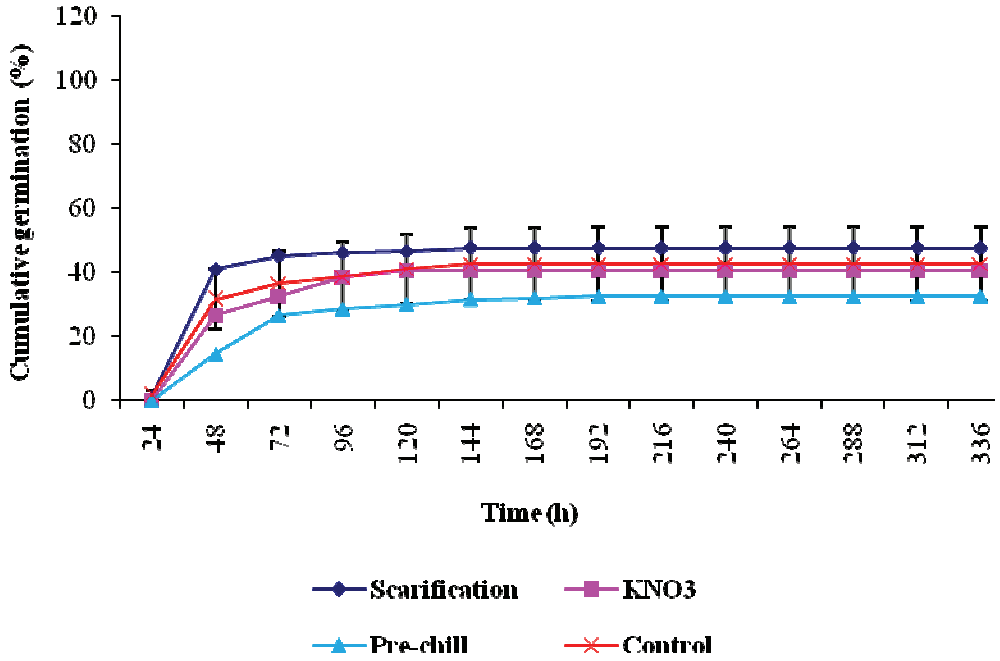

Figure 6.22: Cumulative germination percentage of *Cleome gynandra* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Germination of *C. gynandra* seed commenced after 24 h in the scarification and control treatments and after 48 h in the pre-chill and KNO_3 treatments. After 48 h, scarification improved the onset of germination of seed of *C. gynandra* significantly ($p \leq 0.05$) compared to the other treatments and pre-chilling seed significantly reduced ($p \leq 0.05$) onset of germination from 31.5% (control) to 14.5%. After 72 h, germination percentage of scarified seed (45%) did not differ significantly from that in the control (36.5%). After 96 h, germination of pre-chilled seeds remained significantly lower ($p \leq 0.05$) than seeds that were scarified, but did not differ statistically ($p > 0.05$) from the other treatments.

After 120 h, differences among treatments were no longer statistically different and this persisted until final germination percentage of 47.5%, 42.5%, 40.5% and 32.5% was attained for scarification, control, KNO_3 (after 144 h) and pre-chill (after 168 h), respectively.

***Corchorus olitorius* L.**

In Figure 6.23, the effects of dormancy pre-sowing treatments on the germination of *Corchorus olitorius* seed are shown.

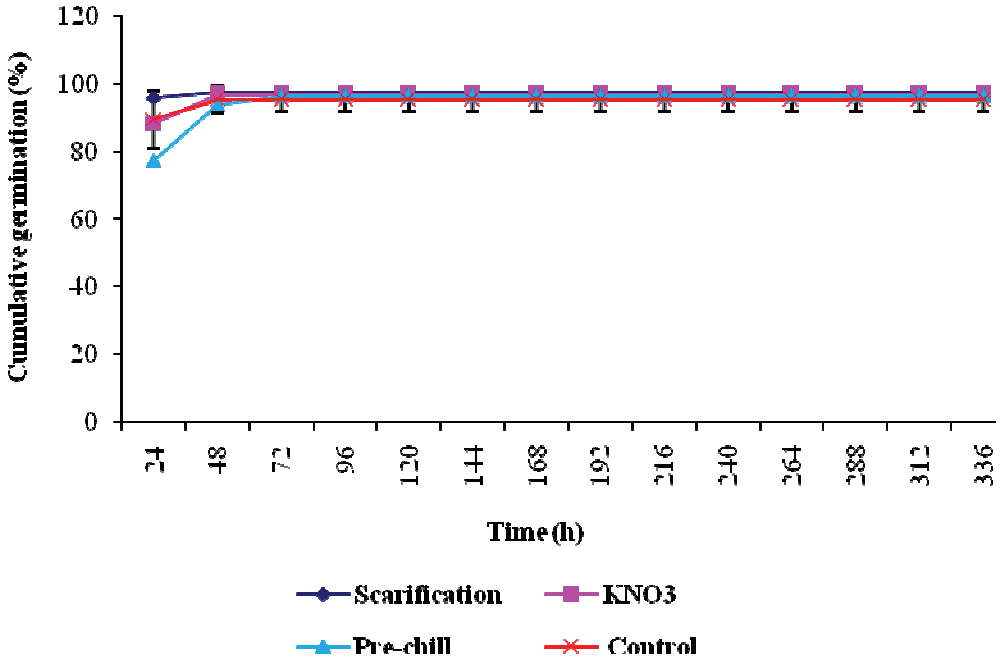

Figure 6.23: Cumulative germination percentage of *Corchorus olitorius* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Commencement of germination of the seed of *C. olitorius* occurred after 24 h of incubation. When seed of *C. olitorius* was pre-chilled, the germination percentage after 24 h (77.5%) was significantly lower ($p \leq 0.05$) than the 89.5% in the control. From 48 h onwards, differences among all treatments were not significant. Final germination was attained after 48 h in the scarification, KNO_3 and control treatments, and after 72 h in the pre-chill treatment. Final germination percentage was 97.5% (scarified), 97.0% (KNO_3), 96.5% (pre-chilled) and 95.5% (control).

***Cucurbita maxima* Duchesne**

Effects of dormancy pre-sowing treatments on the germination of *Cucurbita maxima* seed are shown in Figure 6.24.

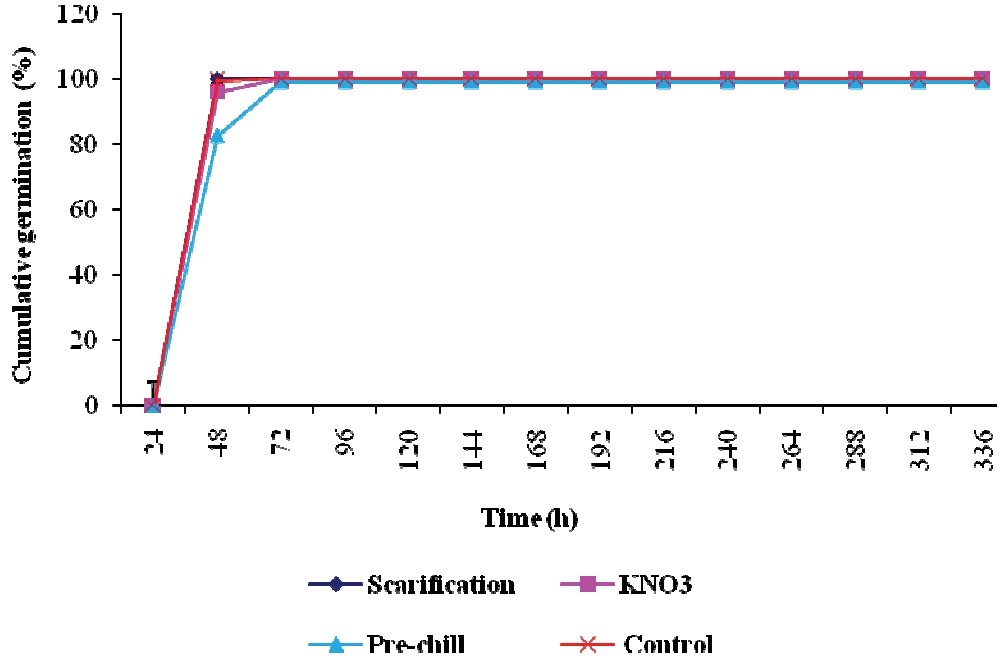

Figure 6.24: Cumulative germination percentage of *Cucurbita maxima* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Commencement of germination in the seed of *C. maxima* occurred after 48 h of incubation in all treatments. After 48 h, germination of *C. maxima* seed was significantly lower ($p \leq 0.05$) in the pre-chilling treatment (82.5%) than the 99.5% in the control. From 72 h onwards, treatment effects were no longer significant. Final germination was 100% for scarified seed and was reached after 48 h. Final germination was reached after 72 h for KNO_3, pre-chill and control. Final germination percentage was 100% in the KNO_3 and control treatments, and 99.0% for pre-chilled seed.

***Solanum retroflexum* Dun.**

Effects of the dormancy pre-sowing treatments on germination of *S. retroflexum* are shown in Figure 6.25. Germination of *S. retroflexum* seed commenced after 48 h of incubation in the scarification treatment and after 72 h of incubation in all other dormancy pre-sowing treatments. Germination percentage after 48 h was significantly improved ($p \leq 0.05$) from zero to 1.5%, when seed of *S. retroflexum* was scarified. After 72 h, germination percentage of seed imbibed with KNO_3 (20.5%) was significantly higher ($p \leq 0.05$) than in the control (7%) and thereafter, KNO_3 treatment significantly improved ($p \leq 0.05$) germination percentage until final germination percentage was attained after 336 h of incubation. From 120-192 h there was no significant difference between scarification and the control. However, after 216 h, germination percentage in the scarification treatment was significantly ($p \leq 0.05$) lower than in the control.

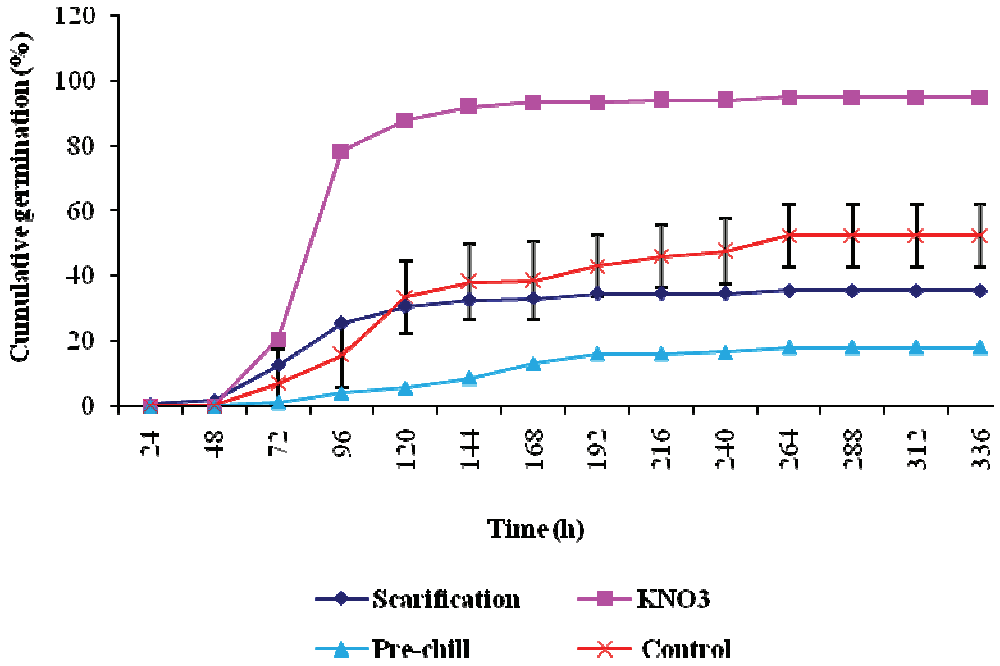

Figure 6.25: Cumulative germination percentage of *Solanum retroflexum* subjected to different dormancy pre-treatments for 14 days (336 h) at 25 ºC

Relative to the control, pre-chilling seed of *S. retroflexum* significantly reduced ($p \leq 0.05$) germination percentage throughout the incubation period. Germination percentage after 216 h of scarified (34.5%) and pre-chilled (16.0%) seed was significantly reduced ($p \leq 0.05$) than the 46% in the control. Final germination percentage was reached after 264 h in all treatments and was 95.0% (KNO_3), 52.5% (control), 35.5% (scarification) and 18.0% (pre-chill).

***Vigna unguiculata* (L.) Walp.**

Effects of the dormancy pre-sowing treatments on the germination of *V. unguiculata* seed are shown in Figure 6.26. Germination of *V. unguiculata* seed commenced after 24 h in all treatments. After 24 h, germination of pre-chilled seed (24%) was significantly lower ($p \leq 0.05$) than that of seed in the control treatment (64%). However, after 48 h, there were no more significant differences among treatments and final germination percentages of 97.5% (KNO_3), 95.5% (scarification), 95.0% (pre-chill) and 94.5% (control) were reached after 120 h, 48 h, 120 h and 72 h of incubation, respectively.

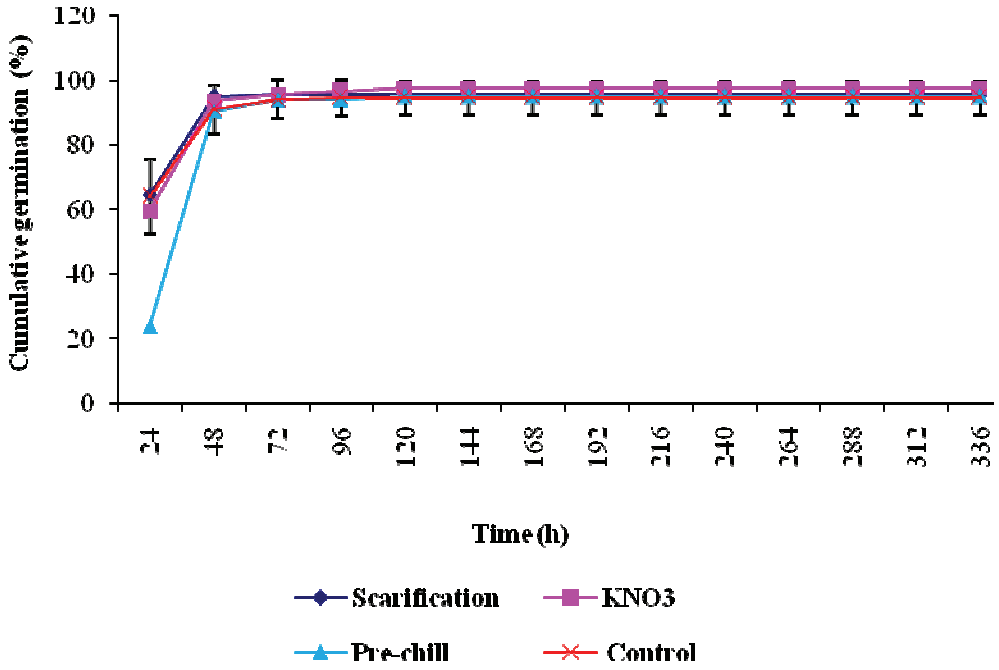

Figure 6.26: Cumulative germination percentage of *Vigna unguiculata* subjected to different dormancy pre-treatments for 14 days (336 h) at 25ºC

6.3.4 Discussion

Results from this study showed that scarification positively improved onset and final germination of *A. cruentus* and *B. rapa* subsp. *chinensis* seed, but negatively affected that of *S. retroflexum*. Pre-chilling had a positive effect on onset and final germination of *A. cruentus* but negatively affected germination of *B. rapa* subsp. *chinensis*, *C. gynandra* and *S. retroflexum.* KNO_3 positively affected both onset and final germination of *B. rapa* subsp. *chinensis* and *S. retroflexum*. Dormancy treatments did not have a significant effect on the final germination percentage of *B. vulgaris* var. *cicla*, *C. lanatus*, *C. olitorius*, *C. maxima* and *V. unguiculata*.

The significant improvement in germination when seed of *A. cruentus* and *B. rapa* subsp. *chinensis* was scarified suggested that the cause of dormancy in these plant species might be attributed to hard seed coats acting as physical barriers to water absorption. According to Materechera and Materechera (2001), during the early stage of germination, the rate of oxygen uptake by a seed is proportional to the moisture content of the cotyledons, which is responsible for germination rate. Scarifying the seed probably ruptured the seed coat thus permitting the water imbibition rate to increase. Therefore, success with using the abrasion technique is probably dependent on the thoroughness of abrasion. In this study, seeds of *A. cruentus* and *B. rapa* subsp. *chinensis* were abraded on the surface of sandpaper, which appears to have been sufficient to produce adequate scarification, explaining the improved germination. It could be concluded that scarification increased the water imbibitions rate of the seeds and hence improved their final germination percentage. Although this study has attempted to document this process in detail, it was still impossible to determine the level of abrasion required to achieve optimum results. Therefore, future studies need to establish the degree of abrading required to break seed dormancy most effectively.

When seed of *C. gynandra* was abraded with sandpaper, the rate of germination was accelerated. However, final germination percentage attained was not significantly different from seed that was pre-chilled, imbibed with KNO_3 or left untreated. These findings partly support the conclusions of Ochoudho and Modi (2005) and Ochuodho *et al*. as cited by Ekpong (2009), that scarification improves germination of *Cleome* seed. However, since final germination percentage was not significantly improved, the cause of dormancy might

not be due to the seed coat acting as a physical barrier to water absorption for effecting final germination. Furthermore, since pre-chilling did not improve final germination percentage of *Cleome* seed, embryo dormancy can also be ruled out (Ekpong, 2009).

Scarification could have improved the onset of germination of *S. retroflexum* by increasing the rate of imbibition leading to rupturing of the seed coat, thus permitting germination to commence (Bewley, 1997; Materechera and Materechera, 2001; Mwamburi *et al*., 2005). However, final germination percentage of scarified seed was significantly lower than in the other seed dormancy treatments and the control, suggesting that abrasion could have caused damage to the seed (possibly the embryo) resulting in less seeds germinating. Slow germination rate of seeds with physical dormancy could be of benefit during dry periods, acting as a delaying mechanism responsible for inhibiting simultaneous germination, thus enabling survival of the species under harsh conditions. On the other hand, seed without any physical dormancy could be responsible for maintenance of high population levels during favourable periods (Bewley, 1997).

Pre-chilling was able to break dormancy and increase germination of *A. cruentus* only. Pre-chilling seed of this specie could have assisted in releasing embryo dormancy inhibitors, thus triggering germination. This could include increasing the level and responsiveness of endogenous gibberellins, and possibly decreasing the abscisic level (Stidham *et al*., 1980; Çetinbaş and Koyuncu, 2006; Nadjafi *et al*., 2006; Ekpong, 2009). Contrary to the findings of this study, Aufhammer *et al*. (1998) reported that dormancy of *A. cruentus* could not be broken by pre-chilling. Pre-chilling is believed to simulate cold winter conditions for seeds with internal dormancy. In untreated seed, oxygen does not diffuse through the seed coat, which leads to germination failure of the embryo. However, at cold temperatures, more oxygen diffuses in soluble water thus better satisfying the oxygen requirements of the embryo (Seed dormancy and treatments, S.a).

Although pre-chilling seed is known to break dormancy of viable seed and enhance germination, it is also known to cause lethal effects on viable seed (Ekpong, 2009; Nkomo and Kambizi, 2009). Seed of *B. rapa* subsp. *chinensis*, *C. gynandra* and *S. retroflexum* showed significantly lower germination when pre-chilled. Ochoudho and Modi (2005:53) also reported low germination when seeds of *C. gynandra* were pre-chilled. Ekpong (2009) stated that the decrease in germination of pre-chilled seeds might be due to water trapped in tissues between the embryo and seed coat, creating an oxygen barrier. Seed of *C. lanatus*, *C. olitorius*, *C. maxima* and *V. unguiculata* appeared not to possess any dormancy mechanism that inhibited germination. When compared with the other treatments (scarification and KNO_3), pre-chilling *C. lanatus*, *C. olitorius*, *C. maxima* and *V. unguiculata* seed delayed onset of germination, but this effect was temporary.

The results obtained for seed of *C. olitorius* contradicted the work of Nkomo and Kambizi (2009), who stated that final germination of *C. olitorius* seed was highest after 84 h of pre-chilling. They also stated that experiments involving pre-chilling of seeds, for plant species that mostly grow in summer, record the highest germination percentages when seeds are pre-chilled for approximately seven days. In this study, there were no significant differences in germination percentage between pre-chilled seed and untreated seed after 72 h (3 days).

Both the onset and final germination percentage of *B. rapa* subsp. *chinensis* and *S. retroflexum* were significantly improved when seed of these species was imbibed with KNO_3. According to Fenner and Thompson (2005), nitrate (NO^-_3) is known to stimulate germination, especially in weedy species. The use of KNO_3 has been an important seed treatment in laboratories for many years without a clear explanation of the specific action in the seed (Çetinbaş and Koyuncu, 2006). It is believed that physiological dormancy in seeds is closely related to the proportion between inhibitors and growth regulators (Çetinbaş and Koyuncu, 2006). Imbibing seed with KNO_3 and a combination of gibberellic acid could stimulate germination by increasing the level and responsiveness of endogenous gibberellins, while substantially decreasing abscisic levels (Stidham *et al*., 1980; Ekpong, 2009). This could explain why germination of *B. rapa* subsp. *chinensis* and

S. retroflexum seed improved by imbibing them in KNO_3. The response to KNO_3 by these two species could be interpreted as a dormancy-breaking mechanism, thus promoting germinability of the seeds. Fenner and Thompson (2005) however, stated that the germination response to nitrate is highly dependent on other environmental factors, such as light. This is in agreement with results published by Hilton (1984), Ochuodho and Modi (2007) and Mollard and Insausti (2009), who reported that the effect of KNO_3 on seed germination is dependent on both light and its quality. During this study, when the effect of light on both *B. rapa* subsp. *chinensis* and *S. retroflexum* was investigated, it was found that a significantly higher germination percentage was obtained in both species under alternating light than in darkness. It appears therefore, that seed germination of *B. rapa* subsp. *chinensis* and *S. retroflexum* could be optimised by imbibing seed with KNO_3 and germinating the seed under alternating light conditions.

Ekpong (2009) reported that when seed of *C. gynandra* was germinated with KNO_3 and gibberellins at various concentrations, the germination percentage was significantly increased relative to the control. This is contrary to findings of this study, where the KNO_3 treatment had no significant effect. Ochoudho and Modi (2005) also reported that imbibing with KNO_3 did not significantly improve germination percentage of *C. gynandra* seed. Final germination percentages attained were below 50% in all treatments and were similar to those attained by Ekpong (2009).

6.3.5 Conclusion

The findings of this study indicated that *A. cruentus, B. rapa* subsp. *chinensis, C. gynandra* and *S. retroflexum* exhibited some form of dormancy within their seed. Seed of these vegetables demonstrated;

- Physical dormancy whereby the seed coat acted as a physical barrier for water uptake and gaseous exchange.
- Embryo dormancy in which the seed when shed probably was immature thus a period of growth or differentiation was required.
- Physiological dormancy whereby germination was stimulated once a chemical change took place in the seed.

The results also suggested that *A. cruentus* and *B. rapa* subsp. *chinensis* had some form of physical dormancy that could inhibit successful germination. Scarification positively improved the onset and final germination of these two species. Although germination onset of *C. gynandra* and *S. retroflexum* seed was improved by scarification, the treatment significantly reduced final germination of *S. retroflexum*, but not of *C. gynandra*.

The findings suggest possible embryo dormancy in seed of *A. cruentus*, because the pre-chilling treatment positively affected the germination of this specie. No embryo dormancy is expected in *B. rapa* subsp. *chinensis, C. gynandra* and *S. retroflexum* seed, because pre-chilling significantly reduced germination percentage, while for *C. lanatus, C. olitorius, C. maxima* and *V. unguiculata*, only germination onset was significantly reduced, not final germination percentage. The pre-chill requirement suggests that *A. cruentus* seed probably needs a form of overwintering before being used for planting. The pre-chilling treatment conditions may actually be simulating the events that occur during winter before the onset of summer.

The germination percentage of *B. rapa* subsp. *chinensis* and *S. retroflexum* seed was significantly improved when imbibed with KNO_3, whilst the onset of germination of *C. lanatus* seed was significantly reduced when seed was imbibed with KNO_3. Soaking seed with potassium nitrate (KNO_3) prior to planting to eliminate dormancy that could be associated with chemical inhibitors within the seed, thus improving germination by enhancing growth regulators within the seed, could be useful for *S. retroflexum* Dun. and *B. rapa* subsp. *chinensis*. Abrasion of seed with sandpaper before planting could be used to improve germination and

consequently, stand establishment of *A. cruentus* L., *B. rapa* subsp. *chinensis* and *C. gynandra*, but should be avoided for *S. retroflexum* Dun. seed.

6.4 EFFECT OF LIGHT ON GERMINATION

6.4.1 Introduction

Germination, being the resumption of the embryo growth to extend beyond the coverings surrounding the embryo will not proceed unless favourable environmental conditions such as light, temperature, water and oxygen prevail (Manoto *et al*., 2004). Light requirements in seed germination differ from species to species (Mpati, 2006). Fenner and Thompson (2005) stated that requirements of seeds to light prevent the occurrence of germination in places and times not favourable to seedling establishment. Therefore, this enables the seed to have some control over where and when germination takes place, and prevents stored seed reserves from weakening before penetrating the soil surface. Exposing seeds to conditions of light helps break dormancy of some seeds thus inducing germination (Bewley and Black, 1994; Khan and Gulzar, 2003; Ochuodho and Modi, 2005).

The germination of light-requiring seeds is triggered by disturbance when soil is turned over (cultivation), gaps in plant canopy and decline in water depths (Kettenring *et al*., 2006). These mostly occur in forest settings where seeds will often not germinate until an opening in the canopy allows them to receive sufficient light for the growing seedling. In addition, many weeds are light sensitive germinators, this is the reason why cultivating lands bring up seeds from deeper layers, which germinate. According to Fenner and Thompson (2005), the presence of light or its absence may trigger the germination process, inhibits germination in some seeds that are buried too deeply or in others not buried at all. Seeds that have thin seed coats, light penetrate into the dormant embryo.

In many species, germination is stimulated once hydrated seeds are exposed to light, which is perceived through photoreceptors, especially originating from the phytochrome family (Bradford and Nonogaki, 2007). The responses of seeds to light prevent the occurrence of germination in places and times not favourable to seedling establishment. This enables the seed to have some control over where and when germination takes place (Fenner and Thompson, 2005).

The objective of this study was to determine the influence of light on selected African leafy vegetables namely: *A. cruentus* (amaranth), *B. rapa* subsp. *chinensis* (Chinese cabbage), *C. gynandra* (spider flower), *C. lanatus* (bitter melon), *C. maxima* (pumpkin), *S. retroflexum* (nightshade), *C. olitorius* (Jew's mallow), *V. unguiculata* (cowpea) and *B. vulgaris* var. *cicla* (Swiss chard) was used as a control.

6.4.2 Materials and methods

Seed germination

A similar methodology for seed treatment and diagnosis of germination was followed as explained in 4.1.2: Materials and Methods. Two experiments were undertaken to investigate the effect of light on germination of selected African leafy vegetables: (1) alternating light/dark and continuous darkness, with evaluation every 24 h, and (2) alternating light/dark and continuous darkness, with evaluation only after 240 h. The rationale for incubating the seeds for 240 h was that during the first experiment maximum germination was reached after 240 h. The experiments were designed in such a way that seeds exposed to alternating light conditions received light for 8 h and dark for 16 h daily. In experiment one, the dark treatment only received normal light during evaluations every 24 h for approximately 10 to 15 minutes. In experiment two no daily evaluations were done; seeds were kept in the respective incubators for 10 days (240 h) without evaluation and germination count was done only after 240 h.

Statistical analysis

Analysis of variance (ANOVA) was used to test for differences between the two treatments (light and dark), nine species and the interaction between treatments and species. The data was acceptably normal with heterogeneous treatment variances. Therefore treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the 1% level of significance (Snedecor and Cochran, 1980). Data was analysed using the statistical program GenStat® (Payne *et al*., 2007).

6.4.3 Results

Experiment one: Evaluation every 24 h

***Amaranthus cruentus* L.**

The effect of light on the germination of *A. cruentus* seed is shown in Figure 6.27. The onset of germination for *A. cruentus* was accelerated by exposing the seed to alternating light. Differences in germination percentage between the dark and the alternating light treatment were statistically significant ($p \leq 0.01$) after 24 h, 48 h and 72 h. At 24 h, 48 h and 72 h, mean germination percentage was 7.5%, 10.5% and 41% in darkness compared to 24%, 79.5% and 80.5% in alternating light, respectively, but subsequently germination in darkness increased rapidly. After 96 h the germination percentage in darkness had reached 86% compared to 80.5% in alternating light. Statistically, the difference between the final germination percentage of 99.5% in darkness and 81.5% in alternating light was not significant ($p > 0.01$).

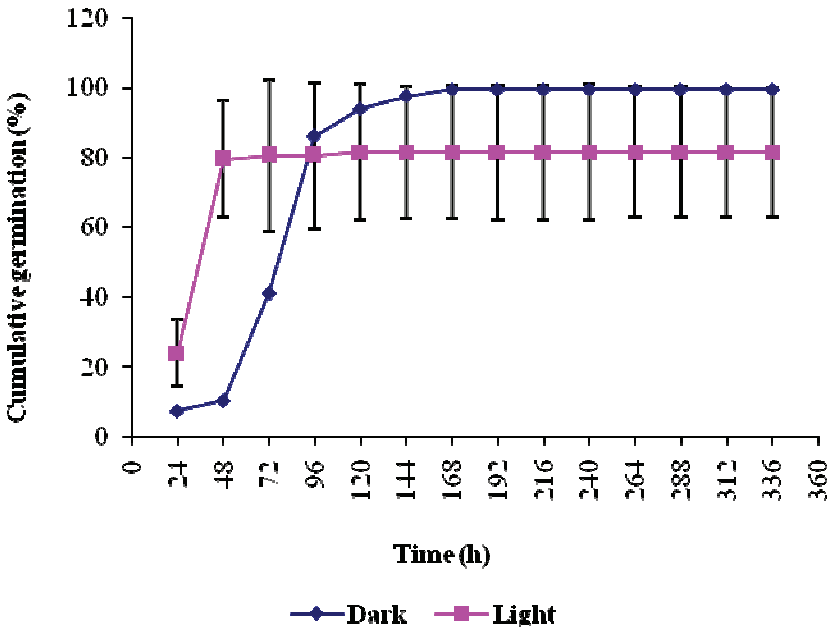

Figure 6.27: Percentage germination of *Amaranthus cruentus* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25ºC

Beta vulgaris* L. var. *cicla

The effect of light on the germination of *Beta vulgaris* var. *cicla* seed is shown in Figure 6.28.

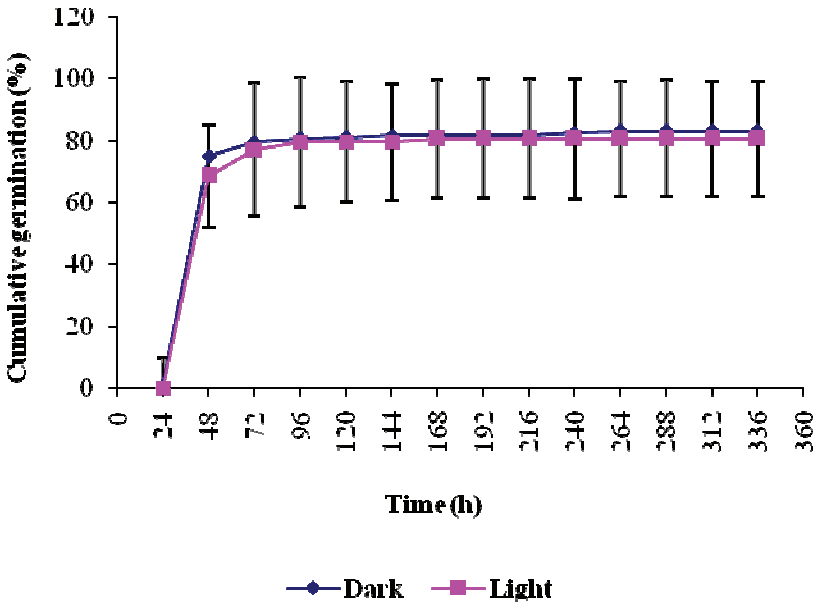

Figure 6.28: Percentage germination of *Beta vulgaris* var. *cicla* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

Commencement of germination of the seed of *B. vulgaris* var. *cicla* occurred after 24 h of incubation when exposed to darkness and after 48 h when exposed to alternating light. After 48 h, the effect of light on germination of *B. vulgaris* var. *cicla* was very minor, with 68.5% of seed germinated in alternating light compared to 75% in darkness. The difference in the final germination percentage of 83% in darkness and 80.5% in alternating light was statistically not significant.

Brassica rapa* L. subsp. *chinensis

The effect of light on the germination of *Brassica rapa* subsp. *chinensis* seed is shown in Figure 6.29.

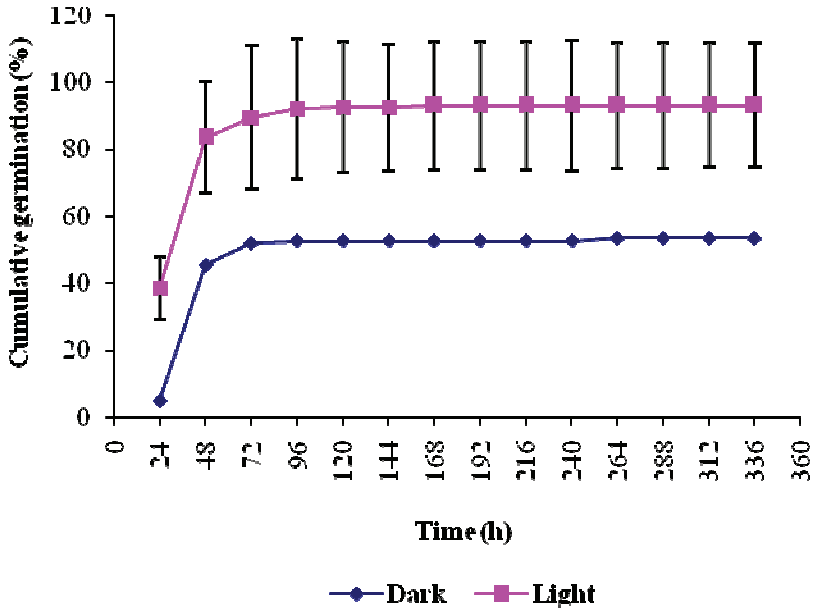

Figure 6.29: Percentage germination of *Brassica rapa* subsp. *chinensis* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

Germination percentage of *B. rapa* subsp. *chinensis* seeds after 24 h was significantly ($p \leq 0.01$) improved by exposure to alternating light. Only 5% germination occurred in darkness after 24 h, compared to 38.5% under alternating light conditions. Treatment differences continued to be significant until final germination was attained. The final germination percentage obtained under alternating light conditions was 93% compared to 53.5% under darkness.

***Citrillus lanatus* L.**

The effect of light on the germination of *Citrillus lanatus* seed is shown in Figure 6.30. From 48 h to 72 h, germination was significantly ($p \leq 0.01$) improved when seed was germinated in continuous darkness as compared to alternating light. After 48 h, 86% of the seed in complete darkness had germinated, whereas only 10% of seed exposed to alternating light had germinated. After 72 h, treatment means converged. The same final mean germination percentage of 97% was reached in both treatments after 168 h.

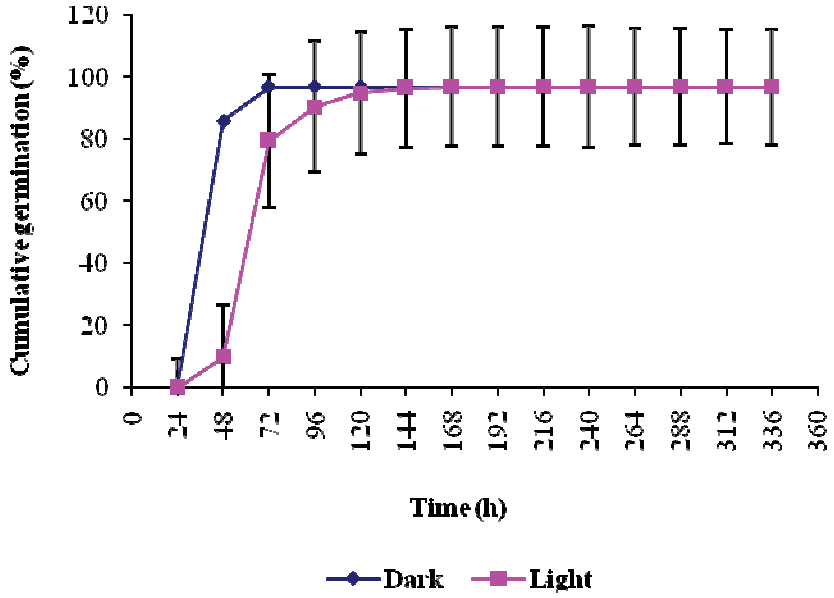

Figure 6.30: Percentage germination of *Citrillus lanatus* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

***Cleome gynandra* L.**

Germination of *Cleome gynandra* seed as affected by light is shown in Figure 6.31. Commencement of germination of the seed of *C. gynandra* occurred after 24 h of incubation when seed was exposed to darkness and after 48 h in the alternating light treatment. The rate at which germination occurred in both treatments was not significantly different throughout the entire 336 h but tended to be higher in darkness than in alternating light conditions with 37% seeds germinated after 48 h in the dark, and only with 28.5% seeds germinated in alternating light. After 72 h, germination remained slightly higher in darkness (41.5%) than in alternating light (37%) but the effect of light on germination disappeared completely after 336 h when the same final germination percentage of 43.5% was reached in both treatments.

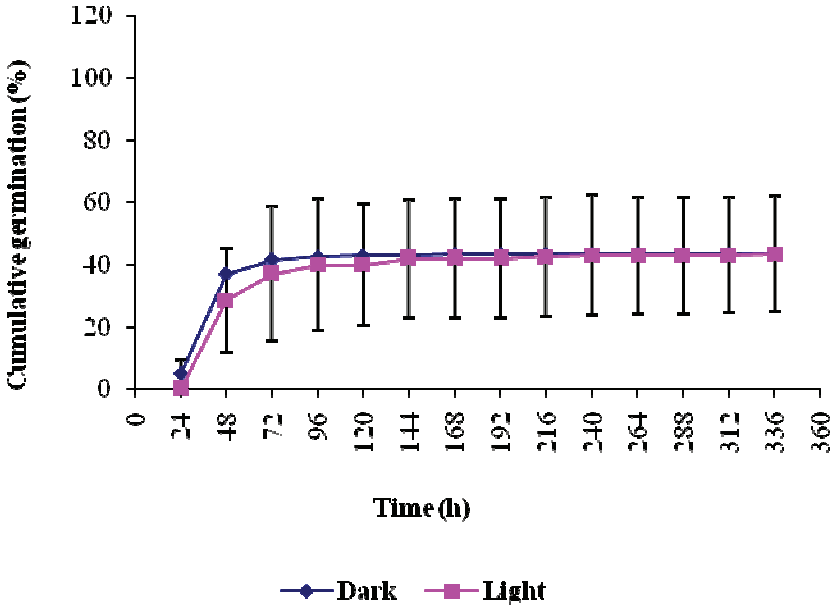

Figure 6.31: Percentage germination of *Cleome gynandra* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

***Corchorus olitorius* L.**

The effect of light on the germination of *Corchorus olitorius* seed is shown in Figure 6.32.

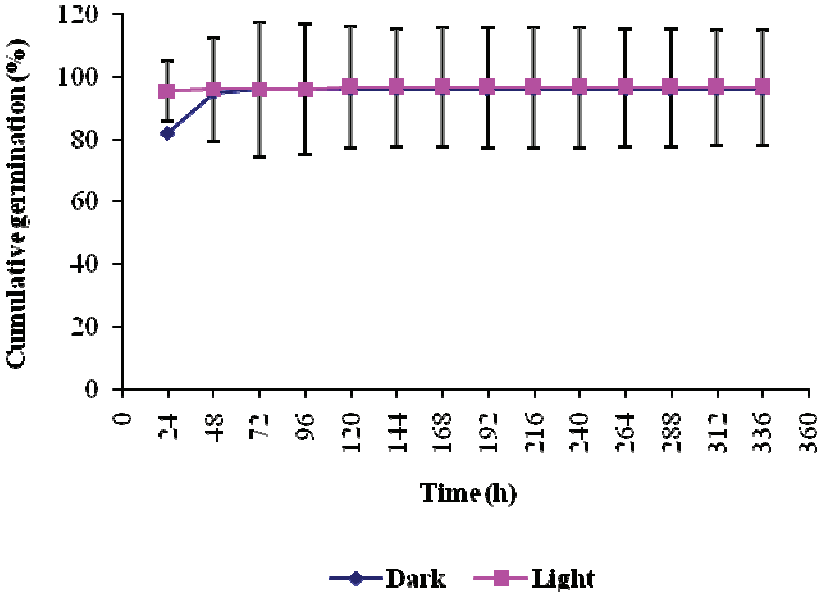

Figure 6.32: Percentage germination of *Corchorus olitorius* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

Alternating light positively affected the onset of germination of *Corchorus olitorius* seed. After 24 h, the rate at which germination occurred was significantly higher ($p \leq 0.01$) under alternating light conditions than under complete darkness. The mean germination percentage was 95.5% in alternating light and only 82% in complete darkness. However, significance of the treatment differences completely disappeared from 48 h

onwards when the final germination percentages of 96.5% and 96% were reached under alternating light and darkness, respectively.

***Cucurbita maxima* Duchesne**

The effect of light on the germination of *Cucurbita maxima* seed is shown in Figure 6.33. .

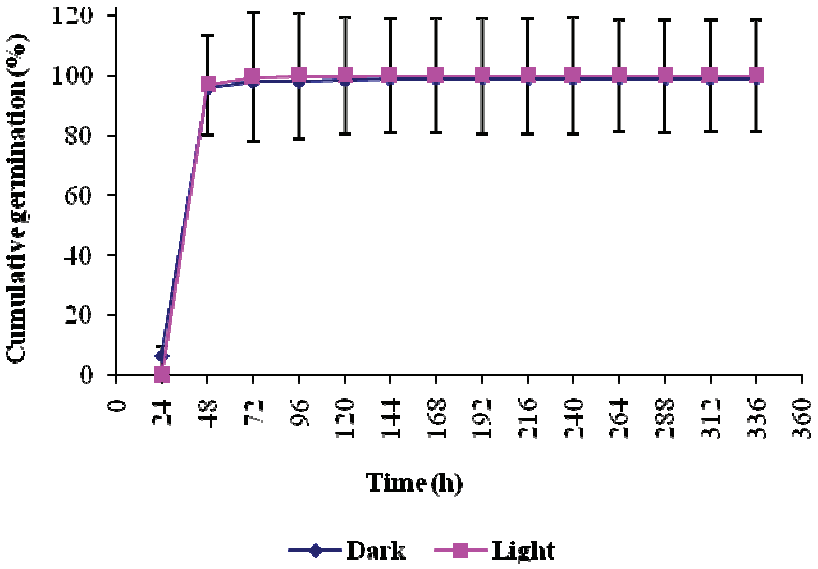

Figure 6.33: Percentage germination of *Cucurbita maxima* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

Germination of *Cucurbita maxima* commenced after 24 h of incubation in the darkness treatment and after 48 h in the alternating light treatment. After 24 h, 10% of the seed had germinated in darkness and none (0%) in alternating light. But, statistically this initial effect of light on germination was not significant ($p>0.01$). Treatment effects weakened even more, and the final germination percentage attained in both treatments was not significantly different, even though it tended to be slightly higher in alternating light than in darkness.

***Solanum retroflexum* Dun.**

The effect of light on the germination of *S. retroflexum* seed is shown in Figure 6.34. In both the alternating light and the continuous darkness treatments, the germination of *S. retroflexum* seeds only occurred after 72 h and was below 10% at that stage. The germination percentage over the entire 336 h period remained low in both treatments but tended to be higher in alternating light than in darkness. The difference between the two treatments tended to increase over time but statistically the final mean germination percentage of 49.5% under alternating light was not significantly different ($p>0.01$) from the 34% obtained in the dark treatment.

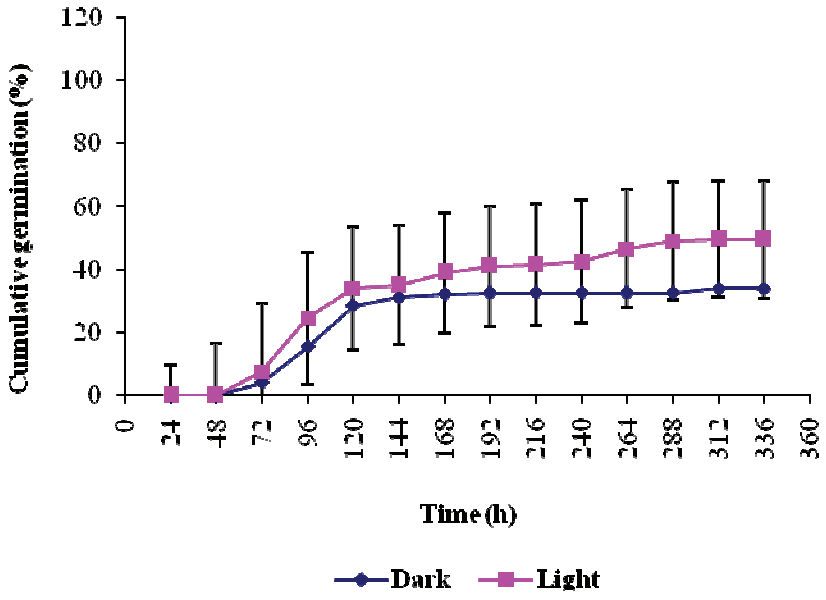

Figure 6.34: Percentage germination of *Solanum retroflexum* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

***Vigna unguiculata* (L.) Walp.**

The effect of light on the germination of *V. unguiculata* seed is shown in Figure 6.35. After 24 h, germination percentage was significantly higher ($p \leq 0.01$) in the continuous darkness (84%) treatment than in the alternating light (69.5%) treatment. Thereafter, treatment effect disappeared. The final mean germination of 95% in darkness tended to be higher than the 94% attained in the alternating light treatment, but statistically this difference was not significant ($p > 0.01$).

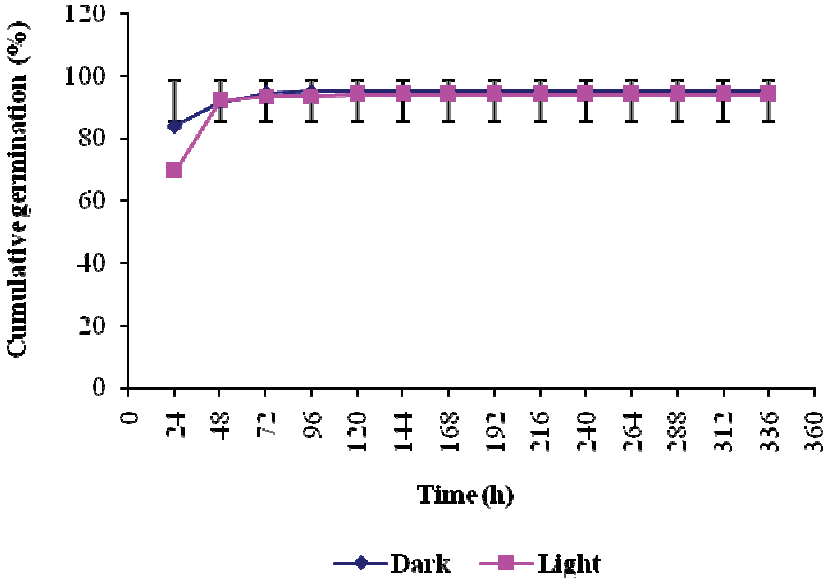

Figure 6.35: Percentage germination of *Vigna unguiculata* under constant darkness and alternating light (8 h light/16 h dark) for 14 days (336 h) at 25 ºC

Experiment two: Evaluation after 240 h

The effect of light on the germination of the selected African leafy vegetables evaluated after 240 h of incubation is shown in Figure 6.36. In terms of the effect of light on final germination percentage, seeds could be divided into two groups, namely,

- Those which were indifferent to the presence or absence of light.
- Those which were significantly improved by exposure to light.
- The first group contained the seeds of species that were indifferent to the presence or absence of light for final germination percentage.

