SCREENING OF COWPEA, BAMBARA GROUNDNUT AND *AMARANTHUS* GERMPLASM FOR DROUGHT TOLERANCE AND TESTING OF THE SELECTED PLANT MATERIAL IN PARTICIPATION WITH TARGETED COMMUNITIES

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Water Research Commission

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Report to the Water Research Commission on the Project
"Selection for drought tolerance in the germplasm of *Vigna unguiculata* (cowpea), *Vigna subterranea* (bambara groundnut) and *Amaranthus* spp. (marog)"

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Executive summary

Introduction

Water scarcity in South Africa has emphasized the need to improve water use efficiency. The challenge for farmers and researchers is to find ways to increase the crop output per unit of water.

Mechanisms used by plants to cope with changes in the environment are widely reported. Amongst them are root architecture, leaf morphology, physiological characteristics and others associated with the developmental biology. These components include cellular, developmental as well as biochemical traits. By selecting for these traits, it is possible to improve complex traits such as yield under stress conditions. Usually a combination of these attributes is present in crops that produce good yields under drought conditions. In environments where water is a limiting factor, water use efficiency is of the utmost importance.

Drought is a very common abiotic stress condition, thus economically important crops with high levels of drought tolerance are of great value. Under field conditions, drought severity, timing and duration vary from year to year and a cultivar, which is successful in one year, might fail in another year. The unpredictable and variable forms in which drought stress manifests itself, complicates the selection of superior plant material as well as the breeding programmes. Significant potential exists for the improvement of crop productivity by selecting plants that are better equipped to cope with unfavourable environmental conditions, such as drought.

One approach to improve crop performance is to select for genotypes that have improved yield during water deficit conditions. The ability of some plants to maintain a higher yield under drought than others is of great value. Average losses of some major crop plants due to environmental stresses may amount to 50-80% of their genetically determined productivity. The highest proportion of yield losses can be directly attributed to drought. The drought related responses in plants are of a complex nature and result from genomic

re-organisation and alterations in gene expression. Drought tolerance has shown to be a highly complex trait, influenced by multiple genes.

Project aims

The main objective of this study was to evaluate the drought tolerance of vegetable crops grown in environments where the crop yields are influenced by limited water supply.

(1) **Germplasm** of cowpeas (*Vigna unguiculata*), bambara groundnut (*Vigna subterranea)* and marog (*Amaranthus* spp.) was collected in different climatic areas. These crops are already well adapted to harsh climatic and growth conditions, but the aim was to select some lines with higher levels of drought tolerance.

(2) A problem that needed to be solved in this study was to find **selection methods** that could be used to screen large numbers of plants for drought tolerance and still give accurate results. The screening methods needed to be practical, fast, cheap and reliable.

(3) The possibility of multiplication and **screening** of the germplasm ***in vitro*** was investigated.

(4) The selected lines were evaluated under different environmental conditions by **community farmer participation** to establish the value of the plants in the communities.

(5) **Capacity building** formed an important part of this project.

The distribution of the desired genotype(s) to the farmers will improve in the end crop productivity and quality and will thus be of value for millions of people that are dependent on indigenous crops as source vitamins and proteins. It will also have a positive impact on subsistence farmers in areas with low precipitation or variable rainfall patterns.

Major results

1. Collection of germplasm

Germplasm of cowpea, bambara groundnut and *Amaranthus* germplasm was collected by personnel of the University of Zululand and the Sustainable Rural Livelihood (SRL) unit of ARC-Roodeplaat. The SRL unit collected seed in Gauteng (Soshanguve), Northern Province (Polokwane and Bushbuckridge), Mpumalanga and KwaZulu-Natal (Ladysmith).

Some *Amaranthus* seeds were also collected in Venda. The personnel of the University of Zululand collected seeds from various street markets and communities around the University, Empangeni, Mahlabatini, Pietermaritzburg, Richardsbay, Durban and Komatipoort. Some of the seeds that were collected originated from countries like Mozambique, Nigeria, Zimbabwe, Portugal and Ghana.

2. Optimisation of tissue culture and multiplication of selected lines

The tissue culture techniques for *Amaranthus*, bambara groundnut and cowpea were investigated. It was possible to establish a multiplication system for *Amaranthus* plants. The possibility for using *in vitro* plant material for screening amaranth for drought tolerance was also investigated, with the aim to reduce large and expensive glasshouse and field trials. PEG was used to induce drought stress *in vitro* and this was used as an alternative to dry land field trials to measure anti-oxidative stress activity, TTC reduction assays and proline production for amaranth. Due to the high variation within treatments and difficulty to relate to *in vivo* results with the *in vitro* results, it is suggested that *in vitro* screening results should always be first correlated to the results of the field trials. For these crops it is suggested that *in vitro* screening should not be used, seeing that the procedure itself causes the plants to stress and this resulted in very high variation within the treatments, rendering the results insignificant.

3. Physiological and anatomical evaluation of drought tolerance

A multidisciplinary approach was followed to measure the effect of drought stress on the physiology, biochemical and morphology of these plants, and to identify mechanisms that allow the plants to survive severe drought stress. Selected lines/ species as well as plant material collected from the communities were cultivated under optimum greenhouse conditions until the plants were subjected to drought stress by withholding water after which various screening methods were used to determine the levels of drought tolerance.

The study included responses with regard to:

(i) leaf morphology (leaf area)

(ii) changes in water status (relative water content (RWC); leaf water potential (LWP))

(iii) metabolism (enzymes of the anti-oxidative pathway: activity of superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (AP)); cell membrane stability (CMS); free proline concentration; 2,3,5-triphenyl-tetrazolium chloride reduction rate (TTC); total soluble protein; photosystem II (PSII) structure and function (chlorophyll fluorescence kinetics)
(iv) rooting architecture (woodenbox screening: root architecture).

a. Amaranth

Two pathways are evident in the maintenance of turgor in amaranth: the modulation of LWP and RWC. Restoration and repair upon rehydration are critical components of desiccation tolerance, and recovery of leaf area. The lower RWC and LWP values coincide with lower photosynthetic activity and rate of decrease in leaf area during drought stress. Furthermore, the ability of the membrane to retain and selectively transport cellular solutes gives an indication of the cellular membrane function. CMS assay indicates excessive leakage of cellular electrolytes and is usable as an indication of cellular membrane injury. TTC reduction assay measures the dehydrogenase activity of the intact cell. Proline accumulation screening seems to be highly useful for determining genotype variation in drought tolerance in amaranth. This is possible since differences in the onset of proline accumulation, as well as amount of proline accumulated as a result of severe water deficit, were observed in the amaranth species tested. Proline accumulation also serves as a good indicator of the water status of the plant as there is an inverse relationship between proline content and water potential. The amaranth species tested at seedling stage showed strong drought avoidance characteristics during early drought screening. Although the leaves of the species were discarded during severe moisture stress (drought avoidance), the plants recovered very well after rewatering by sprouting again. Amaranth seedling roots exposed to extended periods of moisture stress recovered quickly to re-establish water uptake again upon rewatering.

The local collections that out performed some of the species under the drought conditions were: Community 3 and 4, Krugersdorp, Callaloo, Imbuya and Indigenous I. The best species were: *A. tricolor, A. hybridus, A. hypochondriacus* and *A. candatus.*

b. Cowpea

The screening methods, which showed the highest correlation with each other in cowpeas, were the JIP-test (chlorophyll fluorescence measurements), chlorophyll *a* and *b* levels, RWC, woodenbox technique, yield of the greenhouse plants and root architecture. The determination of the free proline levels and LWP showed a relatively good correlation with the above mentioned screening methods. The screening tests of the enzymes of the antioxidative system (SOD and GR) and the CMS were unable to reveal differences in the drought resistance levels of the cowpea lines.

The drought tolerant control line IT96D-602 was able to maintain a higher relative water content and water potential than the other lines tested over most of the seasons. This improved the viability of the plants, which was evident from the TTC reduction assay and the chlorophyll fluorescence values. The fact that IT96D-602 was experiencing lower stress levels than the other lines was also evident from the lower free proline levels accumulated and the higher Chl *a* and *b* levels maintained under drought stress than the other plants. Some qualities that were detected in IT96D-602, which might have contributed to the water retention, were smaller leaves and a more upright growth form. IT96D-602 also possesses a more extensive root system than the other lines, which helps with water uptake.

The community collected lines: Ghana black eyed bean, Manguzi 1 and 2, Okhaluleni and Phelandaba, performed exceptional well against the drought selected line IT96D-602. This illustrates the potential of the indigenous germplasm.

c. Bambara groundnut

Fifteen bambara groundnut lines were evaluated over four seasons. The proline levels changed little over the stress period, as bambara groundnuts are generally very drought tolerant. The SOD enzyme activity decreases in all lines but SB1-1. The AP activity levels show little change over the drought stress period, except SB1-1 that experienced higher AP levels with increased stress. The RWC of all the lines were 100% before stress was induced and declined to between 50 and 70% after 2-3 weeks without water. After rewatering the RWC recovered to 90% within 4 days. The differences in the LWP and

CMS were not significant between the different lines, and could not be used for distinguishing between different lines. The woodenbox experiments were however able to distinguished between the different lines during the drought stress as well as after recovery. The root architecture boxes were used to show differences between the unstressed lines. Some lines had a good distribution of long roots and some had more roots at the surface. The yield of the plants tested differed significantly between the lines, as well as between the treatments.

It was experienced that the bambara groundnut yield was very low in most lines under stress conditions. The lines SB1-1, SB7-1, SB20-1, AS18 and SB9-1 have the characteristic to yield even under dry conditions. The lines that performed best during the test period were: SB7-1, SB1-1, MAD1, SB9-1, SB20-2A, NB and KwaHgnase.

4. Propagation of selected lines

Selected amaranth, cowpea and bambara groundnut species, lines or selections that recorded high levels of drought tolerance were propagated for distribution to communities for testing of general performance and acceptability.

5. Evaluation of the crop for yield and acceptability through farmer participation

Demonstration trials of *Amaranthus*, bambara groundnut and cowpeas were established in Soshanguve and Bronkhorstspruit in co-operation with farmers in order to introduce the crops to community members and to encourage them to be willing to evaluate it for acceptability and potential income generation. Eleven families from KwaZulu-Natal and Gauteng were involved in the first community trials. The program in Gauteng was later extended to 20 farmers. The farmers had to give feedback on any problems experienced with the cultivation of the three crops, how well the crops were accepted by the communities and also how the proposed crops fitted in with the other crops that they were already cultivating. They were used to harvest *Amaranthus* in the field and therefore the cultivation of this crop was new to them. They felt that amaranthus could be an economically viable crop. The farmers preferred the dual-purpose runner types of cowpea, as they could consume the seeds as well as the leaves. The bambara groundnut is a very

popular crop in especially Gauteng, but the unavailability of seed, especially of improved varieties, and lack of space poses problems. The farmers that participated in this study sold their crops to restaurants, hawkers and urban communities. Acceptability of the new selections was evaluated by palatability tests performed by taste panels consisting of local people who are familiar with the taste of the locally grown cultivars.

6. Capacity building

The adaptation of different physiological and anatomic techniques to screen large numbers of amaranth, bambara groundnut and cowpea plants for drought tolerance were mastered by the researchers during the course of the research project. These screening techniques were again passed on to various participants from Africa through training courses funded by AFRA and ICRO. The farmers through farmer participation in demonstration and community trials also learned a lot about the crops and are much more positive about the cultivation of these crops.

The information gathered through this study was of high scientific value seeing that two Ph.D. theses and two BSc honours degrees were completed. Fourty five papers and posters were also delivered at conferences and other scientific meetings. One scientific paper has been published, two are in press and seven to be submitted shortly.

Conclusions

Germplasm of cowpea, bambara groundnut and amaranth were selected in various places in South Africa. The aim was to evaluate the collected germplasm for tolerance to drought stress. In order to do this, different physiological and phenotypic techniques were used. The information obtained through this study contributes towards a better understanding of the physiological and morphological basis of drought tolerance in neglected crops. The techniques selected were able to distinguish between drought tolerance in the different genotypes tested. It was also noticed that the different species reacted differently to a drought stress. Some of the selections out performed the control drought tolerant lines,

indicating the successful identification of increased drought tolerance in the germplasm screened in this study.

It was found that amaranth appears to tolerate water stress by means of mechanisms of osmotic, metabolic and photosynthetic adjustment. From these results it can be concluded that some of the most important physiological factors which effect drought tolerance in amaranth seems to be: (1) limitation of water loss by reduction of leaf area, (2) ability to maintain a high water potential during water deficit, (3) efficient rooting ability and root/shoot ratio to exploit all available soil moisture, (4) maintenance of water status through osmotic adjustment, (5) scavenging of toxic O_3 species, (6) possibility of adapting photosynthesis to comply with the changing demands of the e^- transport system.