Included in this group were *A. cruentus*, *B.vulgaris* var. *cicla*, *C. gynandra*, *C. olitorius*, *C. maxima* and *V. unguiculata*. The second group contained the seeds of species of which final germination percentage was significantly improved by exposure to light. This group included *B. rapa* subsp. *chinensis*, *C. lanatus* and *S. retroflexum*. In the continuous darkness treatment, the germination percentage of *Brassica rapa* subsp. *chinensis* seed was significantly lower (55%) than in alternating light (87.5%). In the case of *C. lanatus*, the final germination percentage of 89% in alternating light was significantly higher ($p \leq 0.05$) than the 79% recorded in continuous darkness. Final germination percentage of 0.5% for *S. retroflexum* seed when exposed to continuous darkness was significantly lower ($p \leq 0.05$) than the 57.5% in alternating light. Generally, final germination percentage of the seed of all species when germinated in the alternating light treatment tended to be higher than when germinated in the continuous darkness treatment. The seed of *V. unguiculata* was the only exception.

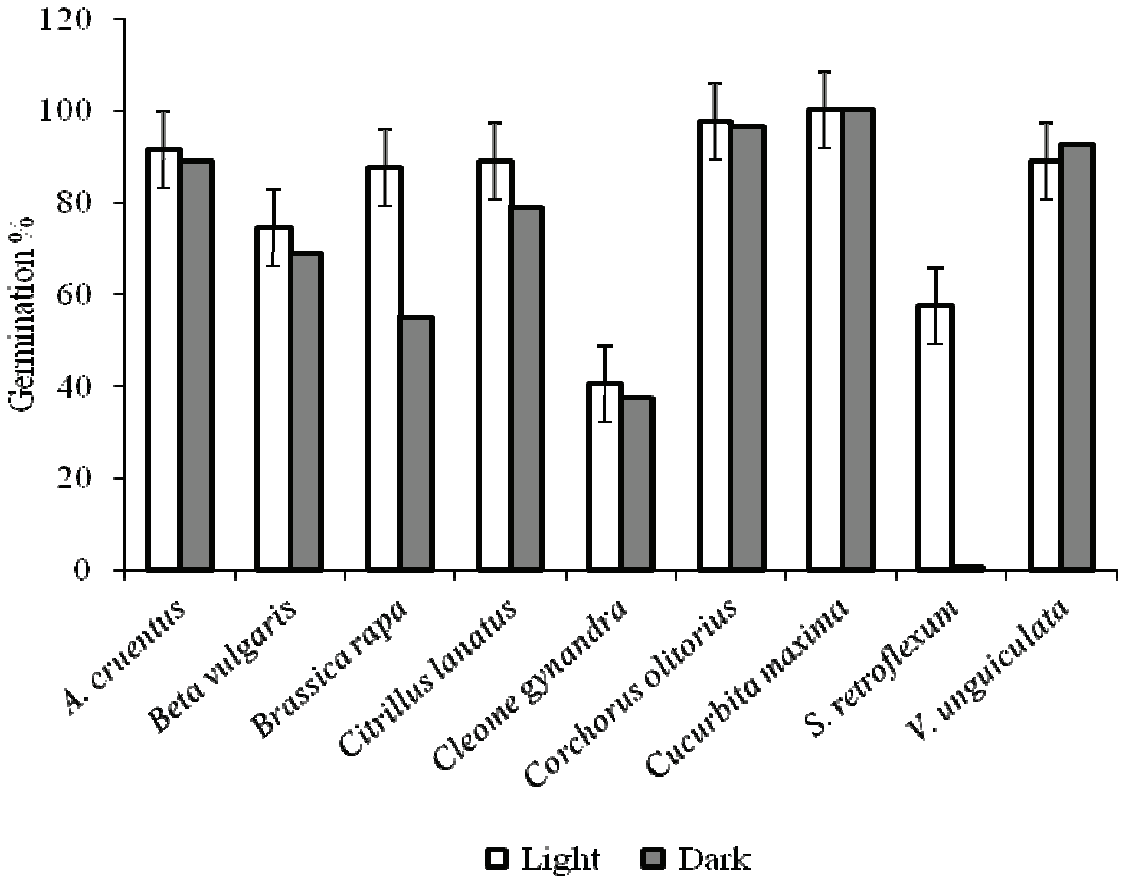

Figure 6.36: Percentage germination of selected African leafy vegetables under constant darkness and alternating light (8 h light/16 h dark) over a period of 10 days (240 h) at 25 ºC

The results obtained in both experiments (24 h and 240 h evaluation) are summarised in Table 6.2.

Table 6.2: Effect of light on the onset and final germination percentage of nine leafy vegetable species

	Effect of light (% germination)		
Leafy vegetable species	**Onset of germination (experiment 1) ($p \leq 0.01$)**	**Final germination (experiment 1) ($p \leq 0.01$)**	**Final germination (experiment 2) ($p \leq 0.05$)**
Amaranthus cruentus	Positive	None	None
Beta vulgaris var. *cicla*	None	None	None
Brassica rapa subsp. *chinensis*	Positive	Positive	Positive
Citrillus lanatus	Negative	None	Positive
Cleome gynandra	None	None	None
Cucurbita maxima	None	None	None
Corchorus olitorius	Positive	None	None
Solanum retroflexum	None	None	Positive
Vigna unguiculata	Negative	None	None

Table 6.2 shows the effect of light on the onset of germination and final germination percentage measured every 24 h over 336 h (experiment 1) and the effect of light on final germination percentage measured after 240 h of incubation of selected ALVs under alternating light (8 h light/ 16 h dark) and constant darkness (experiment 2) at constant temperature of 25ºC.

In the first experiment, light significantly affected the onset of the germination of the seed of five of the nine species, namely, *A. cruentus, B. rapa* subsp. *chinensis, C. lanatus, C. olitorius* and *V. unguiculata*. Treatment significantly affected final germination percentage of *B. rapa* subsp. *chinensis* seed, with seed incubated in alternating light treatment attaining a higher percentage (93%) than that incubated in darkness (53.5%).

In experiment two, alternating light had a statistically significant effect on final germination percentage of the seed of three species, namely, *B. rapa* subsp. *chinensis, C. lanatus* and *S. retroflexum.*

6.4.4 Discussion

This study provided evidence of positive photosensitivity of final germination percentage in three of the nine species that were investigated, namely, *B. rapa* subsp. *chinensis, C. lanatus* and *S. retroflexum*. All three species attained higher final germination percentage under alternating light than under complete darkness. The rate at which onset of seed germination of *A. cruentus, B. rapa* subsp. *chinensis* and *C. olitorius* occurred was significantly increased when exposed to alternating light conditions. Therefore, the onset of germination for these species is positively photosensitive. This results suggests that disturbance by turning soil over and exposing seeds to light (Kettenring *et al*., 2006), or other ways of bringing about a brief illumination (Mpati, 2006) might stimulate germination of these species, an attribute commonly observed among weeds (Taylorson and Borthwick, 1969).

Exposure to alternating light had a significant effect on final germination percentage of *B. rapa* subsp. *chinensis, C. lanatus* and *S. retroflexum* in the 240 h incubation treatment, although only final germination percentage of seed of *B. rapa* subsp. *chinensis* was significantly improved by exposure to the alternating light treatment during the experiment involving 24 h evaluations. The apparent positive effect of light on final germination percentage of these three species suggests that germination of their seed is a photochrome-mediated response. Environmentally induced photosensitivity is usually interpreted as an adaptation that

ensures that seed germinates in places where there is a high probability of seedling establishment (Mpati, 2006). Therefore, it would appear that the seeds of *B. rapa* subsp. *chinensis*, *C. lanatus* and *S. retroflexum* have a preference of germination at or near the soil surface (Bayo and King, 1994). In the case of *B. rapa* subsp. *chinensis*, the preference of its seed to germinate near the soil surface is supported by indigenous knowledge in the Venda region, where the crop is grown widely. According to Tshikalange (2006), people in Venda refer to the land race of *B. rapa* subsp. *chinensis* used in the study as '*dabadaba*', because it germinates like a weed, following soil disturbance.

The germination of the seeds of the remaining six species, *B. vulgaris* var. *cicla* included, was essentially indifferent to light, even though there was some evidence of a minor negative effect on the onset of germination in the case of *C. lanatus* and *V. unguiculata*. In the case of *C. lanatus* and *V. unguiculata*, the onset of seed germination was somewhat delayed when seed was exposed to light. Negative photosensitivity has been attributed to light inhibiting cell elongation by suppressing the expression of selected proteins that enhance germination (Ochoudho *et al*., 2008). Mpati (2006) pointed out that even in species of which seed germination is known to be indifferent to light, there were usually a few individual seeds that were light-sensitive. This could explain the observed minor effect of light on the onset of germination in *C. lanatus* and *V. unguiculata*.

Final germination percentage of *C. gynandra* and *S. retroflexum* seed was very low (below 50%) in both experiments. The low final germination percentage of *S. retroflexum* seeds could possibly be attributed to inadequate removal of sugars and germination inhibitors present in the fruit during extraction of the seed, therefore, necessitating pre-sowing treatments to release seed from dormancy (Jansen van Rensburg *et al*., 2007). Other researchers have reported similar low germination in *C. gynandra* (Böhringer *et al*., 1999; Jansen van Rensburg, 2009, personal communication). *C. gynandra* seed reaches physiological maturity after a period of about 12 months in storage, at which full germination potential is achieved (Böhringer *et al*., 1999; Ochuodho and Modi, 2005). Therefore, it could be possible that the low germination percentage in *C. gynandra* observed in the current study was caused by incomplete physiological maturity status of the seed.

6.4.5 Conclusion

This study showed that African leafy vegetables varied in their response to light during germination. Exposure to alternating light had a significant effect on the onset of germination of several species, either by increasing or reducing the rate at which germination occurred. Based on the onset of germination, the seeds of African leafy vegetables could be divided into three categories:

- Those that were improved by light (positively photosensitive).
- Those that were not inhibited by light (negatively photosensitive).
- Those that were indifferent to the presence or absence of light.

The first group contained the species that were positively photosensitive for onset of germination. Included in this group were *A. cruentus, B. rapa* subsp. *chinensis* and *C. olitorius*. The second group contained the species that were negatively photosensitive for the onset of germination. This group contained *C. lanatus* and *V. unguiculata*. The last group contained species indifferent to light, and included *B. vulgaris* var. *cicla*, *C. gynandra* and *C. maxima*. Effect of light on final germination percentage was only significant for the seed of *B. rapa* subsp. *chinensis, C. lanatus* and *S. retroflexum*. The positive response to light of the seed of these vegetable species could have important implications for the germination of their seeds and establishment of their seedlings. In *C. lanatus* and *V. unguiculata*, the initial negative effect of light on germination was cancelled out by an improvement in the final germination percentage.

From a practical perspective, the germination of *B. rapa* subsp. *chinensis*, *C. lanatus* and *S. retroflexum* is expected to be improved by sowing seed at or close to the soil surface. The seed of these three species could germinate faster when sown very shallow or even when broadcasted on the surface of the soil. Further investigation of the effect of light on germination of these three indigenous vegetables is expected to yield more definitive results.

6.5 GENERAL CONCLUSION

Using laboratory incubation, the response of seed germination to variability in temperature, pre-sowing dormancy treatments and light was examined for eight African leafy vegetables, namely, spider flower (*Cleome gynandra* L.), pigweed (*Amaranthus cruentus* L.), non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*), nightshade (*Solanum retroflexum* Dun.), pumpkin (*Cucurbita maxima* Duchesne), tsamma melon (*Citrillus lanatus* L.), Jew's mallow (*Corchorus olitorius* L.) and cowpea (*Vigna unguiculata* (L.) Walp.).

Amaranthus cruentus seed germinated optimally at 31ºC, with 16ºC as the minimum temperature and 40ºC as the maximum temperature. Improved onset and final germination percentage was observed when the seed of *A. cruentus* was scarified and pre-chilled. Exposure of the seed to light improved onset of germination of *A. cruentus*, but final germination percentage was indifferent to light.

Brassica rapa subsp. *chinensis* seed germinated optimally at 29ºC, with 12ºC being the minimum temperature and 40ºC the maximum temperature. Onset and final germination percentage of *B. rapa* subsp. *chinensis* was improved by scarification and imbibing with potassium nitrate (KNO_3). However, pre-chilling negatively affected germination percentage of this specie. Exposure to light improved onset and final germination percentage of *B. rapa* subsp. *chinensis*.

Cleome gynandra seed germinated optimally at 31ºC, with 12ºC being the minimum temperature and 36ºC, being the maximum temperature. Onset of germination percentage of *C. gynandra* was improved by scarification but final germination percentage was indifferent to the other treatments (pre-chilling and KNO_3) and the control. Germination of *C. gynandra* seed was indifferent to light.

Citrillus lanatus seed germinated optimally at 30ºC, with 20ºC being the minimum temperature and 40ºC, being the maximum temperature. Seed of *C. lanatus* was indifferent to any of the dormancy pre-treatments. Exposure to light tended to negatively affect onset of germination, but improved final germination percentage.

Corchorus olitorius seed germinated optimally at 35ºC, with 12ºC being the minimum temperature and 44ºC, being the maximum temperature. Germination of *C. olitorius* seed was indifferent to any of the dormancy pre-treatments. Exposure to light improved onset of germination, but final germination percentage was indifferent to light exposure.

Cucurbita maxima seed germinated optimally at 32ºC, with 16ºC being the minimum temperature and 40ºC, being the maximum temperature. Seed of *C. maxima* was indifferent to the different dormancy pre-treatments and to light exposure.

Vigna unguiculata seed germinated optimally at 36ºC, with 12ºC being the minimum temperature and 40ºC the maximum temperature. Seed of this specie was indifferent to the dormancy pre-treatments. Exposure to light negatively affected onset of germination, but final germination percentage was indifferent to light.

Solanum retroflexum seed germination percentage was very poor. Therefore, extrapolation of the cardinal temperatures was not possible. Germination percentage never reached 50%. Potassium nitrate (KNO_3) treatment improved germination of *S. retroflexum* seed, while scarification and pre-chilling reduced germination percentage. Improved onset and final germination percentage was observed when the seed of *S. retroflexum* was exposed to light. Previous studies of *C. gynandra* and *S. retroflexum* reported similar difficulty with the germination of these species.

Based on the study results, seeds of *A. cruentus, C. lanatus, C. gynandra, C. olitorius, C. maxima* and *V. unguiculata* germinate better when planted when the temperature is warm. *B. rapa* subsp. *chinensis* seed could be planted when the temperature is still cool.

Some form of seed abrasion to remove seed coat resistance of *A. cruentus* and *B. rapa* subsp. *chinensis* seed could enhance the onset of germination and improve final germination percentage if germination problems are encountered. Overwintering of *A. cruentus* seed could also improve germination. Germination of *B. rapa* subsp. *chinensis* and *S. retroflexum* could be improved by imbibibing its seed with potassium nitrate.

Seeds of *B. rapa* subsp. *chinensis, C. lanatus* and *S. retroflexum* could remain in the soil without germinating when covered by plant canopy, litter or soil, until exposed to adequate light. Therefore, seed of these three species could be planted close to the soil surface in order to improve their germination. For the other species, exposure to light is not a critical factor, and standard procedures for planting seeds can be followed.

A combination of light and potassium nitrate could form part of a strategy aimed at optimum germination for *B. rapa* subsp. *chinensis* and *S. retroflexum* seed, since both this species germinated well when subjected to these treatments.

This study contributed information on the agronomy of African leafy vegetables. By encouraging production and use of these food species, by promoting further work, and by awareness of the use of the nutritional benefits of these crops, some of the food and nutrition problems in Southern Africa and Africa as a whole could be addressed.

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CHAPTER 7: PLANT NUTRIENT REQUIREMENTS OF AFRICAN LEAFY VEGETABLES

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7.1 INTRODUCTION

Plant nutrition is an important growth factor. Typically, crop growth and yield increase as the concentration of nutrients in available forms increases in the soil until an optimum is reached at which growth and yield are maximised (Sonneveld and Voogt, 2009). Low nutrient content of cropped soils is a primary production constraint in smallholder areas of South Africa. The positive interaction effect of nutrients and water on plant growth and yield is well known (Tisdale *et al*., 1985). As a result, to obtain high yields, both the nutrient and the water requirements of crops must be met.

African leafy vegetables (ALVs) have the reputation that they require less plant nutrients than their exotic counterparts, such as Swiss chard, for growth (Jansen van Rensburg *et al*., 2004; Van der Walt *et al*., 2004; Maunder and Meaker, 2007). If this were true, it would make ALVs particularly attractive to smallholders, who often lack the financial resources to apply fertilisers at high rates. Many species of ALVs grow and reproduce naturally on soils that are low in fertility but there is no scientific evidence that they thrive under these conditions. For this reason, studies are needed to determine the plant nutrient requirements of ALVs and to characterise their response to the application of fertilisers.

Among the various plant nutrients that are important for plant growth, nitrogen, phosphorus and to a lesser extent potassium are paramount, particularly in Africa (Sanchez and Swaminathan, 2005). These three nutrients are required in fairly large amounts by crop plants, particularly by species grown for the harvest of leaves, such as Swiss chard (Engelbrecht *et al*., 2010). Crops differ in the way they respond to the availability of the different plant nutrients in the soil, because they vary in terms of the distribution and density of their root systems (Gregory, 1988).

Research aimed at describing the response of ALVs to rates of fertiliser application should consider the sources farmers employ to add nutrients to the soil, because among a range of factors, choice of nutrient source affects the amount and rate of nutrient release (Abbasi *et al*., 2007). In the South African smallholder sector, which at present is largely responsible for the production of ALVs and also forms the target group for recommendations on the production of these vegetable species, animal manure has long been the primary way in which plant nutrients are returned to cultivated soils (Yoganathan *et al*., 1998; Materechera and Modiakgotla, 2006; Mkhabela, 2006; Van Averbeke *et al*., 2008; Materechera, 2010).

Kraal manure produced by ruminants is the dominant type of animal manure used as fertiliser by South African smallholders but increasingly they also make use of poultry litter, which they purchase from large- and small-scale poultry production units (Mkhabela, 2004). Nation-wide, cattle and goats produce the bulk of the manure accumulating in livestock kraals but in the Eastern Cape, sheep are also important (Mkile, 2001). Studies of kraal manure in smallholder areas of the Eastern Cape, North West, KwaZulu-Natal and Limpopo Provinces, indicate that dry ruminant manure (cattle, goats and sheep combined) found in the livestock kraals in these areas contains 1.64% nitrogen (N), 0.36% phosphorus (P) and 1.58% potassium (K), on average. However, the nutrient composition of kraal manure is highly variable for various reasons. Soil particle

content of the manure, which dilutes the concentration of nutrients in the manure, has been identified as the main factor (Yoganathan *et al*., 1998; Materechera and Modiakgotla, 2006).

Poultry manure (excrements only) and poultry litter (excrements combined with bedding material) also vary in composition but less than kraal manure. Important sources of variability are the production system (layer or broiler) and whether or not bedding materials are employed. Type and quantity of bedding material have an effect on the composition of the resulting poultry litter (Wilkinson, 1979).

Dealing with a multitude of crops, which are expected to have different requirements for a range of plant nutrients, makes for a complex reality that is difficult to capture in the design of an experimental study. Moreover, heterogeneity of the sources of nutrients that are used by smallholders to fertilise their soils adds to the complexity. To incorporate some of the main variables in the design, the current study was subdivided into three smaller parts. The structure of this chapter is in accordance with these three parts. The first part deals with the biomass response of four ALVs to rate of application of nitrogen (N), phosphorus (P) and potassium (K) applied in chemical form using greenhouse pot experiments. This component of the study was done by Tshililo Ramusandiwa. The second part deals with the biomass response of four ALVs to application rate of three types of animal manure and was done by Alfred Okorogbona. In the third part, the yield response of eight ALVs to rate of application of nitrogen (N), phosphorus (P) and potassium (K) applied to the soil in the form of chemical fertilisers was investigated under field conditions. This was the work of Mihloti Chabalala. Time and resources did not allow for the investigation of the yield response of these eight vegetables to rate of application of selected types of animal manure under field conditions.

7.2 RESPONSE OF SELECTED AFRICAN LEAFY VEGETABLES IN POTS TO APPLICATION RATE OF NITROGEN, PHOSPHORUS AND POTASSIUM

The objective of this part of the study was to characterise the biomass response of four ALVs to rate of application of nitrogen (N), phosphorus (P) and potassium (K), and compare their biomass response with that of Swiss chard, which served as the exotic green leafy vegetable benchmark. The study was done by means of green house pot experiments.

7.2.1 Materials and methods

When studying the response of crops to important plant nutrients, factorial designs are commonly used, because they enable the study of interaction effects. The main disadvantage of factorial designs is logistic. Typically, large numbers of entries are required when conducting experiments that employ factorial designs and the labour needed to maintain them and to timely execute the various measurements can be prohibitive. Considering that the current study intended to investigate the nutrient response of four ALV species and Swiss chard as the reference crop, the decision was made to use an alternative experimental design in the form of a response surface design. The response surface design that was used in the study was created by Professor H. Groeneveld, retired professor in Statistics and Biometry at the University of Pretoria. The main advantage of response surface designs over complete factorial designs is that they require far fewer entries.

Experimental design

The study consisted of a series of greenhouse pot experiments in which the response surface design consisted of 49 entries representing 41 different N, P and K treatments. The design consisted of three parts. Entries comprising the first part consisted of eight replicates of the expected ‘centre point’. The centre point was chosen close to the optimum that was identified for two ALVs, namely Chinese cabbage (*B. rapa* L. subsp. *chinensis*) and nightshade (*S. retroflexum* Dun.) by Van Averbeke *et al*. (2007a). This treatment consisted of the application of 292.5 mg N kg^{-1} soil, 195 mg P kg^{-1} soil and 195 mg K kg^{-1}soil. The purpose of this part of the design was to estimate error.

The entries in the second part of the design consisted of a full 3 x 3 x 3 factorial arrangement with N, P and K levels equally distributed around the centre level. The purpose of this part of the design was to obtain a good estimate of the optimal combination of N, P and K and to describe as well as possible the biomass response around the centre level. The entries contained in this part of the design are shown in Table 7.1.

Table 7.1: Application rates of nitrogen (N), phosphorus (P) and potassium (K) of the entries that were equally distributed around the centre point of the response surface design used for the study of the nutrient response of selected African leafy vegetable in pots

Nutrient	**Rate of application**		
	Half the centre point rates	**Centre point rates**	**Double the centre point rates**
	---------- (mg kg^{-1} soil)----------		
N	146.3	292.5	585.0
P	97.5	195.0	390.0
K	97.5	195.0	390.0

The entries contained in the third part of the design were aimed at strengthening the predictive model along the edges, namely along the very low and very high N, P and K levels. These entries are shown in Table 7.2.

Table 7.2: Application rates of nitrogen (N), phosphorus (P) and potassium (K) of the entries aimed at strengthening the predictive capability of the response surface design used for the study of the nutrient response of selected African leafy vegetable in pots

Treatment	**Rate of application**		
	N	**P**	**K**
	---------- (mg kg^{-1} soil)----------		
1	0.0	0.0	0.0
2	0.0	195.0	195.0
3	292.5	0.0	195.0
4	292.5	195.0	0.0
5	36.6	195.0	195.0
6	292.5	24.4	195.0
7	292.5	195.0	24.4
8	73.1	195.0	195.0
9	292.5	48.8	195.0
10	292.5	195.0	48.8
11	877.5	195.0	195.0
12	292.5	585.0	195.0
13	292.5	195.0	780.0
14	877.5	585.0	780.0

To cater for possible eventualities resulting in the elimination of entries, for example as a result of seed failing to germinate, it was decided to run each response surface experiment in duplicate, meaning that all 49 entries were replicated simultaneously. Consequently, for each of the test crops the experiment involved 98 pots.

Materials

The pot experiments were conducted in a greenhouse of the Tshwane University of Technology in Pretoria. The fibre glass structure had a wet wall and two fans, which engaged thermostatically when the temperature in the greenhouse reached 30ºC, thus controlling maximum air temperature. In the absence of a heating

device, minimum temperature was not controlled. Plastic pots with a capacity of 7 L and five perforations at the bottom for drainage were used for the experiments. These pots were obtained from Plastilon in Gezina. The chemical fertilisers used to supply N, P and K to the different treatments were limestone ammonium nitrate (28% N), super phosphate (8.3% P) and potassium chloride (50% K). All three fertilisers were manufactured by Sasol and were obtained from Obaro (Pty) Ltd. in Pretoria. The fertiliser granules were crushed using a cast iron pestle and mortar and passed through a sieve with an aperture of 0.5 mm. The size reduction was done to improve uniformity of distribution of the fertilisers in the soil following mixing. The same subsoil (Hutton Hayfield) as was used in the pot experiments conducted by Van Averbeke, Juma and Tshikalange (2007a), was used to fill the pots. The soil consisted of a yellowish red (5YR 4/5 dry), low-fertility, sandy clay loam. It was supplied by JL Coetzer CC and was excavated in Swartspruit, west of Pretoria. To remove the coarse earth fraction and to enhance uniformity, the soil was air-dried, pulverised and screened through a sieve with an aperture of 5 mm. Both sieves were manufactured by Industrial Screen Supply, Inc. Selected chemical and physical properties of the soil used in the pots are shown in Table 7.3. With the exception of clay content, which was determined by the Jowed Laboratory Services C.C. in Pretoria West by means of the pipette method, and the soil pH which was determined in water and in KCl in the Laboratory of the Department of Crop Sciences at Tshwane University of Technology using a Boeco BT 500 pH meter supplied by Lasec SA in Centurion, all other soil analyses were done in duplicates by the Institute for Soil, Climate and Water of the Agricultural Research Council in Arcadia. All soil analyses were done in accordance with procedures described by The Non-Affiliated Soil Analysis Work Committee (1990).

Pigweed (amaranth) (*Amaranthus cruentus* L.), pumpkin (*Cucurbita maxima* Duchesne), cowpeas (*Vigna unguiculata* (L.) Walp.) and tsamma melon (*Citrillus lanatus* Thunb.) were selected for use as test crops, whilst Swiss chard (*Beta vulgaris* L. var. *cicla*) was included as the reference crop. The justification for the selection of crops was dealt with in Chapter 3. Seed of amaranth, tsamma melon, pumpkin (VOPI reference "ex Bushbuckridge") and cowpea (VOPI reference "Fahari") was obtained from the Vegetable and Ornamental Plant Institute (VOPI) of the Agricultural Research Council (ARC) in Roodeplaat. The seed of Swiss chard (variety Ford Hook Giant) was obtained from Hygrotech in Pyramid.

Experimental procedures

The experimental procedures used in the conduct of the experiment were the same as described by Van Averbeke *et al*. (2007a). The equivalent of 8 000 g of oven-dry soil was transferred to each pot. For this purpose, the water content of air-dry soil was determined gravimetrically. The relevant quantities for N, P and K fertilisers were then incorporated into the soil and this mixture was homogenised thoroughly in an enamel basin. The homogenised mixture was then transferred to the designated, labelled pot and water was added to raise the soil water content to field capacity. The pots were then left for one week to allow the fertilisers to react with the soil. During that period, the pots were covered with cling wrap to prevent water from evaporating. All crops were planted using an off-centre planting pattern as described by Juma (2006) and Tshikalange (2006). Amaranth was sown at a density of 10 seeds per pot, cowpea and Swiss chard at a density of five seeds per pot and pumpkin and tsamma melon at a density of four seeds per pot. After planting, the pots were again covered with cling wrap until the seedlings had emerged. Information of dates of planting, final harvest and growing season for the five test crops are shown in Table 7.4.

Planting dates of the different crops were selected to more or less coincide with the typical growing seasons of the crops concerned. Thinning of the plants occurred when the plants had reached the third leaf stage and a single, healthy seedling was left in each pot. Seedlings that were pulled out during thinning were left on top of the soil of the pot, to allow them to decompose and return the nutrients they had removed back to the soil.

Table 7.3: Chemical and physical properties of the soil used in the pot experiments

Soil property	Method	Measurement	Unit
Clay content	Pipette method	23.1	%
pH (H_2O)	1:2.5 soil: water mass ratio	5.7	-
pH (KCI)	1:2.5 soil:1M KCl solution mass ratio	5.3	-
Electrical conductivity	Saturated paste extract	17.5	$mS\ cm^{-1}$
NH_4^+-N	1 M KCl extraction	5.5	$mg\ kg\ soil^{-1}$
NO_3^-N	1 M KCl extraction	3.4	$mg\ kg\ soil^{-1}$
Total N	Digestion	105	$mg\ kg\ soil^{-1}$
Phosphorus	Bray 1 extraction	6.0	$mg\ kg\ soil^{-1}$
Phosphorus	Ambic extraction	3.3	$mg\ kg\ soil^{-1}$
Potassium	1 M ammonium acetate extraction	4.9×10^{-2}	$cmol^{(+)}\ kg\ soil^{-1}$
Calcium	1 M ammonium acetate extraction	1.19	$cmol^{(+)}\ kg\ soil^{-1}$
Magnesium	1 M ammonium acetate extraction	5.2×10^{-1}	$cmol^{(+)}\ kg\ soil^{-1}$
Sodium	1 M ammonium acetate extraction	3×10^{-2}	$cmol^{(+)}\ kg\ soil^{-1}$
Zinc	0.5 M HCl extraction	6.4×10^{-1}	$mg\ kg\ soil^{-1}$
Copper	0.5 M HCl extraction	1.87	$mg\ kg\ soil^{-1}$
Iron	0.5 M HCl extraction	21.96	$mg\ kg\ soil^{-1}$
Manganese	0.5 M HCl extraction	63	$mg\ kg\ soil^{-1}$

Table 7.4: Date of planting and final harvest, duration of the growing period and daily mean temperature during the growing period for the greenhouse experiments conducted with five leafy vegetable species

Leafy vegetable species	Date of planting	Date of final harvest	Duration of growing period	Daily mean temperature during the growing period
			(days)	(^{o}C)
Cucurbita maxima	28 Jan	16 Mar	49	24.6
Citrillus lanatus	09 Feb	12 Apr	63	27.2
Vigna unguiculata	28 Oct	07 Jan	71	26.3
Amaranthus cruentus	15 Dec	13 Feb	60	26.6
Beta vulgaris var. *cicla*	27 Sep	13 Dec	77	21.0

Watering of the pots during growth of the crops ensured that the water content of the soil in the pots remained between field capacity and 60% of total available water content, which was achieved by weighing the pots twice daily, taking into account the mass of the growing plants, and each time adding the mass of water required to raise the water content of the soil in the pots back to field capacity.

Harvesting of the leaves of the different crops followed the protocols were derived from the accounts of local harvesting practices documented by Vorster (2007), complemented with primary data collected in the Vhembe District (Limpopo Province) by Ramusandiwa *et al.* (2009).

Leaf-harvesting by tipping, which refers to the removal of the young shoots of the plant, was used for leaf-harvesting of pigweed. The first harvest of amaranth occurred at the sixth-leaf stage when the seventh leaf started to appear. When the plant had attained this stage, the tip of the main stem, taken as the upper 20 mm, was cut with a pair of scissors. Subsequently, tipping of the upper 20 mm of the main stem was repeated at weekly intervals until the end of the experiment. Harvesting of leaves from branches commenced when the branch had three leaves and the fourth leaf started to appear. As in the case of the main stem, the upper 20 mm of the stem was cut off and this was followed by weekly tipping as in the case of the main stem. The experiment with pigweed was terminated when 50% of the plants in the experiment had started to flower. At termination, the leaves that remained on the stem were considered part of the above-ground biomass as they were regarded as unsuitable for consumption. The leaf yield (edible portion) consisted of the total fresh mass of the tips (stem tip, petioles and leaf blades) that were removed during the successive harvests. At the end of the growing season, the entire plant was removed by cutting the stem with secateurs at the soil surface level and its fresh and oven-dry mass were determined.

Selective and sequential harvesting of leaves, which were about 5 cm in diameter but not yet fully developed, was used for the harvesting of cowpea leaves. The first leaf harvest of cowpea occurred when the main stem had seven leaves. At this stage, counting down from top to base, the third and fourth leaves on the main stem were cut off with scissors, inclusive of the petiole. One week later, the same procedure of harvesting the third and fourth leaf from the top was repeated on the main stem. Harvesting of leaves from branches occurred when the branch had at least four leaves, where upon the third and fourth leaf from the top of the branch were harvested and this process was also repeated on a weekly basis as in the case of the main stem. The latter procedure was then repeated weekly until 50% of the plants in the experiment had started to flower, at which stage the experiment was terminated. At this stage, the entire plant was harvested by cutting the stem with a pair of secateurs at the soil surface level. The bottom three leaves were harvested and contributed to total above-ground biomass while the rest of the leaves were harvested and formed part of the mass of leaves considered suitable for consumption. The leaf yield of cowpea consisted of the mass of the blades of the leaves that were removed during successive harvesting. This meant that the petioles of the harvested leaves

were separated from the blades and the mass of the petioles contributed to total above-ground biomass and not to the mass of leaves considered suitable for consumption.

Selective and sequential harvesting of leaves was also used for the harvesting of leaves of pumpkin and tsamma melon. The long duration of the growing season of both crops combined with their creeping growth habit made them awkward plants to work with, particularly under greenhouse conditions. For this reason, the protocol arbitrarily cut short their growing periods. Harvesting of pumpkin and tsamma melon leaves started when the plant had seven unfolded leaves and the eighth leaf started to appear. At this stage the fourth leaf from the base was harvested. The sixth leaf was the second leaf that was removed from the stem and this was done when the tenth leaf from the main stem started to appear. This was followed by the harvest of the eighth leaf when the twelfth leaf started to appear. At that stage the first three leaves were also harvested. When the fourteenth leaf on the main stem started to appear, the experiment was terminated and all leaves including those on the branches were harvested. Male flowers were harvested on the day they started to open. The stamen and calyx were separated from corolla before weighing. At the end of the growing period, the plant was cut at the base with secateurs and the young fruit was removed from the rest of the plant. Open-flowers were harvested daily in the morning, whereas young fruits were harvested once, at the end of experiment. For the purpose of this study, the mass of leaves considered suitable for consumption of pumpkin and tsamma melon consisted of the mass of the fourth to fourteenth leaf obtained from the main stem and all leaves from the branches. The rest of the plant, including the first three leaves, flowers and young fruit constituted the rest of the biomass.

Although Swiss chard is an important leafy vegetable in South Africa, no detailed harvesting protocol could be retrieved for this crop. As with the other crops, it was decided to opt for selective sequential harvesting of leaves rather than full shoot removal at the end of the predetermined growing period. To identify the characteristics of leaves considered suitable for harvest, bunches of Swiss chard leaves were purchased from Noordvaal Markagente operating at the Tshwane Fresh Produce Market. A total of 69 Swiss chard leaves constituted the sample obtained in this way. The length of these leaves, including the petiole, was determined by means of a ruler. The arithmetic mean was found to be 18 cm and this length was taken as the indicator of readiness for harvest of leaves in the experiment. The first four leaves of the plant were not considered for harvest and harvesting started from the fifth true leaf onwards. Harvest of leaves that had a length of at least 18 cm occurred on a weekly basis. The growing period lasted for 77 days, which was the longest of all five crops that were tested. The final harvest consisted of cutting the entire plant using secateurs at the soil surface level. All leaves shorter than 18 cm were allocated to part of oven-dry biomasses.

For all five crops, the fresh mass of harvested plant parts was determined immediately after these were detached from the stems to avoid reduction in mass due to water loss. Thereafter the plant parts were dried in a forced-draught oven (manufactured by Scientific Engineering and supplied by Labotec in Midrand) at 60^{o}C until constant mass to obtain their oven-dry mass. Biomass measurements involved the use of a portable electronic scale (Scout® Pro SPU123 manufactured by Ohaus) with a capacity of 120 g and an accuracy of 0.001 g. The four indicators used to assess plant response to fertiliser application rate included fresh and oven-dry mass of the plant parts that were considered suitable for consumption as a green leafy vegetable and total fresh and dry above-ground biomass but in this paper only oven-dry total above-ground biomass and fresh mass of leaves are presented and discussed.

Data analysis

Primary statistical analysis of the data was done by means of the regression model developed by Professor H. Groeneveld, which consisted of 16 terms and an intercept. For this chapter, the data were analysed for each of the three nutrients separately which was made possible by the simultaneous duplication of each experiment. The data set used to analyse crop biomass response to variability in the application rate of a single nutrient contained seven treatments only. Each of these treatments consisted of a different application

rate of the nutrient under investigation, whilst the other two nutrients were applied at the centre point rate. Six of the seven treatments were represented by two data points, obtained by the simultaneous duplication of the response surface experiment, whilst the seventh treatment (centre point) was represented by 18 data points. For each of the crop and nutrient permutations, the data (four biomass indicators) obtained from these seven treatments were subjected to an analysis of variance (ANOVA) using Statistical Analysis Software version 9.2, GLM procedure (Statistical Analysis Software Institute, 1999) to determine the statistical significance of treatment effect and the Fisher Protected Least Significance Difference test (α=0.05) was used to separate treatment means. In the analysis of the different data sets, the optimum application rate treatment of the nutrient concerned was defined as the lowest application rate treatment that produced biomass indicator values, which did not differ statistically ($p>0.05$) from those obtained in the treatment that produced the numerically highest biomass indicator values. An overview of the experiment with cowpea is shown in Plate 7.1.

Plate 7.1: Greenhouse pot experiment designed to characterise the biomass response of cowpea to application rate of nitrogen, phosphorus and potassium in chemical form

7.2.2 Results

The effects of application rate of nitrogen, phosphorus and potassium on total oven-dry above-ground biomass and total fresh marketable leaves or edible portion of pigweed, cowpea, pumpkin, tsamma melon and Swiss chard are presented in Tables 7.5, 7.6 and 7.7, respectively.

Table 7.5: Effect of nitrogen application rate on total oven-dry above-ground biomass and total fresh edible portion of pigweed (*Amaranthus cruentus* L.), cowpea (*Vigna unguiculata* (L.) Walp.), pumpkin (*Cucurbita maxima* Duchesne), tsamma melon (*Citrullus lanatus* Thunb.) and Swiss chard (*Beta vulgaris* L. var. *cicla*) when P was applied at 195 mg P kg soil^{-1} and K at the rate of 195 mg K kg soil^{-1}

Rate of application	Pigweed	Cowpea	Pumpkin	Tsamma melon	Swiss chard
(mg N kg soil^{-1})	**----------Total oven-dry above-ground biomass (g pot^{-1})----------**				
0.0	0.0_{cd}	27.8_{cd}	2.3_{c}	4.7_{e}	3.9_{d}
36.6	5.5_{cd}	31.5_{bc}	10.1_{bc}	17.1_{cd}	13.9_{cd}
73.1	14.7_{b}	36.9_{bc}	7.6_{c}	25.8_{bc}	18.3_{bc}
146.3	31.8_{a}	40.5_{ab}	40.0_{a}	37.6_{ab}	27.7_{ab}
292.5	39.3_{a}	49.0_{a}	48.1_{a}	41.3_{a}	34.6_{a}
585.0	8.3_{bc}	19.2_{de}	47.7_{a}	47.1_{a}	37.8_{a}
877.5	0.0_{d}	16.4_{e}	1.0_{b}	7.4_{de}	8.4_{cd}
LSD (p=0.05)	8.0	10.1	12.4	12.0	11.5
	----------Total fresh edible portion (g pot^{-1})----------				
0.0	0.0_{e}	134.4_{cd}	9.0_{d}	10.2_{d}	0.0_{c}
36.6	24.6_{cd}	158.7_{c}	43.2_{cd}	38.9_{cd}	75.7_{c}
73.1	55.5_{b}	185.7_{bc}	56.4_{c}	61.7_{c}	93.2_{bc}
146.3	110.2_{a}	226.3_{ab}	173.4_{a}	136.6_{b}	188.7_{b}
292.5	133.2_{a}	252.8_{a}	217.9_{a}	167.1_{ab}	385.0_{a}
585.0	33.1_{bc}	90.7_{de}	211.2_{a}	204.5_{a}	444.7_{a}
877.5	0.0_{d}	77.1_{e}	116.2_{b}	15.9_{cd}	90.0_{bc}
LSD (p=0.05)	27.2	55.4	47.2	48.0	108.3

Table 7.6: Effect of phosphorus application rate on total oven-dry above-ground biomass and total fresh edible portion of pigweed (*Amaranthus cruentus* L.), cowpea (*Vigna unguiculata* (L.) Walp.), pumpkin (*Cucurbita maxima* Duchesne), tsamma melon (*Citrillus lanatus* Thunb.) and Swiss chard (*Beta vulgaris* L. var. *cicla*) when N was applied at 292.5 mg N kg soil^{-1} and K at the rate of 195 mg K kg soil^{-1}

Rate of P application (mg P kg soil^{-1})	Pigweed	Cowpea	Pumpkin	Tsamma melon	Swiss chard
	----------Total oven-dry above-ground biomass (g pot^{-1})----------				
0.0	1.2_{cd}	0.3_{d}	0.8_{d}	0.7_{c}	0.4_{c}
24.4	5.5_{cd}	8.0_{d}	6.5_{cd}	11.4_{c}	13.9_{b}
48.8	14.7_{b}	24.5_{c}	18.0_{c}	27.5_{b}	34.0_{a}
97.5	31.8_{a}	40.8_{ab}	45.1_{ab}	39.5_{a}	32.9_{a}
195.0	38.8_{a}	49.0_{a}	48.1_{ab}	41.3_{a}	34.6_{a}
390.0	8.3_{bc}	36.2_{b}	54.6_{a}	50.8_{a}	33.0_{a}
585.0	0.0_{d}	21.8_{c}	37.0_{b}	41.9_{a}	28.3_{a}
LSD (p=0.05)	8.0	10.1	14.1	11.7	10.6
	----------Total fresh edible portion (g pot^{-1})----------				
0.0	5.8_{d}	0.0_{d}	1.2_{c}	0.0_{c}	0.0_{c}
24.4	24.6_{cd}	41.1_{d}	29.4_{c}	36.4_{c}	161.4_{b}
48.8	55.5_{b}	168.9_{b}	97.4_{b}	91.5_{b}	407.7_{a}
97.5	110.2_{a}	199.6_{ab}	181.6_{a}	161.0_{a}	354.2_{a}
195.0	130.7_{a}	252.8_{a}	217.9_{a}	167.1_{a}	385.0_{a}
390.0	33.1_{bc}	186.5_{b}	213.9_{a}	200.8_{a}	411.6_{a}
585.0	0.0_{d}	107.0_{c}	185.4_{a}	174.1_{a}	329.8_{a}
LSD (p=0.05)	27.2	61.7	51.1	53.5	95.9

Table 7.7: Effect of potassium application rate on total oven-dry above-ground biomass and total fresh edible portion of pigweed (*Amaranthus cruentus* L.), cowpea (*Vigna unguiculata* (L.) Walp.), pumpkin (*Cucurbita maxima* Duchesne), tsamma melon (*Citrillus lanatus* Thunb.) and Swiss chard (*Beta vulgaris* L. var. *cicla*) when N was applied at 292.5 mg N kg soil^{-1} and P at the rate of 195 mg P kg soil^{-1}

Rate of K application	Pigweed	Cowpea	Pumpkin	Tsamma melon	Swiss chard
(mg K kg soil^{-1})	**----------Total oven-dry above-ground biomass (g pot^{-1})----------**				
0.0	5.7_{e}	12.1_{d}	17.5_{b}	14.0_{d}	17.0_{c}
24.4	8.7_{cd}	20.8_{cd}	22.9_{b}	35.2_{bc}	22.0_{c}
48.8	16.3_{bc}	25.5_{c}	43.2_{a}	42.5_{ab}	19.4_{c}
97.5	22.2_{b}	36.8_{b}	39.7_{a}	46.8_{a}	27.5_{bc}
195.0	38.8_{a}	49.0_{a}	48.1_{a}	41.3_{ab}	34.6_{ab}
390.0	18.4_{b}	41.8_{ab}	48.1_{a}	8.0_{c}	37.8_{ab}
780.0	0.0_{e}	26.0_{c}	24.8_{b}	0.0^{e}	40.4_{a}
LSD (p=0.05)	7.6	9.9	12.7	11.2	10.8
	----------Total fresh edible portion (g pot^{-1})----------				
0.0	26.3_{d}	47.7_{d}	79.7_{d}	55.1_{c}	163.4_{d}
24.4	25.7_{d}	98.2_{cd}	117.7_{cd}	139.2_{b}	238.0_{cd}
48.8	48.2_{cd}	114.2_{c}	151.5_{bc}	161.3_{ab}	240.1_{cd}
97.5	80.6_{b}	190.9_{b}	180.1_{ab}	199.1_{a}	310.8_{bc}
195.0	130.7_{a}	252.8_{a}	217.9_{a}	167.1_{ab}	385.0_{ab}
390.0	60.2_{bc}	205.8_{ab}	215.7_{a}	120.4_{b}	410.8_{a}
780.0	0.0_{e}	120.1_{c}	142.2_{bc}	0.0^{d}	457.2_{a}
LSD (p=0.05)	24.8	55.0	50.0	46.8	96.6

The results in Table 7.5 indicate that when no N was added to the low-fertility soil, biomass production of four of the five crops was extremely limited (less than 5% of the maximum total oven-dry above-ground biomass for pigweed and pumpkin and about 10% for tsamma melon and Swiss chard). Cowpea, which produced 57% of maximum biomass in the absence of any N addition, was the only exception. As the application rate of N was raised, total oven-dry above-ground biomass of all five crops progressively increased. Numerically dry biomass increased up to the rate of 292 mg N kg soil^{-1} for pigweed, cowpea and pumpkin and up to the rate of 585 mg N kg soil^{-1} for tsamma melon and Swiss chard. Increasing the application rate of N to 585 mg N kg soil^{-1} caused a dramatic decline in total oven-dry above-ground biomass of pigweed and cowpea, but for pumpkin, tsamma melon and Swiss chard, the rate had to be increased to 877.5 mg N kg soil^{-1} before a decline in biomass production became evident. The optimum N application rate treatment was 146.4 mg N kg soil^{-1} for all five crops, which is equivalent to the application of 328 kg N ha^{-1}, when worked uniformly into the furrow slice with 2240 t dry mass. The effect of N application rate on the fresh mass of leaves mirrored that observed for total oven-dry above-ground biomass, except for tsamma melon and Swiss chard where the optimum application rate treatment was 292 mg N kg soil^{-1} instead of the 146.4 mg N kg soil^{-1} observed for the other three crops, suggesting that tsamma melon and Swiss chard had slightly higher N requirements than the other three crops.