The most suitable methods for screening large numbers of cowpea plants for drought resistance are: (1) chlorophyll fluorescence (JIP-test), (2) free proline levels and (3) woodenbox screening for drought resistance at the seedling stage and (4) relative water content. One of the strategies used by cowpeas to survive unfavourable conditions is very sensitive stomatal control to minimise water loss.

The most suitable screening methods for bambara groundnut selection are: (1) chlorophyll fluorescence, (2) free proline, (3) woodenbox screening and (4) yield, which all form an important part of the plants survival.

The communities targeted to participate in this project, were generally very enthusiastic about the new crops. The cultivation of the crops was well accepted by the eleven farmers in the test trial and they were all able to find suitable markets for the crops. The initial farmer participation was extended to 20 other farmers, who were generally very enthusiastic about the new crops and willing to grow them. They indicated that they learned a lot during the trials regarding cultivation, utilisation and general nutritional value. Valuable information was gathered on community preferences regarding these crops.

Recommendation for future studies:

- The collections and lines that performed best under drought conditions must be tested against each other in a field trial. Seed from plants selected during this study must be multiplied and distributed to the local community for further evaluation.
- The bambara research must be extended to rain-out shelter trials as the plants did not perform well in pots in the greenhouse. A drought trial with all the best lines tested under controlled conditions with different water regimes will enable us to complete the bambara groundnut research meaningfully.
- Planting of cowpea, bambara groundnut and amaranth in demonstration trials under different water regimes together with soybean, groundnut and spinach, to demonstrate the proposed higher drought tolerance features of the indigenous crops.
- The information obtained through this study can be used in germplasm screening of other indigenous crops like the *Cucurbita, Cleome* and *Solanum* species for drought tolerance. The screening methods identified for the selected crops can be used in future, for instance in the selection of drought tolerant mutants of the above three crops.

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"Selection for drought tolerance in the germplasm of *Vigna unguiculata* (cowpea), *Vigna subterranea* (bambara groundnut) and *Amaranthus* spp. (marog)"

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List of Abbreviations

ABS	Absorption
ABS/RC	The absorption per reaction centre
AOS	Active oxygen species
AP	Ascorbate peroxidase
ATP	Adenine triphosphate
CF	Chlorophyll fluorescence
Chl	Chlorophyll
CMS	Cell membrane stability
CO_2	Carbon dioxide
CS	Cross section
Cu/Zn SOD	Copper and Zinc containing superoxide dismutase
Dww	Days without water
dH_2O	Distilled water
Dl/CS	The flux of the dissipated exitation energy at time zero per CS. (ABS/CS – TR/CS)
EDTA	Ethylenediaminetetraacetic acid
ET	Electron transport
ET/CS	The electron transport flux per CS
F_o	Initial, constant or minimum fluorescence
F_m	Maximum fluorescence
F_p	Point where excitation intensity is high enough to permit the closure of all the reaction centres
F_v	Variable fluorescence
F_v / F_m	Maximum quantum efficiency of primary photochemistry
GR	Glutathione reductase
GSSG	Oxidised glutathione
H_2O_2	Hydrogen peroxide
IAA	Indole acetic acid
IITA	International Institute of Tropical Agriculture

JIP-test	Chl-a fluorescence F_o-J-I-P transient test
KH_2PO_4	Potassium phosphate buffer
KOH	Potassium hydroxide
k_n	The nonphotochemical de-exitation rate constant
k_p	The photochemical de-excitation rate constant
LWP	Leaf water potential
m	Meter
mg	Milligram
min.	Minute
ml	Millilitre
MPa	Megapascal
mm	Millimetre
N	Turnover number of Q_A
nm	Nanometer
NADPH	Nicotinamide adenine dinucleotide hydrogen phosphate
NaOH	Sodium hydroxide
O_2^-	Free oxygen radicals
OA	Osmotic adjustment
OH	Hydroxide
OP	Osmotic potential
PEA	Plant Efficiency Analyser
PEG	Polyethylene glycol
PS I	Photosystem 1
PS II	Photosystem 2
PVPP	Polyvinylpolypyrrolidone
Q_A	Primary quinone acceptor in PS II
Q_B	Secondary quinone acceptor in PS II
RC	Reaction center
RC/CS	The concentration of reaction centres (RC) per cross section (CS) of the sample
rpm	Revolutions per minute

RWC	Relative water content
sec.	second
SOD	Superoxide dismutase
Std.	Standard
TR/CS	The rate of exciton trapping per CS
TR/RC	Exciton trapping flux by the RC
TTC	2,3,5 - triphenyltetrazolium chloride
µg	Microgram
µl	Microlitre
Vit. C	Vitamin C
Φ_{Do}	Quantum efficiency of dissipation
Φ_{Eo}	Quantum efficiency of electron transport
V_I	Intermediate step of fast phase fluorescence rise
V_J	Intermediate step of fast phase fluorescence rise
Φ_{Po}	The maximum quantum yield of primary photochemistry (F_v / F_m)
ψ_0	The efficiency by which a trapped exciton can move an electron into electron transport chain beyond Q_A
ψ_w	Water potential
w.a.p	Weeks after planting
w.a.s	Weeks after seeding
w.o.w.	Without water

Chapter 1

Introduction

1.1 The Importance of Drought Tolerance in Crop Plants

In the lifetime of a plant it may encounter various stress conditions. Two types of stress conditions are recognised, namely biotic and abiotic stress. The focus of this study was on the abiotic stress conditions and specifically water deficit or drought stress. Plants can resist, avoid or escape unfavourable conditions, caused by drought. Drought resistant plants, like succulents, store water in their succulent tissue and keep their water content high by closing their stomata during the day. These plants also posses an extremely thick cuticula to restrict water loss through cuticular transpiration (Salisbury & Ross, 1978). Plants that avoid drought conditions usually have deep extended root systems as well as other properties like smaller leaves, sunken stomata, epidermal hair, etc. One of the methods to escape drought conditions is to have a short growth period (Salisbury & Ross, 1978) as well as CO^2 concentrating mechanisms. These plants grow to maturity and produce at least one set of seeds before the soil moisture has been depleted.

Drought conditions have a direct influence on the growth and development of plants (Larcher, 1980), which subsequently influence the yield. The processes that are directly influenced are the rate of cell division, cell expansion, net photosynthesis, CO_2 assimilation and abscission of leaves (Larcher, 1980). To estimate how well plants can function under stress conditions, many essential systems in the plant can be monitored to determine the level of endurance of the plant. These systems are all linked together to cope with any stress conditions in an effective way. Hall (1993) defines drought resistance as the relative yield of a genotype compared to other genotypes subjected to the same drought stress.

The aims of this project were:

- To apply various physiological and phenotypical screening methods to the three selected crops and to determine which of these methods is suitable for detecting differences between the lines, selections or species of the crops
- To collect germplasm of cowpeas, bambara groundnuts and amaranthus from communities and markets in different areas of South Africa and to compare the levels of drought tolerance of these plants with that of plants with known levels of tolerance.
- To determine if the screening methods can be applied to *in vitro* plants in order to speed up the selection process and to determine if the results can be correlated to results obtained from the plants in the greenhouse.
- To propagate the selected lines and to distribute the seeds to small scale farmers.
- To evaluate the different crops suitability and acceptability through farmer participation
- To transfer technology to farmers and researchers through farmers days, workshops and conferences.
- Capacity building was later added as an aim of the project.

1.2 Literature review

1.2.1 Screening methods

1.2.1.1 Chlorophyll fluorescence and Chlorophyll a and b determination

Photosynthesis is the process by which light energy is absorbed by oxygenic and anoxygenic organisms and converted into redox chemical energy by the synthesis of high energy molecules like ATP and NADPH (Bidwell, 1979; Govindjee, 2000; Pakrasi, 1995). This process of trapping of light energy and the conversion thereof occurs in different stages. The first part (the photochemical process) takes place in the thylakoid membranes of the chloroplasts. Two large pigment-protein complexes namely photosystem I (PSI) and photosystem II (PSII) are situated in the thylakoid membranes

(Pakrasi, 1995). These two complexes have specialised chlorophyll molecules that absorbs the light energy and start a series of redox reactions that result in the production of ATP and NADPH (Pakrasi, 1995). The name chlorophyll (Chl) is commonly used to describe the Mg-ligated protopotphyrin derivatives (Itoh & Iwaki, 2000). These Chl molecules are located inside the reaction center complexes (Hiller & Babcock, 2001; Itoh & Iwaki, 2000). The first protein complex involved when light energy reaches the chloroplasts, is a pigment antenna of PSII. This complex contains chlorophyll *a* and chlorophyll *b* molecules as well as other pigments (Govindjee, 2000; Strasser, 1996). The main role of the Chl *b* molecules is to harvest the light energy and thus act as antenna pigments while the function of the bound forms of chlorophyll *a* (Chl *a*), P680 and P700, is to convert the light energy into chemical redox energy (Govindjee, 2000). Pigments in the antenna system absorb photons and transfer excitation energy to other pigment protein complexes called reaction centres (Gomez & Chitnis, 2000). P680 acts as the reaction centre chlorophyll of PSII and P700 is the reaction centre chlorophyll of PSI (Govindjee, 2000). The P stands for pigment trap and the number gives an indication of wavelength maxima of the first exited state in nanometers (Govindjee, 2000). During the second part of photosynthesis the ATP and NADPH that were produced during the first phase, are utilised for CO_2 fixation and the production of sugars (Strasser, 1996).

When plants experience stress conditions i.e. heat or drought, the photosynthetic process will not function optimally and some of the light energy will be dissipated. Even under optimal conditions some energy is dissipated. This emission takes place mainly in the form of heat but some of the energy is lost in the form of fluorescent light (light with a long wavelength and low energy) (Bidwell, 1979). This is called chlorophyll fluorescence (CF) and can be detected with a fluorometer. Fluorescence mainly originates from Chl *a* of PSII (Govindjee, 1995; Krause & Weis, 1991). Although this Chl *a* fluorescence represents only a fraction of the energy that is dissipated it still provides a lot of information on the primary photosynthetic processes like the absorption of light, energy transfers and photochemical reactions (Krüger, Tsimilli-Michael & Strasser, 1997; Strasser, Srivastava, & Tsimilli-Michael, 2000). Kautsky and Hirsch already described the relationship between photosynthesis and Chl *a* fluorescence in 1931. They reported a

fast rise in the fluorescence emission when dark-adapted leaves are illuminated. The rise reaches a maximum fluorescence after which it decreases until a steady level is reached (Strasser, *et al.*, 2000). The advantage of measuring the CF is that it is a rapid, sensitive, non-destructive and relatively cheap technique that gives the opportunity to study the physiological condition of a plant (Strasser, *et al.*, 2000). It even enables the detection of injury even before visible symptoms appear (Srinivasan, Takeda & Senboku, 1996). This technique has been used to determine differences in the reactions of plants to various stress conditions like heat (Havaux, Ernez & Lannoye, 1988; Havaux, Greppin, & Strasser, 1991), high light intensity (Krüger *et al.*, 1997), cold (Janda *et al.*, 1994; Krause & Somersalo, 1989) and also drought stress (Corlett, 1993; Epron, Dreyer, & Bréda, 1992; Ögren, 1990; Ögren, E. & Öquist, G. 1985; Van Rensburg, Krüger, Eggenberg, & Strasser, 1996).

With the improvement in the technology to detect and quantify the Chl *a* fluorescence in the first few μ s of illumination, it is now possible to determine a precise F_0 and also F_P (Krüger *et al.*, 1997; Strasser *et al.*, 2000). F_0 is defined as the fluorescence value at the onset of illumination when all the reaction centres are open while F_P is the point where the excitation intensity is high enough to permit the closure of all the reaction centres (RC). Under saturating light conditions this is also called the maximum fluorescence (F_M) (Krüger *et al.*, 1997; Strasser *et al.*, 2000). It is also possible to determine intermediate steps between F_0 and F_P (F_M) such as F_J (at ca 2ms) and F_I (at ca 30ms), when the fluorescence kinetics is plotted on a logarithmic scale (Krüger *et al.*, 1997; Neubauer & Schreiber 1987; Strasser *et al.*, 1995). The maximum fluorescence, F_P (F_M), is reached after about 300ms (Strasser *et al.*, 1995). By quantification of the fast polyphasic rise (O-J-I-P) of the direct fluorescence signal by the JIP-test (Krüger *et al.*, 1997), the behaviour of PSII is deconvoluted in several functional and structural parameters. This procedure reveals much more information about the response of the samples than only using a parameter such as $\varphi_{P_0} = Fv / Fm$, which in many cases is rather insensitive.