The results in Table 7.6 show that for each of the five crops, biomass produced in the control treatment was less than 5% of that produced in the treatment that produced maximum biomass. Numerically, both total oven-dry above-ground biomass and fresh mass of the edible portion increased as the application rate of P was raised from the control treatment up to the rate of 48.8 mg P kg soil^{-1} for Swiss chard, 195 mg P kg soil^{-1} for pigweed and cowpea, and 390 mg P kg soil^{-1} for the two cucurbits. For total oven-dry above-ground biomass, the optimum rate treatment was 48.8 mg P kg soil^{-1} in the case of Swiss chard and 97.5 mg P kg soil^{-1} for the other four crops. For the total fresh edible portion, the optimum rate treatment was again 48.8 mg P kg soil^{-1} in the case of Swiss chard, 97.5 5 mg P kg soil^{-1} for the two cucurbits and 195 mg P kg soil^{-1} for pigweed and cowpea. Pigweed and cowpea were the two crops that were most sensitive to supra-optimal application rate of P. This was evident from the substantial drop in the biomass indicator values when the application rate of P was raised from 195 mg P kg soil^{-1} to 390 mg P kg soil^{-1}, especially in the case of pigweed. Biomass production of the other crops tended to decline when the application rate of P was raised from 390 mg P kg soil^{-1} to 580 mg P kg soil^{-1} but statistically this decline was significant only in the case of total oven-dry above-ground biomass of pumpkin.

The results in Table 7.7 show that biomass production in the control treatment, which had a soil test level of 18 mg K kg soil^{-1}, differed among the five crops. Pigweed performed the poorest, producing only 15% to 20% of the maximum when no K was added to the soil. Cowpea produced between 19% and 25% of maximum biomass in the control treatment, tsamma melon between 28% and 30%, pumpkin 36% and Swiss chard performed best, producing between 36% and 42% of maximum biomass, depending on the indicator. Numerically, pigweed, cowpea and pumpkin produced the highest biomass when K was applied at the rate of 195 mg K kg soil^{-1}. Biomass production of tsamma melon peaked when K was applied at the rate of 97.5 mg K kg soil^{-1}, whilst biomass production of Swiss chard continued to increase up to the highest rate of 780 mg K kg soil^{-1}. For total oven-dry above-ground biomass of pigweed, cowpea and Swiss chard, the optimum K application rate treatment was 195 mg K kg soil^{-1}, but for the fresh edible portion of biomass the optimum treatment for pigweed was reduced to 97.5 mg K kg soil^{-1}. Tsamma melon had the lowest optimum K application rate treatment (48.8 mg K kg soil^{-1} for both biomass indicators), followed by pumpkin.

7.2.3 Discussion and conclusion

The findings of this part of the study are summarized in Table 7.8, which shows the identified optimum application rate treatments of N, P and K of the five crops that were tested.

Table 7.8: Optimum rate of application of nitrogen, phosphorus and potassium for pigweed (*Amaranthus cruentus* L.), cowpea (*Vigna unguiculata* (L.) Walp.), pumpkin (*Cucurbita maxima* Duchesne), tsamma melon (*Citrillus lanantus* Thunb.) and Swiss chard (*Beta vulgaris* L. var. *cicla*) obtained when one nutrient element was varied, whilst the other two nutrient elements were kept constant at adequate level (centre point)

Crops	**Application rate**		
	Nitrogen	**Phosphorus**	**Potassium**
	----------(mg kg soil^{-1})----------		
Pigweed	146.3	97.5	195.0
Cowpea	146.3	97.5	195.0
Pumpkin	146.3	97.5	48.8
Tsamma melon	146.3	97.5	48.8
Swiss chard	146.3	48.8	195.0

Table 7.8 shows that there were no differences in the optimum application rate treatment of N among the crops, which was 146.3 mg N kg soil^{-1}. This rate is equivalent to the application of 328 kg N ha^{-1}, when worked uniformly into a furrow slice with 2240 t dry mass. It needs pointing out that in the absence of any addition of N to the soil, cowpea still produced nearly 60% of maximum biomass. The only explanation for this observation was that the cowpea plants obtained nitrogen through symbiotic N fixation, even though neither seed nor soil was inoculated prior to planting.

Adequate P availability was shown to be a critical growth factor for all five crops and in the absence of added P, none of the crops were able to produce biomass of any significance. The optimum P application rate of 97.5 mg P kg soil^{-1} for the four ALV species was higher than the optimum rate of 48.8 mg P kg soil^{-1} identified for Swiss chard.

The optimum K application rate treatment was considerably lower for the two cucurbits (48.8 mg K kg soil^{-1}) than the optimum treatment of 195 mg K kg soil^{-1} recorded for the other three crops. Overall the results obtained in this study indicate that the nutrient requirements for optimum biomass production of the four ALVs that were tested did not differ substantially from those of Swiss chard, the exotic reference crop. Similar findings were reported by Van Averbeke *et al*. (2007a), who tested the *dabadaba* land race of non-heading Chinese cabbage (*B. rapa* L subsp. *chinensis*) and the nightshade species *S. retroflexum* Dun. However, the findings also showed that there were exceptions to this general observation, because cowpea was able to produce substantial biomass in the absence of N addition, and pumpkin and tsamma melon required less K than Swiss chard and other crops for optimum biomass production. From a practical point of view, crop species that have a low requirement for one particular nutrient and not for others, might not present a major advantage, because the N, P and K contents of soils are often correlated, particularly in smallholder settings where the application of animal manure is the main way in which nutrients are added to the soil. Finally, it needs to be kept in mind that the findings of this study were obtained in greenhouse pot experiments, which represent conditions that differ substantially from those encountered in the field. Consequently, field testing is necessary before any conclusive statements can be made about the N, P and K requirements of the ALVs tested in this study. This requirement is attended to in section 7.4 of this chapter.

7.3 RESPONSE OF SELECTED AFRICAN LEAFY VEGETABLES IN POTS TO APPLICATION RATE OF DIFFERENT TYPES OF FERTILISERS

As indicated in the introduction, African smallholders mostly use animal manure to fertilise their cropped fields and gardens. The objective of this part of the study was to determine the biomass response of selected ALVs to application rate of three types of animal manure.

7.3.1 Materials and methods

Four greenhouse pot experiments were conducted using non-heading Chinese cabbage (*B. rapa* L. subsp. *chinensis*), pigweed (*A. cruentus* L.), pumpkin (*C. maxima* Duchesne) and nightshade (*S. retroflexum* Dun.) as test crops. Treatments consisted of different rates of application of the three different types of animal manure which were Promis® and manure from cattle and goat kraals. A control treatment in which no fertiliser was added and two benchmark treatments, referred to as 'medium chemical' and 'high chemical', which involved the application of chemical fertilisers were also included. The treatments were arranged in a complete randomised design and are summarised in Table 7.9. Plate 7.2 shows an overview of the experiment with non-heading Chinese cabbage.

Plate 7.2: Greenhouse pot experiment designed to characterise the biomass response of non-heading Chinese cabbage to application rate of different types of fertilisers

Table 7.9: Summary of the treatments in the pot experiments that explored crop response to application rate of Promis, cattle kraal manure and goat kraal manure using non-heading Chinese cabbage, pigweed, nightshade and pumpkin as test crops

Treatment	Fertiliser	Rate of fertiliser application		Rate of elemental nutrient application					
		(g kg^{-1}soil)	(t ha^{-1})	(mg kg^{-1} soil)			(kg ha^{-1})		
				N	P	K	N	P	K
Control	None	..0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0
MCF	LAN	0.55	1.18	149.1	0.0	0.0	333.8	0.0	0.0
	Supers	1.18	2.63	0.0	98.0	0.0	0.0	219.0	0.0
	KCl	0.20	0.44	0.0	0.0	95.4	0.0	0.0	214.5
HCF	LAN	1.10	2.35	298.1	0.0	0.0	667.5	0.0	0.0
	Supers	2.35	5.26	0.0	195.9	0.0	0.0	438.0	0.0
	KCl	0.40	0.87	0.0	0.0	190.7	0.0	0.0	429.0
PL1	Promis	2.69	6.00	99.8	39.8	48.2	223.7	89.2	108.2
PL2	Promis	5.38	12.00	199.6	79.6	96.4	447.5	178.3	216.5
PL3	Promis	8.07	18.00	299.4	119.4	144.7	671.2	267.5	324.7
PL4	Promis	10.76	24.00	399.3	159.3	192.9	894.9	356.6	432.9
PL5	Promis	15.70	35.00	582.2	232.2	281.3	1305.1	520.1	631.3
PL6	Promis	22.42	50.00	831.8	331.8	401.9	1864.5	743.0	901.9
CKM1	CKM	13.40	30.00	228.0	56.3	225.1	510.5	126.1	504.3
CKM2	CKM	26.80	60.00	455.9	112.6	450.3	1021.0	252.2	1008.6
CKM3	CKM	40.20	90.00	683.9	168.9	675.4	1531.5	378.2	1512.9
CKM4	CKM	67.20	150.00	1139.8	281.4	1125.7	2552.5	630.4	2521.6
CKM5	CKM	80.60	180.00	1367.7	337.7	1350.8	3063.0	756.5	3025.9
CKM6	CKM	94.10	210.00	1595.7	394.0	1575.6	3573.5	882.6	3530.2
GKM1	GKM	13.40	30.00	297.2	57.8	538.0	666.2	129.6	1205.6
GKM2	GKM	26.80	60.00	595.78	115.2	1079.2	1334.9	257.1	2416.9
GKM3	GKM	40.20	90.00	892.6	173.0	1616.4	1999.4	387.2	3621.1
GKM4	GKM	67.20	150.00	1484.2	287.7	2686.8	3324.6	644.7	6018.4
GKM5	GKM	80.60	180.00	1785.7	345.7	3243.7	4000.1	774.4	7244.9
GKM6	GKM	94.10	210.00	2081.2	403.1	3768.9	4661.8	902.8	8442.5

MCF: medium fertiliser treatment; HCF: high fertiliser treatment; PL: poultry litter; CKM: cattle kraal manure; GKM: goat kraal manure

Promis®, the poultry litter used in the study, is a partly composted layer litter that was supplied by National Plant Food cc in Rustenburg. It was produced by layer hens reared by means of a floor system, which involved the application of bedding material. The purpose of this particular layer system was to supply eggs for the production of chicks. At the time National Plant Food cc supplied Promis to the market, it was a registered organic fertiliser that came packaged in plastic bags. The cattle kraal manure was obtained from Mr P Tshiwela and the goat kraal manure from Mr A Ramiatho, both residents of Tshituni Tsha Fhasi village in the Vhembe District of Limpopo Province. The goat kraal from which manure was obtained was covered with a roof protecting the animals against element. The cattle kraal manure was not covered. Before applying the different types of animal manure to the soil in the pots, they were spread on plastic sheets in a cool shady place and air-dried, where after they were homogenised and the particle sizes were reduced by hammer milling and passed through a sieve with an aperture of 6.4 mm in the case of poultry and 1.5 mm in the case of manure from cattle and goat kraals. Information on the composition of the three types of animal manure processed in this way is presented in Table 7.10.

Table 7.10: Chemical and physical properties of the three types of animal manure used in the pot experiments (air-dry basis)

Property	Unit	Promis poultry manure	Cattle kraal manure	Goat kraal manure
pH[1]		6.9	9.2	9.7
Moisture	%	12.1	7.3	9.0
Solids	%	87.9	92.7	91.0
Mineral content	%	15.5	55.0	44.3
Organic matter content	%	72.4	37.7	46.7
Total organic carbon	%	35.5	17.5	25.0
Mineral content (dry)	%	17.7	59.3	48.7
Total Nitrogen	%	3.7	1.7	2.2
Carbon/Nitrogen ratio		9.6	10.3	11.3
Calcium (Ca)	%	2.5	1.9	2.3
Magnesium (Mg)	%	0.7	0.9	1.0
Phosphorus (P)	%	1.5	0.5	0.4
Potassium (K)	%	1.5	1.7	4.0
Sodium (Na)	%	0.5	0.3	0.4
Aluminium (Al)	%	0.1	1.1	1.1
Iron (Fe)	%	0.2	1.1	1.0
Copper (Cu)	mg kg^{-1}	99.1	34.8	39.6
Manganese (Mn).	mg kg^{-1}	759.5	336.8	310.0
Zinc (Zn)	mg kg^{-1}	545.3	99.3	94.3
Electricalconductivity	(m Sm^{-1})	13.4	3.0	8.9

[1] The manure to water ratio used for pH measurement was 1 g manure: 5 m? water.
[2] With the exception of mineral content, all values in the table are reported on an air dry basis and are based on the dried and milled samples provided to the ARC-ISCW, 2008.
[3] The manure to water ratio for electrical conductivity determination was 20 ml manure: 40 ml water in accordance with Peters *et al*., 2003:51.

The experiments were conducted in the same greenhouse as described in section 7.2.1 and the general procedures for preparing the soil and fertiliser mixtures, filling the pots, planting the seed and watering the pots were also the same. After transferring the dry soil/fertiliser mixtures to the pots, the water content of the soil was raised to field capacity. After irrigation, pots were covered with cling wrap and left for 16 days to allow the fertilisers to react with the soil and to allow for nutrient release from the all the different types of animal manure. Planting of the crops was timed to coincide with their normal growing season. Planting dates, final harvest date and duration of the growing period are shown in Table 7.11.

Table 7.11: Planting dates, harvest dates and duration of the growing period of the different crops

Crop	Planting date	Harvest date	Growth period
Chinese cabbage	8 July 2009	11 September 2009	9 weeks
Pigweed	15 September 2009	3 December 2009	12 weeks
Nightshade	15 October 2009	30 January 2010	15 weeks
Pumpkin	11 February 2010	25 March 2010	6 weeks

Harvesting of pigweed and pumpkin was in accordance with the protocol described in section 7.2.1 and harvesting of Chinese cabbage and nightshade was in accordance with the protocol described by Van Averbeke *et al*. (2007a).

Biomass measurements were made using a portable electronic scale with a capacity of 120 g and an accuracy of 0.001 g. The fresh mass of the plant parts was determined immediately after their removal. Oven-dry biomass of plant material was determined by drying the material in a forced-draught oven at 60^{o}C until constant mass. Total oven-dry above-ground biomass was used as the indicator to determine crop response to application rate of the nutrient sources.

The electrical conductivity of air-dried manure and air-dried soil was determined at the end of the experiments. Soil was collected with a core sampler, which had a height of 50 cm and internal diameter of 1.2 cm. Soil sampling was carried out by vertically pressing the core sampler into the soil surface until the bottom of the pot. Thereafter, the core sampler was rotated a few times to collect the soil before pulling the soil up. Soils collected were dried at room temperature in the laboratory for 96 hours and crushed using a mortar and pestle to separate soil particle for laboratory analysis. Two soil samples were collected from each pot in all four experiments and these were bulked. The six bulk samples representing each treatment were then paired to obtain three samples (replicates) representing each treatment.

The electrical conductivity (EC) of manure was determined using the method described by Peters *et al*. (2003). This involved the placement of 20 ml manure and 40 ml distilled water in a 100 ml plastic bottle with screw cap and the shaking of this mixture before determining the EC of the supernatant liquid. Manure was sampled three times for EC measurement. The EC of soil samples collected at the final harvest stage of the vegetable crops was determined by weighing 8 g of soil with a portable electronic scale and placing the weighed soil into a plastic bottle where after 40 cm^{3} of distilled water was added to the bottle using a measuring cylinder and shaken thereafter. The EC was determined using same instrument and procedure as described for animal manure.

Data analysis were subjected to analysis of variance (ANOVA) using the GLM procedure of the Statistical Analysis Software Version 9.2 (Statistical Analysis Software Institute, 1999), to test for treatment effects. Treatment means were separated using the Fishers Protected Least Significant Difference (LSD) test (p=0.05).

7.3.2 Results

The effect of application rates of the different types of animal manure on total oven-dry above-ground biomass of the four vegetables species is shown in Table 7.12.

Table 7.12: Effect of application rate of different fertilisers on total oven-dry above-ground biomass of crops

Treatment	Nutrient source	Application rate	Total oven-dry above-ground biomass			
			Chinese cabbage	Pigweed	Nightshade	Pumpkin
		($t\ ha^{-1}$)		----------($g\ plant^{-1}$)----------		
Control	None	0	0.03 ^{i}	0.00 ^{l}	0.06 ^{l}	11.67 ^{m}
MCF	Chemical fertiliser	1.18 LAN, 2.63 supers,0.44 KCl	30.46 ^{a}	30.88 ^{bc}	46.50 ^{a}	43.99 ^{a}
HCF.	Chemical fertiliser	2.35 LAN, 5.26 supers,0.87 KCl	18.55 ^{c}	35.50 ^{a}	41.61 ^{b}	28.99 ^{e}
PL1	Promis	6	7.08 ^{g}	9.64 ^{ij}	11.42 ^{ij}	15.22 ^{k}
PL2	Promis	12	9.50 ^{f}	19.28 ^{gh}	17.52 ^{gh}	25.23 ^{g}
PL3	Promis	18	11.90 ^{e}	25.12 ^{e}	30.76 ^{e}	21.44 ^{h}
PL4	Promis	24	14.35 ^{d}	23.45 ^{ef}	34.38 ^{d}	17.29 ^{j}
PL5	Promis	35	6.40 ^{g}	21.03 ^{fg}	16.08 ^{h}	13.60 ^{l}
PL6	Promis	50	0.97 ^{i}	19.55 ^{gh}	8.90 ^{k}	8.34 ^{p}
CKM1	Cattle	30	9.47 ^{f}	6.05 ^{k}	10.85 ^{j}	10.82 ^{n}
CKM2	Cattle	60	12.45 ^{de}	10.35 ^{i}	17.94 ^{g}	19.83 ^{i}
CKM3	Cattle	90	13.90 ^{d}	17.46 ^{h}	23.51 ^{f}	27.17 ^{f}
CKM4	Cattle	150	17.53 ^{c}	28.27 ^{cd}	30.87 ^{e}	31.36 ^{d}
CKM5	Cattle	180	18.99 ^{c}	34.01 ^{ab}	37.00 ^{c}	37.83 ^{b}
CKM6	Cattle	210	23.36 ^{b}	26.37 ^{ed}	31.69 ^{e}	32.61 ^{c}
GKM1	Goat	30	4.17 ^{h}	6.65 ^{jk}	12.97 ^{i}	9.19 ^{o}
GKM2	Goat	60	6.52 ^{g}	11.46 ^{i}	16.89 ^{gh}	20.96 ^{h}
GKM3	Goat	90	7.53 ^{fg}	12.76 ^{i}	24.10 ^{f}	12.29 ^{m}
GKM4	Goat	150	1.18 ^{i}	0.00 ^{l}	0.00 ^{l}	0.00 ^{q}
GKM5	Goat	180	0.91 ^{i}	0.00 ^{l}	0.00 ^{l}	0.00 ^{q}
GKM6	Goat	210	0.35 ^{i}	0.00 ^{l}	0.00 ^{l}	0.00 ^{q}
Mean			10.26	16.41	19.67	18.46
LSD** (p=0.05)			1.98	3.13	1.67	0.66
Coefficient of variation (%)			16.88	15.3	7.50	3.12

Treatment means followed by different superscripted letters differed significantly ($p \leq 0.05$); **LSD = least significant difference

Statistically, treatment effect on total oven-dry above-ground biomass was highly significant ($p<0.0001$) for all four crops. The results in Table 7.12 show that without adding any source of nutrients to the soil (control treatment) biomass production was extremely limited for all crops except pumpkins. The medium chemical fertiliser treatment (MCF) produced the highest biomass for Chinese cabbage, nightshade and pumpkin but highest biomass production of pigweed occurred in the high chemical fertiliser treatment.

Chinese cabbage

Relative to the control treatment, addition of Promis increased biomass production of Chinese cabbage, except when Promis was applied at the rate of 50 t ha^{-1} (Table 7.12). The trend was for biomass production to increase progressively as application rate of Promis was increased up to 24 t ha^{-1}, which was the optimum application rate and then for biomass production to decline dramatically when application rate of Promis was raised in excess of 24 t ha^{-1}. Relative to the 24 t Promis ha^{-1} treatment, raising the application rate of Promis to 35 t ha^{-1} reduced biomass significantly ($p \leq 0.05$). The biomass production achieved at the apparent optimum rate of 24 t Promis ha^{-1} was significantly lower than that achieved in both the medium chemical fertiliser treatment (MCF) and the high chemical fertiliser treatment (HCF). The biomass production in the 24 t Promis ha^{-1} amounted to about 47% of that achieved in the medium chemical fertiliser treatment. Amendment of the soil with cattle kraal manure also increased biomass production of Chinese cabbage relative to the control treatment. The trend was for biomass production to increase as application rate of cattle kraal manure was increased over the full range of application rates, suggesting that the optimum rate of application might not have been reached yet. The biomass that was produced in the 210 t cattle kraal manure ha^{-1} treatment was higher than that obtained in the high chemical fertiliser treatment (HCF) but only 77% of that achieved in the medium chemical fertiliser treatment (MCF). Relative to the control treatment, application of goat kraal manure raised biomass production of Chinese cabbage only up to the rate of 90 t goat kraal manure ha^{-1} The trend was for biomass to increase near linearly from the lowest rate of 30 t ha^{-1} up to the rate of 90 t ha^{-1}, which was the optimum application rate for goat kraal manure, and then to decline dramatically when the rate was increased to 150 t ha^{-1}. Generally, biomass production by Chinese cabbage in soils amended with goat kraal manure was disappointing. The biomass production in the 90 t goat kraal manure ha^{-1} treatment was only about 25% of that achieved in the medium chemical fertiliser treatment (MCF).

Pigweed

Compared to the control treatment, applying Promis to the soil increased biomass production of pigweed, irrespective of application rate (Table 7.12). The trend was for biomass production to increase progressively as the application rate of Promis was raised from 6 t ha^{-1} to 18 t ha^{-1} and then for biomass production to decline gradually as application rate was increased above 18 t Promis ha^{-1}. Biomass production of pigweed in the 18 t Promis ha^{-1} treatment amounted to about 71% of that obtained in the high chemical fertiliser treatment, which produced the highest biomass of all treatments included in the experiment. The addition of cattle manure increased biomass production of pigweed relative to the control, irrespective of rate of application. The trend was for biomass production to increase progressively as application rate of cattle kraal manure was raised from 30 t ha^{-1} up to the rate of 180 t ha^{-1} and for biomass production to decline when the application rate of cattle kraal manure was increased from 180 t ha^{-1} to 210 t ha^{-1}. The results suggested that the optimum rate of application was located between the application rates of 150 t ha^{-1} and 210 t ha^{-1}. Biomass production in the 180 t cattle kraal manure ha^{-1} treatment was about 96% of that achieved in the high chemical fertiliser treatment. Relative to the control treatment, application of goat kraal manure increased biomass production of pigweed in a statistically significant way in the 30 t ha^{-1}, 60 t ha^{-1} and 90 t ha^{-1} treatments only and overall the response of the crop to application of goat kraal manure was disappointing. Biomass production in the 90 t goat kraal manure ha^{-1} treatment was only about 36% of that achieved in the high chemical fertiliser treatment.

Nightshade

Relative to the control treatment, adding Promis to the soil increased biomass production of nightshade, regardless of rate of application. The trend was for biomass production to increase gradually and near-linearly as application rate of Promis was increased from 6 t ha^{-1} up to 24 t ha^{-1}, and for biomass production to decline when application rate of Promis was raised in excess of 24 t ha^{-1}. Biomass production in the 24 t Promis ha^{-1} amounted to about 74% of that achieved in the medium chemical fertiliser treatment, which produced the highest biomass of all the treatments used in this experiment. Addition of cattle kraal manure

increased biomass production of nightshade relative to the control, irrespective of rate of application. The trend was for biomass production of nightshade to increase progressively and near-linearly as application rate was increased from 30 t cattle kraal manure ha^{-1} up to 180 t cattle kraal manure ha^{-1}, and then to decline when the rate was increased to 210 t cattle kraal manure ha^{-1}. The decline in the biomass production at the highest application rate of 210 t ha^{-1} of cattle kraal manure relative to the 180 t cattle kraal manure ha^{-1} treatment suggests that the optimum rate of application could be found between the application rates of 150 and 210 t cattle kraal manure ha^{-1}. Biomass production at the application rate of 180 t cattle kraal manure ha^{-1} was about 82% of that achieved in the medium chemical fertiliser treatment. Relative to the control treatment, application of goat kraal manure increased biomass production of nightshade only in the 30 t ha^{-1}, 60 t ha^{-1} and 90 t ha^{-1} application rate treatments. When goat kraal manure was applied at the rates of 150 t ha^{-1}, 180 t ha^{-1} and 210 t ha^{-1}, the biomass produced by nightshade, was statistically not different ($p>0.05$) from that in the control treatment. Biomass production in the 90 t goat kraal manure ha^{-1} treatment was only about 51% of that achieved in the medium chemical fertiliser treatment.

Pumpkin

Relative to the control treatment, addition of Promis increased biomass production of pumpkin in all application rate treatments, except for the 50 t Promis ha^{-1} treatment. The trend was for biomass production to increase as application rate of Promis was increased from 6 t ha^{-1} to 12 t ha^{-1}, and then for biomass production to decline when the application rate of Promis was raised in excess of 12 t ha^{-1}. Biomass production in the 12 t Promis ha^{-1} application rate amounted to about 57% of that achieved in the medium chemical fertiliser treatment, which had the highest biomass production of all the treatments used in the pumpkin experiment. The results in Table 7.12 show that relative to the control treatment, amending the soil with cattle kraal manure increased biomass production of pumpkin, except when cattle kraal manure was applied at the rate of 30 t ha^{-1}, which was the lowest cattle kraal manure application rate treatment. The trend was for biomass production to increase progressively as application rate of cattle kraal manure was increased from 30 t ha^{-1} to 180 t ha^{-1} and for the biomass production to decline when the application rate was raised further to 210 t ha^{-1}, suggesting that the optimum application rate of cattle kraal manure could be found between the rates of 150 t ha^{-1} and 210 t ha^{-1}. Biomass production in the 180 t cattle kraal manure ha^{-1} was about 86% of that achieved in the medium chemical fertiliser treatment. Relative to the control treatment, application of goat kraal manure raised biomass production of pumpkin only at the rates of 60 and 90 t goat kraal manure ha^{-1}. When goat kraal manure was applied at rates above 90 t ha^{-1}, growth of the crop was seriously impaired. Biomass production in the 60 t goat kraal manure ha^{-1} treatment was only about 47% of that achieved in the medium chemical fertiliser treatment.

7.3.3 Discussion and conclusion

The results obtained in this study showed that ALV species differed in their response to application rate of animal manure, which was also the conclusion of Azeez *et al*. (2010). This is also evident in Table 7.13, which shows the application rate treatments at which maximum biomass production of the different crops was achieved.

Table 7.13: Rate of application of different sources of nutrient at which different crops produced maximum biomass

Type of fertiliser	Chinese cabbage	Pigweed	Nightshade	Pumpkin
Chemical fertiliser	Medium	High	Medium	Medium
Promis (t ha^{-1})	24	18	24	12
Cattle kraal manure (t ha^{-1})	210	180	180	180
Goat kraal manure (t ha^{-1})	90	90	90	60

Among the four leafy vegetable species that were tested, Chinese cabbage tended to require the highest rates of application of the different types of fertilisers for maximum biomass production, followed by pigweed, nightshade and lastly pumpkin. The high fertiliser application rate required by Chinese cabbage for optimum growth could be due to its shallow and poorly developed root system, which Van Averbeke and Netshithuthuni (2010) identified as the most likely reason why Chinese cabbage was particularly sensitive to with water stress. Pumpkin, on the other hand, is known for its well developed root system (Philip *et al*., 2009), which appeared to provide the plant with the ability to extract nutrients from low-fertility soils, as indicated by the relatively high biomass produced in the control treatment (Table 7.12). All other plant species investigated in this study appeared to lack this ability.

The findings of this study also supported the notion that animal manure is a resource of variable properties and quality (Malherbe, 1964; Wilkinson, 1979; Yoganathan *et al*., 1998; Chardwick *et al*., 2000; Mkhabela 2004; Materechera and Modiakgotla, 2006; Azeez and Van Averbeke, 2010). Differences in effectiveness of the four types of fertilisers that were used in this study are evident from the data presented in Table 7.14, which shows the effect of nutrient source on maximum relative biomass production of four ALVs.

Table 7.14: Effect of nutrient source on maximum relative biomass production of four African leafy vegetables

Type of fertiliser	**Maximum relative biomass production (%)**				
	Chinese cabbage	**Pigweed**	**Nightshade**	**Pumpkin**	**Mean**
Chemical fertiliser	100	100	100	100	100
Promis	47	71	74	57	62
Cattle manure	77	96	80	86	85
Goat manure	25	36	52	48	40

Chemical fertilisers were most effective, because for all four vegetable species, maximum biomass production was achieved when chemical fertilisers were applied. Cattle kraal manure was next in line. Relative to the biomass achieved when applying chemical fertilisers to the four vegetable species, application of cattle kraal manure enabled biomass production that ranged between 77% and 96%, with an average of 85% across the four crops. Pigweed, known to colonise abandoned livestock kraals (Mingochi and Luchen, 1995), performed best and Chinese cabbage least well when chemical fertilisers were replaced with cattle kraal manure. Third in line in terms of effectiveness was Promis poultry manure. Across the four crops, application of Promis enabled the achievement of 62% of the biomass obtained when chemical fertilisers were applied. Nightshade, known to have its seed distributed by birds (Van Averbeke *et al*., 2007b) responded best to the application of Promis and Chinese cabbage worst. Another reason why nightshade performed better when fertilised with Promis could be that its growing season was the longest. It was observed that the growth performance of nightshade in treatments where Promis was applied at relatively high rates improved as the season progressed, suggesting that the intensity of any negative effects associated with Promis declined over time in the soil. The goat kraal manure used in this study was the least effective fertiliser material. Across the four crops, the optimum application rate treatment of goat manure only produced 40% of the biomass achieved in the optimum chemical fertiliser application rate treatment. Chinese cabbage in particular responded poorly to goat kraal manure. It needs pointing out that the goat kraal manure was three times more saline than the cattle kraal manure, most likely because the kraal was covered, preventing leaching during rainfall events. Over time, this appeared to have resulted in the accumulation of salts.

Figure 7.1 shows that as the rate manure application was raised, soil salinity increased more rapidly in soils amended with goat kraal manure than in soils to which cattle kraal manure was added.

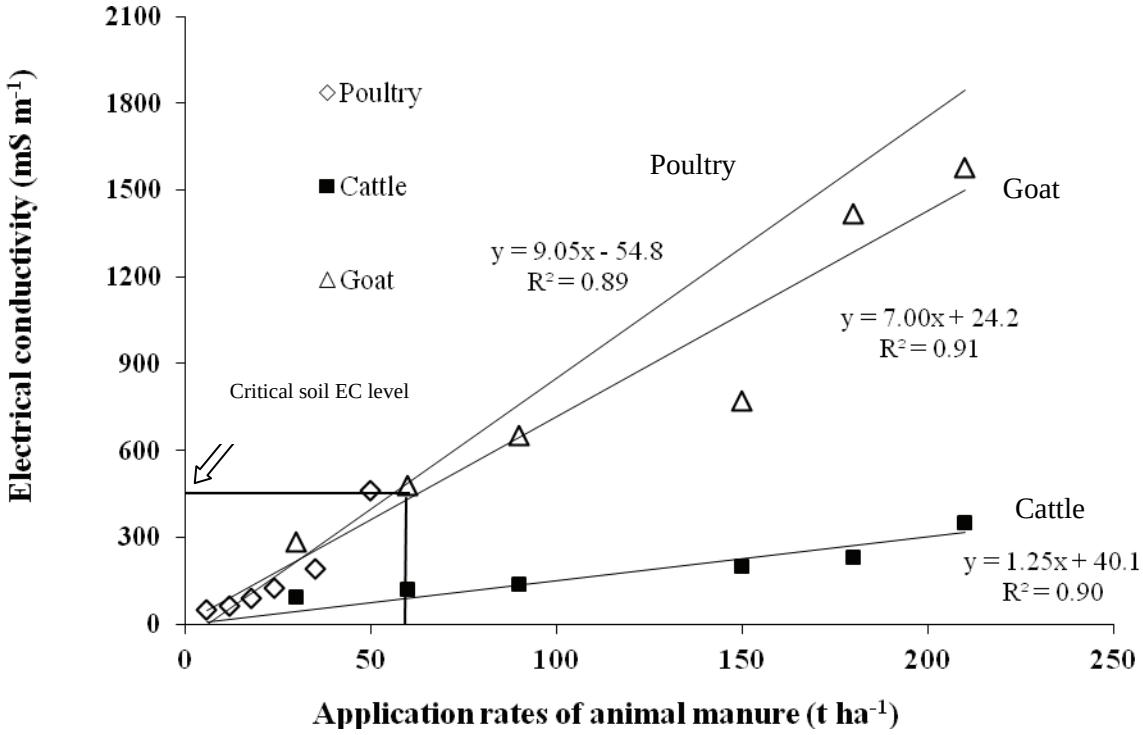

Figure 7.1: Effect of application rate of three types of animal manure on the electrical conductivity of soil

The results in Table 7.12 indicated that with the exception of Chinese cabbage, biomass production of the leafy vegetables in the 30 t ha^{-1} and 60 t ha^{-1} goat kraal manure and cattle kraal manure application treatments were very similar. This suggests that at these two application rates, there was little if any difference in the fertiliser effectiveness of these two kraal manures. However, at the application rate of 90 t ha^{-1}, which tended to be the optimum treatment for goat kraal manure, substantial differences in biomass production were observed between the goat and cattle kraal manure, except in the case of nightshade. This suggests that at the rate of 90 t goat kraal manure ha^{-1}, soil salinity induced by the addition of goat manure had started to materially affect plant growth of pigweed and pumpkin. At the application rate of 90 t goat manure ha^{-1}, the electrical conductivity[2] (EC) of the soil was 653 mSm^{-1}. At the rate of 60 t ha^{-1} the electrical conductivity of the soil was 480 mSm^{-1}. Therefore, the electrical conductivity value of 480 mSm^{-1} could be considered as the threshold value, above which growth of pigweed and pumpkin started to be affected negatively by salinity. Chinese cabbage appeared to have a lower threshold and nightshade a higher threshold.

Figure 7.1 shows that the soil electrical conductivity in the highest Promis application rate treatment was about 450 mSm^{-1} but seemingly negative effects on plant growth resulting from Promis application rates that were too high already occurred at rates as low as 18 t ha^{-1} in the case of pumpkin and pigweed and 24 t ha^{-1} for the other two crops. In other words, the negative effect of supra-optimal application rates of Promis occurred well before the threshold EC value of 480 mSm^{-1} had been reached. This finding confirms the

[2] The EC values were obtained using a mass soil to water ratio of 1:5, which represented an estimated dilution of 18 times the concentration of salts expected to be found in the saturated paste extract of the soil, which is the usual basis for the study of EC effects on growth.

doubts expressed by Fujiwara *et al*. (2009) and Azeez *et al*. (2010) that soil salinity was the primary cause of the negative effect on plant growth of high application rates of poultry manure.

7.4 RESPONSE OF EIGHT AFRICAN LEAFY VEGETABLES GROWN UNDER FIELD CONDITIONS TO DIFFERENT RATES OF CHEMICAL FERTILISER APPLICATION

Whereas pot experiments are a useful tool when initiating nutrient response studies of crops, it has long been known that the nutrient requirements of a crop grown in a pot under greenhouse conditions are not necessarily the same as those of the same crop grown under field conditions (Vandecaveye, 1948; Fried and Broeshart, 1967; Rowell, 1994). Pot experiments employ small quantities of soil. This results in the confinement of root systems and creates artificial growing conditions. Air drying and sieving the soil prior to use in pot experiments results in a flush of mineralisation when the soil is rewetted (Rowell, 1994). When soils with low fertility and organic matter content are used to fill the pots, the application rates of fertilisers needed for optimum growth and yield of the crop tend to be higher than those needed under field conditions. For these reasons, field experiments are necessary to determine the fertiliser requirements of crops more realistically. The main strength of field plot experiments is that the effect of certain agro-chemical measures on crop plants can be studied in the setting where all factors affecting yield, such as climate, soil, plant technology and microbiological activity, are similar to those found in production environments (Davidescu and Davidescu, 1982; FAO, 1998). Field experiments, therefore, provide a much more solid basis for the development of fertiliser recommendations for use by farmers than pot experiments.

The objective of this part of the study was to determine the yield response of eight selected ALVs grown under field conditions to rate of application of nitrogen (N), phosphorus (P) and potassium (K) applied to the soil in the form of chemical fertilisers.

7.4.1 Materials and methods

A series of field experiments using eight different ALVs were conducted at Dzindi Irrigation Scheme (23°1'45"S and 30° 26' 30" E) located in Itsani village, Thulamela Local Municipality, Vhembe District, Limpopo Province, South Africa. Six of the eight test crops were summer crops and two were winter crops. Experiments using the six summer crops were done twice, once during the summer of 2008/09 and then again during the summer of 2009/10. The experiments with the two winter crops were only done once, during the winter of 2010.

Each experiment had three treatments, which were arranged using a complete randomised block design and replicated thrice. Treatments consisted of the control (no fertiliser application), the medium rate of fertiliser application (60 kg N ha^{-1}; 30 kg P ha^{-1} and 40 kg K ha^{-1}), and the high rate of fertiliser application (120 kg N ha^{-1}; 60 kg P ha^{-1} and 80 kg K ha^{-1}). The high fertiliser application rate treatment represented the rate at which maximum biomass production was expected based on the results obtained in the pot experiments. For this purpose, the results of the pot experiments were transformed to quantity of nutrients applied per plant, instead of nutrient concentration, because in the field experiments the fertilisers were band-placed, not broadcast. The medium fertiliser treatment was half the amount of the high fertiliser treatment.

The experimental site was located on Plot 1 of Block 1 of the Scheme on a south-facing, terraced slope of 15% and the soil at the experimental site consisted of a well-drained, clayey Hutton Suurbekom type soil (Soil Classification Working Group, 1991). Prior to the planting of the experiment, the site was had been planted to maize for several years. This crop had been fertilised for optimum yield and as a result, it could be assumed that the soil was of reasonably high fertility. For the six summer crops, seed of pigweed (*A. cruentus* L.); spider flower (*C. gynandra* L.), Jew's mallow (*C. olitorius* L.), cowpea (*V. unguiculata* (L) Walp.); (VOPI reference "Fahari"), pumpkin (*C. maxima* Duchesne) (VOPI reference 'ex Bushbuckridge")

and tsamma melon (*C. lanatus* Thunb.) was obtained from the Vegetable and Ornamental Plant Institute of the Agricultural Research Council at Roodeplaat. For the two winter crops, seed of Chinese cabbage (*B. rapa* L. subsp. *chinensis*) and nightshade (*S. retroflexum* Dun.*)* was obtained from Mr Mabulannga, a smallholder operating at Dzindi.

All crops were seeded directly in the field. At planting, a narrow furrow was opened with a hand hoe along the shoulder of the ridges. Fertilisers to be applied were distributed evenly over the length of this planting furrow and then mixed with soil by dragging a stick along the bottom of the furrow. The water was poured in the furrow at the rate of 2 L per metre row-length and this water was allowed to percolate into the soil. When all the free water had drained into the soil, seed of the crops were sown onto the wet soil using a marked chain to identify the planting stations. At least three seeds were deposited at each station but in the case of small-seeded crops this was raised to 10 seeds. Once sowing was completed, the seed was covered with soil. The thickness of the soil layer that covered the seed was adjusted in accordance with the size of the seed and ranged from about 5 mm in the case of nightshade, Chinese cabbage, pigweed, jute and spider flower to about 20 mm in the case of cow pea and the two cucurbits.

In all experiments, soil water availability was kept at the optimum level. Before planting, the soil received four short furrow irrigations (total of about 80 mm water) to charge the upper part of the profile to field capacity. Following planting; the crops were irrigated twice per week, receiving about 20 mm water per irrigation event, unless abundant rainfall eliminated the need for irrigation. Rainfall was measured daily using a plastic rain gauge that was positioned at the experimental plot at a height of 1.2 m above the soil surface.

Weeds were removed manually or by hand hoeing as they emerged. Pest control measures were limited to the knapsack spray application at planting in the planting furrow after wetting and before sowing of Avalanche, with *alpha-cypermethrin* as the active ingredient, at the rate of 2.5 ml 10 L^{-1} water to control cutworms (Agrotis spp.) and the knapsack spray application on the foliage of the vegetables of Aphox, with *pirimicarb* as the active ingredient, at the rate of 5 g 10 L^{-1} water to control aphids (Aphididae spp.), whenever these were observed during scouting. These control measures were based on recommendations by the Directorate: Food Safety and Quality Assurance (2007).

Specifics for the 2008/09 experiments

In preparation of the 2008/09 experiments, the soil, which had been used the previous year to grow maize that was adequately supplied with nutrients , was ploughed, disked and ridged, mechanically. Gross plot size for the six crops was 2.5 m x 4.5 m. Pigweed, spider flower and Jew's mallow were planted in rows spaced 0.375 m apart using an intra-row spacing of 0.25 m. Pumpkin, tsamma melon and cowpea were planted in rows spaced 0.75 m apart using an intra-row spacing of 0.50 m Table 7.15 shows the dates of planting and harvest that applied to the 2008/09 experiments. All crops were planted simultaneously, one block (replication) per day. The order in which the three blocks of plots were planted was also the order in which they were harvested.

Table 7.15: Dates of planting and final harvest and duration of the growing period of the eight African leafy vegetables used in the 2008-09 fertiliser application rate experiments at Dzindi

Blocks	Planting date	Date of final harvest	Duration of the growing period (days)
Block 1	02 December 2008	28 and 29 January 2009	59
Block 2	03 December 2008	2 and 3 February 2009	64
Block 3	04 December 2008	4 to 6 February 2009	66

Application of chemical fertilisers was done in accordance with the treatments. In the medium and high fertiliser treatments, chemical fertilisers were applied in two stages. During the first stage, the chemical fertiliser mixture 2:3:4 (21) was applied at planting at the rate required to satisfy the full P and K requirement specified in these two treatments but only part of the N requirement. The rest of the N was band-placed 28 days after planting on 31 December 2008. By that time, harvesting had commenced for all six crops. The amount of rainfall (332 mm) and irrigation water (140 mm) received during the growing period was the same for all crops.

Specifics for the 2009/10 experiments

At the start of the 2009/10 experiment, the soil was ploughed, disked and ridged mechanically, in line with standard farmers' practice at Dzindi. Chinese cabbage and nightshade were the first crops that were planted. The gross plot size used for these two crops was 7.5 m x 4.5 m. Planting of the subsequent (summer) crops occurred on the plots that were occupied by Chinese cabbage, whereby each plot was divided into two parts to accommodate two crops. As a result, the gross plot size for the remaining six crops was 3.75 m x 4.5 m. Land preparation for these crops was done by hand and consisted of loosening the soil and reconstructing the ridges.

Plots allocated to a particular fertiliser application rate treatment in the Chinese cabbage experiment maintained that same treatment for all subsequent crops. This meant that the nutrient status of the soil in the plots allocated to the control treatment was expected to decline over the duration of the study, whilst that in plots allocated to the high fertiliser application treatment could possibly have improved.

The eight experiments were planted at different times during the year. The planting dates of the different crops were selected in accordance with their preferences. Date of planting, date of harvest and duration of the growing period are shown in Table 7.16.

Table 7.16: Dates of planting and final harvest and duration of the growing period of the eight African leafy vegetables used in the 2009-10 fertiliser application rate experiments at Dzindi

Crops	Planting date	Date of final harvest	Duration of the growing period (days)
Chinese cabbage	05 June 2009	12 August 2009	68
Nightshade	05 June 2009	24 October 2009	141
Pumpkin	18 September 2009	09 November 2009	52
Tsamma melon	18 September 2009	09 November 2009	52
Jew's mallow	21 October 2009	17 December 2009	57
Cowpea	21 October 2009	17 December 2009	57
Spider flower	09 December 2009	31 January 2010	53
Pigweed (amaranth)	09 December 2009	31 January 2010	53

Application of chemical fertilisers was done in accordance with the treatments but modifications were made to the way the fertilisers were applied, because there was some evidence of fertiliser burn among some seedlings in the high fertiliser treatment in the 2008/09 experiment. This suggested that the fertiliser concentration in the planting furrow of this treatment might have been too high. For this reason, a new strategy of applying fertilisers was developed. In the medium and high fertiliser treatments, chemical fertilisers were applied in three stages. During the first stage, which occurred at planting, the chemical fertiliser mixture 2:3:4 (30) was again applied at the rate required to satisfy the full P and K requirement specified in these two treatments but half of the required amount was broadcast in the old irrigation furrows and worked into the soil by means of a hand hoe. This enriched soil was then used to reconstruct the ridges.

The other half of the chemical fertiliser mixture was distributed evenly at planting in the planting furrow. The outstanding N was band-placed by opening a furrow 10 cm to the side and below the planting row, half after thinning and the other half two weeks later. Data on rainfall and irrigation water that was applied to the crops in the 2009/10 experiments appear in Table 7.17.

The Chinese cabbage experiment was terminated when peduncle elongation has commenced in 50% of the plants. The nightshade experiment ended after the third shoot cutting. All other experiments were terminated about eight weeks after planting but the exact duration of the growing period of each crop is shown in Table 7.16.

Data collection

For all eight ALVs, plant response to fertiliser application rate was determined using four indicators, namely the mass of the edible portion (fresh and oven-dry) and total above-ground biomass (fresh and oven-dry). Biomass data were collected from the plants growing in the net plot. The net plot was obtained by excluding the two outer rows of the gross plot and the first and last plant in each of the remaining four rows. The protocols described by Van Averbeke *et al*. (2007a) were used for the harvest of the edible portion of Chinese cabbage and nightshade and those described in section 7.2.1 for the harvest of the edible portion of pumpkins, tsamma melon, cowpeas and amaranth (pigweed).

Table 7.17: Rainfall and irrigation water received by the eight different African leafy vegetables that featured in the 2009-10 fertiliser application rate experiments at Dzindi

Crop	Rainfall (mm)	Irrigation (mm)	Total (mm)
B. rapa L. subsp. *chinensis*	8	340	348
S. retroflexum Dun.	8	500	508
C. lanatus Thunb.	11	240	251
C. maxima Duchesne.	11	240	251
C. olitorius L.	413	120	533
V. unguiculata (L.) Walp.	413	120	533
A. cruentus L.	154	200	354
C. gynandra L.	154	200	354

For Jew's mallow (*C. olitorius*) the first harvest occurred at the six-leaf stage when the seventh leaf started to appear. When the plant had reached this stage, the tip of the stem, taken as the upper 2 cm, was cut with a pair of scissors. One week after the first harvest, the same procedure was repeated. Harvesting leaves from branches occurred when the branch had three leaves and the fourth leaf started to appear. As in the case of the main stem, the upper 2 cm of the stem was cut off. This weekly procedure was repeated until 50% of the plants in the stand had started to flower. The leaves that remained on the stem were left until end of the growing period to form part of the above-ground biomass that was not edible. The edible portion consisted of the total fresh mass of the tips (stem tip, petioles and leaf blades) that were removed during the successive harvests. At the end of the growing season, the entire plant was removed by cutting the stem with secateurs at the soil surface level and its fresh and oven-dry mass were determined. The first leaf harvest of spider flower (*C. gynandra*) occurred when the plants had reached the four-leaf stage and the fifth leaf started to appear and involved cutting the upper 2 cm of the stem with a pair of scissors. One week later, the same procedure was repeated for the main stem, whilst harvesting from branches occurs by cutting the upper 2 cm of the branches that had at least three fully unfolded leaves. This weekly procedure was repeated until 50% of the plants in the population had reached the flowering stage, when the entire plant was removed by cutting the stem with secateurs at the soil surface level. The leaves that remained on the plants were left until the end of the growing period to form part of the above-ground biomass that was not edible. For all crops, the harvested plant parts were weighed immediately after removal to determine their fresh mass, after which

moisture samples were taken to determine their dry mass. The moisture samples were dried in an oven at 60ºC until constant mass. An Adam portable electronic scale, manufactured by Adam equipment Co. Ltd., with a capacity of 3000 g and an accuracy of 1 g was used for the measurement of fresh biomass and the Scout® Pro RS232, manufactured by Ohaus, with capacity of 600 g and 0.01 g accuracy was used for the measurement of oven-dry biomass.

Data analysis

The data were captured on spread sheets using MS Office Excel ®, and analysed using the SAS statistical software version 9.2 (Statistical Analysis Software Institute, 1999). Statistical analysis of the data involved analysis of variance to identify treatment effects and when these effects were statistically significant ($p \leq 0.05$) the Fisher's Protected Least Significant Difference test ($p=0.05$) was used to separate treatment means. Statistical analysis of the data was done by the Biometric Division of the Agricultural Research Council in Hatfield, Pretoria.

7.4.2 Results

Pictures of the growth of pumpkin, pigweed, spider flower, cowpeas, non-heading Chinese cabbage, nightshade, Jew's mallow and tsamma melon in the high fertiliser treatment of the 2009 and 2009/10 experiments at Dzindi Irrigation Scheme are shown in Plates 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 and 7.10, respectively.

Plate 7.3: Growth of pumpkin (*Cucurbita maxima*) in the high fertiliser treatment of the 2009/10 experiment at Dzindi Irrigation Scheme

Plate 7.4: Growth of pigweed (*Amaranthus cruentus*) in the high fertiliser treatment of the 2009/10 experiment at Dzindi Irrigation Scheme

Plate 7.5: Growth of spider flower (*Cleome gynandra*) in the high fertiliser treatment of the 2009/10 experiment at Dzindi Irrigation Scheme

Plate 7.6: Growth of cowpeas (*Vigna unguiculata*) in the high fertiliser treatment of the 2009/10 experiment at Dzindi Irrigation Scheme

Plate 7.7: Growth of non-heading Chinese cabbage (*Brassica rapa* subsp. *chinensis*) in the high fertiliser treatment of the 2009 experiment at Dzindi Irrigation Scheme

Plate 7.8: Growth of nightshade (*Solanum retroflexum*) in the high fertiliser treatment of the 2009 experiment at Dzindi Irrigation Scheme

Plate 7.9: Growth of Jew's mallow (*Chorchorus olitorius*) in the high fertiliser treatment of the 2009/10 experiment at Dzindi Irrigation Scheme

Plate 7.10: Growth of tsamma melon (*Citrillus lanatus*) in the high fertiliser treatment of the 2008/09 experiment at Dzindi Irrigation Scheme

The effect of rate of chemical fertiliser application on biomass production of the six crops planted in the 2008/09 summer is shown in Table 7.18. On average, pumpkin produced the highest biomass, followed by pigweed, tsamma melon, cowpea, Jew's mallow and spider flower. Statistically, all six crops responded positively to the application of fertiliser in terms of total fresh above-ground biomass. Further significant ($p \leq 0.05$) increases in total fresh above-ground biomass were recorded for cowpea and pigweed when rate of application of fertilisers was raised from the medium to the high level. Pumpkin and tsamma melon also tended to respond positively to raising fertiliser application rate from the medium to the high level but the differences in total fresh above-ground biomass between these two fertiliser treatments were not statistically significant ($p > 0.05$). For pumpkin, the trend for biomass production to increase when fertiliser rate was raised from medium to high was supported by the significant ($p \leq 0.05$) difference in the total oven-dry above-ground biomass between the medium and high fertiliser treatment. In the case of Jew's mallow, the total oven-dry above-ground biomass obtained in the medium and high fertiliser treatments were similar. Total fresh above-ground biomass of spider flower tended to decline when the fertiliser application rate was raised from medium to high.

Table 7.18: Effect of fertiliser application rate on biomass production of six African leafy vegetables (Dzindi, summer 2008-09)

Fertiliser application rate treatment	Pumpkin	Tsamma melon	Cowpea	Jew's mallow	Spider flower	Pigweed
	----------Total fresh mass of edible portion (g m^{-2})----------					
Control	1131^{b}	554^{b}	1331^{b}	441^{c}	440	500^{c}
Medium	4987^{a}	2144^{a}	1453^{b}	1276^{b}	1349	2224^{b}
High	5082^{a}	2695^{a}	1733^{a}	1562^{a}	915	4070^{a}
LSD (p=0.05)	1055	771	156	241	NS	1397
CV%	12.5	18.9	4.6	9.7	36.2	27.2
	----------Total oven-dry mass of edible portion (g m^{-2})----------					
Control	126^{b}	60^{b}	139^{b}	38^{c}	44	41^{c}
Medium	497^{a}	217^{a}	151^{b}	124^{b}	139	277^{b}
High	513^{a}	284^{a}	177^{a}	162^{a}	89	410^{a}
LSD (p=0.05)	103	148	14	22	NS	
CV%	12.0	34.9	3.8	9.2	37.9	11.1
	----------Total fresh above-ground biomass (g m^{-2})----------					
Control	1535^{b}	844^{b}	2073^{c}	1220^{b}	554^{b}	9459^{c}
Medium	7401^{a}	4198^{a}	2734^{b}	3840^{a}	3873^{a}	3708^{b}
High	8724^{a}	5324^{a}	3288^{a}	3951^{a}	3040^{a}	8847^{a}
LSD (p=0.05)	1542	1643	280	610	2117	2028
CV%	11.6	21.0	4.6	9.0	37.5	19.9
	----------Total oven-dry above-ground biomass (g m^{-2})----------					
Control	174^{c}	94^{b}	225^{c}	107^{b}	99	72^{c}
Medium	738^{b}	364^{a}	266^{b}	374^{a}	390	430^{b}
High	957^{a}	480^{a}	337^{a}	405^{a}	304	881^{a}
LSD (p=0.05)	53	215	32	41	NS	87
CV%	3.7	30.4	5.1	6.2	37.8	8.3

Control (0 kg N ha^{-1}, 0 kg P ha^{-1}, 0 kg K ha^{-1}); Medium (60 kg N ha^{-1}, 30 kg P ha^{-1}, 45 kg K ha^{-1}); High (120 kg N ha^{-1}, 60 kg P ha^{-1}, 90 kg K ha^{-1}); NS = treatment effect was not statistically significant (p>0.05)

The effect of rate of chemical fertiliser application on biomass production of the eight crops grown during 2009/10 is shown in Table 7.19. Biomass production differed among the eight crops. Nightshade produced the highest biomass but its growing season was also much longer than that of the other crops, which had growing seasons of comparable duration. Second in line in terms of biomass production was pumpkin, followed by amaranth (pigweed), Chinese cabbage, cowpea, tsamma melon, Jew's mallow and spider flower taking last place. With oven-dry mass of the edible portion of cowpea being the only exception, treatment effects were significant ($p \leq 0.05$) for all four biomass indicators of all eight crops.

Biomass production in the control treatment was substantially lower than in the treatments that received the fertiliser application. Differences in biomass production of cowpea, Jew's mallow, spider flower, pigweed and Chinese cabbage between the medium and the high rate of fertiliser application were not significant (p>0.05) and numerically these differences were also small. However, the tendency was for biomass to increase slightly when the rate of fertiliser application was increased from medium to high. This was not the case for spider flower, because three of the four biomass indicator values obtained in the medium fertiliser application rate treatment were numerically higher than those recorded in the high fertiliser application rate treatment.

Table 7.19: Effect of fertiliser application rate on biomass production of eight African leafy vegetables (Dzindi, 2009-10)

Fertiliser application rate treatment	Pumpkin	Tsamma melon	Cowpea	Jew's mallow	Spider flower	Pigweed	Chinese cabbage	Nightshade
	----------Total fresh mass of edible portion (g m^{-2})----------							
Control	676^{c}	285^{c}	921^{b}	187^{b}	279^{b}	621^{b}	422^{b}	2061^{c}
Medium	2773^{b}	1024^{b}	1702^{a}	614^{a}	672^{a}	2020^{a}	1791^{a}	6781^{b}
High	3445^{a}	1750^{a}	1751^{a}	554^{a}	715^{a}	1712^{a}	2217^{a}	8175^{a}
LSD (p=0.05)	638	592	357	205	258	666	460.4	1355
CV%	12.2	22.5	9.3	26.2	20.5	20.3	13.7	10.5
	----------Total oven-dry mass of edible portion (g m^{-2})----------							
Control	47^{c}	18^{b}	65^{b}	33^{b}	37	80^{b}	41^{b}	282^{b}
Medium	154^{b}	47^{ab}	106^{a}	90^{a}	74	227^{a}	141^{a}	625^{a}
High	181^{a}	84^{a}	100^{a}	70^{ab}	89	255^{a}	144^{a}	792^{a}
LSD (p=0.05)	127	38	21	38	NS	78	56	236
CV%	9.0	32.7	10.2	28.1	27.4	18.4	22.8	18.4
	----------Total fresh above-ground biomass (g m^{-2})----------							
Control	1050^{c}	421^{c}	1521^{b}	352^{b}	631^{b}	1046^{b}	975^{b}	3451^{c}
Medium	4241^{b}	1576^{b}	2907^{a}	1539^{a}	1645^{a}	4433^{a}	3079^{a}	12704^{b}
High	5520^{a}	2781^{a}	2959^{a}	1322^{a}	1697^{a}	3694^{a}	3668^{a}	16185^{a}
LSD (p=0.05)	1 004	811	521	551	572	1021	732	2882
CV%	12.3	25.6	10.8	20.0	19.1	14.7	12.5	11.8
	----------Total oven-dry above-ground biomass (g m^{-2})----------							
Control	76^{b}	36^{b}	119^{b}	55^{b}	72^{b}	124^{b}	112^{b}	433^{c}
Medium	289^{a}	98^{b}	222^{a}	182^{a}	167^{a}	476^{a}	254^{a}	1048^{b}
High	330^{a}	185^{a}	219^{a}	151^{a}	173^{a}	407^{a}	265^{a}	1391^{a}
LSD (p=0.05)	47	79	33	82	79	125	90	342
CV%	9.0	33.7	7.7	22.7	25.3	16.4	19.0	15.8

Control (0 kg N ha^{-1}, 0 kg P ha^{-1}, 0 kg K ha^{-1}); Medium (60 kg N ha^{-1}, 30 kg P ha^{-1}, 40 kg K ha^{-1}); High (120 kg N ha^{-1}, 60 kg P ha^{-1}, 80 kg K ha^{-1}); NS = treatment effect was not statistically significant (p>0.05)

7.4.3 Discussion and conclusion

The field experiment conducted on what was assumed to be a soil of reasonable fertility indicated that ALVs required additions of the important plant nutrients to produce optimally. The results clearly showed that the notion that these crops are well adapted to conditions of low soil fertility commonly encountered in smallholder fields was not really valid. However, the results also showed that some leafy vegetable species had lower fertility requirements than others. Jew's mallow and spider flower produced optimally in the medium fertiliser application rate treatment, which consisted of the application of 60 kg N ha^{-1}, 30 kg P ha^{-1}, 40 kg K ha^{-1}. Nightshade, pumpkin and tsamma melon required the high rate of fertiliser application treatment (120 kg N ha^{-1}, 60 kg P ha^{-1}, 80 kg K ha^{-1}) to produce optimally. For nightshade and pumpkin this could be because they were also the crops that produced the highest biomass.

7.5 CONCLUSIONS

This study was aimed at determining the plant nutrient requirements of a selection of important ALVs and to characterise their response to the application of fertilisers. The results that were obtained warrant a few general conclusions.

Firstly, the study showed that all eight crop species responded positively to the application of fertilisers. The greenhouse pot studies indicated that at least four of the eight ALVs that were tested had nutrient requirements that were not dissimilar of those of Swiss chard. Earlier work by Van Averbeke *et al*. (2007a), in which the fertiliser response of non-heading Chinese cabbage and nightshade was investigated, showed that this conclusion also applied to these two crops. These findings question the notion that ALVs have lower nutrient requirement for optimum growth than exotic leafy vegetables, such as Swiss chard.

Secondly, the results support the proposition that ALVs differ in terms of their nutrient requirements. In the field experiments, which included all eight crops, spider flower and Jew's mallow tended to have lower fertiliser requirements than the other crops, whilst pumpkin and nightshade had the highest nutrient requirements.

Thirdly, application of nutrients to ALVs must be managed carefully. In the case of chemical fertilisers, it appeared safe to apply 20 kg N ha^{-1}, 30 kg P $ha^{-1}$and 40 kg K ha^{-1} in the form of a (2:3:4) fertiliser mixture in the planting furrow. To avoid fertiliser burn, this rate of application should not be exceeded when using band application at planting. When higher rates need to be used, as in the case of low fertility soils, the remainder of the fertiliser mixture should be applied broadcast. Application of additional N during the growing season at rates of about 20 to 30 kg N ha^{-1} as band placed top dressing worked well. The use of two topdressings of N at the indicated rates appeared to be best practice.

Finally, the study showed that application of animal manure also had positive effects on biomass production of a selection of four ALVs. Differences in the quality of animal manure were identified. Salt content was identified as an important attribute that affected the response of these crops to application rate of animal manure. Factors other than salinity appeared to be responsible for biomass reductions when Promis® poultry manure was applied at high rates. To what extent these findings arising from greenhouse experiments in pots would also apply in the field was not investigated and this warrants additional research.

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CHAPTER 8: DISEASE AND PEST MANAGEMENT OF AFRICAN LEAFY VEGETABLES

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8.1 INTRODUCTION

The worldwide use of broad-spectrum pesticides, fungicides, nematocides and bactericides has revolutionized pest and disease management. There is growing concern however about environmental contamination, human health risks and the continuing frustration over the ability of pests and diseases to develop resistance to these chemicals. The global modern approach is Integrated Pest Management (IPM) which promotes both a healthier, balanced ecosystem as well as agricultural productivity (Dobson et al., 2002; Quarles, 2008). This approach to pest and disease management includes the use of organic production where micro-organisms, biological products, resistant cultivars and cultural control form the integral part of pest and disease management, with chemical control limited to the minimum and only as the last resort in pest and disease management (Elwell and Maas, 1995; Slabbert and Ehlers, 2005). There are currently no registered agro-chemicals available for most of the ALVs. Organic vegetable production is based on similar practices that do use very little or no synthetic chemicals (pesticides, fertilizers, and other agrochemicals) unless those chemicals are composed of natural products, largely unaltered extractions from plants, animals, microbes, or minerals. The main emphasis for organic production is based on cultivation practices of crops.

Organic agricultural methods are internationally regulated and legally enforced by many nations, based in large part on the standards set by the International Federation of Organic Agriculture Movements (IFOAM), an international umbrella organization for organic organizations established in 1972. IFOAM defines the overarching goal of organic farming as follows:
"Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved" (IFOAM, 2010).

8.2 METHODOLOGY

This report refers to the most important pests and diseases on the selected African leafy vegetables, which were identified during field trials done by ARC – Roodeplaat, as well as through a literature study. This report also refers to the control of pests and diseases in general on ALVs, with emphasis on organic control with suitable non-chemical control strategies in ALV production. The components of pest and disease control strategies for ALVs were investigated through a literature study for the following phases: selection of seed and plant material, field production, harvesting and storage. Because of a lack of literature available on pest and disease control for many of the traditional ALVs under investigation, comparisons have been made with pest and diseases prevailing in conventional vegetable production of some of the major vegetable crops. This report consists of a literature study of two parts:
1) The most important diseases and pests occurring on ALVs
2) Control strategies for diseases and pests on ALVs

8.3 MAJOR PESTS AND DISEASES OF SELECTED AFRICAN LEAFY VEGETABLES

8.3.1 *Amaranthus cruentus*

Important pests

Amaranth is susceptible to a number of insects (Grubben, 1976; El-Aydam and Burki, 1997), although amaranths are able to recover after feeding by most leaf-chewing insects (Brenner *et al.*, 2000). Tarnished plant bugs, leaf miners, flea beetle, grasshopper, caterpillars and amaranth weevil, are potentially significant insect pests of amaranth. The tarnished plant bug (*Lygus*), a sucking insect which often reaches high populations in the seed head during the critical seed fill stage, affect yields. Flea beetles damage young leaf tissue. The adult amaranth weevil feeds on leaves, but the larval stage is more damaging because they bore into the central tissue of roots and occasionally stems, causing rotting and potentially lodging (Alternative Field Crops Manual, 2000).

Important diseases

Researchers and growers have observed little in the way of major disease problems. Disease problems may develop in large monoculture production systems. Damping-off of young seedlings caused by *Pythium* can be a problem under some environmental conditions (Brenner *et al.*, 2000), and *Rhizoctonia* and stem canker, caused by *Phorma* or *Rhizoctonia* (Alternative Field Crops Manual, 2000). *A. tricolor* also seems to be very susceptible to *Phomopsis*, which colonizes leaves and stems and causes dieback (Bansal, 1996).

8.3.2 *Brassica rapa* L. subsp. *chinensis*

Important pests

Turnip aphid, root maggot and flea beetles are the most injurious insect pests in the cabbage family. *Brassicae* may be parasitized by *Orobanche cernua* (Alternative Field Crops Manual, 2000).

Important diseases

Clubroot (*Plasmodiophora brassicae*), TuMV virus and soft rot are the most serious diseases (Talekar and Griggs, 1981:10). Other fungi reported to attack *Brassicae* include: *Albugo candids, Alternaria* spp., *Fusarium oxysporum, Pythium ultimum, Rhizoctonia sp.* and *Sclerotinia* spp. The following bacteria can cause disease: *Streptomyces scabies, Agrobacterium tumefaciens, Bacterium aroideae, Erwinia carotovora, E. aroideae, Pectobacterium carotovorum, Pseudomonas maculicola, P. madrasensis, Xanthomonas* spp. Viruses isolated from turnips include: beet mild yellowing, beet ringspot, cabbage blackspot, cauliflower mosaic, crinkle mosaic, cucumber mosaic, turnip mosaic and curly top. Nematodes attacking turnips include: *Belonolaimus longicaudatus, Ditylenchus dipsaci, Helicotylenchus* spp., *Meloidogyne* spp., *Nacobbus aberrans, Pratylenchus* spp. *and Trichodorus christiei* (Alternative Field Crops Manual, 2000).

8.3.3 *Citrillus lanatus* (Thunb.)

Important pests

Some of the most important pests mentioned for related species of the watermelon family are aphids, blister beetles pickleworm, spider mites, spotted and striped cucumber beetle and thrips (Sudha and Lakshmanan, 2007). The melon fly (*Daucus* sp.) affects young fruit, while the major pests of stored seeds are *Trilobium casteneum* and *Lasioderma serricone* (Schippers, 2000).

Important diseases

Watermelon is susceptible to a wide range of diseases: Cercospora leaf spot (*Cercospora citrullina*), Downy mildew (*Pseudoperonospora cubensis*), which does not reliably appear in fields, but its occurrence should always be a cause for concern because of its ability to quickly increase and become uncontrollable.

Alternaria leaf blight (*Alternaria cucumerina*), gummy stem blight (*Didymella bryoniae*), powdery mildew, *Acidovorax avenae* subsp. c*itrulli*, Bacterial rind necrosis and Belly rot can be caused by several species of soilborne fungi. Viruses includes the squash leaf curl virus, transmitted by whiteflies, pimples and ringspots (tobacco ringspot virus) (Sudha and Lakshmanan, 2007). Damping off (*Marcrophomina phaseolina*) can cause serious seedling losses, while *Anthracnose*, which is seed transmissible, must be controlled (Schippers, 2000).

8.3.4 *Cleome gynandra* L.

Important pests

In general literature refers to *Cleome* as being pest and disease resistant, although a number of pests are reported to attack the crop in general, including beetles and Harlequin bugs *(Bagroda hilaris)* (Mingochi and Luchen). Other pests reported are following: pentatomids (*Acrosternumgramineum* and *Agonoselis nubilis*) and their parasitoids; locusts (*Schistocera gregaria*); nematodes (*Meloidogyne* spp.); flea beetles (*Phyllotreta mashonana*); greenvegetable bugs (*Nezara* spp.); cabbage sawfly (*Athalia* spp.); cotton jassids (*Empoasca*spp.) and hurricane bugs (*Bagrada* spp.). The hurricane bug renders stand establishment virtually impossible (Chweya and Mnzava, 1997; The Australian New Crops Newsletter, 1999). Aphids can cause leaf damage (Schippers, 2000).

Important diseases

C. gynandra is host to the mildew fungus (powdery mildews *Sphaerotheca fuliginea*, *Oidiopsis taurica* and *Cercospora uramensis*) (Chweya and Mnzava, 1997; Schippers, 2000)

8.3.5 *Corchorus olitorius* L.

Important pests

Corchorus tridens is susceptible to red spider mites (*Tetranychus urticae*), yellow mites, leaf beetles (*Podagrica sjostedji*), sweet potato butterfly (*Acraea acerata* and *Acraea terpsichore*) and root-knot nematode (*Meloidogyne* spp) (Mnzava, 2004). Leaf-eating grasshoppers, *zonocerus variegates*, caterpillars (*Acraea* family) and the armyworm (*Spodoptera littoralis*) can greatly reduce yields (Schippers, 2000). Furthermore flea beetles (*Podagrica* spp.), black beeltes (*Epithrix torvi*) and red spider mites (*Tetranychus cinnabarinus*) can cause crop losses (Schippers, 2000).

Important diseases

Major diseases include wilting by *Sclerotium rolfsii* and leaf spot by *Cercospora corchori* (Schippers, 2000; Mnzava, 2004). Others diseases reported to occasional cause disease are black leafspot (*Curvularia* sp.) and stemblight (*Macrophomina phaseoli*). Powdery mildew (*Erysiphe* sp.) can be more serious during the dry season (Schippers, 2000).

8.3.6 *Cucurbita maxima* Duchesne

Important pests

Major insect pests of the pumpkins and squash includes squash bugs, cucumber beetles, squash vine borers, aphids and mites (Alternative Field Crop Manual, 2000).

Important diseases

Defoliation for relish may continue for several months where interference from pests and diseases is low because plants continue producing new leaves sometimes long after the rainy season is over (Mingochi and Luchen). Some of the major diseases of squash and pumpkin include: *Anthracnose*, bacterial leaf and fruit

spot, bacterial wilt (carried by the striped and spotted cucumber beetle), black rot, downy mildew, powdery mildew and *Phytophthora* blight. (http://www.hort.purdue.edu/rhodcv/hort410/squash/sq00001.html).

8.3.7 *Solanum retroflexum* Dun.

Important pests

Insect damage by chewing insects are often noticed on the leaves (Schippers, 2000). Ants, black aphids, caterpillars, and occasionally grasshoppers, can cause damage to the leaves. Beetles such as small black beetles (possible flea beetles), as well as *Lagria* sp., *Podagrica* sp. and *Epilachna* sp. are some of the commonly found beetles on nightshade (Schippers, 2000).

Important diseases

Leaf blight (Vanitha *et al.*, 2005), rotting caused by *Phytophthora infestans* (Dead, Shaw and Cooke, 2004), powdery mildew (*Leveillula taurica*) (Sudha and Lakshmanan, 2007). Nematodes can cause plant damage, as well as pathogen damage caused by *Alternaria solani* (early blight), *Cladosporium oxysporum* (grey mold), *Cercospora nigrescens* (eye spot), *Leveillula taurica* (powdery mildew), *Ralstonia solanacearum* (bacterial wilt), while leaf curl virus, and yellow vein virus, the latter transmitted by the whitefly (*Bemisia tabaci*) (Schippers, 2000).

8.3.8 *Vigna unguiculata* (L.) Walp.

Important pests

Insects pests reported are Mexican bean beetle, bean leaf beetles, cowpea curculio, aphids, green stink bug, and to a lesser extend also the maize stalk borer (and maybe others), and weevils (when in storage) (Davis *et al.*, 2003). Important pests also include the cowpea aphid (*Aphis craccivora*), various leaf hoppers, the Egyptian leaf worm (*Spodopteris littoralis*), larvae of the African bollworm (*Heliothis armigera*) and the cowpea leaf beetle (*Ootheca mutabilis*) (Schippers, 2000).

Important diseases

Diseases reported are fusarium wilt, bacterial canker, southern stem blight, cowpea mosaic virus (and several other less prominent viruses), *Cercospora* leaf spot, rust and powdery mildew. The rootknot nematode and damping off can also be a problem (Davis *et al.*, 2003). Other diseases reported are brown blotch (*Colletotrichum capsici*), Septoria leaf spot (*Septoria vignae*), stem cancer (*Macrophomina phaseolina*) and bacterial blight (*Xanthomonas campestris*), scab (*Sphaceloma* sp.), brown rust (*Uromyces appendiculatus*) and web blight (*Rhizoctonia solani*) (Schippers, 2000).

8.4 GENERAL STRATEGIES FOR ORGANIC CONTROL OF PESTS AND DISEASES IN AFRICAN LEAFY VEGETABLES

Very little quantitative information on pest and diseases on specific traditional ALVs is available, even more so concerning organic ALV production. Therefore a general approach on organic pest and disease control in conventional agriculture has been considered to provide novel approaches for pest and disease control in ALVs.

The management of pests and diseases rarely relies on a single control practice; usually a variety of tactics are integrated to maintain pests and diseases at acceptable levels. The goal of organic pest and disease control is not to eliminate all pests and diseases; some pests and diseases are tolerable and essential so that their natural enemies remain in the crop. Rather, the aim is to reduce pest and diseases populations to less than damaging numbers. The control tactics used in organic pest and disease management include pest and diseases resistant or tolerant plants, cultural, physical, mechanical and biological control methods, and

minimal chemical control. Chemical control will only include natural, safe extracts and products, which would be readily available to most ALV farmers. Applying multiple control tactics minimizes the chance that insects and pathogens will adapt to any one tactic. The focus of this report is on organic control.
Currently the ALVs end-user can be broadly divided into two groups:

- Home gardens, usually located adjacent to homes, typically combines production of different crops, vegetables and livestock. Diversity in size, form and function make it difficult to define home gardens, but their place in the farming systems of the rural landscape is readily recognized. Home gardeners will usually only utilize immediate recourses available to control pests and diseases.
- The market-orientated smallholders typically practices monoculturing of crops on larger scale. These farmers usually have additional recourses available such as sprayers, storing facilities, and will buy chemicals if necessary to control pests and diseases. This report will however not deal with chemical control.

The approach for organic pest and disease management in ALVs will therefore be basically similar for the two groups mentioned above. Components of pest and disease control strategies for ALVs was investigated for the following three phases: selection of seed and plant material, field production, as well as harvesting, drying and storage of seed.

8.4.1 Selection of seed/plant material

Purchase certified seeds or seeds collected from dry, disease free regions. Pests and pathogens should be prevented from entering the production system as far as possible (Slabbert and Ehlers, 2005). Only healthy seed that has been selected from the previous harvest, stored away from possible insect and pathogen contamination, which has no visual pest or disease symptoms, should be used as propagative material. This will decrease the disease incidence. Farmers and growers who produce their own seedlings should follow strict guidelines to insure quality planting material. Sow disease-free seed in a clean, disease free growth medium. Growth flats previously used in transplant production should be disinfected with copper sulfate or by exposure for 15-20 minutes in hot water (minimum of 50ºC) (Organic Vegetable IPM Guide, 2006).

8.4.2 Field production

Plant pests and diseases occur over a reasonably wide range of environmental factors. The degree of occurrence of a pest or disease as well as the seriousness of the pest or disease is influenced and determined by the deviation from the optimum for pest and disease development for each specific pest and disease, of each factor. These factors include host plant, specific pest or disease, environment and human involvement. This entails a complex interaction of all the factors involved (Slabbert and Ehlers, 2004).

Pest and disease management entails the selection and use of applicable techniques to reduce disease occurrence to a tolerable level. The applicability of a technique is determined by various types of information: the insect or pathogen involved, the epidemiological characteristics of the agro-eco system as well as the effectiveness of the specific technique. Pests and diseases may increase to an intolerable level if any of this information is unavailable. The definition of ‘tolerable level’ is complex, and depends on disease dynamics, economics as well as social and health factors. Techniques that can be used by farmers to reduce pests and diseases in crop production includes:

Cultural control

Site selection

Site selection will be very important for the recourse poor farmer. A farmer should avoid areas of planting where knowledge previous crop failures due to soil borne insects and diseases (such as *Fusarium* and *Verticillium* wilt, bacterial soft rot, clubroot, nematodes) are known. Many of these spores and eggs are very tolerant and will survive in the soil for many years, even in the absence of a host (Agrios, 2005). The farmer is advised to select a planting field away from such areas. Other planting areas that can create risks are pastures, foothills, riverbanks, grasslands, and other areas that support weeds and natural vegetation – these areas are often reservoirs for pathogens and should be avoided (Koike *et al*., 2008). To reduce chances of damping-off, root rot, and other problems associated with wet soils, choose a well-drained site. If drainage is a problem, plant on raised beds to promote drainage and faster warming of soil. Irrigation should be done with care, and soil should not be too wet, as this will encourage pathogen growth and colonization. Correct irrigation practises and good drainage conditions encourage faster seed germination, seedling emergence, and young plants more resistant to seedling disease infection. Similarly, transplants are less susceptible to root disease problems when grown in raised beds (Organic Vegetable IPM Guide, 2006).

Weed control

Certain insects are known to transmit viruses from infected to healthy plants. Perennial weeds in and around the field should be destroyed before planting, since these often act as overwintering hosts for harmful viruses. In spring, aphids, thrips, and other insects feed on virus-contaminated weeds, pick up virus particles, and carry them to healthy plants that are then spread in secondary cycles within the planting (Organic Vegetable IPM Guide, 2006).

Soil test to determine fertility needs

Plants fertilized according to recommendations based on a soil test are not as likely to have disease problems as are those plants that are low in nutrition or receive an imbalance of nutrients. Excess levels of nitrogen have been associated with some foliage diseases as well as increased *Rhizoctonia* seedling disease of peas, beans, and other vegetables. Although the exact role of individual nutrients in disease development is not clearly understood, it does appear that fertilizers play a role in an overall organic disease management program. Fertilizers may not prevent pests and diseases, but a healthy, well-fertilized plant is less susceptible to pests and disease than one growing in soil lacking required nutrients (Organic Vegetable IPM Guide, 2006; Slabbert and Ehlers, 2005).

Sanitation

Sanitation refers to keeping the area clean of plants or materials that may harbour pests. Examples include removal of weeds in greenhouses that may harbour mites, aphids, or whiteflies; destruction of crop residues that may be overwintering sites for insects and pathogens (Agrios, 2005). Farm equipment and shoes that can spread pests and disease between fields should be cleaned daily. Farmers should also keep their implements, example spades, pliers, clean from any plant or soil residue that could harbour and spread pests and diseases. This can be done by simply dipping the tools into a 1% JIK^{TM} solution for 5 minutes before use (Slabbert and Ehlers, 2005). Washing the soil off farm equipment before moving it from one field to another may help to prevent the movement of pathogens present in the soil, such as nematodes or fungal spores. Examine plants at least twice a week for symptoms of pests and disease. Look for leaf damage, discolouration such as yellowing, leaf spots, stunting, fruit rots, misshaped leaves, wilting, cankers and stem damage or rotting. If you detect only a small amount of disease, removal of infected foliage or plant will help reduce the amount of inoculum that may be spread to disease-free plants on the same or nearby plants. Remove and destroy badly diseased plants, including the roots, since remedial treatment of such plants is not effective. In some cases, removal of surrounding soil is necessary. Do not place diseased plants and plant parts in cull piles near production sites (Organic Vegetable IPM Guide, 2006). Infected plant material can also be buried or burned (Slabbert and Ehlers, 2004).

The planting of several crops in close proximity is called polyculture (as opposed to monoculture, which increases pest and disease incidence), a practice that makes it difficult for pests and diseases to multiply when the host crop is in abundance. Field crops can be planted in parallel strips, a practice called strip cropping, which creates the habitat diversity and reduces the chances of specific pests and diseases to increase into uncontrollable numbers (Mahr and Ridgway, 1993).

Trap cropping

Trap cropping is the practice whereby an insect or disease's preferred host crop is planted near the vegetable crop to be protected, e.g. planting a border row with a trap crop. The insects are attracted to the trap crop which is then removed and destroyed. For example, pickleworms will concentrate in squash planted near cucumbers, and the squash plants can be destroyed (Mahr and Ridgway, 1993). A carefully considered time of planting will help avoid some pest problems such as seed corn maggot. Marigold planted near crops will repel or kill nematodes because of the specific organic substance secreted by the plants. (Agrios, 2005; Mahr and Ridgway, 1993; Slabbert and Ehlers, 2004).

Crop rotation

Crop rotation replaces a crop that is susceptible to a serious pest or disease with another crop that is not susceptible, on a rotating basis. For example, maize rootworm larvae can be starved out by following maize with one to two years of a non-host crop such as soybeans, lucerne, or oats. This will ensure the reduction in the inoculum (eggs, larvae, spores), thereby decreasing the incidence of the pest or disease (Mahr and Ridgway, 1993). Rotation is not effective for all pathogenic microorganisms, and certain pathogens (*Pythium*, *Fusarium*, and *Rhizoctonia*, for example) have specialized structures, such as thick-walled resting spores, or can subsist as saprophytes for many years in the absence of host plants.

Table 8.1 lists vegetables susceptible to similar diseases and can be used as a basis for setting up a crop rotation program (Organic Vegetable IPM Guide, 2006). For example, if tomatoes or peppers (from the family Solanaceae) were grown this season, switch the site to production of vegetables in other groupings for 3 more years before returning to tomatoes or peppers.

Table 8.1: Grouping of vegetables, based on susceptibility to similar diseases (Organic Vegetable IPM Guide, 2006)

Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**	**Group G**
Watermelon	Cabbage	Pepper (all types)	Beets	Beans	Onions	Sweet maize
Cucumber	Cauliflower	Tomato	Swiss chard	English peas	Shallots	
Squash	Broccoli	Eggplant	Spinach	Snow peas	Garlic	
Cantaloupe	Brussels sprouts	Irish potato		Southern peas	Leek	
Pumpkin	Mustard					
Gourds	Turnips					
Proposed grouping for the selected African leafy vegetables						
Group A	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**	**Group G**
Citrillus lanatus *Cucurbita maxima*	*Brassica rapa* subsp. *chinensis*	*Solanum retroflexum*	*Amaranthus cruentus* *Cleome gynandra*	*Vigna unguiculata*		

Mechanical control

Mechanical control removes or kills pests. Mechanical control methods can be rapid and effective, but many are mostly suited for small acute pest and disease problems, and are popular with smaller vegetable gardens. Importantly, mechanical controls have relatively little impact on natural enemies and other non-target organisms, and are therefore well suited for use with biological control in an integrated pest management approach. Simple techniques such as cultivation or tillage expose many soil insects to desiccation or predation by birds. Hand-picking can be used for large foliage feeders such as the potato beetle, bean beetle, and tomato hornworm. Shaking of plants will also dislodge many pests. For example, some beetles can be removed from plants by shaking plants with a padded stick and collecting the insects on a white sheet as they fall out of the trees. A strong spray of water will dislodge aphids and mites from vegetable plants (Mahr and Ridgway, 1993).

Biological control

Before biological control will advance, much more emphasis needs to be placed on investigating indigenous natural enemies and their impact on the pests and diseases they attack (Hoffmann and Frodsham, 1993). With this information it may be possible to foster or enhance the efficacy of natural enemies through manipulation of the crop habitat, changes in cultural practices, or changes in pesticide, fungicide, nematocide and bactericide application practices, if the farmer is using synthetic chemicals. New natural enemies can also be introduced into the field, but the development of such a successful biological control programs will be challenging (Hoffmann and Frodsham, 1993) especially in the current socio-economic situation in communities in South Africa.

Beneficial insects and organisms

Beneficial insects and organisms do not harm animals, people or plants. It should be kept in mind that it also takes time before a beneficial can resolve pest and disease problems. Table 8.2 contains a listing of predators and some parasites used in the control of vegetable pests in general during vegetable production.

Table 8.2: List of predators and parasites used to control vegetables pests (Day, 2008; ABICO Organics, S.a.)

BENEFICIAL ORGANISM	INSECTS CONTROLLED	REMARKS
Lady beetles	Aphids and other soft-bodied insects.	Lady beetles may leave the garden to find other prey.
Lacewings	Aphids, scales, mealy bugs and other soft-bodied insects.	Most are *Chrysoperla* spp.
Predatory mites	Mostly spider mites.	*Phytoseiulus persimilus* will work in most situations.
Predatory nematodes	Many ground dwelling and boring insect pests, beetles, parasitic nematodes.	These nematodes will actively seek host prey and do not harm plants or humans.
Parasitic wasps	Many insect pests on the foliage including cater-pillars and whiteflies.	*Trichogramma* wasps work well on many caterpillars. *Encarsia formosa* works on whiteflies.

Microorganisms

Numerous non-pathogenic microorganisms, mostly soil bacteria and fungi, e.g. *Bacillus* and *Trichoderma* spp., have been found to be antagonistic against various soil pathogenic fungi, bacteria and nematodes. These microorganisms can produce antibiotics, compete for food/nutrients, or directly parasitize on insects and pathogens, thereby destroying or inhibiting the insect or pathogen (Agrios, 2005). Pest and disease control using microorganisms has been successfully used, and certain organic products containing such microorganisms are available commercially. This technique could only be successful in a resource poor farming system if the farmer can afford to buy these products, and has the practical know-how of using it. Farmers can however improve the natural occurrence of the beneficial micro-organism population in soils by never using harsh chemicals or sterilants (e.g. Methyl bromide for nematodes) to control insects or pathogens, since the beneficial micro-organisms, which form an indispensable part of the microbial-plant-pest-disease interaction, will be destroyed. Plant debris can be reworked back into the soil to improve the growth of beneficial micro-organisms (Agrios, 2005; Slabbert and Ehlers, 2005).

Amendments and extracts

Proven materials for organic use are limited (Koike *et al*., 2008), although there are some examples in literature which have been traditionally used by farmers, or tested by researchers. Plant extracts or botanicals can be used as an organic, non-toxic insecticide, bactericide, nematocide or fungicide. Botanicals or plant extracts are applied to plant foliage, or into the soil. Plant extracts, used as organic insecticides, combat insects upon contact or through ingestion, as a repellent or it can be used to lure insects into a trap (Hoffmann and Frodsham, 1993).

Garlic is an example of a safe plant extract with a wide application in pest and disease control (Prakash and Rao, 1997; Viayalakshmiet, 1999). Leaf spray can also be made from plants belonging to the solanaceae family, such as tomato, potato and tobacco, since these plants have significant amounts of toxic compounds called alkaloids in their leaves (Richardson, 2005). Not only are alkaloids toxic to pests, but these sprays also act as natural pest enemy attraction that follow powerful chemicals in these plants as cues in searching for prey (Richardson, 2005).

Many organic farmers are familiar with the use of aromatic herbs to repel pests from vegetable plants (Richardson, 2005). The essential oils and alcohol in herbal extracts reduce the number of eggs laid, as well as feeding damage caused by insects such as cutworms, caterpillars and cabbageworms. Pepper dusts and sprays contain a compound capsaicin, that repels insects researchers have found that as little as 7 grams of capsaicin sprinkled around an onion plant reduced the number of root maggot eggs with 75% compared to the control plant (Richardson, 2005).

Nicotine has been one of the most popular insecticides since the 1880s, ad is still widely used (Richardson, 2005). Home-made nicotine tea only retain its toxicity for a few hours after spraying, making it less nonhazardous to bees and lady beetles because of its short persistence. It should however be used with care on edible plants since more concentrated sprays could remain active in the leaves for several weeks. Care should also be taken for the transmittance of Tobacco Mosaic Virus (TMV) to other plants of the nightshade family. Homemade sprays may be stored safely for up to one month, providing they are in sterile, glass, screw top containers (Richardson, 2005).

According to Ricardson (2005), spraying early in the morning or at evening when it is cooler, will give better results of disease and pest control. Spraying in the heat of the day can cause burning of the leaves, or the plant can react negatively on the botanical spray, known as phytotoxicity. The author also states that it is better to always perform a test on a small portion of the plants first, wait 24 hours for any negative reaction, and proceed if there was no plant damage. It is important also to always protect your skin and face when applying plant extracts since some of these ingredients can be irritating to your skin and mucous membranes

(Richardson, 2005). Plant extracts will have to be applied often (2-7 day intervals) since their activity and effectiveness can be greatly influenced by rain and ultraviolet radiation. A key factor will be the safety of the plant extract products for humans, animals and the environment, as well as availability to farmers, and ease of application (Koike *et al.*, 2005).

Other organic dusts reported is pure poultry droppings which seems to be work effectively for nematode control, while traditionally the use of wood ash on leaves can control flea beetles (Schippers, 2000). Tobacco and coffee extracts, beer traps for snails, or sprays made from rosemary, tobacco or rhubarb, can be effectively applied against aphids (various) or black spot (*Diplocarpon*) (Kaplan, 2011).

Sprays made from the leaves of *C. gynandra* are known for their insecticidal and insect-repellent characteristics, and may cause reduction in aphid and thrips populations considerably. Farmers control the melon fly (*Daucus* sp.) using a sugary bait with a pesticide or fungicide added to it (Schippers, 2000).

Oils, plant extracts and other natural products such as bicarbonate (Koike *et al.*, 2005), have to various degrees been able to control pests and diseases. Before applying such an organic product, the farmer should always first test the sensitivity of the plants, and reliability thereof on a small number of plants. Oils pose few risks to people or to most desirable species, including beneficial natural enemies of insect pests. This allows oils to integrate well with biological controls. Toxicity is minimal, at least compared to alternative pesticides, and oils quickly dissipate through evaporation, leaving little residue. Oils also are easy to apply with existing spray equipment and can be mixed with many other pesticides to extend their performance (Peet, 2005)

The main limitation of spray oils is their small but real potential to cause plant injury (phytotoxicity) in some situations. Oils also can stain some surfaces, particularly dark-coloured house paints. Some of the newer spray oils can largely eliminate these problems if they are properly applied. Oils are sometimes applied to prevent transmission of viruses. Many viruses spread by aphids (non-persistent viruses), as well as some that are mechanically transmitted by people, can be inhibited by oil applications. Oils used to inhibit virus transmission are sometimes called "stylet oils," a reference to the piercing and sucking mouthparts (stylets) of aphids that transmit these viruses (Peet, 2005).

Vegetable oils

Vegetable oils also can be used as insecticides, although the type of oil can greatly affect its activity. Cottonseed oil is generally considered the most insecticidal of the vegetable oils. Soybean oil, the most commonly available vegetable oil used in cooking, has often provided fair to good control of some insects and mites (Peet, 2005).

Plant extracts

Plant extracts from seeds of the neem tree (*Azadirachta indica*) have recently attracted attention as a source of pest management products. Extensive research has been done and several neem-derived insecticides have been developed. A number of compounds found in neem seeds, notably azadirachtin, have proven useful as insecticides. However, the oil fraction of neem seed extracts, which is mostly free of azadirachtin and related terpenoid compounds, also has demonstrated effects as a fungicide and insecticide. At least one product is currently on the market. Many over-the-counter products sold in nurseries that mention neem contain the oils of neem seed extracts (Peet, 2005). Examples of beneficial plant extracts in pest control are given in Table 8.3.

Table 8.3: Examples of plant extracts and amendments used in pest control (Day, 2008; ABICO Organics, S.a.)

PRODUCT	INSECTS CONTROLLED	REMARKS
Insecticidal soap	Works well on soft-bodied insects, particularly aphids, mites and mealy bugs.	This product, a fatty acid soap, is available under many trade names
Rotenone	Many garden insect pests including Colorado potato beetles, flea beetles, aphids, weevils, and Mexican bean beetles.	Usually sold as a dust, but some formulations can be mixed in water.
Pyrethrin	A broad spectrum insecticide, works on a wide variety of insects.	Usually sold mixed with other botanical insecticides such as rotenone.
Sabadilla	Stink bugs, cucumber beetles, caterpillars, loopers, leafhoppers and thrips.	A product from the seed of a plant related to lilies.
Pyrethrum/diatomaceous earth	Many insects including white flies, aphids, caterpillars, fly maggots and fire ants.	See label for precautions.

Plant extract from several *Brassica* spp., e.g. the mustard plant, may benefit the soil in several ways. Chemical substances released in the soil during the decomposition of broccoli and other crucifer crops residues suppress the growth of *Verticillium.* Nematode effects can be largely attributed to bio-fumigation. As the mustard bran breaks down, it releases a nematocidal gas called allyl-isothiocyanate. This gas is similar to that released by certain synthetic soil fumigants sometimes used in turf renovation. The gas dissolves in water and is moved into the soil profile by irrigation. Data thus far indicate that it may be effective against some nematode species. The material also has some fertility effects, adding N to the soil (Koike *et al.*, 2005).

Organically Approved Chemicals

Alternatives to synthetic products for control of vegetable diseases include such materials as sulfur and copper containing fungicides. Spraying or dusting with sulfur is an old remedy that still works on rusts, powdery mildews, and leaf spots on tomato and other vegetable crops. Bordeaux mixture is a fungicide effective for a number of common fungal and bacterial blights and leaf spots on vegetable crops.

Physical control

Hot water treatment of propagative plant material, e.g. seed, bulbs and vines, has for many years been traditionally used to kill pests and diseases inside the plant material. For example, hot water treatment of wheat seed was for many years the only practical method of killing the loose smut fungus, *Ustilago nuda*, which occurs systemically in the seeds. Seeds are treated for 11 minutes at 52ºC. Conventional fungicides were not capable of penetrating the seed in order to kill the fungus. Hot water treatment of nursery stock, e.g. flower bulbs, held at 43ºC for 3 hours, is also used to kill nematodes which may occur in such material, e.g. *Ditylenchus dipsaci* in the bulbs of various ornamental plants and *Radopholus similis* in citrus rootstocks (Agrios, 2005; Slabbert and Ehlers, 2005).

Barriers and traps

There are many simple, easy and cheap ways of reducing the occurrence of pests and diseases in vegetable gardening, such as the use of barriers. Covering vegetables with a fine mesh will stop them being attacked by flying pests. This works well for carrot rootfly and pea moth. Fine mesh is also an all-inclusive way of

protecting your cabbages from other insects such as flea beetles, leaf weevils, birds, cabbage white butterflies and white fly (Garden Organic, 2005).

Other barriers include cabbage collars and bottle cloches. Placing a collar of carpet underlay around the neck of a young cabbage will prevent the cabbage root fly from laying its eggs at the base of the cabbage. Placing a bottle cloche, a clear plastic drinks bottle with the top and bottom removed, over newly planted vegetables will prevent them being eaten by slugs or other insect that move on the soil such as stalk borers (Garden Organic, 2005).

Small gauge chicken wire is always useful. Placing it over your newly sown peas can stop them being eaten by mice while they are germinating or being scratched up by cats. Wrap it around your flowering bulbs to prevent rodents from digging them up (Garden Organic, 2005).

Netting can be very useful at preventing bird damage to fruit and vegetables. There is also a humming line that can be wound around canes criss-crossed over your vegetables to prevent bird attacks. Netting can also prevent cabbage white butterflies from laying there eggs on your brassicae (Garden Organic, 2005).

Barriers can also be used to prevent diseases. For example, peach leaf curl is a devastating fungus that can simply be prevented by placing a barrier of polythene sheeting over a trained peach tree in the winter. This simple barrier prevents the spores splashing up onto the plant (Garden Organic, 2005).

A codling moth trap, for example, uses a pheromone placed on a sticky floor. The male moth is attracted to the trap thinking it is a female. On landing he gets stuck in the glue. There are similar traps for pear and plum moths (Garden Organic, 2005).