1.2.1.2 Changes in the Free Proline Concentration

The production of proline is not limited to plants but this amino acid is also found in humans, animals (Kowaloff, Phang, Granger & Downing, 1977) and even lower life forms like insects (Balboni, 1978), yeast and blue green algae (Brandriss *et al.*, 1994). In humans and animals the proline acts as an endogenous source of metabolic fuel. In plants on the other hand the role of proline is not clearly defined and it seems that different plants use this amino acid in different ways. One of the functions of proline that has been widely reported on, is to assist with osmoregulation during stress periods (Delauney & Verma, 1993; Verbruggen, Villarroel &Van Montagu, 1993; Williamson & Slocum, 1992). Proline assists in the protection of the membranes by increasing the osmotic pressure in the cells (Salisbury & Ross, 1978). The production of proline is initiated by a reduction in the water potential of the cells (Aspinall & Paleg, 1981) and can be the result of a number of abiotic stress conditions (Arora & Saradhi, 1995; De Ronde, Van der Mescht & Steyn, 2000; Hare & Cress, 1997 and references therein). According to Aspinall & Paleg (1981), this reaction begins rapidly in barley after the tissue have been exposed to dehydration. Kuznetsov & Shevyakova (1997) report an increase in the proline concentration of salt tolerant *Nicotiana sylvestris* cells after only two hours of high temperature and salinity and a subsequent decrease after six hours, probably because of the trans-methylation of proline to form derivates like N-methylproline, prolinebetaine and hydroxyprolinebetaine. Hare & Cress (1997) however questions the role of proline as an osmoregulant seeing that the leaf osmotic potentials of transgenic tobacco plants were unaffected by osmotic stress.

Another possible role for proline accumulated during a stress period may be to act as an organic nitrogen reserve which can be released during the recovery period (Taylor, 1996). The improved energy status of the plant will accelerate the recovery after a stress period (Lawlor, 1995). According to Phang (1985) proline also has a regulatory role in some enzyme systems and also acts as a transport mechanism because it crosses cellular and organellar barriers readily.

The study of the interaction between proline and drought stress started way back in 1954 when Kemble and Mac Pherson noticed an accumulation of proline in wilted plant tissue. Since then it has been reported that a variety of other organic solutes like glycine betaine (Le Rudulier *et al.*, 1984; McCue & Hanson, 1990), polyols such as glycerol, mannitol, sorbitol and pinitol (Adams *et al.*, 1992; Delauney & Verma, 1993; Ford, 1984) can also accumulate to act as osmoregulators. The accumulations of proline in plants during drought stress have been reported by different authors and for different crops like cotton (De Ronde, Van der Mescht & Steyn, 2000), rice (Chou, Chen & Kao, 1991), wheat (Munns, Brady & Barlow, 1979), soybeans (Moftah & Michel, 1987; Kohl *et al.*, 1991), maize (Ober & Sharp, 1994), tobacco (Szoke *et al.*, 1992), potatoes (Corcuera, Hintz & Pahlich, 1989), tomatoes (Rhodes, Handa & Bressan, 1986) and a host of other crops.

The interpretation of the role of the accumulation of proline differs between different crops. In cotton, De Ronde, Van der Mescht & Steyn (2000) concluded that the plants that produced more proline at an earlier stage of the stress period, were more drought tolerant than those with a lower proline production at a later stage. Levy (1983) on the other hand reports that the accumulation of proline in potato leaves was triggered by severe stress conditions and that high levels of proline in the tubers indicate drought susceptibility and not drought resistance. The determination of the free proline concentration can therefore be used as screening criterion for drought tolerance but the interpretation of the results should take the mechanisms used by the plant to cope with the stress condition into consideration.

1.2.1.3 2.3.5-Triphenyltetrazolium Chloride Reduction

Plants use different defence mechanisms to cope with environmental stress conditions. Determining the viability of cells under these adverse conditions can give an indication of how well the plant is coping with the stress condition. The viability of the cells can be determined in various ways. Some of the methods used to determine viability are to measure regrowth (Ishikawa, Robertson & Gusta, 1995), 2,3,5-triphenyltetrazolium chloride reduction assay (TTC) (De Ronde & Van der Mescht, 1997), vital staining (Chen

& Gusta, 1982), protoplasmic streaming (Larcher, 1980), plasmolysis (Palta, Levitt & Stadelmann, 1977), leakage of ions (Inaba & Crandall, 1988) and measurement of ultra violet absorbing compounds (Wiest, Good & Steponkus, 1976). These methods vary in accuracy and convenience. Ishikawa *et al.*, (1995) compared a few of these methods and came to the conclusion that testing the regrowth is the most sensitive test but it is time consuming while the TTC reduction assay was most convenient. Calkins and Swanson (1990) concluded that none of the tests are completely reliable and that a combination of tests should be used. This is also recommended by Ishikawa *et al.*, (1995).

The TTC reduction assay can be performed under uniform laboratory conditions and can provide answers in much less time than is possible with yield trials (Schaff, Clayberg & Milliken, 1987). The TTC reduction assay measures the ability of individual cells to function physiologically (De Ronde & Van der Mescht, 1997). Normal viable cells can reduce the tetrazolium salt in the mitochondria where electrons from the electron transport chain are accepted by the tetrazolium salt via the dehydrogenase pathway (Anonymous, 2001; Delpech, 2001; Nachlas, Margulies & Seligman, 1960). The result of the reduction of the tetrazolium salt is a red pigment called formazan (Figure 1). The intensity of the red colour of the formazan can be quantified spectrophotometrically.

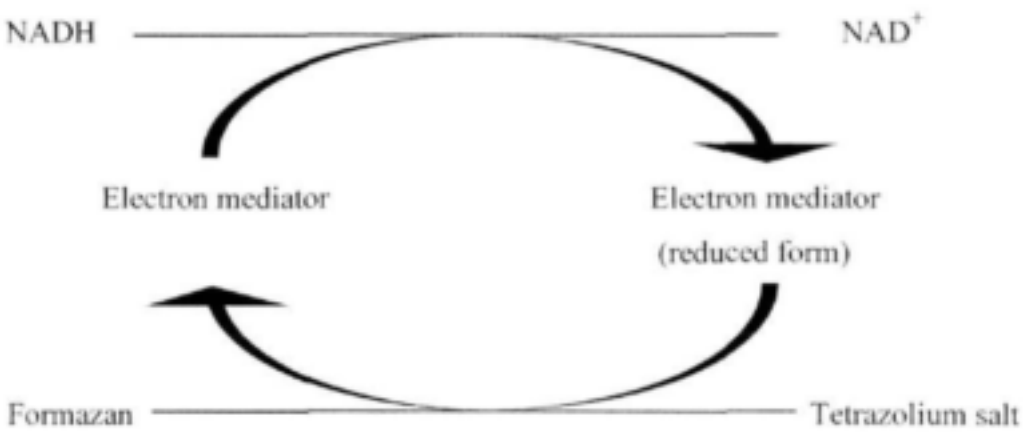

Figure 1. The reduction of 2,3,5-triphenyltetrazolium chloride to form a red pigment called formazan (Ishiyama, 2001).

De Ronde & Van der Mescht, (1997) have shown in a study on cotton that acclimation to drought stress as measured by the reduction of TTC can be used as a screening technique. They could show that formazan production was relatively lower in stressed leaves of drought sensitive cotton cultivars compared to the leaves of the control treatment. The tolerant cultivars on the other hand had higher dehydrogenase activity and therefore produced higher levels of formazan compared to the control treatment. Nachlas *et al.*, (1960) found similar tendencies with drought stressed as well as heat stressed plants.

This phenomenon is possibly due to the fact that the tolerant plants had the ability to activate defence mechanisms against the stress conditions during the pre-stress period. When the severe stress condition was introduced, these plants were able to survive the unfavourable conditions for a longer period and were still able to reduce the TTC salts (De Ronde & Van der Mescht, 1997). The sensitive plants did not possess this quality. The TTC reduction assay has also been used by Chen, Shen & Li, (1982), Fokar, Nguyen & Blum, (1998), Gibon, Sulpice & Larher (2000) and Towil & Mazur, (1974) to distinguish between different resistance levels to abiotic stress conditions.

1.2.1.4 Protein synthesis

Water limitations result in disturbance to the normal capacity of the plant to carry out protein synthesis. A feature of drought tolerance may be the ability to discontinue protein synthesis at moderate water deficits and resume protein synthesis on return to full turgor (Bewley, 1981). A decrease in poly-ribosome abundance is correlated with the decreased protein synthesis. Coincident with this decrease in total protein, is an increase in free amino acids. Much of the amino acid accumulation is due to the reduction in protein synthesis, but in some cases biosynthesis of particular non-protein amino acids is stimulated (e.g. betaine, proline) (Nilsen & Orchutt, 1996). During drought stress there is a complete down-regulation of most genes and an up-regulation of stress associated genes (Van der Mescht & de Ronde, 1993). Total soluble proteins can be *in vitro* labelled and fractionate by SDS Page to study the changes in the protein patterns due to stress.

1.2.1.5 Enzymes of the Antioxidative System

The drought related responses in plants are of a complex nature and result from genomic re-organisation and alterations in gene expression over thouthands of years (Edreva, 1992). A central response during drought stress is the increased concentration of absicisic acid that stimulates the closure of stomatal guard cells to reduce water loss. The availability of CO_2 for photosynthesis is thus reduced and consequently also the demand for reducing power, resulting in the misdirecting of electrons in the photo systems. This process leads to the formation of active oxygen species (AOS) (Bowler, Van Montagu & Inze, 1992). The excess production of AOSs such as superoxide can cause serious damage to plant cells. This over production usually occurs during adverse environmental conditions like high or low temperatures. The combination of these conditions with drought, high light intensities, ultraviolet light, herbicides or air pollutants can aggravate this situation (Inzé & Van Montagu, 1995). Plants use enzymatic and non-enzymatic protection mechanisms to counter this increase in AOS. The production of superoxide dismutase (SOD), ascorbate peroxidase (AP) and glutathione reductase (GR) is one of the methods used by some plants to reduce the high concentration of these potentially lethal AOSs.

Thus, as drought and other physiological stresses cause oxidative injury, high antioxidant capacity or increased levels of antioxidants can prevent cell damage and may correlate with stress tolerance. SOD is a well-described enzymatic antioxidant which breaks down the superoxide radical to hydrogen peroxide and dioxygen (Monk, Fagerstedt & Crawford, 1989; Van Camp *et al.*, 1994). The hydrogen peroxide resulting from this reaction is potentially toxic to cells and is removed by GR, dehydroascorbate reductase and ascorbate peroxidase through the Halliwell-Asada pathway. GR co-operate with SOD to remove superoxide radicals mainly in chloroplasts but also in the mitochondria and cytoplasm. According to Bowler *et al.* (1992) GR has a regulatory function due to the dependence of it's activity on the availability of NADPH. Additionally, this increase in GR enhances NADP availability and electrons can now be accepted from ferredoxin, thereby reducing superoxide formation. Peroxidises are also involved in reactions with a number of organic hydro peroxides. The reactions involve the acceptor molecules with

simultaneous reduction of the per oxidic substrate namely ascorbate (Larson, 1988). AP activity is mainly found in the chloroplasts (Bowler *et al.*, 1992).

However, it is possible that mechanisms that reduce oxidative stress may play a secondary role during drought tolerance (Bowler *et al.*, 1992). This may complicate a direct correlation between the increased concentrations of an enzyme and drought tolerance. Correlation between the simultaneous increase in two or more enzymes involved during the minimization of oxidative injury and known drought tolerance may enhance our understanding of drought tolerance. Malan, Greyling and Gressel, (1990), found a correlation between drought tolerance in maize inbreds and CuZn SOD and GR activities. Increased activity of one enzyme alone did not confer drought tolerance.

1.2.1.6 Relative Water Content

Relative water content (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. The RWC estimates the percent water in a leaf as a fraction of the total volumetric water that the leaf hold at full turgor. It is normally measured in terms of fresh weight, turgessant weight and dry weight (Beadle, Ludlow & Honeysett, 1987). It accounts for the effect of osmotic adjustment in affecting plant water status during plant stress. Two plants with the same leaf water potential can have different RWC if they differ for osmotic adjustment. The differences that are noticed in the physiological and biochemical processes can thus be attributed to the differences in the water status (Blum, personal communication).

1.2.1.7 Leaf Water Potential

Differences in the water potential between the atmosphere and the soil result in water movement through the plant from the soil to the atmosphere. Water flow can also be influenced by resistance regulated by stomata in the leaves, by the conductive system of the plant, or by the resistance of cells and cell walls between soil and the root xylem

vessel. As water transpires from the leaf, leaf water potential (LWP) becoming more negative. If soil water is available, it will flow into the leaf resulting in only a small reduction in LWP. As the soil water becomes scarce, LWP must be further reduced in order to create the necessary gradient differential, which would drive the water up from the drying soil to the leaf (Blum, 2001).

To measure the LWP a pressure chamber can be used. By applying pressure to the leaf, the water interface which, due to negative pressure withdrew can be returned to the level it was before detachment (Beadle, Ludlow & Honeysett, 1987), and this gives an indication of the tension in the xylem of the intact plant. The osmotic potential of the xylem sap are normally less than 0.02 MPa and therefore the hydrostatic pressure in the xylem is equal to the water potential (Beadle, Ludlow & Honeysett, 1987). LWP is determined early in the morning to avoid the measurement of any stress that may occur in the control plants due to slow transpiration. The relationship between RWC and LWP during a stress period represents the effect of osmotic adjustment.

1.2.1.8 Cell membrane stability

One of the challenges a plant has to overcome to survive in harsh conditions is maintaining the water status (Taylor, 1996). Apart from certain physiological responses as described before, the plant can also make some structural and physiological adaptations. This could include thickened wax cuticles, CAM metabolism, reduced leaf area and maximal water use efficiency (Taylor, 1996). The reduction in the water potential of the plant can lead to the delay of developmental events (Blum, 1996) and eventually also a loss in productivity. Maintaining water status is of the utmost importance to plants.

The function of the cell membrane is to retain and selectively transport cellular solutes. The critical role of cell membrane stability (CMS) under conditions of moisture stress is a major component of drought tolerance (Bewley, 1979). Various environmental stresses, such as drought, cold, heat, salinity and mineral deficiency or toxicity were found to

affect cellular membrane function (Blum, 1989). The ability of the membrane to retain and selectively transport cellular solutes gives an indication of the cellular membrane function. Excessive leakage of electrolytes would be an indication of membrane injury. This electrolyte leakage can be determined by measuring the electro-conductivity of the electrolytes in a solution (Blum & Ebercon, 1981). Apart from the regulation of the transport of cellular solutes the stability of the cell membranes may also play a major role in maintaining the water status of the plant.

CMS measurements have mainly been used for the determination of heat tolerance (Blum & Ebercon, 1981; Srinivasan, Takeda & Senboku, 1996; Fokar, Nguyen & Blum, 1998) or cold tolerance (Chan, Sanxter & Couey, 1985; Frumanski & Buescher, 1979; Lewist & Workman, 1963). Malan, Greyling and Gressel (1990) also used this technique to determine the damage caused by drought and chemical stress to maize inbred lines.

1.2.1.9 Leaf area

The leaf area also plays an important role in the maintenance of the water balance. The leaf area influences the amount of light energy that can be absorbed to provide the plant with chemical energy but it also determines the area exposed which is responsible for the loss of moisture. Leaf area is determined by the phenology, stem morphology, rates of emergence and also the potential leaf size (Blum, 1996). Plasticity in the leaf area would mean that the plant maintains control over water-use in drought-stress conditions (Blum, 1996). Garrity, Sullivan and Watts (1984) found that drought stressed sorghum plants reduced the canopy photosynthesis by decreasing the leaf area instead of using stomatal control. Gwathmey and Hall (1992) found that cowpeas reduce the leaf area of stressed plants by either senescence, abscission or the cessation of leaf expansion. These methods can be regarded as ways to avoid drought stress. Sivakumar, Ntare and Roberts (1996) compared the leaf area of cowpea plants with different growth forms and growth cycles to determine the yield of these plants under field conditions. These experiments were conducted in years with good rainfall where drought stress wasn't a factor. The authors speculated on the benefits of plants with a short growth season and smaller leaves during

a drought stress period. The restriction of new growth is also an important factor in limiting leaf area (Akyempong, 1986).

1.2.1.10 Woodenbox screening at the seedlings stage

Drought spells can occur at any stage of the development of the plant. It is therefore important to screen the plants for drought resistance at different developmental stages. The development of the root system of a plant also plays an important role in the survival of the plant. It is therefore crucial to look at root characteristics and to use screening methods that are both relatively cheap and easy to use but still give reliable answers, especially when a lot of plants need to be screened (Singh, Mai-Kodomi & Terao, 1999b). This screening can be done in the field during the dry season (Watanabe, 1997; Watanabe & Terao, 1997) or in rain shelters. The other option is to use pots (Watanabe, 1997) or woodenboxes (Singh, Mai-Kodomi & Terao, 1999a) for screening in the greenhouse.

Seedling mortality is a common problem as a result of drought, thus a screening method for seedling resistance can be of great value. The woodenbox screening method can discriminate between tolerant and susceptible cowpea plants at seedling stage (Singh, Mai-Komomi & Terao, 1999a). It is a method that allows screening for shoot drought tolerance eliminating the root effect and permitting non-destructive identification of drought tolerant plants at the seedling stage. Singh, Mai-Komomi & Terao (1999b) observed a close correlation between woodenbox screening, field screening and pot screening in 12 cowpea cultivars. Two types of drought tolerance mechanisms were observed in cowpea using this method (Mai-Kodomi *et al.*, 1999). The plants using the 1st method, stopped growth alltogether and conserved moisture in all the plant tissue, untill the whole plant died off. The plants using the 2nd method continue to keep the growing tip turgid by mobilizing the moisture from the unifoliate to the trifoliate leaves. This same group demonstrated that segregating plant populations for drought tolerance can successfully be evaluated through this method and the survivors can then be transplanted afterwards.

A woodenbox is a box made from wood (1300 x 650 x 150mm) and lined with plastic. The box is the filled with a sandy soil mixture. Lines of different cultivars or selections are then planted in the box and once the seeds have germinated and the seedlings are well established all watering is stopped and a drought stress is applied until ⅔ of the seedlings have died. The advantage of this method is that, because the water can move freely in the box, major differences in the available water can be avoided. Another advantage is that only the most tolerant plants survive the stress period and these plants can be replanted in pots and allowed to form seeds.

1.2.1.11 Root architecture determination in 2-D rooting boxes

The architecture of the root systems of plants can contribute considerably towards the drought resistance of the plants. Breeding for improved root systems is known to have a beneficial impact on yield (Blum, 1982; Parsons, 1979; Robertson, Hall & Foster, 1985). According to Blum (2001) the root architecture can change as stress develops. Some plants improve deeper root growth under stress conditions to explore deep soil moisture. The study of root systems creates certain problems. The methods that are currently used to study roots and root structures all pose some practical problems (Bohm, 1979). Nilsen and Orcutt (1996) found it more difficult to determine productivity in roots than in any other organ due to the potentially large sampling error. Methods like paper rolls or paper sheets can only be used for the study of young root systems. Roots growing in aeroponic of hydroponic systems (Koukourikou & Porlingis, 1997) do not experience any resistance while growing, which can have an effect on the structure. Soil columns can give an impression of the length and volume of the roots but not the layout (Chen & Gabelman, 2000), while root boxes can provide such information. Care has to be taken while exposing the roots, not to disturb the growth pattern. Field trials also have the problem to uncover the roots and sometimes indirect methods are used to gather information on the root systems (Robertson, Hall & Foster, 1985). Some information can be obtained from each of these methods and the best method has to be selected for the purpose.

1.2.1.12 Yield

Hall (1993) defines drought resistance as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. This definition stresses the importance of the yield of the different lines. It is of no use to have a plant that can withstand extreme drought conditions but does not produce a crop. Turk, Hall and Asbell (1980) also stressed the importance of yield under stress conditions.

A wide variety of mechanisms and adaptations are available to protect plants from adverse conditions. Not all plants use the same strategy to cope with such conditions, which complicate the screening for drought tolerance. Until the strategy used by each group of plants is understood, a wide variety of methods should be implemented and as the strategy of the plant becomes clearer, the screening tests can be narrowed down to only a few. Because the defence mechanisms used by plant are complicated, it is seldom possible to use only one screening method to determine the levels of drought tolerance. It is therefore advisable to use combinations of screening tests.

Chapter 2

Materials and Methods

2.1 Plant material

2.1.1 Cowpea *(Vigna unguiculata)*

Over the duration of the research project various cowpea lines from various sources were screened in comparison with each other (Table 2.1). Some of the lines were obtained form the International Institute of Tropical Agriculture (IITA) in Nigeria. These lines included the control lines IT96D-602 (drought tolerant) and TVu7778 (susceptible). Some seeds were also obtained from other African countries like Ghana, Zimbabwe and Nigeria. Two local South African lines, Encore and Chappy have also been included. Seeds were also collected from the people living in the communities in KwaZulu-Natal

and Northern Gauteng. These seeds were named after the area where they were collected. Lastly some seeds were obtained from the University of California, Riverside, America.

Table 2.1 Cowpea lines screened for drought tolerance over a five year screening period. The drought tolerant control line IT96D-602 was included in every season.

Name	Origin	Seed colour	Growth form
First and second season			
IT96D-602	Drought tolerant control line IITA, Nigeria	Light brown to beige	Upright
IT92K-258-9	IITA, Nigeria	Greenish beige seed colour	Winding
IT90K-59	IITA, Nigeria	Light brown seeds	Winding
Chappy	Local S.A. line	Beige	Winding
TVu7778	IITA, Nigeria	Red brown to light brown seeds with darker specs	Winding
Third season			
IT96D-602	Drought tolerant control line IITA, Nigeria	Light brown to beige	Upright
Okhalweni area	Seeds collected in the communities	Dark grey	Winding
Ghana black eyed bean	Seeds sold in local markets in Zululand	White seeds with black eye	Winding
Encore	A local ARC cowpea line	Beige	Winding
Nigeria brown drum bean	Seeds obtained from Nigeria	Light brown	Winding
Zimbabwe black eyed bean	Seeds sold in local markets in Zululand	White seeds with black eye	Winding
Hluhluwa area	Seeds collected in the communities	Light brown with purple flecks	Winding
Mpenbeni ward	Seeds collected in the communities	Purple seeds with small light brown flecks	Winding
Okhaluleni area	Seeds collected in the communities	Small sized light brown	Winding

Name	Origin	Seed colour	Growth form
Fourth season			
IT96D-602	Drought tolerant control line IITA, Nigeria	Light brown to beige	Upright
Manguzi 1 BS	Seeds collected in at Manguzi in KwaZulu-Natal,	Dark brown seeds with spots	Winding
Manguzi 2 Red	Seeds collected in at Manguzi in KwaZulu-Natal	Red	Winding
Phelandaba	Seeds collected in at Phelandaba in KwaZulu-Natal	Big light brown	Winding
IT97K-209-4	Drought tolerant seeds obtained from IITA, Nigeria	White	Winding
IT97K-338-7	Drought tolerant seeds obtained from IITA, Nigeria	White	Winding
IT97U-819-118	Drought tolerant seeds obtained from IITA, Nigeria	White	Winding
IT93K-452-1	Drought tolerant seeds obtained from IITA, Nigeria	White	Winding
IT97K-608-14	Drought tolerant seeds obtained from IITA, Nigeria	White	Winding
Fifth season			
IT96D-602	Drought tolerant control line from IITA, Nigeria	Big seed Light brown to beige	Upright
Phelandaba	Seeds collected at Phelandaba	Big light brown	Winding
Okhaluleni	Seeds collected at Manguzi in KwaZulu-Natal,	Small sized seed light brown	Winding
IT97K-608-14	Drought tolerant seeds obtained from IITA, Nigeria	Medium to small sized seed white with black eye	Winding
IT97K-499-38	Drought tolerant seeds obtained from IITA, Nigeria	Medium sized seed white with black eye	Winding
IT93K129-4	Drought tolerant seeds obtained from IITA, Nigeria	Medium sized seed beige	Upright
M 217	Drought tolerant seeds obtained from IITA, Nigeria	Medium sized seed beige	Upright
00-11-161	Seeds from Prof. Hall, Univ. of California, Riverside, USA	Big seed white	Winding
00-11-145	Seeds from Prof. Hall, Univ. of California, Riverside, USA	Big seed white with black eye	Upright

2.1.2 Bambara Groundnut (*Vigna subterranea*)

The bambara groundnut lines that were used during this research project came from the seed bank of the Vegetable and Ornamental Plants Institute (VOPI). During the last season of the project, some lines that were collected in the communities were also included. The lines are listed in Table 2.2.

Table 2.2 Bambara groundnut lines that were screened for drought tolerance over four seasons.