Grease bands, painted around the trunks of apple trees in autumn are a popular way of preventing the wingless female winter moth from climbing up the tree to mate. Sticky glue is also very useful for glasshouse staging if you have a problem with ants. Sticky yellow bits of card hung up in the glasshouse can help reduce the population of whitefly (Garden Organic, 2005).

Disease forecasting

To limit the number of applications of chemical control, farmers should make use of disease forecasting. Only spray when the disease development conditions are favourable, e.g. cool, wet weather could promote the development of late blight and cause epidemic proportions, if preventative measures, e.g. contact fungicides, are not applied in time (Arios, 2005; Slabbert and Ehlers, 2005).

8.4.3 Harvesting, drying and storage of seed

Harvesting, drying and storage of seed are some of the most critical stages in leafy vegetable production. Without careful handling of the seed, it is possible to lose or damage the majority of the seed. Seed should only be harvested when the seed heads are dry, because if heads are too wet, the seeds become sticky and adhere to the inflorescence Shattering during the cutting process can also cause losses, so adjustments should be made to minimize shattering of the heads (Alternative Field Crops Manual, 2000).

Seeds from fields or areas where diseases are prevalent should not be kept for the next growth season, and all infected material should be removed and destroyed before storage. Farmers should ensure that seed and other plant material are harvested during dry conditions, and storage of seed and plant material should be in cool, dark, dry environmental conditions to prevent the growth of fungi (Agrios, 2005).

It is important to remove the seed from the heads and to clean the seed to remove plant and foreign material to reduce the chance of molding during storage. Cleaning can be done using for example a wire mesh screen, to separate seed of the same size. Maximum moisture for storing grain is approximately 11%. Seed can be dried by exposure to dry, well aerated conditions during warm days. The optimum way to store the seed after cleaning and drying is in wooden storage bins or in heavy duty paper bags. This will prevent any moisture built up inside the containers during conditions of high humidity. Containers with seed should be stored in a dry, cool place until sowing the following growth season (Alternative Field Crops Manual, 2000).

During harvesting and storage the carry-over of pests and disease inoculums should be minimized or eliminated by various techniques. For example the inoculums of aphids (that curl leaves in spring because of viral infections); caterpillars that winter as eggs on the plant (leafrollers, caterpillars); mites that winter on the plant (e.g. conifer-infesting species) and scale insects can be reduced by enforcing strict sanitation rules during harvesting and storage. Incidence of many fungal, nematode and bacterial diseases, such as powdery mildew, soft rot and dry rot in the next growth season will also be reduced in this manner (Peet, 2005).

To ensure that seed is clean from insect eggs, weeds, seeds or pathogen spores before storing, the seed can be exposed to solarisation, by spreading the seed on a clean surface outside during high sunlight conditions, covering it with a transparent polyethylene sheet, and leaving it for approximately 2-5 days (time of treatment depends on pathogen or insect or weed seeds to be killed). Temperatures of 50-52 ˚C should be reached under the polyethylene cover, which should be sufficient to kill the insect eggs (pers. comm., Dr. B.B. Singh).

8.5 DISCUSSION AND CONCLUSION

Very little quantitative information on pest and diseases on specific traditional ALVs is available, even more so concerning organic ALVs production. Therefore a general approach in literature on pest and disease control in conventional agriculture has been considered to provide novel approaches for pest and disease control in ALVs. Disease and pest problems occur throughout the production cycle of vegetables, from seed and propagation to the growing crop and its harvest and storage. Approaches for pest and disease control are considered at each stage. Cultural control such as sanitation and crop rotation are considered the cornerstone of pest and disease control, while recent developments in understanding interactions between soil micro-organisms, plant pests and pathogens, and the interaction with environment offer the opportunity to manage specific disease problems in a more environmental friendly manner. The use of physical and mechanical control, soil solarisation and barrier or trap crops appear to offer good prospects for improving pest and disease control. The use of covers and mulches should provide a range of benefits particularly if used for successive crops. Biological control agents either natural occurring (e.g. suppressive soils) or introduced may be advantageous against particularly damaging or persistent problems. Similarly, plant extracts require further evaluation, specifically in community setups, for specific uses in disease and pest control. Furthermore good agricultural practices, including site selection, fertigation, sanitation, irrigation practices, weed control as well as harvesting, drying and storage of seed will all contribute to reducing pests and disease incidence to a tolerable level during ALV production.

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Human Nutrition and African Leafy Vegetables

CHAPTER 9: SELECTED VITAMIN AND MINERAL CONTENT OF EIGHT AFRICAN LEAFY VEGETABLES AND THEIR POTENTIAL CONTRIBUTION TO INDIVIDUAL NUTRIENT REQUIREMENTS

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9.1 INTRODUCTION

In South Africa in 2005, 64% of 1-9-year-old children were vitamin A deficient, 28% were anaemic and 6% were iron depleted (Labadarios, 2007). Compared to the South African Vitamin A Consultative Group (SAVACG) study that was done in 1994 (Labadarios *et al*., 1995), both the vitamin A and iron status of South African children appears to have deteriorated. The National Food Consumption Survey (NFCS) that was done in 1999 showed that the great majority of 1-9-year-old children consume a diet deficient in energy and of poor nutrient density, and half of the children consumed less than half of the recommended level for several of the micronutrients (Labadarios *et al*., 2000). With regard to the anthropometric status of South African children, stunting is the major problem, affecting 18% of 1-9-year-old children, while 9% were underweight and 4.5% were wasted in 2005. Severe wasting is not common (<1%) at the national level (Labadarios, 2007).

Nutritional deficiencies in energy and protein, as well as iodine, iron, zinc and vitamin A can contribute to poor growth of children (WHO, 1999). Allen (1994) reviewed the literature on nutritional influences on linear growth and concluded that poor growth may be a consequence of multiple deficiencies that result when children consume diets of poor quality.

A low intake of fruits and vegetables was identified as one of the main deficiencies in the South African diet (Vorster *et al*., 1997). The NFCS of 1999 showed that, for 1-9-year-old children, fruit is low on the list of commonly consumed food items in many provinces (Labadarios *et al*., 2000). In an earlier survey, only 28% of adults reported a daily consumption of four or more portions of fruits and vegetables (Langenhoven *et al*., 1995). A meta-analysis of dietary surveys done in South Africa from 1983 to 2000 showed that less than 50% of 1-5-year-old rural and urban children consumed items from the vegetable group, while 12-18% rural and 27-44% urban South Africans consumed fruit (Nel and Steyn, 2002). Black South Africans have a lower consumption of fruits and vegetables than Coloured, Indian and White South Africans, and consumption of fruits and vegetables is lower in rural than in urban areas (Rose *et al*., 2002). An estimated 11.1 million males and 12.5 million females over 15 years of age were affected by a low intake of fruits and vegetables in South Africa in 2000 (Schneider *et al*., 2007).

Increased consumption of fruits and vegetables is promoted because of its potential to improve health status. Dietary diversification is one of the strategies supported by the South African Department of Health's Integrated Nutrition Programme (Department of Health, 2002). The South African Food-Based Dietary Guidelines encourage South Africans to, among others, "eat plenty of fruit and vegetables every day" (Love and Sayed, 2001). An intake of five portions of fruits and vegetables are promoted through the 5-a-day for better health programme. The World Health Organization recommends a daily intake of at least 400 g of fruits and vegetables per person (WHO, 1990), which is about double the amount consumed by the average South African (Rose *et al*., 2002).

Increasing the intake of fruits and vegetables could potentially reduce vitamin A deficiency (Faber *et al*., 2002a) and have a large impact on reducing many non-communicable diseases (Lock *et al*., 2005). However, affordability, availability and seasonal fluctuations may negatively impact on fruit and vegetable intake (Love *et al*., 2001). For example, monitoring year-round availability of fruits and vegetables in five local shops in a rural village in KwaZulu-Natal during 2004 showed that β-carotene (provitamin A)-rich fruits and vegetables were seldom available (Faber and Laubscher, 2008).

Rural communities in South Africa are nutritionally more vulnerable than those in the urban areas (Labadarios *et al*., 1995; Labadarios *et al*., 2000). A fundamental strategy to address micronutrient deficiencies in resource-poor communities is to increase the availability of, access to, and ultimately consumption of foods that are rich sources of micronutrients. This can potentially be achieved through food production at household level. One of the recommendations of the NFCS of 1999 was that "In rural or appropriate settings, the importance of home-based crops and livestock can make to the children's diet should be strengthened and promoted as feasible and appropriate" (Labadarios *et al*., 2000).

Home-gardening has been shown to be an important means to improve the intake of vegetables and fruit that are rich in provitamin A carotenoids, particularly for resource-poor households and in countries where plant foods are the main source of vitamin A (Berti *et al*., 2004). In South Africa, for example, a home-garden project that focused on β-carotene rich fruits and vegetables resulted in an increased intake of vitamin A and various other micronutrients (Faber *et al*., 2002b) and ultimately serum retinol concentrations in children, which is an indicator of vitamin A status (Faber *et al*., 2002a).

It has been recommended that local crop production systems should aim to increase the use of under-exploited natural resources such as traditional food crops (FAO, 1997). ALVs grow on soils of limited fertility; are relatively drought tolerant; provide good cover; and can be harvested within a short period of time (Chapters 4, 6 and 7 of this report; Shiundu, 2002). Flyman and Afolayan (2006) argued that wild vegetables could make an important contribution to combating micronutrient malnutrition as well as providing food security.

The NFCS of 1999 showed that green leafy vegetables were the 16th most frequently consumed food item for 1-9-year-old South African children (12% of the children consumed it during the recall period). The highest consumption was in Limpopo (previously known as the Northern Province), with green leaves being the 4th most frequently consumed food item and 46% of the children consumed it during the recall period (Labadarios *et al*., 2000). A smaller study in Limpopo showed that green leafy vegetables were the 5th most frequently consumed food item of the adult population (Steyn *et al*., 2001a). Frequent consumers of wild, green leafy vegetables in Limpopo were shown to eat it at least once or twice per week and the cooked portion sizes commonly ranged between 45 to 105 grams and 80 to 270 grams (Nesamvuni *et al*., 2001; Steyn *et al*., 2001b). *A. hybridus* L. and *A. thunbergii* were the most commonly eaten plants (Steyn *et al*., 2001b). In Venda (the most northerly region of Limpopo) the leaves are mainly harvested during summer and the surplus is stored for later consumption in either a dried-cooked or dried-raw form for at least six months (Nesamvuni *et al*., 2001).

9.2 AIM OF THE STUDY

Agronomic research at the Tshwane University of Technology (TUT) was aimed at characterizing eight important African leafy vegetable (ALVs) crops in terms of their response to water stress and nutrient availability using standardized procedures. The eight ALV species that were selected are:

i) Non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*)
ii) Black nightshade (*Solanum retroflexum* Dun)

iii) (Pigweed (amaranth) (*Amaranthus cruentus* L.)
iv) Jew's mallow (*Corchorus olitorius* L.)
v) Cowpeas (*Vigna unguiculata* L. Walp.)
vi) Pumpkin (*Cucurbita maxima*)
vii) Tsamma melon (bitter watermelon) (*Citrillus lanatus* (Thunberg))
viii) Spider flower (*Cleome gynandra* L.)

The aim of the present study was to determine the nutrient content of these eight selected ALV crops grown under optimal or adequate agricultural practices.

9.3 METHODS

9.3.1 Sampling and preparation of samples for analysis

Appropriate ALV crop sampling protocols were developed by project team members of the Agricultural Research Council-Livestock Business Division: Animal Products Institute (ARC – LBD:API) and the Nutritional Intervention Research Unit (NIRU) of the Medical Research Council (MRC) responsible for the nutrient analysis, in collaboration with the horticulturists growing the ALVs. This ensured that a representative sample of the food in question was taken for nutrient analysis. It was decided to grow the ALV crops under optimal or adequate agricultural input/practices and to analyse raw plant material in order to obtain good baseline values characterising each ALV species. These nutrient values will provide a good indication of the best ALV candidates, or any combination of two or more ALVs, for addressing micronutrient malnutrition although nutrient retention studies have not been done yet.

Chinese cabbage and black nightshade material were obtained from the irrigation schemes in the farmers' fields in the Vhembe district in Limpopo Province. Amaranth, Jew's mallow, cowpeas, pumpkin and bitter watermelon were obtained from field plots at Roodeplaat where it was cultivated. The leaves/plants were harvested early in the morning, before 10:00 am. Three lots of each ALV crop were harvested on the same day from the same field, so as to obtain representative sample batches for each ALV. The plants / leaves were sprinkled with water and each lot of plants loosely packed in a marked black plastic bag that was pierced with little holes to avoid "sweating" of the leaves and to allow some airflow. The bags were transported to the Meat Industry Centre of the ARC – LBD:API on the same day where it was kept in a cold storage room at +4°C overnight for processing the next day.

The edible leaves of each ALV sample batch were processed at the Sensory Analysis laboratory of the ARC – LBD:API. Each sample lot was processed separately. For each lot of plants healthy edible leaves were removed from the base/stem and combined (the number of leaves per plant varied for the different ALVs). In all cases only the lamina (leaf blade) that was removed from the petiole was taken for analysis except for Jew's mallow, pumpkin and spider flower leaves for which the lamina attached to the petiole was taken. The leaves were thoroughly washed with tap water to remove soil debris, followed by washing with distilled water. The leaves were air-dried on absorbent paper at room temperature for two hours. The leaves were cut into smaller pieces and homogenised using a household food processor; from this, an aliquot of adequate amount that was needed for all the nutrient analyses was further homogenised to a finer texture using a hand-held stick blender. Aliquots were transferred to marked plastic containers with screw caps. The containers were frozen at -20ºC until required for analysis and dispatched to Cape Town for β-carotene analysis. All nutrient analysis was therefore done on aliquots obtained from the same homogenized and representative sample.

Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*)
The sampling was done from one single field (irrigation strip) that had a homogeneous soil (based on the soil survey done), which is typical for the horticultural scheme. It can be ascertained that the selected strip was well fertilized because farmers were able to recall the amount of fertilizer they applied to the particular strip needed for optimum growth of Chinese cabbage.

Three lots each of 10 whole, healthy, mature cabbage plants from different positions across the same irrigation field-strip were randomly selected and harvested. The plants were cut at the base/stem of the plant just above soil level, keeping the head intact (not removing the leaves). During processing, five healthy edible leaves per plant were removed from the petiole and combined.

Black nightshade (*Solanum retroflexum* Dun)
Three lots of 40 plants each were randomly selected and harvested from different positions across the same irrigation field-strip. During processing five to 10 healthy edible leaves, without the petiole, from the top as well as form the middle and the bottom of each plant were removed and combined.

Amaranth (*Amaranthus cruentus* L.)
Three lots of 50 plants each were randomly selected and harvested from different positions across the same field plot. At the laboratory the plants were sprinkled with water and transferred to buckets half-filled with water and kept in a cold storage room at +4°C overnight for processing the next day. During processing 10 to 15 healthy edible leaves, without the petiole, from the top part of each plant were removed and combined.

Jew's mallow (*Corchorus olitorius* L.)
Three lots of 40 plants each were randomly selected and harvested from different positions across the same field plot. At the laboratory the plants were sprinkled with water and transferred to buckets half-filled with water and kept in a cold storage room at +4°C overnight for processing the next day. During processing 10 to 15 healthy edible leaves, lamina with petiole, from the top part of each plant were removed and combined.

Cowpeas (*Vigna unguiculata* (L.) Walp.)
Three lots of 50 plants each were randomly selected and harvested from different positions across the same field plot. At the laboratory the plants were sprinkled with water and transferred to buckets half-filled with water and kept in a cold storage room at +4°C overnight for processing the next day. During processing five to seven healthy edible leaves, without the petiole, from the top part of each vine were removed and combined.

Pumpkin (*Cucurbita maxima*)
Three lots (each weighing approximately 550 g) of healthy fresh edible leaves, lamina with petiole, were randomly selected and harvested from vines across the same field plot. The leaves were transported in labelled brown paper bags on the same day to the Meat Industry Centre of the ARC – LBD:API and each lot was immediately processed separately.

Tsamma melon (*Citrillus lanatus* (Thunberg)
Three lots (each weighing approximately 1300 g) of healthy fresh edible leaves were randomly selected and harvested from vines from the same field plot. The leaves were transported in labelled brown paper bags on the same day to the Meat Industry Centre of the ARC – LBD:API and processed immediately. The petiole was removed from the lamina during processing.

Spider flower *(Cleome gynandra* L.)
Three lots (each weighing approximately 3500 g) of stems containing healthy fresh edible leaves were randomly selected and harvested from plants from the same field plot. It was transported in labelled plastic bags on the same day to the Meat Industry Centre of the ARC – LBD:API. At the laboratory each lot was rinsed with water, drained and placed in a black plastic bag and kept in a cold storage room at +4 °C overnight for processing the next day. During processing the healthy edible leaves, lamina with petiole, were removed from the stems and combined (approximately 900 g sample per batch).

9.3.2 Nutrient Analyses

Key nutrients, important in the diet of the nutritionally vulnerable, were determined for the various ALVs. The analyses were done on a double blind basis in a South African National Accreditation Services (SANAS) accredited laboratory. Reference samples form part of the daily routine in these laboratories to assure the quality of results. Accepted standardised techniques were used for nutrient analysis. After initial standardisation of techniques during a pilot study, the vegetables were treated identically for each test, throughout the project.

Proximate analysis
Total fat content was determined using a 2-g freeze-dried aliquot and the AOAC method 960.39 (AOAC, 2005). The Tecator Soxtec System 1034 extraction unit with reagent petroleum ether (40 to 60ºC) was used for the extraction.

Moisture content was determined by measuring the weight loss of a 5 g aliquot of fresh material using the AOAC method 950.46 (AOAC, 2005).

Total ash (inorganic matter) content was determined using the AOAC method 920.153 (AOAC, 2005). The organic matter of the samples was removed by heating at 550ºC overnight; the remaining residue is inorganic matter (ash).

Protein content was determined using the Dumas Combustion method, AOAC 992.15 (AOAC, 2005). The sample was combusted at approximately 1100 to 1350ºC, and 10 cm^3 of the sample gas was analysed. A thermal conductivity cell detects the difference in thermal conductivity caused by the presence of Nitrogen. A conversion factor of 6.25 was used in the calculation of the protein content. Duplicate samples were analysed.

Total carbohydrate content was calculated by subtracting the percentage of water, protein, fat and ash from 100 to obtain the percentage of carbohydrate “by difference”. It includes all the non-carbohydrate material not analysed in the other proximate analyses and the cumulative errors from the other measurements (Greenfield and Southgate, 2003).

Dietary fibre content was determined using the Official Method 985.29, (AOAC, 1995).

The energy content (kJ/100g) was calculated using the percentage (g/100g) protein multiplied by 17, percentage total carbohydrates multiplied by 17, plus the percentage fat, multiplied by 37 (Greenfield and Southgate, 2003).

Minerals
The following minerals were determined: sodium, potassium, calcium, manganese, magnesium, copper, phosphorus, iron and zinc. Freeze-dried samples were ashed, dissolved with hydrochloric acid and analysed with an Ion Chromatograph (IC) by a sub-contracted laboratory.

Water soluble vitamins

Thiamine and riboflavin content were determined by High-Performance Liquid Chromatography (HPLC) and fluorescence detection (Fellman *et al*., 1992). The method for folic acid analysis is based on the observation that specific bacteria, yeasts and fungi are able to grow and form metabolic products only in the presence of vitamins of the B-group. When the vitamin, or substrate containing this vitamin, is added, growth takes place which is dependent on the amount of vitamin. Therefore, the amount of vitamin present can be detected by measuring the turbidity resulting from growth. A pure vitamin preparation of known activity is used in a parallel test and serves as the reference standard (Barton-Wright, 1952). Vitamin C content was determined by HPLC and fluorescence detection (Dodson, Young and Soliman, 1992).

β-Carotene

Carotenoids were extracted from 2 to 3 g homogenised aliquots with tetrahydrofuran:methanol (1:1, v/v) and β-carotene content determined with HPLC (Low and van Jaarsveld, 2008; Kimura and Rodriguez-Amaya, 2002). Analysis was done in duplicate.

9.3.3 Data analysis

The nutrient data obtained from the analyses were entered on a spreadsheet using Microsoft Excel (2000). The vitamin A value in µg Retinol Activity Equivalents (RAE) was calculated as the sum of (all-*trans*-β-carotene (µg) + *cis*-β-carotene (µg) × 0.5) divided by 12 assuming RAE to be 12 µg β-carotene:1µg retinol (Trumbo *et al*., 2001). In order to determine the nutrient contribution from ALVs to nutrient requirements, nutrient loss during food preparation needs to be taken into consideration. Nutrients retained after cooking were estimated using the nutrient retention factors given for "veg, greens, boiled, little water, drain" (USDA Table of nutrient retention factors, 2007) for the individual nutrients. Means and standard deviations were calculated and rounded off to the number of decimal places given for the specific nutrient in the SAFOODs database.

An average portion size was set at 130 g boiled leaves for adult females and 90 g for young children (Faber *et al*., 2007). The amount of raw leaves needed to yield an average cooked portion was calculated using a yield factor of 1.3, based on preliminary indications from cooking experiments in our laboratory. The nutrient contribution of an average portion of cooked leaves to the nutrient requirements was calculated and expressed as a percentage of both the recommended dietary allowance (RDA) and estimated average requirement (EAR) for 4-8-year-old children and 19-30-year-old females (Trumbo *et al*., 2001; Otten *et al*., 2006).

9.4 RESULTS

The means and standard deviations of nutrients per 100 g raw leaves for each ALV are given in Table 9.1. The calculated nutrients retained for 100 g raw leaves after preparation are given in Table 9.2. It should be noted that the retention factors that were used to calculate the amount of nutrients retained are rough estimates, as the nutrient retention is affected by various factors such as the cooking method, cooking temperature and cooking time (Greenfield and Southgate, 2003).

In Tables 9.3 and 9.4 the estimated amount of nutrients retained after preparation and taking the yield factor into account are expressed as a percentage of the nutrient requirements for two age groups, namely 4-8-year-old children and 19-30-year-old females. We used these two age groups because young children and women of child-bearing age are nutritionally most vulnerable. Table 9.3 shows the nutrient content as a percentage of the RDA. The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement for nearly all (97 to 98%) healthy individuals in a particular life stage and gender group. Table 9.4 shows the nutrient content as a percentage of the EAR. The EAR is a daily nutrient intake value that is

estimated to meet the requirement of half of the healthy individuals in a life stage and gender group (Trumbo *et al*., 2001; Otten *et al*., 2006). It should be noted that there is no EAR for energy, calcium, potassium and sodium.

Table 9.1: Nutrient composition per 100 g edible portion of raw African leafy vegetables

	Unit	Chinese cabbage leaves	Black nightshade leaves	Amaranth leaves	Jew's mallow leaves	Cowpea leaves	Pumpkin leaves	Bitter watermelon leaves	Spider flower leaves
PROXIMATE ANALYSIS:									
Moisture	g	92.2 (0.2)	89.5 (3.5)	82.0 (0.8)	79.6 (1.8)	82.4 (1.0)	85.6 (1.2)	81.3 (0.5)	87.5 (0.7)
Protein	g	2.5 (0.2)	0.5 (0.2)	4.2 (0.0)	3.2 (0.2)	4.7 (0.1)	2.9 (0.2)	3.5 (0.1)	5.0 (0.2)
Ash	g	1.00 (0.02)	1.32 (0.41)	2.38 (0.06)	1.81 (0.14)	1.76 (0.07)	1.51 (0.14)	1.66 (0.05)	1.46 (0.09)
Fat	g	0.2 (0.1)	0.4 (0.1)	0.3 (0.0)	0.1 (0.0)	0.6 (0.0)	0.2 (0.0)	0.4 (0.1)	0.3 (0.0)
Energy	kJ	120 (5)	162 (53)	272 (13)	319 (28)	280 (17)	222 (18)	296 (10)	195 (10)
Dietary fibre	g	2.2 (0.1)	2.5 (0.9)	6.7 (0.2)	10.8 (1.1)	5.8 (0.0)	3.0 (1.3)	3.8 (0.2)	3.1 (0.2)
Carbohydrates	g	4.1 (0.1)	8.2 (2.9)	11.2 (0.8)	15.3 (1.4)	10.5 (0.9)	9.8 (1.0)	13.1 (0.5)	5.7 (0.3)
MINERALS:									
Calcium	mg	152 (5)	199 (62)	443 (25)	310 (31)	398 (33)	177 (11)	212 (20)	232 (10)
Magnesium	mg	42 (1)	92 (27)	242 (19)	87 (8)	62 (4)	67 (5)	59 (2)	76 (3)
Phosphorus	mg	32 (5)	36 (11)	81 (5)	118 (10)	51 (1)	102 (8)	119 (2)	138 (4)
Sodium	mg	29 (8)	8 (3)	10 (1)	11 (1)	10 (0)	12 (1)	9 (0)	15 (1)
Potassium	mg	165 (29)	257 (77)	459 (47)	407 (46)	238 (27)	351 (43)	260 (12)	374 (34)
Copper	mg	0.07 (0.01)	0.16 (0.05)	0.17 (0.02)	0.19 (0.03)	0.14 (0.01)	0.21 (0.03)	0.20 (0.01)	0.25 (0.01)
Zinc	mg	0.30 (0.06)	0.56 (0.17)	0.70 (0.05)	0.57 (0.04)	0.42 (0.03)	0.75 (0.06)	0.74 (0.04)	1.04 (0.05)
Iron	mg	1.4 (0.3)	7.2 (3.1)	5.1 (0.7)	3.6 (0.6)	4.7 (0.7)	9.2 (1.6)	6.4 (1.4)	2.1 (0.1)
Manganese	mg	0.35 (0.04)	2.08 (0.52)	2.34 (0.24)	0.79 (0.10)	2.69 (0.03)	0.54 (0.05)	0.76 (0.06)	0.58 (0.04)

Table 9.1 (continued)**:** Nutrient composition per 100 g edible portion of raw African leafy vegetables

	Unit	Chinese cabbage leaves	Black nightshade leaves	Amaranth leaves	Jew's mallow leaves	Cowpea leaves	Pumpkin leaves	Bitter watermelon leaves	Spider flower leaves
VITAMINS:									
Thiamine	mg	0.04 (0.02)	0.08 (0.02)	0.04 (0.00)	0.02 (0.00)	0.07 (0.01)	0.04 (0.01)	0.01 (0.01)	0.06 (0.01)
Riboflavin	mg	0.06 (0.00)	0.17 (0.03)	0.05 (0.00)	0.03 (0.00)	0.08 (0.01)	0.10 (0.01)	0.10 (0.01)	0.21 (0.01)
Folic acid	µg	92 (16)	56 (15)	75 (13)	45 (14)	105 (6)	47 (12)	68 (7)	121 (9)
Vitamin C	mg	8 (2)	5 (3)	2 (0)	1 (0)	9 (0)	2 (1)	10 (0)	2 (0)
Total β-Carotene	µg	3593 (47)	5566 (32)	7138 (492)	4307 (120)	7031 (186)	4247 (190)	4956 (182)	5936 (300)
All-*trans*-β-Carotene	µg	2823 (34)	4568 (36)	5757 (406)	3578 (115)	5857 (168)	3547 (178)	4043 (166)	4477 (204)
Vitamin A	µg RAE	267 (3)	422 (3)	537 (37)	329 (10)	537 (15)	325 (15)	375 (14)	434 (21)

Means and standard deviations of three sample lots analysed individually. Single analysis was done for proximates, minerals and vitamins except protein and β-carotene which were analysed in duplicate. Retinol activity equivalents (RAE): conversion factor of 12:1 for all-*trans*-β-carotene and 24:1 for *cis*-β-carotene (total β-carotene – all-*trans*-β-carotene) (Trumbo *et al*., 2001).

Table 9.2: Estimated[1] amount of nutrients retained after cooking 100 g raw African leafy vegetables

	Unit	Chinese cabbage leaves	Black nightshade leaves	Amaranth leaves	Jew's mallow leaves	Cowpea leaves	Pumpkin leaves	Bitter watermelon leaves	Spider Flower leaves
Energy	kJ	120	162	272	319	280	222	296	195
Protein	g	2.5	0.5	4.2	3.2	4.7	2.9	3.5	5.0
Calcium	mg	144	189	421	295	378	168	201	220
Magnesium	mg	40	87	230	82	59	64	56	72
Phosphorus	mg	29	33	73	106	45	92	107	125
Sodium	mg	27	8	9	10	10	11	9	14
Potassium	mg	148	232	413	366	214	316	234	337
Copper	µg	63	152	161	181	129	196	188	238
Zinc	mg	0.28	0.53	0.66	0.54	0.40	0.71	0.70	0.99
Iron	mg	1.4	6.9	4.9	3.4	4.5	8.8	6.1	2.0
Manganese	mg	0.35	2.08	2.34	0.79	2.69	0.54	0.76	0.58
Thiamine	mg	0.03	0.07	0.03	0.02	0.06	0.03	0.01	0.05
Riboflavin	mg	0.06	0.16	0.05	0.03	0.08	0.09	0.09	0.20
Folic acid	µg	60	37	49	29	68	30	44	79
Vitamin C	mg	5	3	1	1	5	1	6	1
Vitamin A	µg RAE [a]	254	401	510	312	510	309	356	412

[1] The nutrient content in cooked African leafy vegetables was calculated from the raw values using the following nutrient retention factors: Protein, Energy and Manganese = 1 (assumption); Calcium, Magnesium, Sodium, Copper, Iron, Zinc, Riboflavin and β-Carotene = 0.95; Phosphorus and Potassium = 0.90; Thiamine = 0.85; Folic acid = 0.65; Vitamin C = 0.60 (USDA Table of nutrient retention factors, 2007)

[a] RAE (Retinol Activity Equivalents) = 1 µg RAE = 1 µg Retinol = 12 µg all-*trans*-β-carotene and 24 µg *cis*-β-carotene

Source for nutrient requirements: Trumbo *et al*., 2001; Otten *et al*., 2006

Table 9.3: Estimated nutrient contribution of an average portion size[1] of African leafy vegetables to the recommended dietary allowance (RDA) for children aged 4-8 years and women aged 19-30 years

	Unit	Chinese cabbage leaves	Black nightshade leaves	Amaranth leaves	Jew's mallow leaves	Cowpea leaves	Pumpkin leaves	Bitter watermelon leaves	Spider Flower leaves
Energy	% EER 4-8 y [a]	1	2	3	3	3	2	3	2
	% EER 19-30 y	1	2	3	3	3	2	3	2
Protein	% RDA 4-8 y [b]	9	2	15	12	17	11	13	19
	% RDA 19-30 y	5	1	9	7	10	6	8	11
Calcium	% AI 4-8 y [c]	13	17	37	26	33	15	18	19
	% AI 19-30 y	14	19	42	29	38	17	20	22
Magnesium	% RDA 4-8 y	22	47	124	44	32	34	30	39
	% RDA 19-30 y	13	28	74	27	19	21	18	23
Phosphorus	% RDA 4-8 y	4	5	10	15	6	13	15	17
	% RDA 19-30 y	4	5	10	15	6	13	15	18
Sodium	% AI 4-8 y	2	0	1	1	1	1	1	1
	% AI 19-30 y	2	1	1	1	1	1	1	1
Potassium	% AI 4-8 y	3	4	8	7	4	6	4	6
	% AI 19-30 y	3	5	9	8	5	7	5	7
Copper	% RDA 4-8 y	10	24	26	29	20	31	30	38
	% RDA 19-30 y	7	17	18	20	14	22	21	26
Zinc	% RDA 4-8 y	4	7	9	8	6	10	10	14
	% RDA 19-30 y	4	7	8	7	5	9	9	12
Iron	% RDA 4-8 y	9	48	34	24	31	61	43	14
	% RDA 19-30 y	8	38	27	19	25	49	34	11
Manganese	% AI 4-8 y	16	97	109	37	125	25	35	27
	% AI 19-30 y	19	115	130	44	149	30	42	32
Thiamine	% RDA 4-8 y	4	8	4	2	7	4	1	6
	% RDA 19-30 y	3	6	3	2	5	3	1	5
Riboflavin	% RDA 4-8 y	7	19	6	3	9	11	11	23
	% RDA 19-30 y	5	15	4	3	7	8	8	18
Folic acid	% RDA 4-8 y	21	13	17	10	24	11	15	27
	% RDA 19-30 y	15	9	12	7	17	8	11	20
Vitamin C	% RDA 4-8 y	13	8	3	2	15	3	17	4
	% RDA 19-30 y	6	4	1	1	7	1	8	2
Vitamin A	% RDA 4-8 y	44	70	89	55	89	54	62	72
	% RDA 19-30 y	36	57	73	45	73	44	51	59

[1] 90 g cooked African leafy vegetables for young children and 130 g cooked African leafy vegetables for females using a yield factor of 1.3 from raw to cooked

[a] EER = estimated energy requirements; EER (kJ) for 4-8 years is the mean of 7316 (EER for boys) and 6896 (EER for girls) = 7106

[b] RDA = recommended dietary allowance, which is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98%) healthy individuals in a particular life stage and gender group

[c] Adequate Intake (AI) as there is no RDA; the AI is a recommended intake value that is assumed to be adequate

Source for nutrient requirements: Trumbo *et al*., 2001; Otten *et al*., 2006

Table 9.4: Estimated nutrient contribution of an average portion size[1] of African leafy vegetables to the estimated average requirement (EAR) for children aged 4-8 years and women aged 19-30 years

	Unit	Chinese cabbage leaves	Black nightshade leaves	Amaranth leaves	Jew's mallow leaves	Cowpea leaves	Pumpkin leaves	Bitter watermelon leaves	Spider flower leaves
Protein [a]	% EAR 4-8 y [b]	11	3	19	15	22	14	16	24
	% EAR 19-30 y	6	1	11	8	12	8	9	13
Magnesium	% EAR 4-8 y	26	55	146	52	37	41	36	46
	% EAR 19-30 y	16	34	90	32	23	25	22	28
Phosphorus	% EAR 4-8 y	5	6	13	18	8	16	18	22
	% EAR 19-30 y	5	6	13	18	8	16	18	21
Copper	% EAR 4-8 y	13	31	33	37	26	40	39	49
	% EAR 19-30 y	9	22	23	26	18	28	27	34
Zinc	% EAR 4-8 y	5	9	12	9	7	12	12	17
	% EAR 19-30 y	4	8	10	8	6	10	10	15
Iron	% EAR 4-8 y	23	117	83	58	76	150	104	33
	% EAR 19-30 y	17	85	60	42	55	108	75	24
Thiamine	% EAR 4-8 y	4	9	5	2	8	4	2	7
	% EAR 19-30 y	3	7	4	2	7	3	1	6
Riboflavin	% EAR 4-8 y	8	23	7	4	11	13	13	28
	% EAR 19-30 y	6	18	5	3	9	10	10	22
Folic acid	% EAR 4-8 y	26	16	21	13	30	13	19	34
	% EAR 19-30 y	19	11	15	9	21	9	14	25
Vitamin C	% EAR 4-8 y	15	9	3	2	17	3	19	5
	% EAR 19-30 y	8	5	2	1	9	2	10	2
Vitamin A	% EAR 4-8 y	65	102	130	79	130	79	91	105
	% EAR 19-30 y	51	80	102	62	102	62	71	82

[1] 90 g cooked African leafy vegetables for young children and 130 g cooked African leafy vegetables for females using a yield factor of 1.3 from raw to cooked

[a] EAR = estimated average requirement; EAR for protein is based on a 20 kg child and 58 kg female

[b] The EAR is a daily nutrient intake value that is estimated to meet the requirement of half of the healthy individuals in a life stage and gender group

Source for nutrient requirements: Otten *et al.*, 2006

When comparing the estimated nutrient contribution of an average portion size of ALVs to the RDA of 19-30-year-old women, the following emerges. ALVs are low in energy content (<5% of the requirement). In general, ALVs provide 10% or less of the RDA for 19-30-year-old females for protein (except for spider flower), zinc (except for spider flower), thiamine, riboflavin (except for black nightshade and spider flower) and vitamin C. Phosphorous, copper and folic acid content range from about 4 to 26% of the RDA. Calcium and magnesium contents for all the ALVs are above 10% of the requirement, with the two best sources for calcium being cowpeas and amaranth and the best source for magnesium being amaranth. Iron content shows a large variation, with Chinese cabbage having the lowest content (<10% of the RDA), black nightshade and bitter watermelon both providing at least a third of the RDA, and pumpkin leaves providing nearly 50% of the RDA. Chinese cabbage has the lowest vitamin A content (36% of the RDA) and together with Jew's

mallow and pumpkin leaves provide more than a third of the RDA, whereas black nightshade, amaranth, cowpea, bitter watermelon and spider flower leaves provide more than 50% of the RDA.

When comparing the estimated nutrient contribution of an average portion size of ALVs to the EAR of 19-30-year-old women, the following emerges. In general, ALVs provide a maximum of 13% of the EAR for 19-30-year-old females for protein, and 10% or less for zinc (except for spider flower), thiamine, riboflavin (except for black nightshade and spider flower) and vitamin C. Phosphorous, copper and folic acid content range from about 5 to 34% of the EAR. Magnesium content varies between 16 and 34% of the EAR, with the exception of amaranth, which provides 90% of the EAR. All the ALVs, except Chinese cabbage, spider flower and Jew's mallow, provide at least 50% of the EAR for iron. All the ALVs provide at least 50% of the EAR for vitamin A.

9.5 DISCUSSION

In terms of the major nutritional deficiencies in the South African population, the vitamin A, iron and zinc content are of specific interest. The ALVs provide substantial amounts of vitamin A and an average cooked portion size (90 g for young children; 130 g for females) provide a significant proportion of the EAR for vitamin A. Chinese cabbage provides the least amount of vitamin A, while amaranth and cowpeas provide the largest amount of vitamin A. With the exception of Chinese cabbage, spider flower and Jew's mallow, all the ALVs provide at least half of the EAR for iron for 19-30-year-old females. The ALVs are not a good source of zinc.

Sixty-four percent of South African children are vitamin A deficient according to the 2005 NFCS-BF (Labadarios, 2007). Vitamin A deficiency reduces the child's ability to fight infections (Ross, 1996). Children who are vitamin A deficient have a higher risk for diseases, such as diarrhoea (Fawzi *et al*., 1995). Consumption of cooked and pureed green leafy vegetables (containing 750-850 µg RE, which is equal to 375-425 µg RAE, per unknown portion size) was shown to have a beneficial effect on improving vitamin A status (Takyi, 1999; Haskell *et al*., 2004, 2005). Table 10.2 shows that after cooking 100 g leaves (based on nutrient retention factors), ALVs exceeding 375 µg RAE are amaranth, cowpeas, black nightshade and spider flower leaves; amaranth and cowpeas exceed 425 µg RAE. Using fat, such as cooking oil, during preparation of ALVs will have a beneficial effect on vitamin A status because fat enhances carotenoid absorption for bioconversion to vitamin A (Jayarajan *et al*., 1980).

Several of the ALVs provide a significant amount of iron. Although dark-green leafy vegetables contain relatively large amounts of iron, they also contain oxalates, phytates and polyphenols that inhibit the absorption of non-heme iron. The bioavailability of non-heme iron in plant foods is therefore low and the potential contribution of plant foods towards controlling iron deficiency in developing countries has been questioned (De Pee *et al*., 1996). Agricultural interventions to increase the supply and intake of iron from plant foods are not popular. Instead, the production and consumption of animal foods are usually encouraged because of the high bioavailability of heme iron from animal foods (Ruel, 2001).

The iron content of pumpkin leaves in the present study was the highest followed by black nightshade and bitter watermelon and appears to be a very good source of non-heme iron compared to the other ALVs analysed. The iron content of raw pumpkin leaves, for example, in the SAFOODS database is 2.2 mg/100g (Kruger *et al*., 1998).

When compared to the nutrient values given for certain green leafy vegetables in the SAFOODS database (Kruger at al., 1998), differences between the nutrient content in the present study and the SAFOODS database values are observed. From a public health perspective, the most significant difference is probably the higher β-carotene content observed in the present study for most of the ALVs. The present study further

showed much lower values for vitamin C content. Comparing nutrient content of leaves from different data sources should be done cautiously as the nutrient content of raw plant foods vary widely and is affected by factors such as variety or cultivar; part of the plant consumed; stage of maturity; geographic site of production or climate; harvesting and post-harvest handling conditions; and storage. Common errors introduced during sampling procedures and analytical methods can also affect the nutrient values that are reported.

The energy and fat content of the ALVs is low. The leaves contain between 2.2 and 10.8 g fibre per 100 g raw edible portion. Because of these characteristics, ALVs are important elements of a dietary pattern to treat and prevent obesity. More than 50% of South African adult females are either overweight or obese (Department of Health, 2004). In many South African communities maternal obesity and child malnutrition co-exist within the same community, and often the same household (Faber *et al*., 2001). ALVs can therefore be potentially beneficial in terms of addressing maternal obesity and childhood micronutrient malnutrition, particularly vitamin A and, to some extent, iron.

9.6 CONCLUSION

Generally, the ALVs investigated in the present study contribute significantly towards the nutrient requirements for several of the nutrients, particularly vitamin A and iron, which are critical nutrients in the South African population. Based on the average portion size and the contribution towards the RDA for 4-8 year old children, amaranth and cowpeas are the best sources of vitamin A (>75% RDA), followed in a declining order by spider flower, black nightshade, bitter watermelon, Jew's mallow and pumpkin leaves (50-75% RDA), with Chinese cabbage the lowest source of vitamin A (25-50% RDA). Based on the contribution towards the RDA for 4-8 year old children, pumpkin leaves are the best source for iron (50-75% RDA), followed in a declining order by black nightshade, bitter watermelon, amaranth and cowpea (25-50% RDA), with Jew's mallow, spider flower and Chinese cabbage providing the least amount of iron (<25% RDA).

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CHAPTER 10: ANTIOXIDANT PROPERTIES OF SELECTED AFRICAN LEAFY VEGETABLES

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10.1 INTRODUCTION

Consumption of vegetables has been associated with a reduction in the incidences of chronic diseases of lifestyle such as cardiovascular diseases, coronary heart diseases and various types of cancer. With increasing urbanization and westernization of dietary and other socio-behavioural attitudes in South Africa, it is estimated that the burden of these diseases will increase to epidemic proportions (Addo *et al*., 2007; Parkin *et al*., 2008). Similar to other countries in sub-Saharan Africa, the magnitude of these diseases has been under-recognized and under-prioritized in South Africa because of competing health priorities such as HIV/AIDS and tuberculosis (Parkin *et al*., 2008).

African leafy vegetables (ALVs) contribute significantly to household food security and add variety to cereal-based staple diets (van den Heever, 1997). The consumption pattern of these vegetables across the country is however highly variable and depends on factors such as poverty status, degree of urbanization, distance to fresh produce markets and season of year (Jansen van Rensburg *et al*., 2007). Over the years, the frequency of consumption of ALVs has decreased. A possible reason for the decrease is that ALVs remain seasonal in rural areas and, unlike the exotic vegetables, are not readily available in the urban areas. Furthermore, these vegetables are often considered to be inferior in their taste and nutritional value compared to exotic vegetables such as spinach (*Spinacea oleracea* L.) and cabbage (*B. oleracea* subsp. *capitata*) (Weinberger and Msuya, 2004). This perception is prevalent despite the fact that several studies have indicated that ALVs contain micronutrient levels as high as or even higher than those found in most of their exotic counterparts (Uusiku *et al*., 2010).

ALVs contain phenolic compounds that have been shown to have antioxidant properties (Salawu *et al*., 2008 and 2009). These vegetables are usually cooked and less commonly stirfried and steamed before consumption. The level of antioxidant and radical scavenging activity after cooking is dependent on a number of factors including the type of vegetable, duration of boiling, boiling temperature, bioavailability of phenolics, localization and stability at high temperatures (Jimenez-Monreal *et al*., 2009). Differences in tissue hardness and phenolic profile of each vegetable are also major contributors to antioxidant activity (Yamaguchi *et al*., 2001). Published data on the effect of processing on phenolic contents and antioxidant activity of leafy vegetables in general are inconsistent and seem to depend on the plant species, as well as the type of assay used for analysis. Although studies on the effect of thermal processing on the phenolic composition and antioxidant activity of ALVs are limited, the trends may be similar to those reported for leafy vegetables originating elsewhere. Salawu *et al*. (2009) found that almost all phenolic constituents were stable after 10 minutes of boiling four ALVs, while Salawu *et al*. (2008) found a decreased total phenolic content in *C. olitorius* after boiling for 15 minutes.

In a study based on secondary intake data, Louwrens *et al*. (2009) reported that South Africans only consumed about half of their total antioxidant requirement. Understanding the antioxidant properties of

ALVs is therefore crucial for reducing pathogenesis related to CDL in South Africa. Currently, the available data on the phenolic composition and antioxidant activity of some ALVs (Lindsey *et al*., 2002; Odhav *et al*., 2007; van der Walt *et al*., 2009) is fragmented and incomplete. Different methods of analysis and standards have been used to analyze the antioxidant content and activity of these vegetables, therefore in most cases, the results cannot be compared. The effect of boiling on these parameters in ALVs has also not yet been well established. Knowledge on the above stated issues is required so that the potential of ALVs as a source of antioxidant phenolics can be evaluated. The results of such investigation may form the basis for promoting the utilization of these vegetables in managing CDL, as well as the cultivation and commercialization of these vegetables in this country.

10.2 AIMS OF THE STUDY

The ALVs selected were amaranth (*A. cruentus* L.), Jew's mallow (*C. olitorius* L.), cowpea (*V. unguiculata* L. Walp.) and pumpkin (*C. maxima*).

i) (a) To determine the total phenolic content, total flavonoid content and total antioxidant activity of selected ALVs.
(b) To identify and quantify selected flavonoids in extracts of ALVs using high-performance liquid chromatography (HPLC).

ii) To determine the effect of boiling and extraction solvent on the total phenolic content, total flavonoid content and total antioxidant activity of selected ALVs.

iii) To determine the ability of extracts of raw and boiled ALVs to protect against AAPH-induced oxidative damage in biological molecules (erythrocytes and plasmid DNA) and cell cultures (SC-1 mouse fibroblast and human adenocarcinoma colon cancer (Caco-2) cells).

10.3 METHODS

10.3.1 Green leafy vegetable samples and their preparation

The selected ALVs (i.e. amaranth, Jew's mallow, pumpkin and cowpea) were grown, harvested and collected from the Vegetable and Ornamental Plant Institute of the Agricultural Research Council (ARC) at Roodeplaat, Gauteng Province, South Africa. The vegetables were covered in black plastic bags and transported in a cooler box to the University of Pretoria.

The vegetables were washed with plenty of water to remove any soil or dirt. The consignment of each type of vegetable was divided into four, one was kept raw and the other cooked in boiling water (750 g to 1800 ml water) for 30 minutes. The cooking time was chosen as a mean of the period that these ALVs are traditionally cooked, usually in water from 10 to 60 minutes. After boiling, samples were drained, freeze-dried, ground and homogenized in a blender before passing through a 500 µm mesh sieve. The ground vegetables were finally packaged in air-tight Ziploc plastic bags and stored at -20˚C in the dark until analysis. For each type of vegetable, three independent samples were collected and processed immediately.

10.3.2 Crude plant extracts

Crude extracts were prepared for analysis by adding ground vegetable samples to distilled water at 0.01 $g.ml^{-1}$ concentration. The extraction was carried out for 1 hour at room temperature using a magnetic stirrer. The extraction mixture was filtered through Whatman No. 4 filter paper, and the filtrate used for analysis. Analyses were determined using three independent samples, each one analysed in triplicate after the crude extracts were obtained.

10.3.3 Antioxidant Analyses

Determination of total phenolic content (TPC)

TPC of each sample was determined using the Folin-Ciocalteu (F-C) method as described by Amin *et al*. (2006). To water extracts (0.1 ml), 0.75 ml of F-C reagent was added and mixed thoroughly. The mixture was allowed to stand for 5 minutes at room temperature before addition of 0.75 ml of sodium carbonate solution (60 $g.L^{-1}$). Ten millilitres of distilled water was then added and the contents were thoroughly mixed again and were allowed to stand at room temperature for 90 minutes before the absorbance of the mixture was measured at 760 nm, using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). Gallic acid was used as a standard and TPC results were expressed as gallic acid equivalents per gram ($GAE.g^{-1}$).

Determination of total flavonoid content (TFC)

TFC was measured using a modified aluminium chloride colorimetric assay of Zhishen *et al*. (1999). Catechin was used as a standard. A 30 µl aliquot of vegetable extracts or catechin solutions (0-0.167 $mg.ml^{-1}$) were placed in 96-well microplates. To each well, 30 µl 2.5% sodium nitrite, 20 µl 2.5% aluminium chloride and 100 µl 2% sodium hydroxide were added. The solution was mixed well and the absorbance was measured at 450 nm with a BioTek ELx800 plate reader (Analytical and Diagnostic Products, Weltevreden Park, South Africa). For each assay run, a blank with 30 µl phosphate buffer solution (PBS) (pH 7.4) was included to correct the absorbance values and TFC results were expressed as mg catechin equivalents (CE)/g.

Determination of antioxidant activity

ABTS radical scavenging assay

The ABTS radical scavenging assay used in this study followed the method of Awika *et al*. (2003). The $ABTS^{\bullet+}$ was freshly generated by adding 3 mM of potassium peroxodisulfate solution to 8 mM ABTS and the mixture was left to react in the dark for at least 12 hours at room temperature. The working solution was prepared by diluting $ABTS^{\bullet+}$ stock solution with 0.2 M PBS (pH 7.4). Trolox (water-soluble vitamin E analogue) was used as a standard, at a concentration range of 0 to 1000 µM. A volume of 2.9 ml of the working solution was added to 0.1 ml of ALV extracts or trolox. The reaction mixtures were left to stand at room temperature and the absorbance readings were taken at 734 nm after 30 minutes. Antioxidant activity was expressed as µmol Trolox Equivalents ($TE).g^{-1}$.

DPPH radical scavenging assay

The scavenging activity of vegetable extracts on DPPH radicals was measured according to the method of Awika *et al*. (2003) with some modifications. The stock solution was freshly prepared by dissolving 24 mg of DPPH in 100 ml methanol and was sonified for about 20 min to ensure that all DPPH had dissolved. A working solution was prepared by further diluting 10 ml stock solution with 50 ml methanol. In a 96-well plate, 285 µl of the working solution was added to a 15 µl volume of ALV extracts and the plate was left to stand in the dark for 15 minutes. Trolox (0-800 µM) was used as standard and the absorbance was measured at 570 nm, using the BioTek ELx800 plate reader (Analytical and Diagnostic Products, Weltevreden Park, South Africa). The results were expressed as µmol $TE.g^{-1}$.

ORAC assay

ORAC assays were carried out on a FLUOstar OPTIMA plate reader and procedures were based on a modified method of Ou *et al*. (2002). AAPH was used as a peroxyl radical generator, trolox as standard and fluorescein as a fluorescent probe. PBS was used as blank. Briefly, ALV extracts were diluted 15-fold with PBS. Fluorescein working solution (0.139 M) and AAPH (0.11 µM) were added to diluted extracts or Trolox (0-800 µM) concentration series. The prepared microplate was placed into the plate reader (Analytical and Diagnostic Products, Weltevreden Park, South Africa) and incubated at 37˚C and fluorescence was measured every 5 minutes for 4 hours. The assay protocol included the following parameters: a position delay of 0.5 seconds, a measurement start time of 0.0 seconds, 10 flashes per cycle, 300 seconds cycle time, 485 nm for

the excitation filter and 520 nm for the emission filter. The ORAC values of the samples were calculated by integrating the net area under the decay curves (AUC), using the Origin software Version 6.0 (Microcal, TM). The results were expressed as μmol TE.g^{-1}.

Quantification and identification of selected flavonoids in extracts of ALVs using HPLC

The reversed-phase HPLC analysis was conducted using the method of Kim *et al*. (2007). The sample extracts were filtered through 0.2 μm Millipore Millex filters prior to HPLC injection. The HPLC system consisted of a Waters 1525 binary HPLC pump and a Waters 2487 dual wavelength absorbance detector. The separation was accomplished by means of an YMC-Pack ODS AM-303 (250 mm x 4.6 mm i.d., 5 μm particle size) column. Breeze^{TM} software was used to monitor the separation process and after analysis a chromatogram was obtained for each sample extract.

The injection volume for all samples was 20 μl with the analysis conducted at a flow rate of 0.8 ml/minutes and monitored at 280 nm. The mobile phase consisted of 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B). The linear gradient of the solvents was as follows: solvent B was increased from 8 to 10% in 2 minutes, then increased to 30% in 25 minutes, followed by an increase to 90% in 23 minutes, then increase to 100% in 2 minutes, kept at 100% of B for 4 minutes, and returned to the initial condition. Running time was 60 minutes and the column temperature was held at 25ºC during the run.

The flavonoid standards (myricetin, rutin, kaempferol, luteolin, apigenin, epicatechin and quercetin) were prepared in dimethylsulphoxide (DMSO) at concentrations of 150, 100, 50, 25, 20, 10 and 5 ppm (mg.L^{-1}). Standards of 20 μl aliquots were chromatographed singly and as mixtures by injection into the HPLC system. Standard calibration curves were obtained for each compound by plotting peak areas versus concentrations. Regression equations that showed high degree of linearity ($R^2 \geq 0.984$) were obtained for each flavonoid from the calibration curves. Flavonoids in the samples were identified by comparing the retention time of the unknown with those of the standard flavonoids. The concentrations of the identified flavonoids were calculated using the regression equations obtained and expressed as mg.g^{-1} of sample on dry basis.

In-vitro biological assays

Preparation of erythrocytes suspension

After informed consents were obtained, blood was obtained from healthy volunteers via vein-puncture into tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Erythrocytes were isolated by centrifugation at 2750 x g for 3 min and washed at least four times with isotonic phosphate buffer solution (iso-PBS; 0.137 M NaCl, 3 mM KCl, 1.9 mM NaH_2PO_4 and 8.1 mM Na_2HPO_4, pH 7.4) to remove plasma, platelets and buffy coat.

Induction of oxidative stress

Induction of haemolysis was carried out based on the modified method of Ko *et al*. (1997). Briefly, 0.04 mg.ml^{-1} of ALV extracts was added to 10 μl of erythrocytes and the volume was made up to 150 μl with iso-PBS before incubation at 37ºC for 20 minutes. AAPH at a concentration of 4.4 x 10^{-3} μM was added and the erythrocytes were further incubated at 37ºC for 90 minutes. The extent of haemolysis was determined at 570 nm in the supernatant following centrifugation at 2750 x g for 3 minutes. Blanks included the same volume of erythrocytes in iso-PBS. The percentage protection (%P) against haemolysis was calculated using the formula: %P = 100 – [(absorbance of sample/control) x 100].

pBR 322 plasmid DNA damage assay

Procedures for this assay were based on a modified method of Wei *et al*. (2006). Briefly, the reaction mixtures containing 2.5 μg of pBR 322 plasmid DNA, 5 x 10^{-5} mg/ml of ALV extracts, 5.5 x 10^{-4} μM AAPH were made to 22.5 μl with BPS (pH 7.4) and incubated at 37ºC for 90 minutes. For the control samples, PBS

replaced the ALV extract treatments. After incubation, samples were mixed with an equal amount (22.5 µl) of gel loading buffer (0.13% bromophenol blue and 40% sucrose) and the reaction mixtures were immediately loaded into a 1% agarose gel in 40 mM Tris, 20 mM glacial acetic acid and 0.5 M EDTA (pH 8.0) (TAE) containing 0.5 $\mu g.ml^{-1}$ ethidium bromide. Electrophoresis was undertaken in TAE buffer for 2 hours at 60 V. The DNA bands were visualized by fluorescence under ultraviolet light.

Imaging and data analysis

Gel imaging and data analysis followed the method of Benherlal and Arumughan (2008). Stained gels were placed on a UV trans-illuminator at 254 nm and photographed to tagged image file format (TIFF) with a 4 mega-pixel digital camera (Canon power shot, Japan). The image captured in RGB format was imported into the image processing Gel Pro Analyzer, version 3.0 software (Media Cybernetics, Silver Spring, MD, USA) to quantify the density of the supercoiled DNA. The image was transformed to 8-bit grayscale format. The grayscale image was then normalized based on the blank lane by adjusting the brightness and contrast tool. The fluorescent region of each lane was then selected using free-hand selection tool and measured the average intensity of all pixels in the selected region. The numeric value of a pixel was between 0 and 255 based on their signal strength. The values of all pixels in the selected area were computed and the average value per pixel was determined for each lane. Average fluorescence in the blank lane was considered 100% protection (0% DNA damage) and the difference between the blank and treated DNA was taken as the extent of damage expressed as a percentage.

In-vitro cellular assays

DCF antioxidant protection assay

SC-1 mouse fibroblast and Caco-2 cells were plated at a concentration of 2×10^4 per well of a 96-well plate (Analytical and Diagnostic Products, Weltevreden Park, South Africa). The plate was then incubated at 37ºC for 24 h in a humidified CO_2 water-jacketed incubator (Forma Scientific, Ohio, USA). The DCFH-DA probe was initially dissolved in PBS (pH 7.4) to prepare a 200 µmol DCF-DA working solution.

Antioxidant activity

Cells were loaded with 20 µmol DCFH-DA working solution and incubated at 37ºC for 1 hour. 0.11 µM AAPH was then added together with the 0.2 $mg.ml^{-1}$ ALV extracts. Fluorescence readings were taken, and the results expressed as % damage, with 0.11 µM AAPH alone causing 100% damage.

Total cellular protection

Cells were loaded with 20 µmol DCFH-DA working solution and 0.2 $mg.ml^{-1}$ ALV extracts was added before incubation at 37ºC for 1 hour. This was followed by the addition of 0.11 µM AAPH. Fluorescence readings were taken, and the results expressed as % total cellular protection (100% damage caused by AAPH).

Intracellular protection

Cells loaded with 20 µmol DCFH-DA working solution together with 0.2 $mg.ml^{-1}$ ALV extracts were incubated at 37ºC for 1 hour. The cells were then washed with PBS (pH 7.4) to remove the ALV extracts. Cells were then exposed to 0.11 µM AAPH before fluorescence was measured. The results were expressed as % intracellular protection (100% damage caused by AAPH).

For all assays, fluorescence was measured using a FLUOstar OPTIMA plate reader (BMG Labtechnologies, Offenburg, Germany) immediately and every 2 minutes up to 1 hour.

10.3.4 Data Analysis

All experiments were repeated at least three times and the results were expressed as mean ± SD (for Tables) or SEM (for Figures). The data was subjected to analysis of variance (ANOVA), using samples and cooking time as independent variables and the values determined as dependent variables. Fisher's least significant difference (LSD) test was used for comparison of means using Statistica software Version 9.0 (StatSoft, Tulsa, OK).

10.4 RESULTS

10.4.1 Antioxidant content and activity of raw ALVs

Table 10.1 shows the TPC, TFC and antioxidant activity of extracts of the raw ALVs. Data was expressed as fresh weight (fw) basis to provide an indication of the TPC, TFC and antioxidant activity of the ALVs as is when harvested. Extracts from Jew's mallow had highest levels of TPC, TFC and ORAC values among all samples. TPC ranged between 2.1-3.0 mg GAE.g^{-1}, fw, while TFC ranged between 1.5-2.7 mg CE.g^{-1}, fw. The level of antioxidant activity for each ALV was dependent on the type of antioxidant assay. Antioxidant activity for extracts from the ALVs ranged between 44.7-75.3, 24.2-83.6 and 20.0-59.5 μmol TE.g^{-1}, fw for the ABTS, DPPH and ORAC assays, respectively (Table 10.1). Cowpea, pumpkin and Jew's mallow had highest antioxidant activity when determined using the ABTS, DPPH and ORAC assays, respectively.

10.4.2 Effect of boiling on the antioxidant content and activity of ALVs

Table 10.2 shows the effect of boiling on TPC, TFC and antioxidant activity of extracts of this material. Boiling reduced TPC and TFC of all ALVs, with the greatest effect observed in extracts of pumpkin with a 79% and 76% decrease in TPC and TFC, respectively. Generally, TPC and TFC contents of extracts from boiled ALVs were lower than for those of raw samples. Extracts of Jew's mallow were an exception due to a significant increase in TPC and no significant difference in TFC values of raw and boiled samples. Although there was a general decrease in levels of TPC and TFC after boiling, the ALVs retained appreciable levels of antioxidants.

In general, the trends in antioxidant activity were similar to those observed for TPC and TFC (Table 10.2). The three assays (ABTS, DPPH and ORAC) showed that there was a decrease in antioxidant activity of extracts of the ALVs after boiling. Jew's mallow appeared to deviate from this trend where its extracts from boiled leaves had higher antioxidant activity than the raw leaves according to the three assays. Extracts from boiled amaranth also had higher antioxidant activity than the raw amaranth leaves according to the DPPH assay.

10.4.3 Levels of flavonoids in raw ALVs

Almost all flavonoids were detected in the extracts of raw GLVs, except for luteolin, apigenin and quercetin in extracts of Jew's mallow (Table 10.3). Among the extracts, epicatechin was the most abundant flavonoid in amaranth, while rutin was most abundant in pumpkin. Pumpkin had the highest amounts of total flavonoids (12.01 mg.g^{-1}, dw or 1.36 mg.g^{-1}, fw), while jute mallow had the least amounts with 0.18 mg.g^{-1}, dw (0.02 mg.g^{-1}, fw).

10.4.4 Effect of boiling on flavonoid contents of ALVs

The contents of rutin in extracts of amaranth and Jew's mallow, as well as myricetin in Jew's mallow increased significantly ($p < 0.05$) as a result of boiling (Table 10.3).

Table 10.1: Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant activity of water extracts of raw African leafy vegetables (ALVs)

GLVs	TPC (mg GAE[1].g^{-1}, fw)	TFC (mg CE[2].g^{-1}, fw)	ABTS (µmol TE[3].g^{-1}, fw)	DPPH (µmol TE[3].g^{-1}, fw)	ORAC (µmol TE[3].g^{-1}, fw)
Amaranth	2.1 b[4] (0.0)[5]	1.5 b (0.0)	44.7 b (1.3)	24.2 b (2.2)	20.0 b (1.9)
Jew's mallow	3.0 c (0.0)	2.7 c (0.0)	58.7 c (1.1)	45.1 c (5.4)	59.5 d (7.1)
Cowpea	2.4 b (0.1)	1.5 b (0.1)	75.3 d (5.2)	81.5 d (8.3)	33.0 c (3.4)
Pumpkin	2.7 bc (0.0)	1.6 b (0.0)	62.1 c (2.5)	83.6 d (9.5)	36.0 c (4.1)

[1] GAE, gallic acid equivalents.
[2] CE, catechin equivalents.
[3] TE, trolox equivalents.
[4] Means with different letters in the same column are significantly different ($p < 0.05$).
[5] Standard deviation in parentheses.

Table 10.2: Effect of boiling on total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant activity of water extracts of African leafy vegetables (ALVs)

	Raw	Boiled
	Pigweed (amaranth)	
TPC[1]	22.7 b[6] (0.2)[7]	11.3 a (0.1)
TFC[2]	16.3 b (0.7)	6.9 a (0.7)
ABTS[3]	481.2 b (14.4)	416.7 a (13.9)
DPPH[4]	260.4 a (9.0)	525.2 b (64.9)
ORAC[5]	215.5 b (8.3)	110.1 a (8.4)
	Jew's mallow	
TPC[a]	25.0 a (0.4)	27.3 b (0.4)
TFC[b]	22.5 a (0.7)	24.7 b (2.4)
ABTS[c]	489.3 a (9.0)	578.7 b (18.6)
DPPH[d]	376.5 a (9.0)	887.0 b (65.0)
ORAC[e]	495.7 a (14.6)	612.0 b (24.3)
	Cowpea	
TPC[a]	23.7 b (0.9)	13.4 a (0.3)
TFC[b]	14.7 b (1.2)	6.8 a (0.7)
ABTS[c]	737.8 b (50.8)	471.4 a (16.4)
DPPH[d]	799.9 b (97.7)	351.4 a (32.8)
ORAC[e]	323.8 b (14.2)	253.2 a (11.7)
	Pumpkin	
TPC[a]	23.8 b (0.1)	4.9 a (0.0)
TFC[b]	14.3 b (0.7)	3.5 a (0.6)
ABTS[c]	545.1 b (22.0)	342.9 a (19.2)
DPPH[d]	733.1 b (85.3)	171.1 a (10.2)
ORAC[e]	315.5 b (16.4)	82.7 a (8.1)

[1] Expressed as mg gallic acid equivalents.g^{-1}, dry weight basis.

[2] Expressed as mg catechin equivalents.g^{-1}, dry weight basis.

[3] Expressed as µmol trolox equivalents.g^{-1}, dry weight basis.

[4] Expressed as µmol trolox equivalents.g^{-1}, dry weight basis.

[5] Expressed as µmol trolox equivalents.g^{-1}, dry weight basis.

[6] Means with different letters in the same row are significantly different ($p < 0.05$).

[7] Standard deviation in parentheses.

Table 10.3: Effect of boiling on levels of flavonoids (mg.g^{-1}, dry weight) in water extracts of selected African leafy vegetables (ALVs)

GLVs	Flavanol	Flavonols				Flavones		Total
extracts	Epicatechin	Rutin	Myricetin	Quercetin	Kaempferol	Luteolin	Apigenin	Flavonoids
amaranth								
raw	7.47 b[1] (0.56)[2]	1.64 a (0.78)	0.01 a (0.00)	0.01 a (0.00)	0.01 a (0.00)	0.01 a (0.00)	0.01 a (0.00)	9.16 a (2.78)
boiled	3.85 a (0.59)	7.50 b (0.11)	0.03 a (0.01)	0.01 a (0.00)	0.01 a (0.00)	0.01 a (0.00)	0.02 a (0.00)	11.44 b (2.96)
jute mallow								
raw	0.02 a (0.00)	0.13 a (0.04)	0.02 a (0.00)	n.d.	0.01 a (0.00)	n.d.	n.d.	0.18 a (0.06)
boiled	0.34 a (0.00)	1.59 b (0.02)	0.72 b (0.01)	n.d.	n.d.	n.d.	n.d	2.65 b (0.64)
cowpea								
raw	1.16 b (0.04)	0.86 a (0.37)	0.15 a (0.00)	0.01 a (0.00)	0.02 a (0.00)	0.02 a (0.00)	0.01 a (0.00)	2.23 b (0.48)
boiled	0.06 a (0.00)	0.21 a (0.04)	n.d.	0.01 a (0.00)	n.d.	n.d.	n.d.	0.28 a (0.10)
pumpkin								
raw	1.29 b (0.03)	3.64 b (0.63)	1.70 b (0.45)	0.76 b (0.08)	1.36b (0.57)	1.64 b (0.09)	2.12 b (0.90)	12.01 b (0.95)
boiled	0.79 a (0.01)	1.14 a (0.50)	0.23 a (0.03)	0.07 a (0.02)	0.01 a (0.00)	0.06 a (0.01)	0.01 a (0.00)	2.31 a (0.45)

n.d.: not detected.

1 Means with different letters in the same column are significantly different ($p < 0.05$).

2 Standard deviation in parentheses.

Extracts of boiled amaranth and Jew's mallow also had higher total flavonoids than extracts of raw samples (Table 10.3). The contents of epicatechin in amaranth, cowpea and pumpkin, rutin in pumpkin, as well as myricetin, quercetin, kaempferol, luteolin and apigenin in pumpkin decreased significantly ($p < 0.05$) after boiling. The content of total flavonoids of extracts from boiled cowpea and pumpkin was also less than that of raw samples.

10.4.5 Protection of erythrocytes by ALVs against oxidative damage

Among raw samples, all ALV extracts with the exception of Jew's mallow, significantly protected erythrocytes from AAPH-induced haemolysis (Figure 10.1). Extracts of raw Jew's mallow contributed to the damage of erythrocytes membrane and therefore offered no protection against AAPH induced haemolysis, while extracts from raw pumpkin offered the highest protection than all the other extracts. Among extracts from boiled ALV samples, amaranth and Jew's mallow exhibited highest protection against haemolysis, while pumpkin and cowpea offered least protections. There was no difference in % protection against haemolysis between extracts of raw and boiled amaranth, although the results from the antioxidant activity assays in Table 10.2 suggested that boiling reduced antioxidant activity in this vegetable.

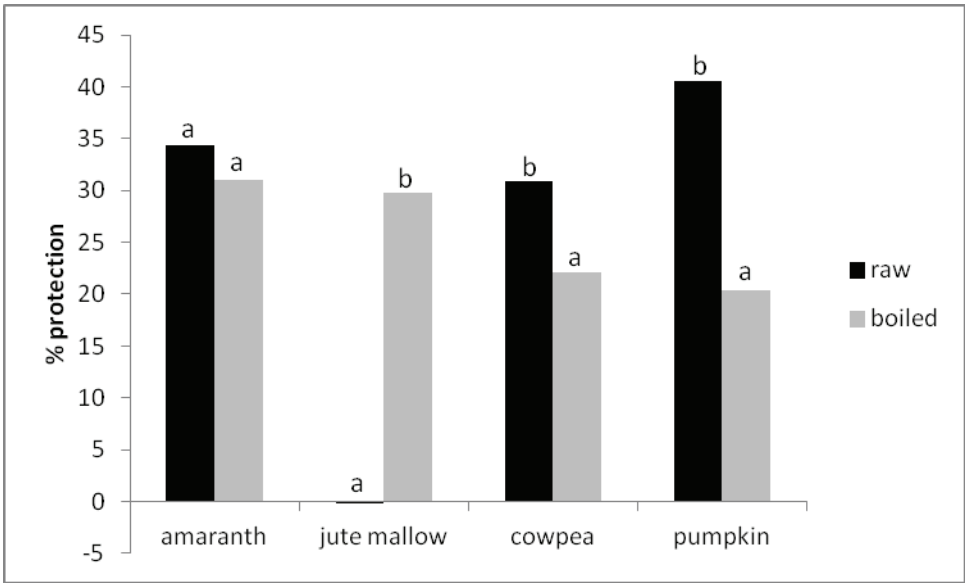

Figure 10.1: Protection against AAPH-induced damage of erythrocytes by leafy vegetable extracts. For each vegetable, means with different letters are significantly different ($p < 0.05$)

10.4.6 Protection of plasmid DNA by ALVs against oxidative damage

Figure 10.2 shows the quantitative % protection of the ALV extracts against oxidative damage on pBR 322 plasmid DNA. Among the extracts of raw samples, cowpea offered highest % protection, while amaranth offered least protection against AAPH-induced oxidative damage on the plasmid DNA. The protective effects of extracts from boiled samples were dependent on the type of vegetables. Boiling reduced the protective effects of cowpea and pumpkin and increased those of amaranth and Jew's mallow. Extracts of cowpea gave better protection among raw ALVs, while extracts of Jew's mallow offered highest protection among extracts of boiled samples. The results of this assay seem to be in agreement with those of total antioxidant activity assays discussed above, possibly indicating that the protective effects of ALVs against plasmid DNA damage may be attributed to their antioxidant content and activity.

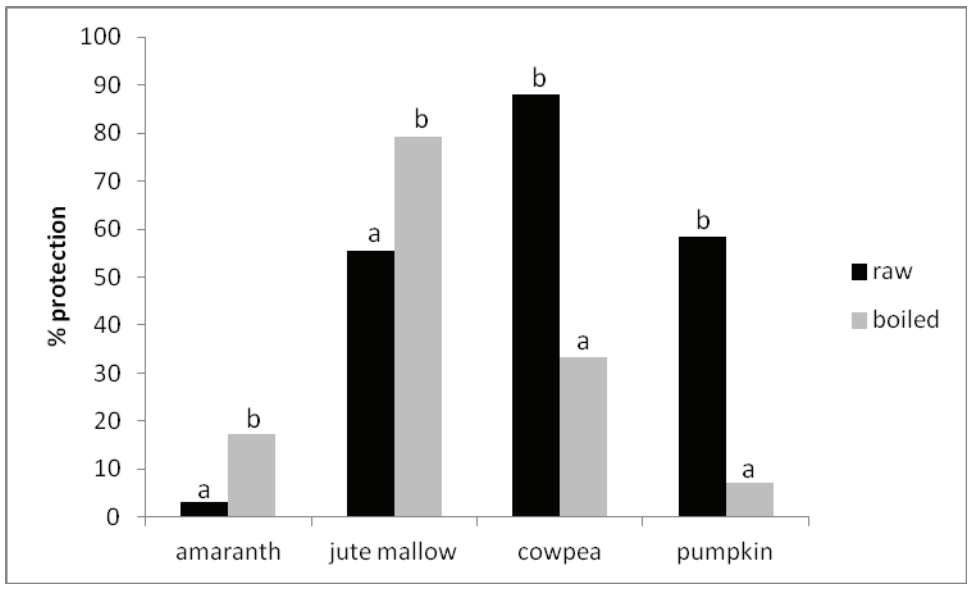

Figure 10.2: Protection against AAPH-induced damage of pBR 322 plasmid DNA by green leafy vegetable extracts. For each vegetable, means with different letters are significantly different ($p < 0.05$)

10.4.7 Comparison of total and intracellular antioxidant protection assays

Table 10.4 shows the percentage total and intracellular ability of ALVs to protect against oxidative damage. In the SC-1 cell line, high levels of total cellular protection were observed for all raw and boiled ALVs. In contrast, in the Caco-2 cell line, total cellular protective effects were observed for raw and boiled Jew's mallow and pumpkin and only for the boiled amaranth and cowpea. In both cell lines, pumpkin showed the best total protection, while cowpea the least. Table 10.4 further suggests that most of these GLVs were not able to provide good protection against damage, intracellularly.

Table 10.4: Percentage ability of water extracts of African leafy vegetables to protect cells against AAPH induced oxidative damage

Cell line	Cellular protection	amaranth		Jew's mallow		cowpea		pumpkin	
		raw	boiled	raw	boiled	raw	boiled	raw	boiled
SC-1	total	86.2 a[1] (2.2)[2]	96.4 b (0.8)	81.1 a(1.6)	87.6 a(1.4)	92.6 b(0.6)	82.3 a(3.1)	92.9 b(1.3)	87.4 a(1.7)
	intracellular	7.7 b (0.4)	-14.5 a (0.7)	-3.1 b(0.2)	-15.0 a(0.2)	25.8 b(0.5)	18.9 a(1.1)	9.2 a(0.8)	6.7 a(1.2)
Caco-2	total	-20.0 a (1.6)	76.4 b (0.0)	49.8 a(3.9)	81.5 b (2.6)	-39.9 a(4.0)	71.7 b(0.8)	58.6 a(2.1)	83.8 b(0.3)
	intracellular	12.1 a (0.4)	12.9 a (0.5)	7.9 b (0.7)	1.0 a (0.1)	13.3 b (1.2)	-15.0 a(2.0)	2.4 a (0.2)	4.1 a (0.4)

[1] Means with different letters for each vegetable per assay are significantly different ($p < 0.05$).

[2] Standard deviation in parentheses.

10.5 DISCUSSION

It is difficult to conduct a direct comparison of antioxidant content and activities reported in ALVs in the literature due to variations in factors such as sample preparation, extraction methods (Hayouni *et al*., 2007), assay types, maturity factors (Pandjaitan *et al*., 2005) and genetics (Cho *et al*., 2008). The values found in this present study fall within the wide range of TPC levels reported for these vegetables in the literature although in most instances there are differences in sample preparation and extraction methods used. Among the ALVs, the TPC values found in the literature include 9.6 mg GAE.g^{-1}, dw for water extracts (Oboh *et al*., 2008) and a range of 10.6 to 21.8 mg GAE.g^{-1}, dw for 80% methanol extracts (van der Walt *et al*., 2009) of *Amaranthus* species, which are less than the 22.7 mg GAE.g^{-1}, dw for extracts of amaranth found in this study (Table 10.2). Van der Walt *et al*. (2009) also reported TPC value of 29.1 mg GAE.g^{-1}, dw for 80% methanol extracts of cowpea leaves, which is higher than the 23.7 mg GAE.g^{-1}, dw for the water extracts of cowpea reported in this study. Salawu *et al*. (2008) reported TPC values of 42.3 mg GAE.g^{-1}, dw for 70% ethanol extracts of jute mallow, which is more than the 25.0 mg GAE.g^{-1} for water extracts of Jew's mallow found in this study.

Compared to this study, Yang *et al*. (2006) reported higher ABTS radical scavenging values of 79, 65 and 147 μmol TE.g^{-1}, dw for *Cucurbita* spp., *C. olitorius* and *Amaranthus* sp., respectively. Nevertheless, the antioxidant activity of ALVs used in this study is within the range reported for Mauritian endemic plants (Soobrattee *et al*., 2008). All these results are however lower than the approx. 2 mmol TE.g^{-1}, dw reported for water extracts of *A. cruentus* by Oboh *et al*. (2008). Using the DPPH assay, the antioxidant activity of *Amaranthus* sp. was also determined by Yang *et al*. (2006) and was found to be 4.0 μmol TE.g^{-1}, fw which is less than the 24.2 μmol TE.g^{-1}, fw found in the present study.

Information regarding TFC and ORAC values of ALVs or other vegetables similar to these vegetables in the literature is limited and therefore no comparisons were made. However, this data shows that the ALVs under study have appreciable levels of phenolics and antioxidant activity.

Studies of flavonoid composition of ALV species similar to the ones used in this study and grown in sub-Saharan Africa are also limited. It is therefore difficult to make direct comparison of flavonoid levels found in this study with those in the literature from leafy vegetables grown elsewhere. Factors such as variations in sample preparations and extraction methods, as well as differences in genotypes, agronomic, environmental and climatic growth conditions used are also critical in making comparisons (Cho *et al*., 2008; Luthria, 2006). Flavonoid contents of acid extracts from Taiwanese leafy vegetable species similar to the ones being studied here have previously been reported by Yang *et al*. (2008). The researchers did not detect these flavonoids in amaranth extracts. They reported 0.01, 0.60 and 1.05 mg.g^{-1}, fw quercetin in pumpkin, Jew's mallow and cowpea, respectively, as well as 0.02, 0.04 and 0.11 mg.g^{-1}, fw kaempferol in pumpkin, Jew's mallow and cowpea, respectively. These values are in the range of what the present study found. However, in contrast to the results observed in the present study, Yang *et al*. (2008) did not detect the flavones (luteolin and apigenin) in these vegetables. Salawu *et al*. (2009) found that the dicaffeoyl quinic acids (12.1 mg.g^{-1}, dw) and two quercetin monoglycosides (up to 5 mg.g^{-1}, dw) were the dominant compounds in the 70% ethanol extracts of Jew's mallow grown in Nigeria. Overall, each plant species seemed to possess a specific phenolic composition fingerprint based on its flavonoid compounds.

Regarding the effect of boiling on TPC, TFC, antioxidant activity and specific flavonoids; TPC and TFC contents of extracts from boiled ALVs were lower than for those of raw samples. Extracts of Jew's mallow were exception where there were no significant differences in TFC content between raw and boiled samples. Although there was a general decrease in levels of TPC and TFC after boiling, the ALVs retained appreciable levels of antioxidants. In general, the trends in antioxidant activity were similar to those

observed for TPC and TFC. The three assays (ABTS, DPPH and ORAC) showed that there was a decrease in antioxidant activity of extracts of the ALVs after boiling.

Boiling decreases the phenolic and antioxidant content of vegetables. The decrease may have resulted from leaching of vegetable antioxidants into the cooking medium (Roy *et al*., 2007; Wachtel-Galor *et al*., 2008) or may be due to oxidation of polyphenol components by polyphenol oxidase in vegetables (Yamaguchi *et al*., 2003). Amin *et al*. (2006) found that different varieties of the same *Amaranthus* species differed significantly regarding TPC content and the effect of blanching on TPC. Salawu *et al*. (2008) reported both decrease and increase in phenolic content of several ALVs after boiling. The trend of antioxidant and radical scavenging activity after boiling is dependent on a number of factors including the type of vegetable, type and duration of boiling, boiling temperature, bioavailability of phenolics, localization and the stability of high temperatures (Jimenez-Monreal *et al*., 2009). Differences in tissue hardness and phenolic profile of each vegetable are also major contributors to antioxidant activity (Yamaguchi *et al*., 2001). Yamaguchi *et al*. (2001) found both decreases and increases in flavonoid content after cooking. An increase in total flavonoid levels is attributed to new products with phenolic properties forming as a result of exposure to heat (Ruiz-Rodriguez *et al*., 2008), liberation of high amounts of individual flavonoids due to thermal destruction of vegetable cell wall structures and sub-cellular compartments (Yamaguchi *et al*., 2001), as well as suppression of the oxidation of phenolics by thermal inactivation of polyphenol oxidase in boiled vegetables (Yamaguchi *et al*., 2003).

ALVs contain appreciably high antioxidant content and activity. However, Wolfe and Liu (2007) argued that cellular antioxidant activity assays are more biologically relevant methods than the popular chemical antioxidant activity assays because the former account for some aspects of uptake, metabolism, and location of antioxidant compounds within the cells. Little is known about the functional role of the ALV extracts on oxidative damage as well as their toxicity level in biological molecules and cell cultures.

Effective protection of erythrocytes against free radical-induced oxidative haemolysis was reportedly offered by extracts of raw Indian *S. nigrum* L. and *S. torvum* L. (Loganayaki *et al*., 2010), Taiwanese *Bidens pilosa* (Yang *et al*., 2006) and some Portuguese plants (Gião *et al*., 2010). Inhibition of haemolysis by plant extracts has been associated with their respective antioxidant properties, especially the number and position of phenolic OH groups (Edenharder and Grunhage, 2003), binding of the flavonoids to the plasma membrane (Blasa *et al*., 2007), ability of flavonoids to penetrate lipid bilayers (Lopez-Revuelta *et al*., 2005), H-atom abstraction from the phenolic groups (Deng *et al*., 2006), as well as iron chelation by the polyphenols (Grinberg *et al*., 1997).

The damage to the cell membrane caused by extracts of raw Jew's mallow (Figure 10.1) could be due to either the presence of glycosylated flavonoids as observed by Kitagawa *et al*. (2004) or the presence of polysaccharides similar to a gel found in the extracts, which made it slimy. Polyphenols can create hydrogen bonds between their OH groups and H atoms of polysaccharides in the cell wall, leading to a gel-like structure that can encapsulate phenolic compounds and make their extraction difficult (Freitas *et al*., 2003). The results of this study indicate that the protection of cell membrane by ALV extracts may be dependent on the types of phenolics present in the extracts, as well as other constituents of the extracts including their synergistic effects, and not necessarily on the concentrations of phenolics and levels of total antioxidant activity.

Significant protection of DNA against degradation initiated with reactive oxygen species has been reported using extracts of Portuguese wild plants (Gião *et al*., 2008), Korean *Cnidium officinale* (Jeong *et al*., 2009) and some Indian plants (Benherlal and Arumughan, 2008). Protection of DNA from oxidative damage has

been attributed to iron chelation properties of extracts (Benherlal and Arumughan, 2008; Melidou *et al*., 2005), as well as the number and position of the phenolic OH groups, presence of oxo group at proximal carbon positions, and the presence of C2-C3 double bond of individual flavonoids in the extracts (Kumar and Chattopadhyay, 2007; Melidou *et al*., 2005).

Besides being a function of structure, concentration and localisation (cell membrane vs. cytoplasm), the observed effects in the cell culture assay was a function of cellular factors such as the cell line specific rate of absorption, metabolism, conjugation excretion as well as the density of the cells plated. Furthermore, other mechanisms involved in the protection against oxidative damage include stimulation of other biological mechanisms utilized by cells to protect themselves from reactive oxygen species such as endogenous antioxidant enzyme systems (Youdim *et al*., 2000). Protection of cell cultures against oxidative damage by plant extracts has been attributed to elevated glutathione peroxidase content in the cells after extract supplementations (Aherne, Kerry and O′Brien, 2007), presence of superoxide dismutase and glutathione peroxidase enzymes in the membranes (Cadenas and Davies, 2000), the ability of the extracts to act as reducing agent, free radical scavengers, and quenchers of singlet O_2 formation (Amarowics *et al*., 2004), binding of redox-active iron from specific intracellular locations (such as endosomal and lysosomal cell compartments) as well as the number and structural positions of phenolic OH groups of individual flavonoids present in the extracts (Melidou *et al*., 2005).

10.6 CONCLUSION

The chemical assays used in this study allowed for the rapid identification of ALV extracts with antioxidant activity that gave merit for further investigations using both biological and cellular systems. This study indicates that ALVs have appreciable antioxidant content and activity even after boiling. ALVs also offered good protection against AAPH-induced oxidative damage in biological and cellular models, although the level of these protections was mainly dependent on the type of ALV, as well as type of assay and cell lines used. The comparison of values found in this study with those in the literature is relevant and provide a better understanding of the importance of these vegetables in contributing to dietary diversification. The data obtained from this study can be used to encourage the consumption, cultivation and commercialization of these ALVs. In turn, this will assist in addressing the problem of low antioxidant status identified by Louwrens *et al*. (2009) possibly leading to the reduction of diseases associated with oxidative stress, most of which are reportedly increasing in sub-Saharan Africa.

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CHAPTER 11: AVAILABILITY AND HOUSEHOLD CONSUMPTION OF AFRICAN LEAFY VEGETABLES AT SELECTED SITES IN KWAZULU-NATAL AND LIMPOPO PROVINCES

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11.1 INTRODUCTION

A low intake of vegetables and fruit is among the top ten risk factors contributing to mortality worldwide (Ezzati *et al*., 2002). The World Health Organization (WHO) recommends a daily intake of more than 400 g of vegetables and fruit per person to protect against diet-related chronic diseases (WHO, 2003:56). This is about double the amount consumed by the average South African (Rose *et al*., 2002:33). A recent study showed that vitamin A rich fruit and vegetables, together with eggs and legumes, were the least consumed foods by South African adults (Labadarios *et al*., 2011). Increasing the intake of vegetables and fruit is therefore needed. However, rural and urban South African women in KwaZulu-Natal and Western Cape Provinces considered affordability, and to a lesser extent availability, as major constraints for the consumption of vegetables and fruit (Love *et al*., 2001).

A low intake of vegetables and fruit, particularly those rich in provitamin A carotenoids, contributes towards the high prevalence of vitamin A deficiency. For children younger than five years worldwide, an estimated 190 million are vitamin A deficient (WHO, 2009:10) and, in 2004, an estimated 0.6 million died because of vitamin A deficiency (Black *et al*., 2008). In South Africa, 63.6% of 1- to 9-year old children were shown to be vitamin A deficient in 2005 (Labadarios, 2007:428).

Micronutrient supplementation and food fortification are short and medium term strategies to address micronutrient malnutrition, but in the long term dietary diversification through a food-based approach involving agriculture has been proposed as one of the more sustainable options (Kiess *et al*., 2001; Ruel, 2008; Ruel and Levin, 2000:3). Dietary diversification has to widen its scope to include indigenous crops, such as wild-growing green leafy vegetables. Although concerns with respect to the bioavailability of vitamin A from green leafy vegetables have been raised (de Pee *et al*., 1995; Khan *et al*., 2007), consumption of cooked and pureed green leafy vegetables was shown to have a beneficial effect on improving vitamin A status (Takyi *et al*., 1999; Haskell *et al*., 2004; Haskell *et al*., 2005). It stands to reason that wild green leafy vegetables could have a similar beneficial effect, particularly in rural resource-poor settings.

The potential value for food security and rural development of gathering wild foods, growing locally adapted varieties and eating from the local ecosystem, is recognized by an international initiative (under the umbrella of the Convention of Biological Diversity) lead by the Food and Agriculture Organization of the United Nations (FAO), together with the Biodiversity International (formerly IPGRI, International Plant Genetic Resources Institute), with the overall aim to promote the sustainable use of biodiversity in programmes contributing to food security and human nutrition (Toledo and Burlingham, 2006). Smith and Eyzaguirre

(2007) argued that in Sub-Saharan African countries African leafy vegetables (ALVs) could play an important role in the WHO global initiative on increased consumption of vegetables and fruit.

In South Africa, dark green leafy vegetables are commonly consumed, particularly in rural areas, and can potentially make a significant contribution to dietary intake (Faber *et al.*, 2007; Steyn *et al.*, 2006). *Morogo/imifino* are traditional terms used for a collection of various dark-green leaves that are eaten as a vegetable; the leaves either grow wild or come from vegetables such as pumpkin, beetroot and sweetpotato. In this report, the term African leafy vegetables is used as the collective of leafy vegetables that usually grow wild (Jansen van Rensburg *et al.*, 2007). People in rural areas usually have free access to ALVs, where they can harvest them from either the wild, fallow land or home gardens. Urban people do not have these opportunities, as they have limited access to fallow land and wild spaces, and often less space is available for home gardens.

The potential effect of ALVs on the nutritional status of a particular population depends on many factors. From the model developed by Johns and Sthapit (2004) for developing countries (Figure 11.1) it is evident that these factors interact, yet, among others, the following could play a role: species that are geographically and seasonally available (related to biodiversity); species that are known and socio-culturally acceptable/popular as food; and the frequency of consumption and the amount consumed (related to socio-cultural traditions). All of this is affected by the specific (rural/urban) context.

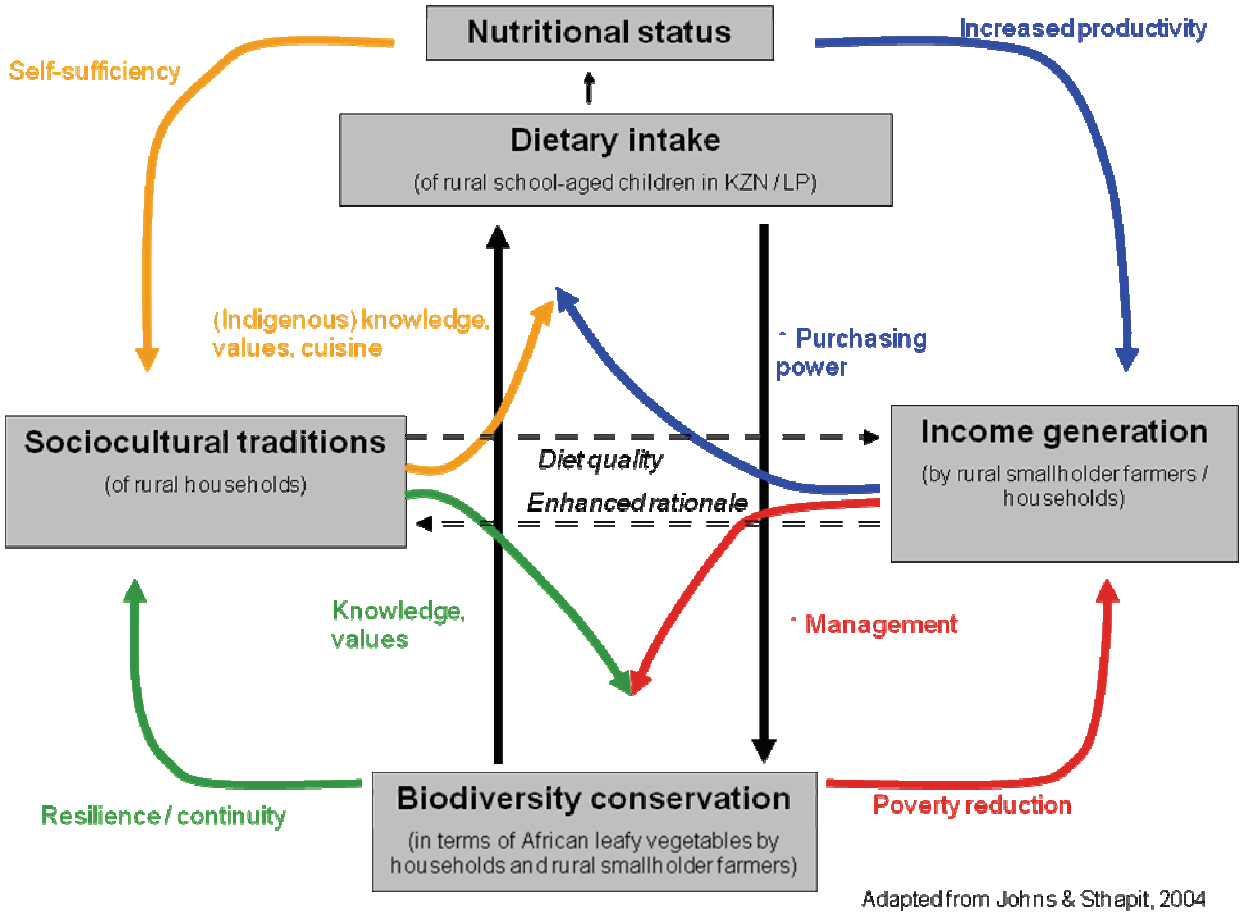

Figure 11.1: Synergy between nutrition, socio-cultural traditions, biodiversity conservation and income generation (adapted from Johns and Sthapit, 2004

11.2 AIM OF THE STUDY

The aim of this study was to determine the availability of, access to and nutrition-related uses of ALVs in two rural and one urban site in South Africa; to determine the contribution of ALVs to total nutrient intake of primary school children and their caregivers in one rural and one urban site; and to analyse a prominent ALV for β-carotene content.

11.3 OVERALL APPROACH

The study was done after the first summer rains, from October to December 2008 at three sites; a rural site in Limpopo province, and a rural and an urban site in the KwaZulu-Natal province. KwaZulu-Natal and Limpopo are the two provinces with the highest prevalence of vitamin A deficiency (Labadarios, 2007).

In the Limpopo province (where the term *morogo* is used), the rural villages selected for this study were located in the Greater Sekhukhune district municipality and within the local municipality of Makhuduthamaga. Greater Sekhukhune forms part of one of the Integrated Sustainable Rural Development (ISRD) nodes. ISRD nodes consist of districts and local municipalities that have been prioritized by the South African government for development as they are among the poorest areas in the country, and are characterized by poor infrastructure, limited resources and economic depression. Geographically the study site is a plateau area that is relatively eroded with generally shallow soils and limited vegetation cover.

In the KwaZulu-Natal province (where the term *imifino* is used), data were collected at both a rural and an urban site. The rural site, a mountainous village falling under the KwaXimba tribal authority bordering The Valley of a Thousand Hills just north of Pinetown, has a rugged terrain, is relatively inaccessible, and has steep slopes with mainly shallow soils that limit cultivation. A home-garden project promoting, among other, dark-green leafy vegetables was done in the area from 1998 to 2002 (Faber *et al*., 2002). The low socio-economic urban site is situated in the Mariannhill area, Pinetown, approximately 20 km south of the rural site, and is more densely populated.

Support and permission for the study were obtained from the local tribal authority in the respective study sites.