Name	Origin	Colour
First and second season		
SB1-1	Control line, VOPI seed bank	Light brown with purple specs
SB7-1	VOPI seed bank	Wine red
SB9-1	VOPI seed bank	Light brown to beige with slightly darker hilum
SB20-1	VOPI seed bank	Beige
MAD-1	VOPI seed bank	Beige with purple specs

Name	Origin	Colour
Third season		
SB1-1	Control line, VOPI seed bank	Light brown with purple specs
SB8-1 (4) *	VOPI seed bank	Brown with purple spots similar to SB1-1
Swazi V5B (6)	VOPI seed bank	Cream to light brown
SB20-2A (9)	VOPI seed bank	Beige with small brown ring around hilum
AS 18 (11)	VOPI seed bank	Beige
AS 17 (13)	VOPI seed bank	Beige with black or red brown spots on sides of hilum
SB2-1C (15)	VOPI seed bank	Beige with black ring around hilum
SB9-1 (16)	VOPI seed bank	Light brown to beige with slightly darker hilum
Fourth season		
SB1-1	Control line, VOPI seed bank	Light brown with purple specks
Swazi V5B (6)	VOPI seed bank	Light brown
AS 17 (13)	VOPI seed bank	Beige with black spots on both sides of hilum
SB9-1 (16)	VOPI seed bank	Light brown to beige with slightly darker hilum
KwaHgnase	collected in the communities	Brown
NB	collected in the communities	Beige with black ring around hilum
NLB	collected in the communities	Light brown
NR	collected in the communities	Red

* SRL units code number

2.1.3 *Amaranthus*

Amaranthus germplasm collected during the growth seasons from 1998 to 2002 were used as explant material. The plant material that was included in the study on drought tolerance mechanisms for this report is listed in Table 2.3.

Table 2.3 *Amaranthus* species, cultivars and collections screened for drought resistance.

Name	Origin	Additional info
First season		
A. hypochondriacus	USA	Select for high production
A. hybridus	USA	Select for high production
A. tricolor	USA	Select for high production
Second season		
A. albus	USA	
A. hybridus	USA	
A. hypochondriacus	USA	Grain amaranth
A. tricolor	USA	Leafy amaranth with big leaves
A. tricolor I, II III	Taiwan, India, China	
A. hypochondriacus I, II	Nepal, unknown	Big leaves, good production
A. spinosus,	Zimbabwe	
A. hybridus I, II	Greece, America	
A. albus	Canada	
A. graecizans spp thellungianus	Mauritania	
A. fimbriatus	Mexico	
A. cruentus	Mexico	
Third season		
Community 1	KwaZulu-Natal	
Community 2	KwaZulu-Natal	
Community 3	KwaZulu-Natal	
Community 4	KwaZulu-Natal	
Amaranthus sp. "MacDonald"	MacDonald seed company	
A. cruentus		
A. hypochondriacus 1	Nigeria	
A. hypochondriacus 2	Nigeria	
A. hypochondriacus 3	Nigeria	
A. hypochondriacus 4		
A. tricolor		
A. hybridus		

Name	Origin	Additional info
Fourth season		
A. cruentus (Anna)		
Community 1	Ficksburg	
Community 2	Krugersdorp	Big leaves, good production
Community 3	Venda	
Community 4	Callaloo	
Community 5	Local	
A. cruentus (Amar)	Mayford	
A. hybridus	Krugersdorp	
Fifth season		
A. tricolor I	USA	
A. tricolor II	ARC Roodeplaat	
A. tricolor III	ARC Roodeplaat	
A. palmeri	ARC Roodeplaat	
A. dubius	ARC Roodeplaat	
A. cruentus	ARC Roodeplaat	
Imbuya	ARC Roodeplaat	Red stem, selected as leafy vegetable on taste
Imbuya	ARC Roodeplaat	Green stem, selected as leafy vegetable on taste
Indigenous I	KwaZulu-Natal	
Indigenous II	KwaZulu-Natal	
Six season		
A. cruentus	Mexico	
A. hybridus	Zimbabwe	
A. hibridus	Pennsylvania	
A. hypochondriacus	Nepal	
A. lividus	India	
A. candatus	India	
A. hibridus	Mexico	
A. candatus	US	
A. hypochondriacus	Mexico	

2.2 Screening methods

Cowpea and bambara groundnut seed was treated with the rhizobium strain *Bradyrhizobium* sp. for nodulation before planting. The plants were planted in 25 cm

pots containing a soil mixture of potting soil, vermiculite and sand (5:2:2). The plants were kept in a greenhouse at an 18 - 28°C, night - day temperature regime and were watered twice a week.

After respectively a 45 - 50 day (cowpea, bambara groundnut) or a 30 - 35 day growth period (amaranth), the plants of the stress treatment received one last watering after which they were allowed to dry out until the plants were so badly wilted that it was not possible to collect any more data. Measurements were taken every second day as the stress intensified. When the decision was taken to end the experiment the plants were rewatered and one last measurement was taken two or three days later to measure the recovery potential of the plants.

2.2.1 Chlorophyll fluorescence

Chlorophyll-a fluorescence transients were measured using a Plant Efficiency Analyser (PEA, Hansatech Ltd. King's Lynn, Norfolk, UK). The fluorescence transients were induced by a red light of 600 W m^{-2} intensity (excitation intensity) provided by 6 light-emitting diodes. Leaves were covered for 1 hour, using leaf clips, whereafter the measurements were taken using the PEA. During the first second of illumination, the following data were stored: F_m (maximal fluorescence intensity when all the reaction centres (RC's) are closed); F_O (fluorescence intensity at 50 µs when all RC's are open); F_J (fluorescence intensity at 100µs, 300 µs and 2 ms); and F_I (fluorescence intensity at 30ms); the time t_{Fmax} to reach F_M and the area between the fluorescence transient and the level of F_M. These transients were quantified using the Biolizer program. This data was used to calculate the phenomenological and biophysical expressions. The JIP-test (Strasser & Strasser, 1995) refers to the main steps for F_O-J-I-P. The energy fluxes of ABS, trapping and ET through PSII as well as the flux ratios and yields were calculated.

2.2.2 Chlorophyll *a* & *b* Determination

The method of Coombs *et al.* (1987) was used to determine the Chl *a* and *b* concentrations. Samples of 25µg freeze-dried leaves and 0.01g calcium carbonate

($CaCO_3$) were pulverised in liquid nitrogen before 5 ml, 80% acetone was added and the mixture was homogenised. The pulverised mixture was centrifuged at 14 000 r.p.m. for 15 min. at 4°C. The supernatant was transferred to 2.5 ml eppendorfs. The absorbance of the samples was determined at the following wavelengths: 663, 645 and 652 nm. The whole procedure was conducted in darkness seeing that light will cause the rapid destruction of the chlorophyll.

The Chl *a* and *b* concentration was determined using the following formulae:

mg Chl *a* / g dry mass = [12.7 (D663) – 2.69 (D645)] x V / (1000 x W)

mg Chl *b* / g dry mass = [22.9 (D645) – 4.68 (D663)] x V / (1000 x W)

mg total Chl / g dry mass = [20.2 (D645) + 8.02 (D663)] x V / (1000 x W)

mg total Chl / g dry mass = (D652 x 1000) / 34.5 x V / (1000 x W)

D = optical density of the Chl extract at a certain wavelength

V = final volume of the 80% acetone-chlorophyll extract

W = Weight in gram of the tissue initially used

The concentration of the Chl molecules was expressed as µg / ml

2.2.3 Changes in the Free Proline Concentration

The calorimetric method of Bates, Waldren & Teare (1973) was used to determine the proline concentrations. Samples of 50µg freeze-dried leaves were pulverised in liquid nitrogen before 5ml, 3% sulphosalicylic acid was added (Table 2.1.1.1). The pulverised mixture was centrifuged at 13 000 r.p.m. for 15 min. at 4°C. 1 ml of the supernatant was combined with 1 ml acid ninhydrin and 1 ml acetic acid. The samples were mixed well and placed in a waterbath at 100°C for one hour, where after the reaction was terminated on ice. Toluene (2 ml) was added to the reaction solution and the mixture was vortexed for 15 seconds. Two hundred µl of the toluene phase were transferred to heat resistant Elisa plates. The absorbance of the solutions was determined with a multiscan reader at a wavelength of 520nm. The proline concentration was determined using a standard curve and the concentration was expressed as µg proline/g dry weight.

Table 2.1.1.1: The extraction method of proline from leaf material

Buffers	Method	Remark
3% Sulphosalicylic acid	30g Sulphosalicylic acid 1000ml	Prepare previous day
Acid ninhydrin	Mix 1.25 gram ninhydrin, 30 ml glacial acetic acid; Heat Cool down, add 20 ml phosphoric acid	Prepare fresh daily
PROCEDURE:		
Add 5 ml 3% sulphosalicylic acid to sample that has been ground to a powder, mix well with pestle		
Centrifuge at 20°C, 13 000rpm for 10 minutes		
Mix 2 ml of the supernatant, 2 ml acid ninhydrin and 2 ml acetic acid in a test tube		
Vortex for 30 seconds, and incubate at 100°C for one hour		
Cool down in ice water, and add 4 ml toluene to each test tube, vortex for 30 seconds		
Load four replicates of 200µl for each sample into an Elisa™ plate and keep covered to prevent evaporation, load toluene as blank		
Read at 520nm on Titertek™		

2.2.4 2.3.5-Triphenyltetrazolium Chloride Reduction

The 2,3,5-Triphenyltetrazolium Chloride reduction assay (TTC) as described by De Ronde *et al.* (1995) was used with minor modifications. Leaves collected from the third and fourth node of healthy, unstressed plants were used for this experiment. Leaf disks with a diameter of 7 mm were punched from these leaves with a cork borer. Five leaf disks were used for each treatment. The leaf discs that were used for the heat stress experiment received a moderate heat stress of 40°C for a period of 3 hours, followed by a severe heat stress of 50°C up to 2.5 hours. The control group was initially kept at 29°C for 3 hours and then also received a severe stress of 50°C up to 2.5 hours. Leaf discs (control and stressed treatment) were sampled every 30 minutes over a period of 150 minutes.

Mannitol was used as an osmoticum for drought simulation. A drought acclimation treatment was given using a 0.5 M mannitol (-1.24MPa) solution for a period of 3 hours, followed by a severe osmotic shock in a 1.0 M mannitol solution. The leaf discs of the control treatment were initially kept in a 0.5 M sodium phosphate buffer for 3 hours after

which it also received a severe shock of 1.0 M mannitol. As with the heat treatment, leaf discs were sampled every 30 minutes over a period of 150 minutes.

After receiving the heat or drought treatments the leaf discs were vacuum infiltrated with a TTC solution (8g/l) and left to stand in total darkness for 18 hours. The glass bottles containing the leaf discs were then rinsed with distilled water, 3ml alcohol was added and the samples were heated to rupture the cells. The formazan was resuspended in alcohol and 100µl of the suspension were pipetted into micro plates. The absorbancy values were determined spectrophotometrically on a titertec multiscan micro plate reader at 485nm.

2.2.5 Protein synthesis

The leaf from the third apical node was sampled every 2nd day from drought stressed and non-stressed control plants. Leaf samples were quick frozen after harvesting, and samples were vacuum dried. The procedure continued until the plants were severely stressed. Four replicates were analysed for each sample.

A standard protein curve was first established, using albumin bovine serum (ABS) in fractions of 5µg, up to 85 µg in a dilution series with water and 200 µl Biorad protein assay (colouring). Thereafter 10µl of a monster was added to 790µl H_2O and 200µl Biorad protein assay. The samples were left at room temperature of approximately 22°C for 30 minutes, where after the absorption was measured at 595nm using a spectrophotometer. The spectrophotometer was calibrated with a mixture of 800 µl H_2O and 200µl Biorad protein assay. The absorption of the samples was measured as a fraction in µg/ml against the standard protein curve.

2.2.6 Enzymes of the Antioxidative System

2.2.6.1 Preparation of Enzyme Extract

For the preparation of the enzyme extract the extraction method of Malan *et al.* (1990) was used with minor modifications. 40 mg leaf tissue was homogenized with liquid nitrogen and resuspended in 1 ml of a 50 mM potassium phosphate extraction buffer

containing 0.1 mM EDTA and 2% polyvinylpyrrolidone. The pH of the extraction buffer was adjusted to 7. The homogenized leaf tissue and extraction buffer was centrifuged at 13 000 r.p.m. for 20 minutes at 4°C. The supernatant was transferred to eppendorfs which were embedded in ice and this enzyme extract was used for all the subsequent experiments.