The study included the following components:

i) Qualitative explorative survey on the availability and use of ALVs at the two rural sites through observation, semi-structured interviews with key informants, and focus group discussions in the two rural sites (Section 11.4)
ii) Quantitave household-level survey on the procurement and consumption of *morogo / imifino* at the two rural and one urban site (Section 11.5)
iii) Quantified dietary survey on the nutrient contribution of *imifino* to total nutrient intake for children and caregivers in the two sites in KwaZulu-Natal (Section 11.6)
iv) β-carotene analysis of fresh, boiled and fried amaranth (Section 11.7)

The study was approved by the Ethics Committee: Faculty of Natural and Agricultural Sciences of the University of Pretoria (EC080826-036). Data on the nutrient contribution of *imifino* to total nutrient intake for children and caregivers in the two sites in KwaZulu-Natal was collected during a survey that investigated the availability of, access to, and consumption of fruit and vegetables in urban households in KwaZulu-Natal as part of a larger project “School gardens to address vitamin A”, which was approved by the Ethics

Committee of the Medical Research Council. Respondents who were interviewed gave written consent after the purpose and nature of the study were explained to them.

11.4 AVAILABILITY AND USE OF AFRICAN LEAFY VEGETABLES BASED ON OBSERVATION, SEMI-STRUCTURED INTERVIEWS AND FOCUS GROUP DISCUSSIONS

11.4.1 Methods

Observations and key informant interviews at the rural sites

A spokesperson at each rural site was requested to identify key informants who were considered knowledgeable regarding the traditional uses of ALVs. These key informants assisted during field walks to identify ALVs available in the village. Photographs were taken of the plants in their natural habitat and the local name of the plant was recorded. A sample of each plant was collected for identification of the scientific and popular English name by a horticulturist from the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC – VOPI) with assistance from the South African National Biodiversity Institute.

Semi-structured interviews were conducted with the key informants. This comprised of six females, aged 38 and 49 years at the rural Limpopo site, and eight females between the ages of 38 and 60 years at the rural KwaZulu-Natal site. The interview guide covered the availability of and access to the ALVs, beneficial traits and constraints related to the consumption and production (where applicable), their household use, and beliefs and practices.

Focus group discussions at the rural sites

The key informants recruited participants for the focus group discussions. In total, eight focus group discussion meetings were held; two with younger women (18 to 30 years) and two with older women (45 years and older) in each of the two rural sites. Purposive sampling was used to select between eight and twelve women who were considered knowledgeable on ALVs per focus group. Pictures taken during the site visits and a picture atlas portraying ALVs were used as an aid during the focus group discussions.

During each focus group discussion the participants identified the four most dominant ALVs in their areas. The source (cultivated, gathered from the wild, purchased) and information on the household's use were obtained for each of these plants. Aspects that were discussed included the parts of the plants that are used, preparation, processing and processing methods, beneficial traits, medicinal values, cultural beliefs and constraints for consumption, foods usually consumed with, acceptability in terms of taste, and knowledge/perceptions on nutritional benefits. For plants that were cultivated (if any), additional aspects were discussed, such as cultivation practices and constraints, susceptibility to pests and diseases, water use, marketability, and the family members responsible for cultivation.

Each focus group discussion lasted approximately one hour. A facilitator chaired the discussion using a discussion guide that was compiled specifically for the study. A fieldworker recorded the session and took notes. The information was captured by transcription, translated and checked.

11.4.2 Results

Dominant African leafy vegetables

A wide range of different ALVs was identified during the field walks (16 different plants at the rural Limpopo site and 20 different plants at the rural KwaZulu-Natal site). This was narrowed down during the

focus group discussions to ten and six plants for the two rural sites, respectively (Table 11.1). At the rural Limpopo site, spider plant (*Cleome gynandra*) was the dominant ALVs (mentioned during all four group meetings), followed by amaranth (*Amaranthus spp*) (mentioned during three group meetings) and wild watermelon (*C. lanatus*) leaves (mentioned during two group meetings). The remaining seven plants were each mentioned during one group meeting. At the rural KwaZulu-Natal site, amaranth and blackjack (*Bidens spinosa*) were both mentioned during all four group meetings. The remaining four plants were each mentioned during two group meetings.

Table 11.1: The four most popular African leafy vegetables identified per focus group discussion at the two rural sites

	Rural Limpopo site				**Rural KwaZulu-Natal site**			
	FGD1	**FGD2**	**FGD3**	**GD4**	**FGD1**	**FGD2**	**FGD3**	**FGD4**
Amaranth		✓	✓	✓	✓	✓	✓	✓
Spider plant	✓	✓	✓	✓				
Blackjack					✓	✓	✓	✓
Bitter watermelon		✓	✓					
Uwata [a]					✓	✓		
Imbobela [a]						✓		✓
White goosefoot							✓	✓
Stinging nettle					✓		✓	
Repelele [a]	✓							
Letlawane [a]	✓							
Devil's thorn	✓							
Purslane		✓						
Pumpkin			✓					
Morning glory				✓				
Lehlanye [a]				✓				

[a] common English names not identified
FGD – focus group discussion

Qualitative information from observations, key informants and focus group discussions at the rural sites

Information on the dominant ALVs (spider plant, amaranth, wild watermelon and blackjack)[3] as obtained from the key informants and focus group discussions at the two rural sites is summarised jointly below.

Amaranth *(Amaranthus spp)*

Rural Limpopo site

Various amaranth species are used as leafy vegetables. At the rural Limpopo site amaranth is known as *thepe*. The plants grow in the wild, planting fields and households' yards. Young amaranth leaves are handpicked by older women, every two to five days in summer. Washed leaves may be dried in the shade and stored for up to one year for consumption during winter. Cooked leaves may also be dried and stored.

During preparation, the leaves are boiled in a little water, with no water remaining after cooking; tomatoes and salt, and sometimes onions and minestrone soup powder are added. The respondents mentioned that the leaves are never fried. Amaranth is usually eaten with stiff maize meal porridge.

[3] Results from the quantitative household survey (section 10.5) confirmed that spider plant, amaranth and wild watermelon leaves were the most frequently consumed at the rural Limpopo site, and amaranth (and to a lesser extent blackjack) the most frequently consumed at the rural KwaZulu-Natal site.

Amaranth is perceived as nutritious ("has vitamins", "prevents us from going hungry", "feel very strong") and being good for the complexion ("freshens our skins"). Some respondents said that amaranth is good for constipation ("it makes the stomach loose"). This was echoed by others who said that if the first plants of the season are eaten with charcoal it prevents constipation or stomach problems. Two of the groups felt that the leaves could be sold for a source of income. Amaranth was not seen as the most popular *morogo*. It was regarded as less popular than spider plant, but better tasting than spinach.

Rural site in KwaZulu-Natal
At the rural KwaZulu-Natal site amaranth is known as *imbuya*. The plants grow in the wild, planting fields and households' yards. Amaranth is not cultivated. The leaves are picked weekly in summer after the first rains, mostly by older women. The leaves are stored in a fridge if electricity is available. The respondents mentioned that the leaves are not dried.

During preparation, the leaves are usually washed, chopped and steamed in the water left over from washing until just tender, and then fried in oil. The leaves form their own juice during boiling – this is discarded after boiling. Less often the leaves are boiled in salted water. Oil and onions, and to a lesser degree curry powder and soup powder, are added during preparation. Amaranth is usually eaten with either stiff maize meal porridge or homemade bread.

Amaranth was seen as nutritious ("gives us vitamins which is very important"), filling ("fills us up") and an energy booster ("gives energy as a booster"). The leaves are available throughout summer and hence popular. Knowledge of the plant was passed down over generations. It was seen as an integral part of nature helping with soil fertility. The respondents did not mention any cultural beliefs associated with amaranth. They did note that individuals eating it were regarded as poor and unable to afford other food. It was seen as having commercial possibilities as some said they had seen it being sold in towns. Amaranth was preferred above spinach.

Spider flower (spider plant) *(Cleome gynandra)*
At the rural Limpopo site spider plant is known as *lerotho*. The plants grow in the wild, mountains, planting fields and households' yards. When collected from the wild, young leaves with stems are regularly (daily up to every third day) hand-picked by older women in summer after the rains (can be harvested until autumn).

Spider plant is cultivated in the planting fields or home-gardens by older women. Seeds are obtained from older plants, dried and stored in a bottle or plastic bag until planted during summer after the rains. Production mainly depends on the rain. Manure is used as fertilizer. The plants are susceptible to various pests, such as locusts and worms. The cultivated leaves are sold within the community.

Washed leaves may be dried in either the shade or the sun, and stored in a packet for consumption in winter. The dried leaves are usually stored for three to four months, and in some cases for up to one year.

During preparation, the leaves are boiled with a little water for roughly an hour to an hour and a half. Food items added during preparation are mostly tomatoes and salt, and sometimes onions, oil and minestrone soup powder. No or little water remains after cooking, but if water is retained it may be used for soup. Whereas they do not fry the fresh leaves, they do fry the dried leaves in oil and add tomatoes and salt. The leaves are usually eaten with maize meal porridge, sorghum porridge or peanut butter. Sometimes spider plant and amaranth leaves are mixed.

The respondents valued spider plant because it occurs naturally and does not have to be bought; it was recognized as the "best" *morogo* on account of its taste ("it is the best and very nice") and was regarded as very nutritious; "having it is just like having meat". It tastes better than spinach (which has a "bad taste"); "Once it starts to rain everybody is happy because then the *lerotho* is coming". Respondents said it could be grown commercially. They learnt about its usage from their grandparents. According to one of the groups it sometimes helps with constipation. One group reported that when spider plant is eaten for the first time, they need to eat charcoal (from a fire) before they eat the leaves to prevent constipation or upset stomachs.

Tsamma melon (bitter watermelon) *(Citrillus lanatus)*
At the rural Limpopo site wild watermelon is known as *mochacha*. The plants grow in the wild. The leaves, which are available in summer, are picked daily by older woman. Leaves of any age are consumed. The leaves are usually boiled, and salt and tomato are added. The leaves may be dried and kept for consumption during winter.

Bitter watermelon is cultivated in community gardens by older women. The respondent was not sure whether the plants are susceptible to pests and diseases. The plants need little water. No fertilizer is used. The cultivated plants are used for household consumption; they are not sold. Although wild watermelon is cultivated, one of the respondents mentioned that a lot of will power is needed to cultivate the plants, as some people do not really see the benefit of cultivating something that grows in abundance in the wild.

Blackjack *(Bidens spinosa)*
At the rural KwaZulu-Natal site blackjack is known as *uqadolo*. The plants grow in fertile soil in the wild and in planting fields and gardens. They are also found on the riverside throughout the year. Blackjack is not cultivated. The leaves are picked weekly by older women from the wild in summer, or from plants growing next to the river in winter. According to the participants of the focus group discussions, blackjack cannot be stored for more than one day and is therefore consumed on the day it is harvested. According to the key informant, however, leaves could be stored in a fridge until consumed.

During preparation, the fresh leaves are boiled in no or a little water for up to an hour because it is "harder" than other varieties of *imifino*. It is then fried in oil. Onions and salt are added during preparation. Any water remaining after boiling is discarded. According to the key informant, only parts of the stem and leaf (not the entire leaf) are used. Blackjack is usually eaten with stiff maize meal porridge and sometimes with beans (legumes). One of the groups mentioned that blackjack cannot be eaten on its own – it must be mixed with other *imifino*.

Blackjack is appreciated because it is naturally available, particularly during winter when most of the other *imifino* is not available. It was considered nutritious. Respondents noted that children with flu drank the leave-extract, or ill children were bathed in it. It was also said to help with high blood pressure. Some of the respondents said they preferred blackjack over spinach because of its taste, while others said that spinach could be planted and thus, was better. It was not seen as having commercial value.

Other plants identified during the field-walks and key-informant interviews
Amadumbe *(Colocasia esculenta)*
At the rural KwaZulu-Natal site amadumbe leaves are known as *dumbekhulu*. The plants grow on the riverside. The leaves, which are available throughout the year, are picked weekly by older women and stored in a fridge until consumed. During preparation, the leaves are boiled for almost an hour, drained, boiled for another hour, drained, and then fried in oil. It is believed that amadumbe will prevent bad spirits. Amadumbe is better known as taro in African communities.

Devil's thorn *(Tribulus terrestres)*
At the rural Limpopo site devil's thorn is known as *tshehlo*. The plants grow in the planting fields, households' yards and mountains. During preparation, the young leaves are added to boiling water, with no water remaining after cooking. Tomatoes and salt are added during preparation. Washed leaves may be dried in the sun to be used during winter. Devil's thorn leaves are usually eaten with sorghum porridge.

Devil's thorn is cultivated in the planting fields by older women. No fertilizer is used. The plants are susceptible to insects. A shortage of water is a constraint for production. The cultivated leaves are not marketable. Devil's thorn leaves are believed to be good for the complexion. Devil's thorn was not seen as the most popular *morogo* and is thus not seen as being commercially viable.

Gallant soldier *(Galinsoga parviflora)*
At the rural KwaZulu-Natal site gallant soldier is known as *shukeyana*. The plants grow in the planting fields and community or home-gardens. The leaves are picked either weekly or every second week in summer, by older and younger women. During preparation, the young leaves are boiled for about 15 to 20 minutes, and then fried in oil. The leaves are not stored.

Wild jute *(Corchorus asplenifoluis)*
At the rural Limpopo site wild jute is known as *thelele*. The plants grow in the wild. The leaves, which are available throughout the year, are picked daily by older women. Leaves of any age are consumed. During preparation, leaves are boiled for approximately 5 minutes. The leaves may be dried in winter and stored for consumption. It is believed that consumption of wild jute during the final months of pregnancy will ensure that the baby and the mother are healthy. A distinct characteristic of wild jute is its mucilaginous texture after being cooked.

At the rural KwaZulu-Natal site wild jute is known as *unamunamu*. The plants grow in the wild, next to the road, and in the planting fields. The leaves, which are available throughout the year, are picked weekly by older women and stored in a fridge until consumed. Leaves of any age are consumed. During preparation, the leaves are boiled, and then fried in oil for about 15 minutes.

Morning glory *(Convolvulus* spp.*)*
At the rural Limpopo site morning glory is known as *lefswe*. The plants grow in the wild. The plants are available after the first spring rains until autumn. The young leaves are picked by older woman. The leaves are left in the shade for a day before cooking. During preparation, the leaves are boiled in a little water for about 5 minutes, with no water remaining after cooking. Tomatoes, salt and amaranth leaves are added during preparation. Morning glory leaves are usually eaten with maize meal porridge. It does not taste good; even spinach tastes better. They do however value it because it is the first *morogo* of the season. It is not seen as being commercially viable because of its taste.

At the rural KwaZulu-Natal site morning glory is known as *umkhoka*. The plants grow in the wild. The leaves, which are available in summer, are picked every second week by older women and stored in a fridge until consumed. During preparation, the leaves are boiled for about 5 to 10 minutes, and then fried in oil.

Nightshade *(Solanum retroflexum)*
In the rural KwaZulu-Natal site nightshade is known as *umsobo*. The plants grow on the riverside. The leaves, which are available throughout the year, are picked every 3 to 4 days by older and younger women. During preparation, the young leaves are boiled for about 30 minutes, and then fried in oil. The leaves are not stored.

Pumpkin leaves (*Cucurbita* spp.)
At the rural Limpopo site pumpkin leaves are known as *mphodi*. Pumpkin leaves are a cultivated *morogo* and seeds are planted after the first summer rains. Pumpkin does not grow wild. The young leaves are picked every three to four days. Washed leaves are cut into pieces and dried in the shade; it is stored for use during winter.

During preparation, the leaves are cooked for about 10 minutes; tomatoes, salt and potatoes are added. Pumpkin leaves are mixed with rice or eaten with maize meal porridge.

Pumpkin leaves are regarded as nutritious ("gives us strength") and important because pumpkins are also obtained from the plant. It is regarded as commercially viable ("better than other *morogo*") and better tasting than spinach.

Purslane *(Portulaca quadrifida)*
At the rural Limpopo site purslane is known as *tejane*. The plants grow in the wild. The whole plant is consumed, and is collected during winter by younger women. During preparation, the plants are grinded with a stone to be softened, added to boiling water and then boiled for approximately an hour. Tomatoes, salt and traditional *lengangala* are added during preparation. The left-over water is eaten as soup. The cooked plant is eaten with sorghum porridge.

Purslane is regarded as nutritious. It is beneficial for the complexion ("they become more beautiful"), and individuals with skin rashes are washed with this plant. It is believed that purslane prevents colds, coughs and diarrhoea. Their grandmothers used to give purslane to pregnant women. Consumption of purslane during pregnancy is believed to be beneficial during childbirth. It is not regarded as commercially viable despite its medicinal advantages over other *morogo*.

Stinging nettle *(Utrica urens)*
At the rural KwaZulu-Natal site stinging nettle is known as *imbongozemba* or *imbati*. The plants grow in the wild and on the riverbanks, and are available throughout the year. The leaves are harvested mostly in winter when the leaves are fresh (according to the respondents the leaves are old during summer). The plant has stinging hairs and therefore scissors are often used when picking the leaves. The leaves are picked every second to fourth day. According to the one group the leaves cannot be stored, while the other group (and the key informant) mentioned that the leaves can be stored in a fridge. During preparation, the young leaves are chopped and boiled in water for about 15-30 minutes, where after the water is drained off. The water remaining after boiling may be consumed, especially by children. Salt is added during preparation. Sometimes the boiled leaves are fried in oil and with onions. The cooked leaves are eaten with stiff maize meal porridge, sour porridge or beans. It softens other added *imifino* when cooked with it.

During one of the group discussion it was mentioned that the plant can be transplanted from the wild to the households' gardens during any time of the year.

The water that stinging nettle was boiled in is also used to lower blood pressure, especially in summer when the temperatures are high. It is also believed that it helps to stop bad spirits spreading in the water. It is believed that stinging nettle prevents witch doctors. The people appreciate stringing nettle because it is available in winter. However, it is regarded as a food eaten by people who are too poor to afford other food. It is not seen as having commercial possibilities. Spinach is preferred over stringing nettle.

Thistle *(Sonchus asper; Sonchus oleraceus)*
At the rural Limpopo site thistle is known as *lekgakga and lekgakga red.* The plants grow near water. *Lekgakga* leaves are available during summer, while l*ekgakga red* leaves are available throughout the year provided there is water nearby. Older women pick the leaves twice weekly, or daily for *Lekgakga red*. They consume only the young leaves of *lekgakga* but all the leaves of *lekgakga red*. During preparation, *lekgakga* leaves are boiled with little water (only covers the bottom of the pot) until soft. *Lekgakga red* leaves are boiled for roughly 1½ hour, and salt and tomato are added. It is believed that HIV positive people tend to show improvement when consuming thistle leaves grown in the wild. It is further believed that people will live longer if they consume thistle leaves.

At the rural KwaZulu-Natal site thistle is known as *ihwabuhwabu.* The plants grow in the wild, next to the road, in the planting fields, and in community or home-gardens. The leaves, which are available throughout the year, are picked weekly by older women and are stored in a fridge. During preparation, the leaves are boiled for about 3 minutes, and then fried in oil with onions.

Wandering jew *(Commelina benghalensis)*
At the rural Limpopo site wandering jew is known as *lehopje.* The plants grow everywhere depending on the amount of rain. The leaves, which are available in summer, are picked daily by older women. Leaves of any age are consumed. During preparation, the leaves are boiled for about 10 minutes. Wandering jew is consumed mixed with amaranth. The leaves may be dried and stored for consumption.

At the rural KwaZulu-Natal site wandering jew is known as *idangabane*. The plants grow in the wild, on the riverside or next to other water sources, in the planting fields, and in community or home-gardens. The leaves, which are available throughout the year, are picked weekly by older and younger women and stored in a fridge. Leaves of any age are consumed. During preparation, the leaves are boiled for about 15 minutes, and then fried in oil with onions.

White goosefoot / Lambs quarters / Fat hen *(Chenopodium album)*
At the rural Limpopo site white goosefoot is known as *seruwe.* The plant was originally spotted at Ba-Pakewadi. The plant grows in the mountains. The leaves, which are available in winter, are picked daily by older women. Leaves of any age are consumed. During preparation, the leaves are boiled for about 5 minutes; salt and tomato are added. The leaves may be dried during winter and stored for consumption. It is believed that white goosefoot is good for the treatment of high blood pressure and diabetes. Whether the medicinal value is a medical fact or a belief only time will tell (according to the respondent).

At the rural KwaZulu-Natal site white goosefoot is known as *imbilikicane*. The plants grow in the wild, planting fields, and community or home-gardens. The leaves, which are available in summer, are picked every second week by older women and stored in a fridge. During preparation, young leaves are usually washed, and then steamed in the water left from washing for about 10 minutes, and then fried in oil. Onions, salt and stock cubes are added during preparation. White goosefoot leaves are eaten with homemade bread and stiff maize meal porridge. White goosefoot has a bad smell and is not very tasty; nonetheless, they eat it because their parents advised them to do so. White goosefoot leaves are sometimes mixed with other *imifino*. White goosefoot is not a popular *imifino*; spinach is preferred over it. White goosefoot is regarded as a food for poor people. The leaves cannot be sold. White goosefoot is not cultivated.

Unidentified plants

Rural Limpopo site

Four plants could not unambiguously be identified, namely *bolouwane*, *lehlanye*, *letlawane* and *repelele*.

Bolouwane plants grow in the wild. The leaves are picked in summer daily by older women. During preparation, the young leaves are boiled; salt is added. The leaves may be dried and stored. It is believed that eating the leaves will "make the stomach free". Midwives give the leaves to mothers during childbirth.

Lehlanye plants grow in the wild. The leaves are picked from spring to autumn by older women. During preparation, the young leaves are added to plenty of boiling water, and boiled for about an hour until there is not water remaining; tomatoes and salt are added. *Lehlanye* leaves are eaten with maize meal porridge. Fresh and cooked leaves may be dried in the shade and stored. According to the respondents, *lehlanye* leaves do not have a pleasant taste (it is sour) and are therefore not marketable. *Lehlanye* leaves are less popular than spinach. It is believed that people can live longer if they consume the leaves and that the roots of the plant can be cooked in water, which can then be drunk to prevent bedwetting. *Lehlanye* can be cultivated. No fertilizer is used. Although the plants are drought tolerant, a lack of water was seen as a constraint for production. The plants are susceptible to insects. The cultivated plants have no marketability.

Letlawane leaves are picked after the first summer rains every five days from the cultivated fields. Washed leaves may be dried in the sun and stored for winter usage. The dried leaves are marketed as a source of income. During preparation, the leaves are cooked with a little water for about 10 minutes; tomatoes, salt and onions are added. *Letlawane* leaves are eaten with sorghum porridge. It is regarded as nutritious and a source of energy. Respondents reported that if burnt in a fire, the smoke could be inhaled as a cure for headache. Dried leaves may be mixed with peri-peri and boiled as a cure for stomach ailments. Paste from the dried plants may be applied to wounds. It was neither more nor less popular than other varieties of *morogo*.

Repelele plants grow in the wild, planting fields and home-gardens. The leaves are picked in summer by older and younger women. During preparation, the washed young leaves are added to boiling water, and boiled for approximately 25 minutes. Tomatoes, onion and salt are usually added during preparation. Washed leaves may be dried in the sun and stored for consumption during winter. *Repelele* leaves are eaten with sorghum porridge. *Repelele* has a different taste than spider plant. *Repelele* is liked ("it is nice") and it tastes better than spinach. It is seen as having benefits for the complexion. It is not seen as being commercially viable (it is not that popular). *Repelele* plants are cultivated in the planting fields and home-gardens. Production depends on the rain, and no fertilizer is used. There are no production constraints. The leaves are not marketed.

Rural KwaZulu-Natal site

Eight plants could not unambiguously be identified namely *gqithikazi*, *imbobela*, *umamgoblozi*, *umvemvane*, *uqanga*, *usawotshana*, *usilepi*, and *uwata*.

Gqithikazi plants grow in the wild, on the riverside, in the planting fields, and in community or home-gardens. The leaves, which are available throughout the year, are picked weekly by older and younger women. During preparation, the young leaves are fried in oil with onions for about 10 to 15 minutes. The leaves are not stored.

Imbobela plants grow in the wild, and community or home-gardens. The leaves are picked every second day in summer by older women and stored in a fridge. During preparation, the young leaves are either boiled for about 30 minutes, or it is boiled in its own juice for about 5 minutes after which the juice is discarded and the leaves fried. Peanuts, onions and tomatoes are added during preparation. *Imbobela* is eaten with stiff maize

meal porridge and homemade bread. It cannot be cooked on its one because it has a “slimy taste” and must have other *imifino* added to it. Spinach is better tasting and more highly regarded. It is regarded as a food eaten by people who are poor. It has no commercial value.
Umamgoblozi plants grow on the riverside throughout the year. The leaves are picked every third day by older women and stored in a fridge. During preparation, the young leaves are boiled for about 15 minutes, and then fried in oil.

Umvemvane plants grow in community or home-gardens throughout the year. The leaves are picked every second week by older women and stored in a fridge, or kept in cold water. During preparation, the young leaves are boiled for about 5 minutes; no oil is added.

Uqanga plants grow in the wild, planting fields and home-gardens. The leaves, which are available in summer, are picked weekly by older women and stored in a fridge. The washed leaves are boiled with little water; salt and onions are added. Sometimes the leaves are fried.

Usawotshana plants grow in the wild, planting fields, and community or home-gardens. The leaves, which are available throughout the year, are picked weekly by older women and stored in a fridge. During preparation, the young leaves are boiled, and then fried in oil for about 10 minutes.

Usilepi plants grow in the wild, and in community or home-gardens. The leaves, which are available throughout the year, are picked weekly by older women and stored in a fridge. During preparation, the young leaves are boiled in a little water, salt is added, and then fried with onions and tomatoes.

Uwata plants grow in water and on the riverside throughout the year. While it is available and can be eaten throughout the year, it is normally harvested in winter as other *imifino* are available and harvested from spring to autumn. The leaves are picked weekly by older and younger women. During preparation, the washed young leaves are boiled with salt and pepper for about an hour, the remaining water is discarded, and the leaves are then fried in oil; onions, tomatoes and soup powder are added. Sometimes *uwata* leaves are mixed with other *imfino* types when consumed. The cooked *uwata* is eaten with stiff maize meal porridge. The leaves are not stored; the leaves need to be stored in a fridge.

The respondents valued *uwata* because it is available during winter when most of the other *imifino* is not available. Elderly mothers and grandmothers roasted the plant as medicine for pregnant mothers who had difficulties with their pregnancies. According to the respondents people who eat *uwata* are seen as not having the financial means to buy other food. *Uwata* is preferred over spinach because it is healthier and better tasting. *Uwata* leaves can be sold.

11.5 PROCUREMENT AND CONSUMPTION OF *MOROGO / IMIFINO* BY HOUSEHOLDS BASED ON QUANTITATIVE SURVEYS

11.5.1 Methods

One hundred households per study area at the two rural sites, and 400 households at the urban site were randomly selected. The respondents (one per household) were interviewed in the local language by trained fieldworkers using a structured questionnaire that focused on the procurement, frequency of use and preparation methods of *mororgo / imifino*. Socio-demographic data were collected to set the context in which the study was done. The questionnaire was developed using the guidelines of Gross *et al*. (1997). Data was collected during November and December 2008.

11.5.2 Results

In total, 592 respondents were interviewed (rural Limpopo n = 100; rural KwaZulu-Natal n = 101; urban KwaZulu-Natal n = 391). Their age, educational and household characteristics are given in Table 11.2. The rural KwaZulu-Natal respondents were slightly older and had lower educational levels than the respondents at the other two sites. The urban KwaZulu-Natal site was the best-serviced and the rural Limpopo site the least-serviced area in terms of potable water, electricity and sanitation.

Table 11.2: Age, educational and household characteristics of the respondents who were included in the quantitative questionnaire survey

		Limpopo	**KwaZulu-Natal**	
		Rural	**Rural**	**Urban**
		n = 100	**n = 101**	**n = 391**
Age (years)	mean (SD)	43 (15)	50 (14)	41 (13)
Educational level (%)				
did not attend school		19	39	10
grade 1-7		20	40	33
grade 8-12		59	21	54
higher qualifications		2	0	3
Number of people per household	median	6	7	7
	range	2-11	2-15	2-17
Toilet facilities (%)				
flush toilet, connected to public pipe		0	1	13
flush toilet, not connected to public pipe		1	2	10
pit toilet		87	97	77
none		12	0	0
Source for drinking water (%)				
river / stream / spring / dam		36	0	1
own tap – inside the dwelling		0	15	18
own tap – outside the dwelling		0	54	35
public tap		63	2	44
neighbour's tap		0	29	3
borehole		1	0	0
Electricity available in home (%)		79	94	93
Energy source for cooking food (%)				
electricity		5	49	75
wood, open fire inside dwelling		93	29	1
wood, open fire outside dwelling		0	14	1
gas or paraffin		2	8	19
other		0	0	5

The percentage of households usually collecting ALVs from the wild and the leaves that are usually (regardless of the season) collected by at least 20% of the households are shown in Table 11.3, while Table 11.4 reflects the main source and season of availability. All the households at the rural Limpopo site usually collected ALVs from the wild, versus 66% at the rural KwaZulu-Natal site and 39% at the urban KwaZulu-Natal site; mostly women collected the leaves (Table 11.3).

Table 11.3: African leafy vegetables gathered from the wild

	Limpopo	KwaZulu-Natal	
	Rural	**Rural**	**Urban**
	n = 100	n = 101	n = 391
Percentage of households getting wild growing green leafy vegetables from the wild	100	66	39
Person(s) usually collecting the leaves from the wild [1] (%)	n = 100	n = 67	n = 153
older women	67	87	67
younger women	86	42	46
younger men	1	-	-
children, girls	10	21	11
children, boys	-	-	3
Leaves collected from the wild [2] (%)	n = 100	n = 67	n = 153
amaranth	89	54	37
blackjack	-	49	21
devil's thorn	38	-	-
Jew's mallow	21	-	-
lehlanye	50	-	-
spider plant	86	-	-
wild watermelon	58	-	-

[1] Expressed as a percentage of those households who usually collect African leafy vegetables
[2] African leafy vegetables gathered from the wild by at least 20% of the households

In the rural Limpopo site younger woman than older woman collect ALVs; this is contrary to the belief that the younger people are not interested in the ALVs anymore (or it may also be linked to the poverty in the area). Households at the rural Limpopo site collected a bigger variety of leaves than households at the two KwaZulu-Natal sites. Amaranth and spider plant were the most popular leaves collected at the rural Limpopo site, followed by wild watermelon and *lehlanye* (probably a *Centaurea* species). Amaranth and blackjack were the most popular leaves collected at the two KwaZulu-Natal sites. From Table 11.4 it is clear that ALVs are available mostly during summer, and are typically collected from the wild. Decline in wild spaces and wild populations of ALVs will necessitate the cultivation of ALVs. It also emphasizes the need for sustainable harvesting of ALVs in the wild.

Type of leaves collected by 10-20% of households included the following:

Rural Limpopo site: *letloane;* morning glory; purslane; *repelele*

Urban KwaZulu-Natal site: gallant soldier

Type of leaves collected by 1-10% of households included the following:

Rural Limpopo site: blackjack; *bolouwane*; *dipepetwane*; Jew's mallow; *lehowane*; *maswiana*; *mathetswana*; *mogoti*; *molebedu*; *monawa*; *monyaku*; *motangtang*; *motatane*; *mphodi*; *sekalerotwana*; *sekatheetswana*; *swkalerotwana*; thistle; white goosefoot

Rural KwaZulu-Natal site: *imbobela*; *mamgobhozi*; nightshade; pumpkin; stinging nettle; thistle; *umamdebezi*; *umamgoblozi; ushukeyana*; *uwata;* white goosefoot

Urban KwaZulu-Natal site: *ihwabuhwabu*; *intshungu*; *khelengisi*; nightshade; pumpkin; sweetpotato; *uwata*; white goosefoot

Table 11.4: The main source and season for collecting leaves for African leafy vegetables collected by at least 20% of the households

Plant	Area	n	Source						Season		
			Wild	Planting fields	Community members [1]	Home-garden/ yard	River-side	Next to road	Summer	Winter	Summer and winter
Amaranth	rural Limpopo	89	99	1	-	-	-	-	99	-	1
Amaranth	rural KZN	54	46	13	33	6	2	-	100	-	-
Amaranth	urban KZN	145	64	1	3	5	6	20	93	1	6
Blackjack	rural KZN	49	65	10	16		8		50		50
Blackjack	urban KZN	84	52	2	-	5	7	33	93	6	1
Devils thorn	rural Limpopo	38	97	3					100		
Jew's mallow	rural Limpopo	21	95	5					100		
Lehlanye	rural Limpopo	50	98	2					54	44	2
Spider flower	rural Limpopo	86	99	1					100		
Tsamma melon	rural Limpopo	58	100						100		

All values are given as a percentage of those households that usually collect the specific leaves

[1] from friends, family or neighbour

Table 11.5 reflects the respondents' perceptions of *morogo / imifino*. Most of the respondents thought that *morogo / imifino* was good for them, for a variety of reasons. Thirteen percent of the respondents at the rural KwaZulu-Natal site said that *imifino* is good because it contains vitamin A, probably reflecting the effect of a home-garden project that was done previously in the area (Faber *et al*., 2002). Between 20 and 28% of the respondents at the two KwaZulu-Natal study sites thought that consumption of *imifino* saves money (they do not have to buy it because it is freely available). At the rural Limpopo site there clearly is a transfer of knowledge within the households, as mothers and grandmothers were the main source information on *morogo.* The radio and clinic staff is important sources of information on *imifino* at the KwaZulu-Natal study sites.

Table 11.5: Respondents' perception of *imifino / morogo*

	Limpopo	**KwaZulu-Natal**	
	Rural n = 100	**Rural n = 101**	**Urban n = 308[1]**
	%	%	%
Do you think *imifino / morogo* is good for you?			
Yes	99	92	77
No	1	-	6
Unsure	-	8	17
Apart from being healthy, reasons why *imifino / morogo* is good for people [2]			
gives energy	13	-	4
gives strength	28	11	18
strong bones	-	-	6
prevent illness	20	-	-
contains vitamins	9	8	-
contains vitamin A	-	13	-
saves money	-	28	20
live longer	9	-	-
Information source for the use of *imifino / morogo*			
radio	3	61	62
clinic	22	70	90
community health workers	-	2	13
mother / grandmother	86	28	18
family other than mother / grandmother	2	3	12
newspapers / magazines	2	-	8
friends	-	3	3
project nutrition monitors [3]	-	56	-
nowhere	-	-	7

[1] Missing value because of a printing error in the questionnaire – was corrected after some of the respondents were already interviewed.

[2] Only answers for those reasons given by more than 5% of the respondents are reflected.

[3] A home garden project focusing on β-carotene rich vegetables was done in the area from 1998 to 2002. Nutrition monitors were responsible for the daily activities.

Figure 11.2 shows the frequency of household consumption of *morogo / imifino* during the month prior to the survey. Households at the rural Limpopo site had the highest frequency of consumption (with 40% of the households consuming it at least 4 days per week), while households at the urban KwaZulu-Natal site had the lowest frequency of consumption (with 41% of the households consuming it seldom or never).

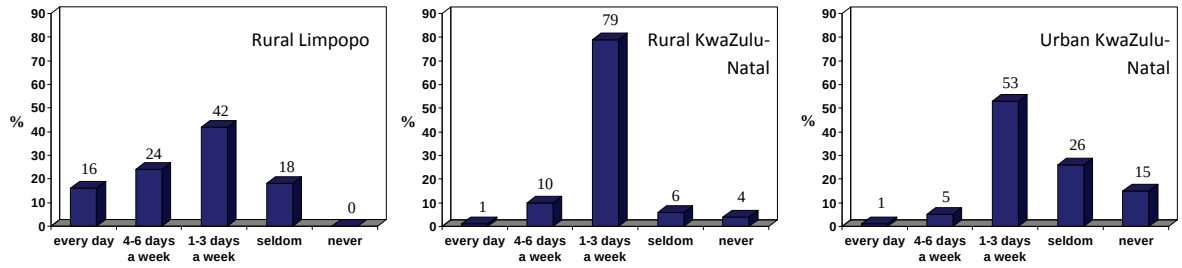

Figure 11.2: Household's frequency of consumption for *morogo / imifino* during October / November 2008 (month prior to the survey)

Household consumption and source of *morogo / imifino* is summarised in Table 11.6. For those households who consumed *morogo / imifino* the previous month, the leaves were obtained from the wild by 94% of the respondents at the rural Limpopo site, versus 53% at the rural KwaZulu-Natal site and 38% at the urban KwaZulu-Natal site. Home-gardens were an important source of *imifino* at the rural KwaZulu-Natal site. Shops and the informal market were important sources for *imifino* at the urban KwaZulu-Natal site.

The number of respondents who consumed *morogo / imifino* the day before the survey and the number of times per day was the highest at the rural Limpopo site. All of the households at the rural Limpopo site, 97% of the households at the rural KwaZulu-Natal site and 78% of the households at the urban KwaZulu-Natal site consumed *morogo / imifino* the week prior to the survey. At the urban KwaZulu-Natal site the two main reasons for not consuming *imifino* the previous week was personal preference (43%) and the unavailability of leaves (32%).

Nearly all (99%) of the households at the rural Limpopo site consumed *imifino / morogo* the day before the survey, versus 30% at the rural KwaZulu-Natal site and 19% at the urban KwaZulu-Natal site. Most of the KwaZulu-Natal households ate *imifino / morogo* once during the day, whereas 58% of the rural Limpopo households ate it at least twice a day. *Imifino / morogo* was consumed during all three main meals and during mid-morning at the rural Limpopo site, whereas at the KwaZulu-Natal sites it was consumed during either lunch or supper.

Using a 5-point hedonic scale with smiley faces showed that the *imifino / morogo* consumed the day before was well-liked by most of the respondents. However, it was the perception of the respondents that the *imifino / morogo* was not that well-liked by their children, except for the rural KwaZulu-Natal site.

Table 11.6: Household consumption and source of *imifino* / *morogo*

	Limpopo	**KwaZulu-Natal**	
	rural	**rural**	**urban**
	n = 100	n = 101	n = 391
Source for *imifino* / *morogo* (%) [1]	n = 100	n = 97	n = 330
bought / informal market	-	2	69
household's yard / home-garden	-	52	29
community garden	1	1	2
Wild	94	53	38
riverside	-	24	7
planting fields	11	15	2
Percentage of respondents who ate *imifino* / *morogo* during the past week	100	97	78
Number of days *imifino*/ *morogo* was consumed the previous week	n = 100	n = 94	n = 257
mean (SD)	3.2 (1.8)	1.8 (0.6)	1.9 (0.9)
Percentage of respondents who ate *imifino* / *morogo* the day before the survey	99	30	19
Number of times *imifino* / *morogo* was consumed the day before (%)	n = 99	n = 30	n = 73
once	41	100	81
twice	38	-	13
three times	20	-	6
Meal during which *imifino* / *morogo* was consumed (%)	n = 99	n = 30	n = 73
breakfast	30	7	9
mid-morning	35	-	4
lunch	31	60	45
mid-afternoon	2	-	4
supper	80	33	63
How much did YOU like the *imifino* / *morogo* that you ate yesterday? (%)	n = 99	n = 30	n = 73
Bad	1	-	-
Indifferent	5	-	3
Nice	18	3	19
very nice	76	97	78
How much do you think your children liked the *imifino* / *morogo* that was eaten yesterday? (%)	n = 99	n = 30	n = 73
very bad	4	-	-
Bad	21	3	13
Indifferent	30	17	27
Nice	23	20	26
very nice	21	60	34

[1] expressed as a percentage of those respondents who consumed green leafy vegetables during the previous month; households could obtain the leaves from more than one source

Imifino / mororgo was consumed the previous day by 99% of the households at the rural Limpopo site, 30% of the households at the rural KwaZulu-Natal site and 24% of the households at the urban KwaZulu-Natal site. Some of the respondents included spinach, beetroot and turnip leaves, particularly at the urban KwaZulu-Natal site. All cases where these leaves were eaten without other ALVs were excluded from

further data analysis. Consumption and preparation of ALVs consumed the day before they survey are summarised in Table 11.7.

Table 11.7: Preparation methods of African leafy vegetables consumed the day before the survey

Rural Limpopo site

For the 100 respondents, consumption of ALVs the previous day was reported for 164 meals.

Leaves mostly consumed were spider plant (51% of the time), wild watermelon (27% of the time) and amaranth (24% of the time), either on their own or in combination with other leaves.

For 98% of the cases the ALVs were collected from the wild.

Ingredients added during preparation were salt (98%), tomato (69%), oil (1%) and peanut butter (1%).

Nearly all of the respondents (98%) added water during preparation, with either only the bottom of the pot (31%), half of the leaves (57%) or all of the leaves (10%) covered with water. Only one respondent changed the water during cooking. Most (95%) of the respondents said that there was no water left after cooking.

Rural KwaZulu-Natal site

For the 101 respondents, consumption of ALVs the previous day was reported for 22 meals.

Leaves mostly consumed were amaranth (82% of the time), either on its own or in combination with other leaves (mostly blackjack).

The leaves were collected from the wild (33%), household's yard (38%), riverside (10%), planting fields (10%), or obtained from other community members (10%).

Ingredients added during preparation were onion (100%), salt (100%); oil (96%), stock cubes (41%), and tomato (18%).

None of the respondents added water during preparation.

Urban KwaZulu-Natal site

For the 391 respondents, consumption of ALVs the previous day was reported for 54 meals.

Leaves mostly consumed were amaranth (87% of the time), either on its own or in combination with other leaves (mostly blackjack).

The leaves were collected from either the wild (35%), household's yard (28%) or next to road (20%); or were obtained from either people who were selling it (9%) or other community members (6%).

Ingredients added during preparation were onion (100%), oil (98%), salt (91%), tomato (46%), stock cubes (20%), and peanut butter (2%). One respondent added bicarbonate of soda to the leaves.

The majority (87%) of the respondents did not add water during preparation. Of the seven respondents who used water during cooking, five changed the water during cooking.

11.6 NUTRIENT CONTRIBUTION OF IMIFINO TO TOTAL NUTRIENT INTAKE FOR CHILDREN AND CAREGIVERS IN A RURAL AND AN URBAN SITE IN KWAZULU-NATAL

11.6.1 Methods

In this phase of the study, consumption of *imifino* was determined for the rural and urban sites in KwaZulu-Natal [quantified consumption data was not collected at the rural Limpopo site]. During March and April 2007, 500 grade 6 and 7 learners were randomly selected from one school at the rural site and four schools at the urban site in the KwaZulu-Natal Province (100 learners per school).

The caregivers of the children were interviewed in the local language (isiZulu) by experienced fieldworkers, with the learner present during the interview. Dietary intake was quantified for the grade 6 and 7 learners and their caregivers using a repeated 24-hour recall. The two repeats were done approximately one week apart, and on different days of the week. The fieldworkers worked from Monday to Friday, and the dietary data collected for the total group therefore covered one weekend and four week days (Sunday through to Thursday). Plastic food models, household utensils, and three-dimensional sponge models were used to quantify and record food consumption for the previous day. In addition, dry oats was used to quantify portion sizes of certain food items, especially cooked food. The caregiver / learner used the dry oats to indicate the quantity resembling the amount of food that was consumed. The fieldworker quantified the dry oats with a measuring cup. Food intake reported in household measures was converted into weight using the MRC Food Quantities Manual (Langenhoven *et al*., 1991). The SAS software package (version 9.1; SAS Institute Inc., Cary, NC) was used to convert food intake to macro- and micro-nutrients, using the SAFOODS 2000 database. The code 3980 (leaves, amaranth, boiled) in the SAFOODS 2000 database was used for *imifino*. Values for fortified maize meal and bread were used – these values reflect the fortification levels as added during the manufacturing process and do not take the effect of processing into account.

The average portion size for *imifino* was calculated as the total weight in grams of all occurrences reported for the recall period, divided by the number of occurrences.

Daily per capita consumption of *imifino* was calculated. The total amount (in grams) consumed over the 2-day recall period was divided by two to obtain the total amount consumed for one day, and this was then divided by the total study population (including both consumers and non-consumers).

The amount of nutrients supplied by *imifino* was calculated and expressed as a percentage of total intake for each nutrient. Research participants who did not consume *imifino* on either of the two recall days were excluded from this part of the data analysis. The results on the nutrients supplied by *imifino* are therefore reported only for those participants who consumed *imifino* over the two-day recall period. These research participants are referred to as “consumers”.

11.6.2 Results

Study population

At the rural site the questionnaire was completed for 99 households. Of the caregivers interviewed, 70% were the mother of the learner. The average age of the caregivers was 38±10 years, and 24% had some secondary school education (grade 8 to 12). Nearly all the households had access to toilet facilities (92% pit toilet), tap water and electricity. The households used electricity (54%), wood (32%) and gas or paraffin (13%) for cooking. Seventy-seven percent of the households received a child grant.

At the urban site the questionnaire was completed for 398 households. Of the caregivers interviewed, 75% were the mother of the learner. The average age of the caregivers was 41 ± 10 years, and 65% had some secondary school education (grade 8 to 12). Nearly all the households had access to toilet facilities (73% pit toilet, 18% flush toilet not connected to pipe, 9% flush toilet connected to pipe), tap water and electricity. The households used electricity (68%), and gas or paraffin (30%) for cooking. Seventy-seven percent of the households received a child grant.

Respondents reporting imifino for the 2-day recall period

The respondents for whom *imifino* where reported for the 2-day dietary recall period are listed in Table 11.8. *Imifino* was consumed by 38 to 37% of the respondents over the 2-day recall period at the rural site, and by 15 to 17% of the respondents at the urban site. Average portion size, average daily consumption and per

capita intake for *imifino* are also given in Table 11.8. The portion sizes differed slightly between the rural and urban sites, but the average per capita intake was substantially higher at the rural sites.

Table 11.8: Respondents for whom *imifino* was reported for the 2-day recall period, average portion size, average daily consumption and per capita intake

	Frequency[1]	**Respondents**		**Average portion size** (g)	**Average daily consumption**[2] (g)	**Consumption / capita / day** (g)
		n	**%**			
Rural site						
Learners (n = 98)	57	37	38	100	76	29
Caregivers (n = 99)	58	39	39	110	82	32
Urban site						
Learners (n = 399)	71	59	15	130	77	11
Caregivers (n = 394)	87	68	17	135	87	15

[1] The total number of times that *imifino* was reported over the 2-day recall period

[2] Average over the 2-day recall period

As the average daily consumption were very similar between the two sites, the data for the two areas were combined for the rest of the data analysis and the data analysis was done only for those respondents who consumed *imifino* during the recall period. Figure 11.3 shows the frequency distribution of *imifino* consumption over the meals. For example, Figure 11.3 shows that for the grade 6 and 7 learners, when *imifino* was eaten, it was 4% of the time for breakfast, 18% of the time for lunch, 27% of the time during mid-afternoon, and 51% of the time for supper. When *imifino* was eaten, approximately half of the time it was eaten for supper over the two age groups.

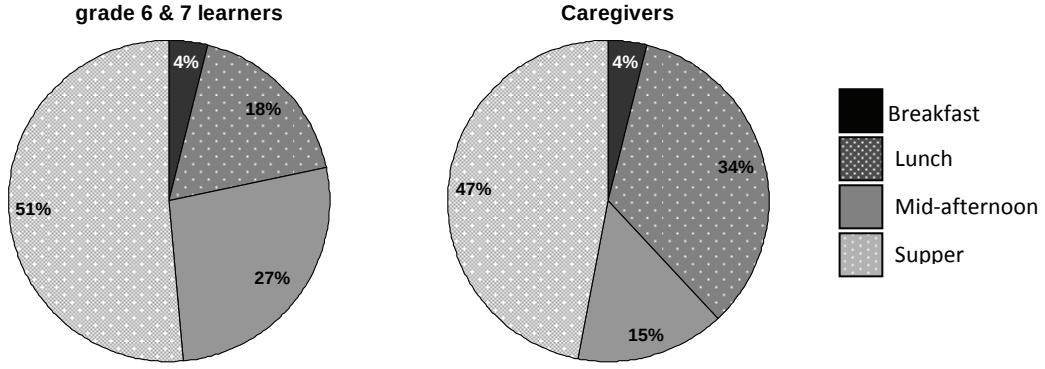

Figure 11.3: Frequency distribution of *imifino* consumption over the different meals

During preparation of *imifino* oil was added 97% of the time. The following foods were eaten in the same meal with *imifino*:

- Starch eaten with *imifino* in the same meal was mostly stiff maize meal porridge (77% of the time), and less often bread (16% of the time), soft porridge (6% of the time) and potato (3% of the time).
- Sausage was eaten in the same meal 8% of the time.
- Pumpkin was the only vegetable eaten in the same meal (7% of the time).
- Banana (2% of the time) and apple (1% of the time) were the only fruit eaten with the meal.
- Drinks taken with the meal were water (63%), tea (9%) and juice (8%).