2.2.6.2 Superoxide Dismutase (SOD)

A reaction solution is prepared containing potassium phosphate, xantine reagents and hydroxyl ammonium chloride. The enzyme extract is added to this mixture after which the mixture is kept at 25°C for 20 min. After this period a mixture of sulfanilic acid and X-naphtylamine is added to the reaction solution. After another waiting period of 20 min., the SOD activity is determined spectrophotometrically at a wavelength of 530nm.

2.2.6.3 Glutathione Reductase (GR)

The enzyme extract that was isolated in the procedure described in point 2.2.6.1 was added to a mixture of glutathione oxidiert (GSSG) (0.25 mM), Tris (50 mM), and EDTA (0.5 mM). NADPH was added to the solution and the level of the GR activity was measured spectrophotometrically by following the oxidation of NADPH at340nm over a period of 1 min. Enzyme activities were expressed as changes in absorbance min^{-1} g^{-1} dry weight.

2.2.6.4 Ascorbate Peroxidase (AP)

A mixture of a 50 mM phosphate buffer and 0.25mM ascorbic acid was prepared to which the enzyme extract was added. A diluted H_2O_2 mixture with an absorbance of 0.32 at 240nm was added to the solution, after which the rate of the reduction in absorbance was measured at 265 nm over a period of one minute. (Dalton *et al.*, 1986)

2.2.7 Relative Water Content

The leaf samples were taken early in the morning. Using a no. 6 cork borer, leaf disks were cut and weighed immediately after harvest (within 30 minutes) to obtain the fresh weight (W). Five disks were used for each replicate. The samples were re-hydrated by putting the disks into 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 desicator, and weighed again to obtain the dry weight (DW). The RWC was measured by calculating the following parameter:

$$RWC=[(W-DW)/(TW-DW)] \times 100$$

2.2.8 Leaf Water Potential

The pre-dawn leaf water potential (LWP) was established by measuring the pressure needed to force xylem sap out of the leaf through the peduncle. This was done by removing the leaf from the plant and placing the leaf inside the pressure chamber with only the peduncle protruding. The pressure was then increased at a rate of 0.03 $Mpa.sec^{-1}$ until the xylem sap is forced out of the leaf. A pressure chamber, PMS-instrument from Oregon, USA was used for the determination of the measurements.

2.2.9 Cell membrane stability

Each sample consisted of 5 leaf disks that were cut with a number 6 corkborer, rinsed well with distilled water, and placed in marked test tubes. Ten ml deionised water was added to each tube, and the tubes were covered with Parafilm^{T}. Four replicates were used per species.

The treatments were as follow:

(i) Heat treatment: Samples were taken from the control plants, leaf disks were incubated at 40°C for 3 hours in a heated water bath, where after it was left for another hour at an

increased temperature of 50°C. Test tubes were brought to room temperature, and the solution conductance was measured using a conductivity meter (T1= conductivity before autoclaving), where after the samples were autoclaved for 15 minutes, and conductivity was measured again (T2 = conductivity after autoclaving).

(ii) Drought treatment: Samples were taken directly from the stressed plants, 10 ml deionised water added, and left for 24 hours. Conductivity was measured (T1), samples autoclaved for 15 minutes, and measured again (T2).

Control tubes were left at room temperature of approximately 20°C for 24 hours. Conductivity was measured (C1). After the measurements were taken, the test tubes were autoclaved, cooled to room temperature, and the conductivity was measured again (C2). The CMS was calculated as the reciprocal of the cell membrane injury after Blum & Ebercon (1981):

$$CMS\ (\%) = [1-(T1/T2)/1-(C1/C2)] \times 100$$

2.2.10 Leaf area

Leaf area was measured for both stress and control leaves. One sample was taken from each plant, using four replicate plants for each treatment. The samples were put into a plastic bag directly after sampling to prevent desiccation or shrinking of the leaves. Leaf area was measured using a leaf area meter (LI-3100™). 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.

2.2.11 Tissue culture techniques

The establishment of *in vitro* amaranth plantlets were investigated.

2.2.11.1 Sterilisation of explants

Seeds were surface sterilised for 15 minutes using 1% NaOCl, and rinsed three times with sterile distilled water. Seeds were germinated on a modified Murashige & Skoog (1967)

(MS) -medium in Petri™ dishes or test tubes in a growth room, and cultured at 21°C at a photoperiod of 12 hours at a photosynthetic photon flux (PPF) of 40 µmol m^{-2} s^{-1} GEC Alsthom cool white light. Subculturing was done using single node segments for *A. hybridus* and *A. hypochondriacus*, and whole plantlets or large segments of plantlets for *A. tricolor*, placing it into 500 ml clear poly-ethylene tubs, containing 100 ml modified MS-medium each. Fifteen to twenty explants were inoculated into each tub.

2.2.11.2 Medium

Media to improve multiplication rate:

MS-medium containing various growth regulator combinations were tested to improve the multiplication rate, according to recommendations on amaranth and related crops in literature: MS-medium containing (in mg/l^{-1}) (i) 0.5 BA; (ii) 1.0 BA; (iii) 1.0 BA + 0.1 NAA; (iv) 1.0 Kinetin. MS-Medium was also tested where the nitrogen (NH_4) concentration was doubled (AMS-medium), since yellowing of the leaves were observed.

Media to initiate somatic embryos:

Aseptic *in vitro* leaves of *A. hybridus* and *A. hypochondriacus* were used as explant material. Whole leaves were used, and fine transversal cuts were made through the leaf lamina after which explants were inoculated onto different mediums for the production of somatic embryos:

(i) AMS-medium containing the growth regulators

BAP (0;0.5; 1.0; 2.0 mg/l) & 2,4-D (0; 0.1; 2.0 mg/l)

(ii) AMS-medium containing the growth regulators

IAA (0; 1.0; 5.0; 10.0; 20.0 mg/l) & GA (0; 1.0; 5.0; 10.0 mg/l)

Pro-embryogenic tissue formed in this manner was subcultured after 4 weeks onto one of the following fresh media:

(i) Back onto fresh original medium used to form pro-embryos

(ii) AMS-medium & 0.5 mg/l BAP

(iii) AMS-medium & 0.5 mg/l BAP & 0.1 mg/l GA

(iv) AMS-medium & 1.0 mg/l BAP & 0.5 mg/l GA

2.2.11.3 In vitro selection pressures

The screening of in vitro plant material for drought tolerance was investigated by the addition of polyethylene glycol (PEG) to the growth media. Selection pressure was applied on *in vitro* plantlets of *A. hybridus* and *A. hypochondriacus* that have been established as described in point 2.2.11.1. Internode segments were used as propagation material. When the *in vitro* plantlets reached an age of 4 weeks, the roots were cut off, and the plantlet was inoculated onto AMS-medium containing 4% PEG 6000. Fifteen plantlets were inoculated onto 10 ml medium in 500 ml clear poly-ethylene tubs, with similar growth conditions as mentioned above. Sample material was taken after 3 weeks growth on PEG-stress induced medium.

Proline analysis of *in vitro* plantlets

Extraction of proline was performed on leaves from these plants as described in the procedure in point 2.2.3.

2.3.5-Triphenyltetrazolium Chloride Reduction

The TTC reduction assay as described in point 2.2.4 was used with leaves collected from the *in vitro* plants.

2.2.12 Woodenbox screening at the seedlings stage

The following procedure was used for the screening of seedlings for early drought tolerance. Seeds of various lines were sown in the wooden boxes, and thinned out to twelve seedlings of each species per treatment. Seedlings were grown in a sandy soil mixture of peat/vermiculite/sand (5:3:3) in wooden boxes (± 80mm x 150 mm x 20mm), and 3 replicate boxes were used. Plants were allowed to germinate and grow for 2 weeks, where after water was withheld. Observations were made of the wilting and dying of the plants until 75% of the plants had reached the permanent wilting point. Plants were then rewatered and the survival and recovery of the plants were also noted. With the bambara groundnut plants a similar experiment was conducted in pots in the greenhouse, the only difference being that the plants were planted in separate pots and not in one box.

2.2.13 Root architecture determination in 2-D rooting boxes

It is not always possible to form a good impression of the three-dimensional development of the plant's root system. If we however allow the plant to develop a two-dimensional root system we can get an impression of how the roots are distributed. The root architecture screening boxes consisted of two flat sides (800mm X 600mm) separated by a piece of wood, 50mm thick. The one side of the box was firmly attached with long nails while the other side was attached in such a way that it could be removed. The box was lined with a thick plastic sheet and filled with a sandy soil mixture. Three healthy seeds were planted in each box and the plants were thinned to one healthy plant shortly after germination. The plants were watered daily. Once the plants have developed a good rooting system, the box was opened, the sandy soil was washed away and the root system was examined. A nailboard was used to keep the roots in their original position while the sand was being washed away.

Seeds from all the selected lines were planted in root architecture boxes and were allowed to grow undisturbed for four weeks. The boxes were opened, the sand carefully removed, and the root architecture was examined. The total length of the roots was measured. For this purpose the woodenbox were divided into three equal parts namely a top, middle and bottom part. All the roots in these three sections were collected and the total root length of the roots in each section was determined with a Geotron root length meter (Model: WLM1).

2.2.14 Yield determination

Assessment of the yield of the plants used in all the experiments is very important. It is not worthwhile to have a plant that can survive adverse conditions but which produces no yield. The ideal plant for subsistence farmers will be a plant which produce a moderate to good yield under optimal conditions but which will produce approximately the same yield under adverse conditions. The yield should also be determined in terms of the type of plant and the purpose for which it is cultivated. Some cowpeas for instance produce all the seeds at the same time which is well suited for commercial production while other

lines produces seeds over a longer period which is better suited for sustainable harvesting. Some farmers also prefer to cultivate their crops to harvest the leaves for human or animal consumption while other prefers to harvest only the seeds. It is therefore necessary to select the appropriate cultivar for the purpose.

Only the yield of the cowpea and bambara groundnut seeds were measured for the purpose of these experiments. The cowpea seeds were allowed to dry out before they were harvested. The seeds were then shelled and the dry weight was determined. The bambara groundnut seeds were harvested when the whole plant had dried out. Because the bambara seeds are formed under the soil, some of the first seeds formed, started to germinate before the mother plant had died down. The number of these seeds was also recorded.

The *Amaranthus* plants selected for these trials were the leafy amaranthus, which were selected for their leaf production. The yield of the seeds was therefore not estimated at the end of each growth season. Only one experiment was conducted were the seed yield of five amaranth species or selections were determined. These were Community 4, *A. tricolor*, Callalloo, *A. hypochondriacus* and Krugersdorp selection.

2.3 Germplasm Collection

2.3.1 Germplasm collected by SRL unit of ARC-Roodeplaat

Cowpea, bambara groundnut and *Amaranthus* seeds were collected from different areas in Gauteng, the Northern Province, Mpumalanga and KwaZulu-Natal. Special focus areas included Polokwane, Bushbuckridge and Ladysmith in KwaZulu-Natal. Some *Amaranthus* seeds were also collected in Thohoyandou (Venda). Seeds were bought in markets of fairs and also form local farmers. During some seasons unfavourable weather prevented the collection of seeds.

2.3.2 Germplasm collected by University of Zululand

Staff members from the University of Zululand collected cowpea, bambara groundnut and *Amaranthus* seeds from various street markets around the University. Seeds were acquired from markets at Empangeni, Mahlabatini, Pietermaritzburg, Richardsbay, Durban and even as far as Komatipoort. Some of the seeds acquired in the shops came from other countries like Mozambique, Nigeria, Zimbabwe and Ghana. Some seeds even came from Portugal. Seeds were also collected from local farmers in the communities but this action was sometimes not possible due to excessive rain.

2.3.3 Germplasm from gene banks at Roodeplaat

Cowpea and bambara seeds from the genebanks of the ARC were also used in some of the trials. This was especially true for the bambara groundnut seeds (Table 2.3.1), where the seed from the communities was such a mixture that it could not be regarded as lines or selections. The seeds from the communities first had to be propagated before they could be used in the selection experiments.

Table 2.3.1 Bambara groundnut seeds from the genebank at Roodeplaat.

Name	Description
SB1-1	Light brown with purple specks
SB7-1	Wine red
SB8-1 (4)*	Brown with purple spots similar to SB1-1
SB9-1 (16)	Light brown to beige with slightly darker hilum
SB20-1	Beige
SB20-2A (9)	Beige with small brown ring around hilum
AS 18 (11)	Cream
AS 17 (13)	Beige with black or red brown spots on sides of hilum
MAD-1	Beige seeds with purple specks
SB2-1C (15)	Beige with black ring around hilum
Swazi V5B (6)	Cream to light brown

* Number in ( ) are SRL allocated numbers

Amaranthus seeds of three species were included in the initial study because of their inclusion in current trials in resource poor communities in Mpumalanga, KwaZulu-Natal, Gauteng and Northern Province. The three species were *A. tricolor, A. hypochondriacus* and *A. hybridus.*

2.4 On-farm Trials

2.4.1 SRL unit of ARC-Roodeplaat

Cowpea

The aim of the cowpea trials that were planted at Roodeplaat was to compare the yield of 13 lines at this locality. The plants were therefore cultivated under optimal conditions and the yield was determined. Some seeds were also planted for seed propagation in order to have enough seeds for distribution in the communities and for further experiments.

Bambara groundnuts

Bambara seeds of the line SB1-1 were planted at Roodeplaat for seed production in order to have enough seeds for the other experiments and also to supply seeds to the communities. The yield of the bambara groundnuts was higher when planted in the field than when planted in pots. The bambara seeds were inoculated with *Rhizobium* spp. prior to planting, therefore no nitrogen fertiliser was required. The trial area was fertilised with 50kg potassium chloride and 25kg super phosphate. The trial was planted in December 1999, at the ARC-Roodeplaat. Only one line, SB1-1, was planted with a plant spacing of 0.5 x 0.1m (200 000plants/ha). Pods were harvested during April 2000. Weeds, such as nut grass, were controlled chemically with Round-up® (3ml/20l water) prior to planting. After planting, weeds were controlled by hand. Pest and disease control was done on a regular basis. The trial was established with initial irrigation of 20mm per week. Supplementary irrigation was terminated in December 2000, after which the rain was sufficient.

2.4.2 University of Zululand

Cowpea

At the University of Zululand five cowpea lines was planted to compare the growth and yield of these plants with that obtained in the communities. The lines that were planted included the black-eyed lines from Ghana, Zimbabwe and Nigeria and also two brown lines from Nigeria. These lined were selected on the grounds of seed availability.

Trials for the drought screening of plants at the seedling stage were also conducted at the University. The lines that were selected for these experiments included plant material obtained from the researchers of Roodeplaat as well as plant material that was collected in KwaZulu-Natal. The seed colour varied from brown (Mozambique, Nigeria), grey and Reddish brown (Empangeni) mixed colour (Mahlabathini, Okhalweni and Nibela) to white (Portugal, Ghana and Nigeria, Zimbabwe). Twenty-four cowpea lines were tested for drought tolerance using six wooden boxes, which accommodates twelve rows in each box. Each line was replicated three times. The boxes were kept on benches in a rain protected shelter and filled with a 1: 1mixture of sand and topsoil.

The boxes were then filled up to 12cm depth leaving about 3cm space for watering. A ruler was used to make equidistant holes in straight rows 10cm apart with a hill to hill distance of 5cm within the rows. The seeds were planted and were then irrigated to ensure even germination. After germination and full expansion of the unifoilate leaves i.e. 10 to 12 days after planting watering was stopped.

A daily count of permanently wilted plants in each variety was made until all the plants of the susceptible cowpea lines were dead. Watering was then resumed to ascertain recovery for each variety. Based on the days taken to wilting and percent recovery the varieties were then rated as drought tolerant or drought susceptible.

Taste evaluation of cowpea

The palatability of the cowpea (Nigeria brown and Zimbabwe) seeds was evaluated by a taste panel consisting of local people who are familiar with the taste of the indigenous

Zulu cowpeas and also dry beans. The cowpeas were prepared by cooking with maize. The taste panel completed a questionnaire, comparing the taste of the cowpeas from Nigeria and Zimbabwe with locally planted varieties and with sugar beans.

Rural households in Zululand cook cowpea seeds with dry maize during the off-season when green mealies are not available to supplement the family food. Some of the dishes include; '*isitambu*' (cowpea cooked together with maize) '*umbajiya*' (cowpea cake) and also used in stew. Apart from the above mentioned preparation methods, cowpea seeds are also boiled and eaten alone, boiled with rice or with potato (Sweet or Irish) and can also be used in salad.

Bambara

The two lines of bambara groundnut and maize were planted in November 1998 at the experimental farm of the Department of Agriculture, University of Zululand. The bambara groundnut lines were planted with a spacing of 15 cm in plots of 3m X 3m, with 3 replicates. The bambaras were either planted as sole crop or intercropped with maize, following a complete randomised block design.

Twelve Bambara groundnut lines were grown at the orchard farm unit of the Department of Zululand. The aim was to multiply the seeds for further evaluation. Seeds were sown at a spacing of 45cm between the rows and 20cm within the rows. Before planting, 2:3:2 compound fertiliser was applied at the final stage of land preparation. The experimental design used was a complete randomised block design. Each treatment was replicated three times.

The greenhouse evaluation of bambara groundnut in the woodenbox was done as described for cowpea. The aim of the screening was to compare the result of the experiment obtained at the University of Zululand with the results obtained from the woodenboxes at Roodeplaat. Ten lines of bambara groundnut were used for the evaluation.

Amaranthus

The aim of the on-farm trials conducted with *Amaranthus,* was to compare the effect of different fertilisers on the growth form of the different species and cultivars and to determine the palatability of these plants.

The on-farm trials that were conducted at the University of Zululand were done with the specie *A. hypochondriacus.* The seeds were first germinated in seedling trays and after 6 weeks the plants were transplanted in the field on the experimental farm. Two experiments were conducted. The first was to compare the effect of use of chicken – and kraal manure on the growth of the *Amaranthus* plants and the second experiment investigated the effect of chemical fertilisers (NPK and superphosphate) on *Amaranthus* plants. The height of the plant as well the number of leaves was noted.

Taste evaluation of *Amaranthus*:

Culinary experiments were also conducted for *Amaranthus* species. The species and selections that were included in the tasting experiments were Calaloo, *A. tricolor* and *A. hypochondriacus.* Three basic and three modified recipes was prepared and a panel of 28 people was used to evaluate the different dishes. The basic recipe consisted of amaranth leaves cooked in salt water while in the modified recipes ingredients like cheese, onion, peanuts and beans were added.

2.5 Community trials

2.5.1 SRL unit of ARC-Roodeplaat

Demonstration trials were established in Soshanguve and Bronkhorstspruit. The aim of the demonstrations was to familiarise the community members with the *Amaranthus*, bambara groundnut and cowpea plants and to determine whether they would be willing to assist the ARC in obtaining information regarding production of these crops in their respective areas. Specific focus areas were yield and acceptability of crops to communities. Two farmers (one in Soshanguve and one in Bronkhorstspruit) were identified through community participation and demonstration plots were established on their farms. In Soshanguve two lines each of *Amaranthus*, bambara groundnut and cowpeas were planted and evaluated for acceptability and potential income generation. The farmer sold all the marketable products of these crops to community members to generate some additional income. He expressed his sincere interest in co-operating with the ARC. In Bronkhorstspruit four cowpea cultivars were planted, namely Bechuana white, Encore, Glenda and Renoster. The same evaluations were done on this farm. The farmer also indicated his interest to co-operate with the ARC in this regard. Processing techniques of these crops (sun drying) were demonstrated during farmers days held in the communities.

In an additional experiment six farmers were identified to take part in a similar trial. These farmers had to give feedback on any problems experienced with the cultivation of the three crops, how well the crops were accepted by the communities and also how the proposed crops fitted in with the other crops that they were already cultivating.

In the last phase of this project the provision of seeds and information dissemination have been extended to 20 other farmers. Some of them planted all three crops while others decided to plant only one or two of the species. Information on the cultivation and marketing of the crop was obtained from the selected farmers.

2.5.2 University of Zululand

Cowpea seeds were distributed to farmers in spring to be planted in summer. Along with the seeds the farmers received information on planting methods and the cultivation of the crop. Seeds of cowpea were distributed to the farmers for planting in September, but because maize is their major crop, the farmers agreed to sow the seeds in November or December 1999. Unfortunately there was an organisational problem at the University, which disrupted the communication channels and hence the seeds were not sown.

In a following experiment five households agreed to co-operate with the researchers from the University of Zululand. These farmers were provided with seeds of the three species. The first lot of *Amaranthus* seeds did not germinate and it had to be replanted. The following criteria were measured during the course of the experiment: Time from planting till flowering and harvesting, yield, acceptability, palatability and also the weight of the leaves and stems.

Amaranth seeds were also distributed to the farmers at the community cooperative farm in Empangeni in August 1999. The uses of the crop were explained and brief training given on the cultivation practices. Information on the time of planting, spacing, thinning, fertilizer application (rate, time and frequency), choice of harvesting techniques and frequency of cutting was provided. Practical demonstration on the method of sowing was carried out on the field. Some of the farmers did sow the seeds in spring.

In a further experiment seeds of five cultivars of amaranth, *A. cruentus, A. hybridus, A. hypochondriacus, Amaranthus* sp. "McDonalds" *and A. tricolour* were sown by the personnel of the University in the community garden at Empangeni in September, 1999. The layout used followed the complete randomised block design, replicated four times. Seeds were drilled at 30 cm apart in 2 m x 1.2 m beds and 30 cm between beds. Compound fertiliser 2:3:2 was applied at the rate of 250 kg / ha at planting and LAN applied at the rate of 200 kg / ha at 2 weeks after seeding. Plants were thinned to 30cm apart. Four plants were tagged in each plot and growth parameters such as plant height and leaf number were determined fortnightly from 6 weeks after planting. Visual

observations were made on the plants, weight of crops removed at thinning was estimated and seed weight determined after harvesting. Analysis of variance was conducted on the data obtained using SPSS student version with Tukey – HSD.

The cooperative community garden, where some of the trials were sown, is located very close to the river. The advantage of this location is that the high water table can support the cultivation of crops during the dry winter season. In summer though there is problem of flooding, especially in the case of heavy continuous rainfall. During December and January 2000, heavy rainfall caused the delay of seed production in favour of vegetative growth. Seed formation was also disrupted by fungal infections.

Chapter 3

Results and Discussion

With all three the crops that were investigated a wide variety of physiological and phenotypical screening methods were initially used to determine the levels of drought tolerance. As the experiment progressed only the screening methods that showed the best correlation were used.

3.1 Cowpea (*Vigna unguiculata*)

3.1.1 Screening methods

3.1.1.1 Chlorophyll fluorescence

The chlorophyll fluorescence method is extremely sensitive. Most of the criteria that were evaluated showed marked differences between the drought tolerant and less tolerant cowpea lines. These differences were evident when the plants were evaluated over the entire stress period as well as when only the values of the stressed and control plants at the height of the stress was compared.

The cowpea lines were ranked at the end of each growth season (Table 3.1.1) and in the 5th season the lines with the highest levels of drought tolerance from the previous years were combined. The drought tolerant line IT96D-602 was included in each experiment as a positive control. Some of the selections, especially Phelandaba and Okhaluleni, out performed this control drought tolerant line, indicating the successful identification of increased drought tolerance in the germplasm screened in this study.