For those research participants who consumed *imifino* during the recall period (consumers), the average daily intake over the 2-day recall period was 76 to 82 g of *imifino*. The contribution of *imifino* to total intake is expressed as a percentage of the total nutrient intake consumers. The median energy contribution from

imifino was low for the learners (65 kJ) and caregivers (83 kJ) and the median contribution for the macronutrients was 1 g or less for each of carbohydrates, protein and fat. Nutrients that contributed at least 5% of total intake for the learners and caregivers are given in Table 11.9. *Imifino* contributed significantly to the total intake of calcium (42%), vitamin A (39 to 41%) and iron (22 to 23%). Although *imifino* contributed 8% to total vitamin C intake, the mean vitamin C intake from *imifino* was very low (0.8 g).

Table 11.9: Nutrient contribution from *imifino* for grade 6 and 7 learners and their caregivers who consumed *imifino* during the recall period; for nutrients for which imifino contributed at least 5% of total intake

Nutrient	Grade 6 and 7 learners (n = 96)			Caregivers (n = 107)		
	Nutrients from *imifino*		% contribution from *imifino*	Nutrients from *imifino*		% contribution from *imifino*
	Range	Mean (95% CI)	Mean (95% CI)	Range	Mean (95% CI)	Mean (95% CI)
Fiber (mg)	1-10	2.3 (2.0; 2.6)	10 (9; 11)	1-10	2.6 (2.9; 2.3)	10 (9; 12)
Calcium (mg)	37-608	144 (126; 162)	42 (39; 45)	34-654	2.6 (142; 178)	42 (39; 45)
Iron (mg)	1-15	3.6 (3.1; 4.0)	22 (20; 24)	1-16	3.9 (3.5; 4.4)	23 (21; 25)
Magnesium (mg)	7-120	28 (25; 32)	11 (10; 12)	7-130	32 (28; 35)	12 (10; 13)
Phosphorus (mg)	8-124	29 (26; 33)	5 (4; 5)	7-133	32 (29; 36)	5 (4; 5)
Zinc (mg)	0-3	0.6 (0.5; 0.7)	6 (6; 7)	0-3	0.69 (0.61; 0.77)	7 (6; 8)
Vitamin A (µg RE)	80-1297	308 (270; 346)	39 (35; 42)	73-1396	341 (303; 380)	41 (38; 44)
Riboflavin (mg)[1]	-	-			0.03	5
	-	-		0.01-0.14	(0.03; 0.04)	(4; 5)
Vitamin C (mg)	0-3	0.8 (0.7; 0.9)	8 (4; 11)	0.2-3.5	0.9 (0.8; 1.0)	8 (6; 10)

[1] *Imifino* contributed <5% of total nutrient intake for riboflavin for learners

11.7 B-CAROTENE CONTENT OF FRIED, BOILED AND FRESH AMARANTH

11.7.1 Methods

Three sample lots of edible leaf parts of wild-growing uncultivated amaranth were collected on the same day at the rural KwaZulu-Natal site. The edible parts consisted of growth tips containing three to five mature leaves with stems (growth shoots) as is customarily collected for consumption by the local community. A sub-sample of these leaves were used for preparation (steamed and then fried) by a community member on the day of collection, while the remainder of the three sample lots of fresh leaves was kept in plastic containers with lids in a refrigerator at 4ºC until it was transported by air to the laboratory (in Cape Town) the following day where it was kept in a refrigerator over-night for analysis of fresh and boiled leaves.

Fresh amaranth
One composite sample of the raw, fresh amaranth was prepared. An equal amount of growth tips from each sample lot was combined and washed with tap water and allowed to air dry on absorbent paper for approximately 2 hours. The leaves were then homogenized with a hand-held stick blender and analysed in duplicate.

Boiled amaranth (prepared in the laboratory)
For each of three composite samples, an equal amount of growth tips from each sample lot was combined and washed with tap water. Approximately 2½ cups of water (± 1.5 cm depth) was brought to the boil in a 24 x 11.5 cm (5.2 L capacity) stainless steel pot. The washed leaves were boiled with the lid on until tender for 15 min. The water was drained through a sieve and after cooling it was transferred to marked plastic containers with screw caps and frozen at -20ºC until analysis. On the day of analysis each thawed composite boiled sample was homogenized with a hand-held stick blender and analysed individually in duplicate.

Fried amaranth (prepared by a community member)
For each composite sample, an equal amount of growth tips from each sample lot was combined and washed with tap water. The washed undrained leaves were steamed without adding water for 10 min, and then fried in sunflower oil for an additional 10 min. After cooling it was transferred to marked plastic containers with screw caps and frozen at -20ºC. It was transported in a cooler box containing ice packs by air to the laboratory (in Cape Town) the following day and kept at -20ºC until analysis. On the day of analysis each thawed composite fried sample was homogenized with a hand-held stick blender and analysed individually in duplicate.

Carotenoid extraction
β-Carotene analysis was done within one week of harvest. An aliquot of between 2.5 to 3 g of the homogenised sample was weighed and the carotenoids extracted with tetrahydrofuran:methanol (1:1, vol/vol), partitioned to petroleum ether and β-carotene content determined with High Performance Liquid Chromatography (HPLC) as previously described by Kimura and Rodriguez (2002) and Low and van Jaarsveld (2008).

11.7.2 Results

The β-carotene content per 100g fresh, boiled and fried amaranth is given in Table 11.10.

Table 11.10: The β-carotene content of fresh, boiled and fried amaranth (per edible portion)

	Total β-carotene ***µg / 100g***	**Trans-β-carotene** ***µg / 100g***	**Vitamin A value** ***µg RAE / 100g***	**Vitamin A value** ***µg RE / 100g***
	mean (SD)	mean (SD)	mean (SD)	mean (SD)
Fresh leaves	6156 (120)	5047 (94)	421 (8)	842 (16)
Boiled leaves	6472 (102)	5151 (85)	429 (7)	859 (14)
Fried leaves	9230 (383)	7526 (302)	627 (25)	1254 (50)

All values are given as the mean, with the standard deviation in brackets
SD, standard deviation
Vitamin A value calculated from all-trans-β-carotene:
12 µg β-carotene = 1 µg retinol activity equivalents (RAE)
6 µg β-carotene = 1 µg retinol activity equivalents (RE)

The mean *trans*-β-carotene content (5047 µg/100 g; 421 µg RAE/100g) of fresh leaves is similar than the fresh Amaranth (5757 µg/100 g; 480 µg RAE/100 g) sampled from ARC – Roodeplaat. The mean vitamin A

value (µg retinol activity equivalents; RAE) of fresh, boiled and fried amaranth was 421 µg RAE/100 g, 429 µg RAE/100 g and 627 µg RAE/100 g, respectively. The higher β-carotene content of fried leaves compared to boiled leaves is understandable considering the higher temperature generated during frying resulting in a greater moisture loss from fried leaves compared to the boiled leaves (Greenfield and Southgate, 2003). Nevertheless, both fried and boiled amaranth provides considerable quantities of vitamin A (µg RAE) per 100 gram.

11.8 DISCUSSION

ALVs were commonly consumed in the study areas, mostly because they are a free source of nutritious food that can be easily accessed and regularly harvested during the growing season. The more frequent consumption and the larger variety of ALVs at the rural Limpopo site as compared to the rural KwaZulu-Natal site were expected. The National Food Consumption Survey of 1999 showed that green leafy vegetables were the 4th most frequently consumed food item for 1- to 9-year old children in the Limpopo Province (previously known as the Northern Province), while in KwaZulu-Natal Province it was the 20th most frequently consumed food item (Labadarios *et al*., 2000:289,292). The higher consumption and the consumption of a larger variety in the Limpopo province may reflect the level of poverty forcing communities to revert to wild vegetables for nutrition.

The quantified dietary data showed that *imifino* contributed significantly to total dietary intake of calcium, iron and vitamin A of learners and their caregivers. Analysis for β-carotene content of both fried and boiled amaranth confirmed the potential of these leaves to contribute significantly to vitamin A requirements of nutritionally vulnerable communities. For example, a portion of 100 g boiled or fried amaranth will provide more than half of the recommended dietary allowance for vitamin A for adult females (Trumbo *et al*., 2001). Wild growing green leafy vegetables are generally richer sources of various micronutrients than are exotic or commercially produced leafy vegetables (Steyn *et al*., 2001; Kobori and Rodriguez-Amaya, 2008; Su *et al*., 2002). The bio-availability of some of the micronutrients in green leafy vegetables is however affected by the oxalates, phytates and nitrates present in the leaves (Steyn *et al*., 2001).

The average portion size for *imifino* consumed by the caregivers was 110 to 135 g. Studies done in the Limpopo Province showed that the cooked portion sizes for green leafy vegetables consumed commonly ranged between 45 to 105 g and 180 to 270 g (Nesamvuni *et al*., 2001; Steyn *et al*., 2001).

Addition of oil during the preparation of green leafy vegetables increases the bio-availability of β-carotene (Hedrén *et al*., 2002). A recent study showed that as little as 2.4 g of fat is needed for optimal bioavailability and effectiveness of plant carotenoids (Ribaya-Mercado *et al*., 2007). At both the KwaZulu-Natal study sites oil was added during preparation, which can be expected to enhance the nutritional benefits from the leaves in terms of vitamin A. However, at the rural Limpopo site no oil was added to the dish, and the leaves were eaten mostly with a starchy staple, which will limit the bio-availability of β-carotene. For maximum benefit the addition of a little fat should thus be encouraged, but, considering the high prevalence of adult obesity and escalating overweight observed in children in the country (SADHS, 2003:277; Labadarios *et al*., 2008:255), this should be done in moderation.

A variety of ALVs was observed at the rural sites, but only a few were frequently consumed. At the rural Limpopo site, spider plant was the most popular ALVs, followed by amaranth and wild watermelon. At the rural KwaZulu-Natal site, amaranth was most frequently consumed, either on its own or in combination with other ALVs, mostly blackjack. Similar to the findings of other South African studies (Steyn *et al*., 2001; Vorster, 2007:59), this study showed that although amaranth is not always the most popular *imifino* / *morogo*, it is always amongst the most important consumed species. According to Vorster (2007:61) some ethnic groups prefer the “stronger” taste of other species, but they use amaranth to supply the bulk.

The respondents reported that the leaves were available mostly during summer, which is in line with the findings of other studies (Steyn *et al*., 2001; Vorster, 2007:82-84). The importance of location on the availability of green leafy vegetables is illustrated by the period of availability of blackjack for the two KwaZulu-Natal sites (which are approximately 20 km apart). At the rural KwaZulu-Natal site, where a river is flowing through the village, blackjack is available during winter growing on the riverside, while in the urban site, where there is no river, it is available only in summer.

This study was done during the rainy/summer months, as most of the wild growing species that are consumed grow in summer (Jansen van Rensburg *et al*., 2007). A study previously done at the rural KwaZulu-Natal site showed that consumption of *imifino* (consumed during the first and last quarter of the year; summer) and spinach, mostly Swiss chard (consumed during the 3rd quarter of the year; winter) complemented each other (Faber *et al*., 2007). The authors therefore concluded that *imifino* should be promoted together with consumption of locally produced or commercially available spinach (a cool weather crop) to ensure a year-round consumption of dark-green leafy vegetables to ensure a sustainable delivery of nutrients to these communities.

Drying the leaves for consumption during winter was observed at the rural Limpopo site, and this practice has been reported previously (Vorster, 2007:87,88; Nesamvuni *et al*., 2001). The leaves are dried either in the sun or in the shade. Tanzanian studies have suggested that the traditional processing practices of sun-drying reduce the concentration of carotenoids (Mosha *et al*., 1997; Mulokozi and Svanberg, 2003). It is therefore important that drying and storage conditions are optimised in order to minimize nutrient loss.

While the rural households obtained the leaves mostly from the wild, urban households relied to a great extent on the informal market sector for access. The trading of *imifino* at some urban informal markets in KwaZulu-Natal has been observed (personal observation), and is confirmed by market surveys done in other parts of South Africa, e.g. Soshanguve and Durban (Pasquini *et al*., 2009). It is not known whether the vendors source the leaves from the wild or from home gardens, and from how far a distance they source it. The trading of ALVs in informal urban markets does however show that these leaves may have commercial value. Although cultivation of ALVs is very limited in Southern Africa, the explorative phase of this study suggested that some of the leaves could be cultivated. Pasquini and co-workers (2009) reported that some farmers do grow ALVs for trading (extra income).

ALVs are often regarded as a poor person's food (Vorster, 2007). This was only the case at the rural KwaZulu-Natal site. Approximately a quarter of the respondents of the quantitative survey thought that consumption of *imifino* is good because "it saves money". They generally regarded ALVs as a poor person's food, and people who ate *imifino* were generally judged as "being poor" and "having no food". The aspect of affordability / cheap should therefore be avoided or used carefully during promotion, as to avoid the perception that ALVs are food for the poor. The emphasis should rather be on the potential nutritional and hence health benefits the consumption of these vegetables could offer.

This study confirms the general belief that information on ALVs is passed down over generations. There are, however, indications from the KwaZulu-Natal sites, that clinics and radio are also an important source of information on *imifino*, pointing to these as potentially worthwhile communication channels for nutrition promotion of these leaves.

Differences between provinces, as well as rural/urban differences within a province in terms of type and source of ALVs, preparation methods, consumption patterns, and preference were observed. The differences between the two rural sites could probably be ascribed to geographical differences, ethnic/cultural difference, knowledge transfer differences and/or preference differences. Data on the use of ALVs should therefore be reported within the context of the area from where the information was collected. It is evident that

information collected by small studies within a specific area cannot be generalised for the overall South-African population.

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Conclusions and Recommendations

CHAPTER 12: NUTRITIONAL VALUE AND WATER USE OF AFRICAN LEAFY VEGETABLES FOR IMPROVED LIVELIHOODS: CONCLUSION AND RECOMMENDATIONS

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The research project reported on in this publication explored the potential to increase the consumption of African leafy vegetables (ALVs) by enhancing access through cultivation as an avenue to improve livelihoods through better nutrition among vulnerable communities in South Africa. The decision to focus on ALVs instead of other crops was justified by the acceptability and reputed high nutritional value and low resource requirements of ALVs, particularly in terms of water and plant nutrients. The primary objectives of the research project were:

- to review existing knowledge of the nutritional status of South Africans and the strategies used to address malnutrition in vulnerable communities;
- to review literature on the use and status of ALVs in contemporary South Africa, including the identification of key plant species and their contribution to household food security;
- to characterise a selection of eight important ALV species in terms of their drought- and heat tolerance and water use to generate knowledge of the agronomic requirements of the eight selected ALV species; and
- to assess human nutritional aspects of the eight ALV species, including their micronutrient and antioxidant content, as well as patterns of availability and consumption of ALVs among households at selected sites in South Africa.

The eight ALV species that were the focus of the empirical work in this research project are listed in Table 12.1.

Table 12.1: African leafy vegetables that were investigated in the current study

Scientific name	English name
Amaranthus cruentus L.	Pigweed (amaranth)
Vigna unguiculata (L.) Walp.	Cowpeas
Corchorus olitorius L.	Jew's mallow, wild jute
Cleome gynandra L.	Spider flower, cat's whiskers, spider plant, bastard mustard
Citrillus lanatus Thunb.	Tsamma melon, bitter watermelon, *egusi* watermelon
Cucurbita maxima Duchesne	Pumpkin
Solanum retroflexum Dun.	Nightshade
Brassica rapa L. subsp. *chinensis*	Non-heading Chinese cabbage

In this concluding chapter the important findings of the project are highlighted, ideas for additional research are formulated and recommendations are made for the promotion of these vegetables, taking into account their possible limitations and the challenges related to their cultivation. In a separate report, specific guidelines for the cultivation of these crops are presented.

12.1 NUTRITIONAL STATUS OF SOUTH AFRICANS AND STRATEGIES TO ADDRESS MALNUTRITION

The review of literature showed that anthropometrically, over-and undernutrition were rife in South Africa, and that under- and over-nutrition co-existed in the same communities and often in the same households. Amongst adult females, overweight and obesity were of concern. Children suffered from various forms of undernutrition, mainly of a chronic nature. In respect of the micronutrient status, undernutrition in vitamin A, iron and zinc stood out. Dietary data confirmed that inadequate intakes were major contributors to the malnutrition that existed among South African children. Increased intake of fruit and vegetables was identified as one of the possible strategies to address under-nutrition and this was where home-production of ALVs could fit in. Increased intake of fruit and vegetables could also be effective in addressing over-nutrition, by forming part of weight management programmes, in line with the general health promotion messages contained in the South African food-based dietary guidelines. Since the rural poor in South Africa are disproportionately affected by the burden of malnutrition, investigating the option of home production of ALVs to improve their diet and nutritional status was considered to be a useful proposition.

12.2 AFRICAN LEAFY VEGETABLES IN SOUTH AFRICA

The review of literature showed that the leaves of a wide array of plant species were consumed by local people. In this report the plant species that were used for this purpose were referred to as African leafy vegetables (ALVs), defined as the collective of leafy vegetable species that form part of the culinary repertoire of particular contemporary African communities. Some ALV species were consumed regularly, while others were only consumed in times of famine. Collecting green leafy plants for use as food continued to be widespread among rural people in South Africa but in some parts there was evidence of decline. Indications were that declining use was associated with the low status of these plants and their association with poverty and lifestyles of the past. Most ALVs were harvested in the wild or from plant populations that occurred spontaneously as weeds in cultivated and fallowed fields. However, several cultivated and semi-cultivated species were also identified. Generally, ALVs were perceived to be well adapted to local agro-ecological conditions and were said to grow under conditions of limited soil water and plant nutrient availability.

12.3 DROUGHT TOLERANCE, HEAT TOLERANCE AND WATER REQUIREMENT OF AFRICAN LEAFY VEGETABLES

The drought and heat tolerance traits of six ALVs were examined using various techniques and compared with those of Swiss chard, which served as the reference crop. Techniques used included relative water content, leaf area, cell membrane stability, cell viability and early drought tolerance. Table 12.2 shows the tolerance ranking of the ALVs and Swiss chard to drought stress and heat stress. The variation among the crops supported existing theory that plant species display different adaptations/avoidance to water and heat stress.

Overall, the results indicated that the ALVs had a higher degree of drought tolerance than Swiss chard and perhaps with the exception of non-heading Chinese cabbage (*B. rapa* subsp. *chinensis*). This is significant considering the general scarcity of water in South Africa and the threat of further drying of the climate in at least some parts of the country as a result of global warming and associated climate change. Several of the ALVs also had a higher degree of heat tolerance than the reference crop. Based on the ranking of the summer crops in Table 12.2, cowpea was the most drought tolerant vegetable, followed by nightshade, pumpkin, tsamma melon, pigweed and lastly Swiss chard.

Table 12.2: Summary of ranking for drought and heat tolerance of African leafy vegetables

	Drought*							Heat*			
	RWC	**LA**	**CMS**	**TTC**	**WB**	**Average**	**Rank**	**CMS**	**TTC**	**Average**	**Rank**
Summer crops											
A. cruentus	5	2	4	6	5	(4.4)	5	2	1	(1.5)	1
C. lanatus	2	4	2	4	6	(3.6)	4	3	6	(4.5)	4
C. maxima	2	4	6	1	3	(3.2)	3	3	2	(2.5)	2
S. retroflexum	4	2	4	2	1	(2.6)	2	5	4	(4.5)	4
V. unguiculata	1	1	1	3	1	(1.4)	1	1	5	(3.0)	3
B. vulgaris var. *cicla*	6	6	2	5	4	(4.6)	6	5	3	(4.0)	6
Winter crops											
B. rapa subsp. *chinensis*	2	2	1	2	1	(1.6)	2	1	2	(1.5)	1
B. vulgaris var. *cicla*	1	1	1	1	2	(1.2)	1	2	1	(1.5)	1

*1 = most drought- or heat tolerant and 6 = most drought- or heat sensitive
RWC = relative water content; LA = leaf area; CMS = cell membrane stability; TTC = 2,3-5-Triphenyl tetrazolium chloride reduction; WB = wooden box (early drought tolerance)

Pigweed (amaranth) was the most heat tolerant crop, followed by pumpkin, cowpea and then Swiss chard. Nightshade and tsamma melon appeared to be less heat tolerant than Swiss chard, which was somewhat surprising in the case of tsamma melon.

From the crop water studies conducted in the field at Roodeplaat, it was evident that all eight ALVs were sensitive to water stress, because biomass production was affected by irrigation treatment. Therefore, production of these crops is dependent on the availability of adequate amounts of water. Full irrigation, whereby the rooting depth was regularly recharged to field capacity was necessary to achieve maximum biomass and high quality produce. Highest crop productivity was obtained when deficit irrigation (50FC and 25FC irrigation treatments) was applied but deficit irrigation treatments compromised the quality of the leaves. The average water requirement for the full growing season, measured over two season in the well-watered treatment (100FC) was 340 mm for pumpkin, tsamma melon and cowpeas, 360 mm for pigweed, 368 mm for Jew's mallow, 381 mm for nightshade, 382 mm for non-heading Chinese cabbage and 463 mm for spider flower.

When the eight crops were well-watered, substantial differences in biomass, water use, crop productivity and water use efficiency were identified among the eight ALV species, as is evident from Table 12.3.

Table 12.3: Average dry matter yield, irrigation water use and crop water productivity of the eight ALVs obtained in the well-water irrigation treatment (100FC) over two growing seasons (2009/10 and 2010/11, ARC Roodeplaat)

African leafy vegetable species	Dry matter yield[1] (t ha^{-1})	Average irrigation water use (mm)	Crop water productivity[1; 2] (kg m^{-3})
Chinese cabbage (*Brassica rapa* subsp. *chinensis*)	1.6	190	0.90
Nightshade (*Solanum retroflexum*)	1.5	240	0.80
Pigweed (amaranth) *(Amaranthus cruentus)*	2.2	413	0.50
Jew's mallow *(Corchorus olitorius)*	1.1	448	0.26
Spider flower (cat's whiskers) (*Cleome gynandra*)	0.9	414	0.20
Cowpea (*Vigna unguiculata)*	1.0	446	0.23
Pumpkin (*Cucurbita maxima)*	0.5	389	0.13
Tsamma melon (bitter water melon) (*Citrillus lanatus)*	0.4	389	0.14

[1]Consumable or marketable portion; [2]dry matter basis

Across the eight crops, dry consumable biomass obtained in the well watered (100FC) treatment of the irrigation experiments at Roodeplaat ranged between 400 kg ha-^{1} and 2 200 kg ha-^{1}; crop water use between 190 mm and 448 mm; crop water productivity based on dry consumable biomass between 0.13 kg m^{-3} and 0.90 kg m^{-3}; and the associated water use effiency between 1.0 and 8.4 kg mm ha^{-1}. Comparing average fresh matter yield (consumable portion) and crop water productivity between obtained in the best (100FC) and least (25FC) watered treatments (Table 12.4), non-heading Chinese cabbage appeared to be the most water-productive of the eight ALVs, possibly because it was the only ALV that was grown during winter. For all crops, water productivity was higher in the least watered treatment (25FC) than in the best watered treatment, probably because less water was lost through evaporation from the soil in the least watered treatment than in the best-watered treatment.

Table 12.4: Average fresh matter yield and crop water productivity of the eight ALVs obtained in the best (100FC) and least (25FC) watered treatments over two growing seasons (2009/10 and 2010/11, ARC Roodeplaat)

	Best watered treatment		**Least watered treatment**	
African leafy vegetable species	Fresh matter yield[1] (kg m^{-2})	Crop water productivity[1; 2] (kg m^{-3})	Fresh matter yield[1] (kg m^{-2})	Crop water productivity[1; 2] (kg m^{-3})
Non-heading Chinese cabbage	1.45	7.7	0.86	18.3
Nightshade	0.67	2.7	0.43	7.3
Pigweed	1.51	3.6	0.96	5.4
Jew's mallow	0.66	1.5	0.25	2.1
Spider flower	0.68	1.6	0.53	3.0
Cowpea	1.05	2.4	0.55	4.3
Pumpkin	0.26	0.7	0.21	2.3
Tsamma melon	0.33	0.8	0.30	3.2

[1]Consumable or marketable portion; [2]fresh matter basis

12.4 AGRONOMIC CHARACTERISATION OF AFRICAN LEAFY VEGETABLES

Information on the seed germination and plant nutrient requirements of the eight ALVs listed in Table 12.1 was generated empirically. Information on the plant protection requirements of these eight crops was obtained by means of a review of literature.

Seed germination

Assessment of the response of germination of the seed of the eight ALVs to variability in temperature, pre-sowing dormancy treatments and light using incubation studies revealed that pigweed (*A. cruentus)* seed germinated optimally at 31ºC, with 16ºC as the minimum temperature and 40ºC as the maximum temperature. Improved onset and final germination percentage was observed when the seed of pigweed was scarified and pre-chilled. Exposure of the seed to light improved onset of germination but final germination percentage was indifferent to light.

The seed of non-heading Chinese cabbage (*Brassica rapa* subsp. *chinensis*) germinated optimally at 29ºC, with 12ºC as minimum and 40ºC as maximum temperature. Onset and final germination percentage was improved by scarification and imbibing with potassium nitrate (KNO_3). Pre-chilling negatively affected germination percentage of Chinese cabbage but exposure to light improved onset and final germination percentage.

Spider flower (*Cleome gynandra*) seed germinated optimally at 31ºC, with 12ºC as the minimum and 36ºC as the maximum temperature. Onset of germination percentage of spider flower was improved by scarification. Final germination percentage was indifferent pre-chilling, KNO_3 and light.

The seed of tsamma melon (*Citrillus lanatus*) germinated optimally at 30ºC, with 20ºC as the minimum and 40ºC as the maximum temperature, was indifferent to any of the dormancy pre-treatments. Exposure to light tended to negatively affect onset of germination, but improved final germination percentage.

The seed of Jew's mallow (*Corchorus olitorius*) germinated optimally at 35ºC, (minimum 12ºC and maximum 44ºC), was indifferent to any of the dormancy pre-treatments, whilst exposure to light improved onset of germination but not final germination percentage.

The seed of pumpkin (*Cucurbita maxima*) germinated optimally at 32ºC, with 16ºC as the minimum and 40ºC as the maximum temperature and was indifferent to the different dormancy pre-treatments and light exposure.

The seed of cowpea (*Vigna unguiculata*) germinated optimally at 36ºC, with 12ºC as minimum and 40ºC as maximum temperature and was indifferent to the dormancy pre-treatments. Exposure to light negatively affected onset of germination, but final germination percentage was indifferent to light.

The germination of the seed of nightshade (*Solanum retroflexum*) was very poor and never reached 50%, which precluded the identification of the cardinal temperatures. Germination was improved by potassium nitrate (KNO_3), whilst scarification and pre-chilling reduced germination percentage. Exposure to light improved onset and final germination percentage.

Plant nutrition

The various plant nutrient studies clearly showed that to grow optimally, African leafy vegetables needed fairly high levels of plant nutrient availability. In the field experiments, all eight crop species that were tested responded positively to the application of fertilisers. Spider flower and Jew's mallow tended to have lower fertiliser requirements than all other crops, whilst pumpkin and nightshade had the highest nutrient requirements. The greenhouse pot studies indicated that at least four of these eight ALVs had nutrient requirements that were similar to those of Swiss chard. Greenhouse pot studies also showed that application of different types of animal manure (ruminant and poultry) had positive effects on biomass production of the four ALVs (pumpkin, pigweed, nightshade and non-heading Chinese cabbage) that were tested. Generally, it was concluded that for optimum growth most ALVs had nutrient requirements that were more or less similar as those of exotic leafy vegetables, such as Swiss chard.

In the greenhouse pot experiments, salt content was identified as an important manure attribute that affected the response of ALVs to application rate of animal manure. Factors other than salinity appeared to be responsible for biomass reductions when poultry manure was applied at high rates. To what extent these findings also applied to conditions in the field could not be ascertained.

From the results obtained in the various experiments it was clearly evident that application of fertilisers in the production of ALVs should be done with the necessary care. When applying chemical fertilisers in the furrow at planting, which is the most effective way of applying this type of fertilisers, it appeared safe to apply 20 kg N ha^{-1}, 30 kg P ha^{-1} and 40 kg K ha^{-1} in the form of a (2:3:4) fertiliser mixture. Indications were that this rate of application in the band at the time of planting the seed should not be exceeded in order to avoid fertiliser burn. When higher rates of application of P and K are required at planting, the remainder should be applied broadcast and worked into the soil. Application of additional N during the growing season, at rates of about 20 to 30 kg N ha^{-1} per application as a band-placed top dressing, worked well. In the field

experiment, the use of two topdressings of N appeared to be good practice. A first approximation of fertiliser recommendations for use in the production of the eight leafy vegetable species that were investigated in the project has been compiled. These fertiliser recommendations form part of a separate publication aimed at providing farmers and other interested parties with production guidelines for these eight ALVs.

Plant nutrition and crop water productivity

Water use and water productivity were an important focus in this research project. However, water alone is insufficient for optimum growth of crops. This was evident from the results obtained in the irrigated field experiments conducted at Dzindi Irrigation Scheme in Vhembe, Limpopo Province (Table 12.5). The data on crop water productivity in Table 12.5 are based on total rainfall and irrigation water applied during the growing season and fresh mass yields of the consumable parts. It is important to note that for certain but not all summer crops, rainfall during the growing season exceeded crop requirement. As a result, deep percolation, known to reduce water productivity, probably occurred. For this reason, the water productivities shown in Table 12.5 should only be used for comparisons between fertiliser treatments and not for water productivity comparisons among crops.

Table 12.5: Average fresh matter yield and crop water productivity of the eight ALVs obtained in the high and the low fertiliser application treatments at Dzindi (2008/09 and 2009/10)

	High fertility treatment		**Low fertility treatment**	
African leafy vegetable species	Fresh matter yield[1] ($kg\ m^{-2}$)	Crop water productivity[1; 2] ($kg\ m^{-3}$)	Fresh matter yield[1] ($kg\ m^{-2}$)	Crop water productivity[1; 2] ($kg\ m^{-3}$)
Non-heading Chinese cabbage	2.22	6.4	0.42	1.2
Nightshade	8.18	16.1	2.06	4.1
Pigweed	2.89	3.4	0.56	1.4
Jew's mallow	1.06	2.2	0.31	0.6
Spider flower	0.82	2.7	0.36	0.8
Cowpea	1.74	3.5	1.13	2.3
Pumpkin	4.26	12.3	0.90	2.5
Tsamma melon	2.22	6.3	0.90	1.2

[1]Consumable or marketable portion; [2]fresh matter basis

Table 12.5 shows the importance of adequate plant nutrition in the production of ALVs. Fertilisers raised yields and, therefore, also crop water productivity. In the low fertility treatment, all crops recorded a crop water productivity below 5 $kg\ m^{-3}$ and three crops had a water productivity smaller than 2 $kg\ m^{-3}$. In the high fertility treatment, two crops (nightshade, and pumpkin) recorded a crop water productivity higher than 10, and all crops had a water productivity greater than 2 $kg\ m^{-3}$.

Plant protection

The review of literature on the pests and diseases of important South African ALVs revealed that little information had been generated on this topic. For most of these ALVs, there were also no agro-chemicals that were registered specifically in South Africa to control the pests and diseases that affected them. As with other types of vegetables, disease and pest problems in ALVs can be expected to occur throughout the production cycle and also after they have been harvested and are in storage. At each stage, suitable ways of controlling pests and diseases need to be considered.

The review clearly showed that cultural control, such as sanitation and crop rotation, needed to be the cornerstone of pest and disease control. Use of physical and mechanical control, soil solarisation, and barrier or trap crops were identified as offering good prospects to achieve acceptable levels of pest and disease

control in the field. Biological control agents, either natural occurring (e.g. suppressive soils) or introduced, had the potential to deal with particularly severe or persistent problems. The use of plant extracts as pesticides was another option that was identified but further evaluation of the effectiveness of these extracts for the control of specific diseases or pests is needed before robust recommendations can be made.

12.5 AFRICAN LEAFY VEGETABLES AND HUMAN NUTRITION

Work on African leafy vegetables in the domain of human nutrition focused on the nutrient content of the eight leafy vegetables listed in Table 12.1, the antioxidant properties of a selection on ALVs, and the contemporary consumption patterns among selected communities in the Limpopo and KwaZulu-Natal Provinces.

Vitamins and minerals

The findings of laboratory analyses indicated that the nutritional value of ALVs was dependent on the type of vegetable. Generally, the ALVs provided substantial amounts of vitamin A. An average cooked portion size (90 g for young children; 130 g for females) provided a significant proportion of the EAR for vitamin A. Chinese cabbage provided the least amount of vitamin A and pigweed and cowpeas the most. Using fat, such as cooking oil, during preparation of ALVs is expected to have a beneficial effect on vitamin A status, because fat enhances carotenoid absorption for bioconversion to vitamin A.

Several of the ALVs provided a significant amount of iron. The iron content of pumpkin leaves was the highest, followed by nightshade and tsamma melon. All but three of the eight ALVs that were tested provided at least half of the EAR for iron for 19-30-year-old females The three exceptions were Chinese cabbage, spider flower and Jew's mallow. Although dark-green leafy vegetables generally contain relatively large amounts of iron, they also contain oxalates, phytates and polyphenols, which inhibit the absorption of non-heme iron. The bioavailability of non-heme iron in plant foods is therefore low and the potential contribution of plant foods towards controlling iron deficiency in developing countries has been questioned.

The ALVs had low energy and fat contents and the leaves contained between 2.2 and 10.8 g fibre per 100 g raw edible portion. These attributes make ALVs ideal elements for inclusion in diets aimed at preventing or treating obesity. ALVs can therefore be potentially beneficial in addressing maternal obesity and childhood micronutrient malnutrition, particularly vitamin A and, to some extent, iron.

The ALVs were not a good source of zinc. Compared to Swiss chard, ALVs had higher contents of micronutrients, except for thiamine, riboflavin, zinc and vitamin C. Some of the analytical results obtained in the current study were within the range of those reported by others but higher contents of ß-carotene and iron and lower contents of vitamin C were recorded in the current study.

Antioxidants

Antioxidant content and activity of four ALVs, namely, pigweed (amaranth), Jew's mallow, pumpkin and cowpea, were all appreciably higher than those of Swiss chard. Boiling affected the antioxidant properties but the extent of this effect was dependent on the type of leafy vegetable. The results showed that ALVs were able to reduce the oxidative damage in erythrocytes, plasmid DNA and cell cultures more effectively than Swiss chard. These results could be significant for disease prevention in vulnerable groups.

Contemporary patterns of acquisition and consumption

The ALV consumption pattern studies showed that ALVs were collected by women and children and that indigenous knowledge pertaining to the identification and collection of these plants was carried over by women, from one generation to the next. Leafy vegetable dishes were prepared from one single plant species or from a combination of different species. The combinations that were used to prepare the dishes were

found to vary daily, depending on the availability of the various plant species used. ALVs were most often cooked and eaten as a relish together with a starchy staple food, usually in the form of a stiff porridge prepared predominantly from maize. When the ALVs were cooked, salt was usually added to enhance the taste. Oil, bicarbonate of soda, tomato and onion were also added, depending on availability and preference. Boiling was the traditional method of cooking but steaming and frying were also occasionally used. Surplus leaves were often dried (and lately frozen) and stored for consumption during the off-season. Boiling time varied greatly among and within communities.

Poor South African households consumed ALVs more often than their wealthier counterparts. These vegetables were part of a safety net that communities used to cope with poverty. Use of ALVs was largely confined to home consumption. Consumption pattern and frequency of consumption were highly variable and appeared to depend on several factors, such as poverty status, degree of urbanization, distance to fresh produce markets and season of the year. Indications were that ALVs were gradually being replaced by exotic vegetables, such as Swiss chard (*Beta vulgaris* var. *cicla*), spinach (*Spinacia oleracea*) and white cabbage (*Brassica olearacea* var. *capitata*). One of the reasons for the declining use of ALVs was their perceived inferiority in terms of taste and nutritional value when compared to exotic vegetables. Preference and popularity of ALV species differed and depended on the type of plant species available, gender and age of consumers, as well as cultural background and geographical location. In this study, consumption of ALVs was more frequent in Limpopo than in KwaZulu-Natal. The number of ALV species used in Limpopo was also greater than in KwaZulu-Natal.

12.6 AFRICAN LEAFY VEGETABLES AND LIVELIHOODS

Household food security and human health enhancement

In contemporary South Africa, the livelihood impact of African leafy vegetables in South Africa is primarily in the domain of household food security. This study demonstrated that ALVs contributed significantly to household food security and added variety to cereal-based staple diets. ALVs were shown to constitute an accessible and affordable source of nutrients during summer but less so during winter. Even though leaves of summer crops were being stored for consumption during winter, in most of South Africa, Swiss chard was the dominant dark-green leafy vegetable that was consumed fresh during this season. The existing practice of storing summer produce for consumption in winter was to dry the leaves in sun or shade. This practice could affect the nutritional quality of the leaves and needs investigation and optimisation to minimize nutrient loss.

Although some of the nutrients found in ALVs were fairly low in concentrations, frequent consumption of these leafy vegetables could improve dietary diversification, increase nutrient intake, improve household food security and to an extent the nutritional status of communities that consume these vegetables. Increased consumption of ALVs could also increase plasma antioxidant capacity, which could provide beneficial effects to individuals with underlying chronic diseases, such as cancer and cardiovascular diseases, as well as communicable diseases such as HIV and TB, which are common in vulnerable groups, such as children and the immune-compromised.

Commercialisation and value chain development

Rural households obtained ALVs mostly from the wild, whilst urban households relied to a great extent on street traders for access. Whether street traders source these vegetables from the wild or from home gardens, and from how far a distance they source them was not investigated. The trading of ALVs in informal urban markets does, however, show that these leaves have commercial value. ALVs, therefore, hold the potential for commercialisation. Considerable information for use in the production of ALVs has been generated under the auspices of this project. As indicated earlier, this information has been summarised in the form of guidelines that are contained in a separate publication obtainable from the Water Research Commission. The creation or expansion of demand for ALVs is expected to enhance their production, creating economic

opportunities for South African farmers, particularly black smallholders. Recent work in the Vhembe District of Limpopo Province documented various types of livelihoods that were linked to fresh produce value chains that included three ALVs. The findings supported the notion that commercialisation of ALVs could improve existing livelihoods and create new livelihood opportunities. Commercialisation of ALVs is also expected to improve access to ALVs, which would increase their contribution to dietary diversification among both rural and urban households.

Knowledge transfer

This study showed that information regarding gathering, cooking and preservation of ALVs was usually transferred from generation to generation along the female line. Clinics and radio were also an important source of information on ALVs. The latter channels of communication could be used more intensively to promote the production, consumption and to communicate the nutritional benefits of consuming ALVs, especially in rural areas. Considering the regional differences, as well as differences between rural and urban areas in terms of the type and source of ALVs, preparation methods, consumption patterns and preference, research data on ALVs should be recorded and reported within specific contexts.

12.7 FINAL CONCLUSION AND RECOMMENDATIONS

12.7.1 Final conclusion

The findings of this project clearly supported the promotion and /or reintroduction of African leafy vegetables as food crops from both an agronomic and a human nutrition perspective, especially in food insecure communities. It was demonstrated that the eight ALVs (amaranth, spider flower, non-heading Chinese cabbage, nightshade, Jew's mallow, cowpea, pumpkin and tsamma melon), which were the focus of this research project, could contribute significantly to nutrient intake and hence improve nutrition and health status of people vulnerable to food insecurity with particular reference to micronutrient intake.

The study demonstrated that cultivation of the eight ALVs posed certain challenges but a lot was learnt on how best to grow them. The results of the study indicated that the widely held belief that ALVs need less water than exotic dark-green leafy vegetables for optimum growth was not necessary valid for all ALV species that were tested. However, on the whole ALVs demonstrated greater drought and heat tolerance than Swiss chard, which was used as the reference crop in this study.

The work reported on in this manuscript was by no means exhaustive of all aspects of ALVs but made a significant contribution to the understanding of the place of ALVs in the agricultural repertoire and diet of people in South Africa. The results from the different components of this project clearly showed that there was not one single plant that could address all human nutrition and agronomic challenges. Consequently, the important message arising from this study could well be that cultivation and consumption of a variety of plants, including different ALVs, was likely to provide the greatest impact on human health and livelihood.

12.7.2 Recommendations

From the results of this study, several recommendations can be made. These recommendations have been arranged thematically.

Knowledge of water use, agronomy and post-harvest technology of African leafy vegetables

Scrutiny of the data on biomass production, leaf yields, water productivity and water use efficiency recorded in the irrigation experiments at VOPI, and the fertiliser experiments at Dzindi, indicates that these indicators were subject to considerable variability. Much of this variability is difficult to explain at this stage, even though differences in harvest protocols could have contributed. Refinement of existing information,

including the information generated by this project, awaits additional studies that are aimed at optimising the various production factors that affect growth and yield of crops, including planting date, spacing, irrigation frequency, plant protection, harvesting method and harvesting frequency to name but a few. However, as has been pointed out by Rudy Schippers, perhaps the most important research and development need is for the selection and reproduction of genotypes with favourable traits from the populations of the different ALVs, which are all characterised by substantial genetic heterogeneity. Research on the processing, storage and preservation of ALVs, aimed at extending their shelf life or to preserve them for future consumption during times of scarcity, such as winter, is needed. Existing processing methods, such as drying, and alternative processing methods need to be explored.

Knowledge of the nutritional attributes of ALVs

With reference to the nutritional attributes of ALVs, one of the key recommendations for further research is to elucidate the bioavailability of the key micronutrients they contain. Preliminary data suggest that availability is comparable to that reported for exotic dark-green leafy vegetables but confirmation is needed to strengthen calls for the promotion of increased use of ALVs. In addition, *in vitro* and *in vivo* studies are needed to investigate the difference in β-carotene uptake from ALVs prepared with and without addition of oil or fat. The anti-nutritional components contained in the ALVs should be further investigated through whole meal studies, as their presence could have inhibitory effects on nutrient availability from other foods in the meal. The protective effect of the antioxidants contained in some ALVs against oxidative damage in erythrocytes, plasmid DNA and cell cultures needs to be broadened as these results may have implications for disease prevention in vulnerable groups where both communicable (HIV and TB) and non-communicable diseases (hypertension and coronary heart disease) are prevalent.

Promoting the production and consumption of ALVs

As indicated, the findings of this study support the promotion of ALVs. Promotion is necessary if only to combat the existing negative perceptions of these plants. Women hold most of the knowledge of harvesting, preparing and consuming ALVs. For this reason, it is recommended that whenever knowledge on ALVs is communicated to promote aspects of these plants, or to re-introduce these plants where they are no longer utilised, women be the target group, or at least form part of the target group. It is also recommended that partnerships between government, NGOs and the research community be established for the promotion of ALV production and consumption. Of special mention here are the production guidelines that were compiled as a separate publication under the auspices of this project. Practices associated with ALVs differ spatially and local preferences should be taken into account when constructing promotion messages. Finally, it is recommended that both variety in taste and nutritional variety be carefully considered when promoting ALVs.

APPENDIX A: OUTPUTS

Capacity building/competency development :

Number of persons who achieved, or are on course for achieving, project-associated post-graduate degrees

Qualification (degree)	Black		White		Total
	Male	Female	Male	Female	
Doctors: Achieved		1			1
On course					
Masters: Achieved	2				2
On course	3				3
Honours: Achieved		1			1
On course					
Other: Achieved	4		1	18	23
On course	3				3
Total: Achieved	12	2	1	18	**33**
On course	6				**6**

- **Knowledge dissemination**

Outcomes:

Medium	Number to date	Anticipated additional number
Refereed publications	6	3
Popular articles	3	
Conference presentations	5	2
Workshops	1	
Other		

Refereed publications

1. Faber M, Oelofse A, van Jaarsveld PJ, Wenhold FAM, Jansen van Rensburg WS. African leafy vegetables consumed by households in the Limpopo and KwaZulu-Natal provinces in South Africa. South African Journal of Clinical Nutrition 2010; 23(1): 30-38.

2. Jansen van Rensburg WS, Van Averbeke W, Slabbert R, Faber M, Van Jaarsveld P, Van Heerden I, Wenhold F, Oelofse A. African Leafy Vegetables in South Africa. Water SA 2007; 33(3): 317-326.

3. Wenhold FAM, Faber M, Van Averbeke W, Oelofse A, Van Jaarsveld P, Jansen van Rensburg WS, Van Heerden I, Slabbert R. Linking smallholder agriculture and water to household food security and nutrition. Water SA 2007; 33(3): 327-336.

4. Uusiku NP, Oelofse A, Duodu GK, Bester MJ, Faber M. Nutritional value of leafy vegetables of sub- Saharan Africa and their potential contribution to human health. Journal of Food Composition and Analysis 2010; 23: 499–509.

5. Beletse YG, CP du Plooy and Daniel Mogotlane. Water use efficiency of four indigenous food crops. African Crop Science Proceedings 2009; 9: 263 – 265.

6. Wenhold F, Faber M. Water in nutritional health of individuals and households: an overview. Water SA 2009; 35(1): 61-71.

Submitted

Mavhunghu NP, Serrem J, Bester MJ, Duodu KG, Oelofse A. Raw and cooked African green leafy vegetables have greater antioxidant and cellular protective properties than spinach. Submitted to American Journal of Clinical Nutrition.

To be submitted

Van Jaarsveld PJ, Faber M, van Heerden I, Wenhold FAM, Jansen van Rensburg W, van Averbeke W. Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes.

Conference presentation

1. Oelofse A, Van Averbeke W, Faber M, Wenhold F, Van Heerden I, Van Rensburg W. The first international symposium on the water use and nutritional value of indigenous crops for improved livelihoods. The first international conference on indigenous vegetables and legumes. Hyderabad, India 12-15 December 2006.

2. Uusiku, N.P., Oelofse, A., Duodu, K.G. & Bester, M.J. The effect of boiling on total phenolics and antioxidant activity of selected South African leafy vegetables. 22nd Biennial Nutrition congress: Pretoria October 2008.

3. Van Jaarsveld PJ, Faber M, van Heerden I, Jansen van Rensburg WS. Selected nutrient content of six African leafy vegetables and its potential contribution to nutrient requirements. Nutrition Congress, 19 – 22 September 2010, Durban.

4. Wenhold F, Faber M, Laurie S. Establishing a school-based vitamin A garden for capacity development, service-learning and research.

5. Faber M, Oelofse A, Van Jaarsveld P, Wenhold F, Jansen van Rensburg W. African leafy vegetables consumed by households in Limpopo and KwaZulu-Natal (South Africa). Faculty of Health Sciences Research Day (2011)

Under-graduate research projects

- Nutritional implications of current vegetable gardening practices in the Makapanstad community

- Community risk of vitamin A deficiency and anthropometric profile of learners at Tau Sebele Middle School

- Factors influencing Tau Sebele Middle School learners' intakes of foods rich in vitamin A

- Awareness, popularity and consumption of selected wild plants by middle school learners