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Table 3.1.1 Ranking of cowpea lines for drought resistance over five growth seasons according to chlorophyll fluorescence measurements. (lowest number = most tolerant plant; highest number = less tolerant plant)

Name	Ranking
First and second season	
IT96D-602	1
IT92K258-9	3
IT90K59	3
Chappy	2
TVu7778	5
Third season	
IT96D-602	6
Okhalweni area	3
Ghana black eyed bean	8
Encore	5
Nigeria brown drum bean	9
Zimbabwe black eyed bean	7
Hluhluwa area	4
Mpenbeni ward	1
Okhaluleni area	2
Fourth season	
IT96D-602	5
Manguzi 1 BS	2
Manguzi 2 Red	5
Phelandaba	7
IT97K209-4	3
IT97K-338-7	4
IT97U-819-118	7
IT93K-452-1	8
IT97K-608-14	1
Fifth season	
IT96D-602	8
Phelandaba	1
Okhaluleni	2
IT97K-608-14	7
IT97K-499-38	4
IT93K129-4	5
M 217	6
00-11-161	3
00-11-145	9

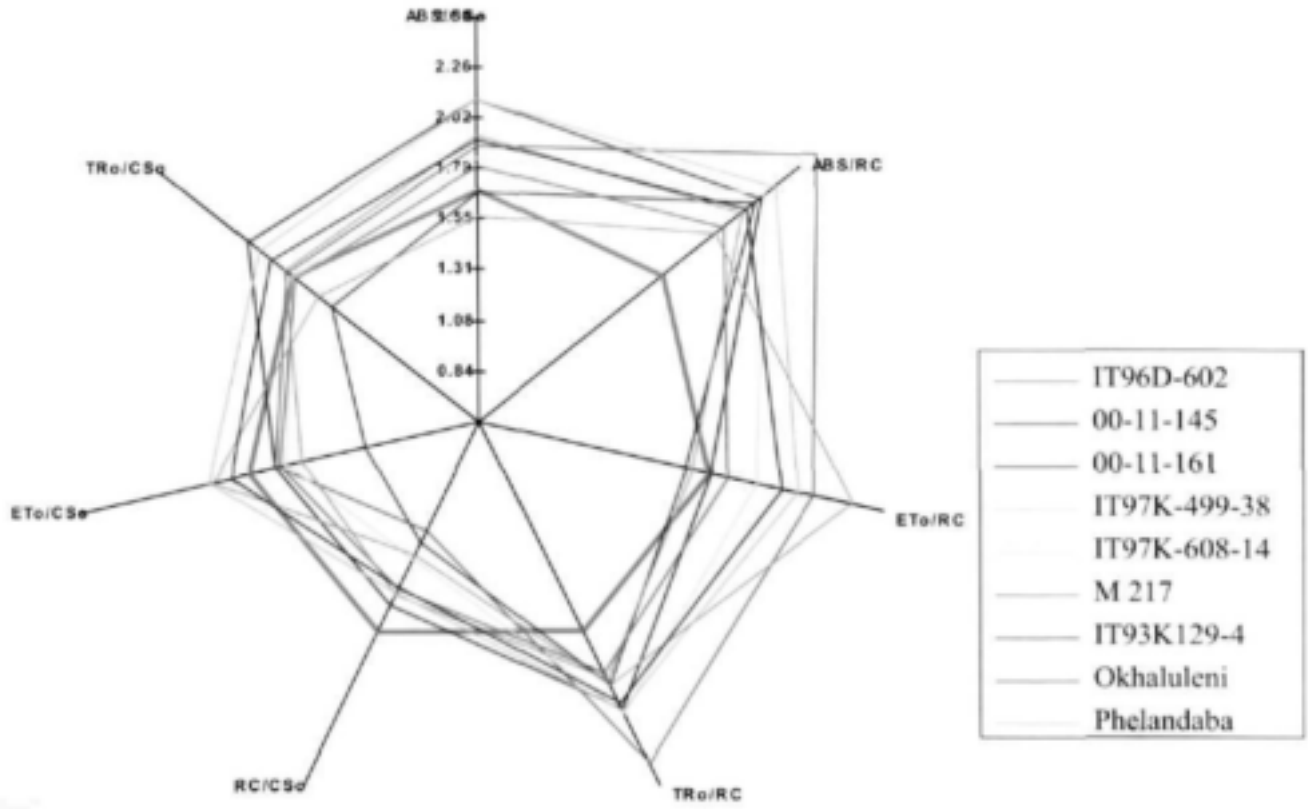

Figure 2 PSII function of 8 cowpea lines under drought stress conditions (5th season). The normalized data of the control lines were used as the reference line (regular red octagon).

When the specific and phenomenological fluxes were compared it was found that the lines compare very favourably indicating that the lines are all drought tolerant lines (Figure 2). The line IT93K129-4 had the highest value for the number of reaction centres per cross section (RC/CS_o) of all the stressed cowpea lines but this value was still lower than the value of the unstressed plants (Figure 2). The lines that had the lowest number of activated RCs were 00-11-161 and 00-11-145. In the cowpea lines where a lower number of RCs were activated the remaining RCs have to absorb, trap and transport much more energy just to have the same output as the RCs of the line IT93K129-4. This is evident in the high value registered for the absorption per reaction centre (ABS/RC) for 00-11-161 (Figure 2).

When the rate of absorption of photons by the antenna in the cross section of the tested sample (ABS/CS_o) were examined it was found that the line Phelandaba had the highest absorption rate and IT97K-608-14 the lowest in comparison to the other lines (Figure 2). Despite the low absorption and trapping rate the line IT97K-608-14 was very efficient to move the electrons into the electron transport chain (ET_o/CS_o).

The line Phelandaba performed in this study as the most tolerant, with highest or second highest absorption (ABS), trapping (TR) and transport (ET) of electrons per CS of all the lines. The line 00-11-145 performed as the least tolerant line with the lowest values compared to all the lines for these three parameters.

The specific energy fluxes of each individual RC of all of the lines were higher than that of the control lines (Figure 2). The line with the lowest ABS/RC value was IT97K-608-14, while the highest value was registered for the line 00-11-161. This line also had the lowest number of active RC/CS and therefore the individual RCs must trap, absorb and transport more energy inorder to provide the plant with enough energy (Figure 2).

3.1.1.2 Changes in the free proline concentration

Contrary to what was found in some other crops, the cowpea plants in which the levels of free proline starts to rise in the latter part of the stress period and at lower concentrations, were found to be more drought tolerant. The proline concentration gives an indication of the level of stress the plant is experiencing and it is not used as a protective measure against drought stress. The cowpea lines were ranked for each season according to the proline concentration but also the time when the proline levels started to rise (Table 3.1.2).

As was the case with the chlorophyll fluorescence measurements, the proline levels of the most drought tolerant lines were tested in the 5th season. The extent of these lines drought tolerance becomes evident when the highest concentration measured during this season is examined. During previous seasons levels of close to 90 µmole proline / g dry weight was

measured while the highest level measured in the last season was only 32.8 µmole proline/g dry weight.

Although cowpeas do not implement proline as a protective measure against drought stress, measuring the proline concentration can still be used as a screening tool for drought tolerance. This is even more valid if used in conjunction with other screening methods. The selections Phelandaba and Okhaluleni performed similar as the drought tolerant control IT96D-602.

Table 3.1.2 Ranking of cowpea lines for drought resistance over five growth seasons according to free proline measurements.

Name	Total stress – total control values	Statistical significance	Ranking
First and second season			
IT96D-602	2.62 ± 1.23	a	1
IT92K258-9	7.56 ± 4.75	b	2
IT90K59	10.10 ± 6.54	b	2
Chappy	6.37 ± 4.98	b	2
TVu7778	9.84 ± 6.51	b	2
Third season			
IT96D-602	14.30 ± 11.83	ab	2
Okhalweni area	71.26 ± 55.88	d	6
Ghana black eyed bean	8.24 ± 6.75	a	1
Encore	126.06 ± 90.25	d	5
Nigeria brown drum bean	118.11 ± 78.93	d	5
Zimbabwe black eyed bean	301.62 ± 213.72	e	6
Hluhluwa area	15.43 ± 6.69	b	3
Mpenbeni ward	29.38 ± 39.14	c	4
Okhaluleni area	9.44 ± 3.96	a	1

Name	Total stress – total control values	Statistical significance	Ranking
Fourth season			
IT96D-602	2.34 ± 0.87	bc	3
Manguzi 1 BS	1.00 ± 0.53	a	1
Manguzi 2 Red	0.75 ± 0.77	a	1
Phelandaba	1.79 ± 0.72	ab	2
IT97K209-4	2.77 ± 2.08	c	4
IT97K-338-7	4.72 ± 4.08	c	4
IT97U-819-118	12.77 ± 8.5	d	5
IT93K-452-1	2.86 ± 1.23	c	4
IT97K-608-14	0.15 ± 1.77	a	1
Fifth season			
IT96D-602	1.37 ± 1.51	a	1
Phelandaba	1.23 ± 0.55	a	1
Okhaluleni	1.46 ± 0.59	a	1
IT97K-608-14	2.44 ± 1.27	b	2
IT97K-499-38	1.12 ± 0.97	a	1
IT93K129-4	4.32 ± 5.53	cd	4
M 217	9.47 ± 6.44	d	5
00-11-161	8.80 ± 5.29	d	5
00-11-145	3.32 ± 2.23	bc	3

1 = most tolerant; 9 = least tolerant

3.1.1.3 2.3.5-Triphenyltetrazolium Chloride Reduction assay

The TTC reduction assay was executed during the first and second seasons. The advantage of this method is that healthy greenhouse plants can be used as starting material and the leaf discs are exposed to the stress treatment. This means that different stress (heat, drought, cold, salt, etc.) can be measured separately (Table 3.1.3). The ranking of the lines according to the TTC reduction assay during drought stress resulted in high variance within the treatments, making it difficult to prove statistically. It was however possible for the heat treatment to distinguish between the lines.

Table 3.1.3 Ranking of cowpea lines for drought resistance according to TTC measurements.

Name	Drought			Heat		
	Total stress – total control values	Statistical significance	Ranking	Total stress – total control values	Statistical significance	Ranking
IT96D-602	0.018 ± 0.041	ns *	1	-0.014 ± 0.045	b	3
IT92K258-9	0.012 ± 0.018	ns	1	0.004 ± 0.011	a	1
IT90K59	0.010 ± 0.014	ns	1	-0.003 ± 0.014	ab	2
Chappy	0.012 ± 0.015	ns	1	-0.009 ± 0.015	b	3
TVu7778	0.006 ± 0.017	ns	1	-0.005 ± 0.019	ab	2

*ns = not significant, 1 = most tolerant; 3 = least tolerant

During the second season an experiment was also conducted to see if a higher mannitol concentration during the pre-stress period would give a better seperation between the pre-stressed and control plants. During this experiment the concentration of the mannitol of the pre-stress treatment was increased to 0.7 mM mannitol. The heat treatment was repeated as described in the first experiment. When the pre-stress treatment was intensified the differences between the lines became less obvious than in the standard procedure. It is therefore recommended to use the standard TTC procedure and not the higher mannitol concentration.

Similar to the other physiological screening methods, the TTC viability assay should not be used alone but instead should be used in parallel with other viability assays. The findings can be supported by biochemical or physiological screening methods. The TTC reduction assay seems to be a good selection method to use for determining heat and drought resistance in cowpeas with the above-mentioned recommendation.

3.1.1.4 Protein Synthesis

Apart from the determination of the free proline concentration the total protein content was also determined. There was no statistical difference between the protein content of the stressed plants and the control plants of the different cowpea lines. Measuring the

total protein content of cowpea plants is not recommended as a screening method for drought tolerance.

3.1.1.5 Enzymes of the Antioxidative System

Although some enzyme activity was recorded in all the cowpea lines, none of these enzyme concentrations increased drastically over the stress period. This would have indicated that such mechanisms play an important role in the defence system of the plant to cope with drought stress. According to the data obtained in this investigation, it would seems that determining the level of the enzymes of the antioxidative system is not a good measure for drought resistance in cowpea (Table 3.1.4).

The enzymes that showed some sort of fluctuation were ascorbate peroxidase (AP) and superoxide dismutase (SOD). The AP levels seemed to rise towards the end of the stress period. It was possible to rank the plants according to their AP and SOD levels. It was assumed that plants with a lower AP and SOD concentration were experiencing less stress and is therefore more drought tolerant. This trend was also found in the free proline levels of stressed plants i.e. the lower the stress induced proline levels the higher the drought tolerance (3.1.1.2).

Tabel 3.1.4 Ranking of five cowpea lines according to the changes in the levels of the enzymes of the antioxidative system.

Name	**Ranking**		
		Statistical significance	**Ranking**
Superoxide Dismutase			
IT96D-602	-0.155 ± 0.406	a	1
IT92K258-9	-0.584 ± 1.811	b	2
IT90K59	-0.617 ± 0.549	b	2
Chappy	1.666 ± 2.293	ab	1
TVu7778	-2.646 ± 2.655	c	3

Name		Statistical significance	Ranking
Ascorbate Peroxidase			
IT96D-602	-0.0001 ± 0.0018	a	1
IT92K258-9	0.0021 ± 0.0050	b	3
IT90K59	0.0014 ± 0.0027	ab	2
Chappy	0.0007 ± 0.0025	ab	2
TVu7778	0.0007 ± 0.0018	ab	2
Glutathione reductase			
IT96D-602	33.13 ± 60.21	ns*	ns*
IT92K258-9	8.25 ± 74.11	ns*	ns*
IT90K59	15.65 ± 57.24	ns*	ns*
Chappy	3.90 ± 61.39	ns*	ns*
TVu7778	20.85 ± 85.35	ns*	ns*

* ns = not significant, 1 = most tolerant; 9 = least tolerant

3.1.1.6 Relative Water Content (RWC)

RWC measurements of stressed and control plants will always be an important criterion seeing that it estimates the current water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity. It is a measure of water deficit in the leaf. Measuring the RWC poses some problems in the number of plants that can be screened but this screening method does not have to be measured pre-dawn as is the case when measuring the LWP. It is however recommended that the measurements be taken at the same time of the day during the course of the experiment.

Table 3.1.5 Ranking of cowpea lines for drought resistance over five growth seasons according to relative water content measurements.

Name	First and second season	Statistical significance	Ranking
IT96D-602	-13.02 ± 6.21	a	1
IT92K258-9	-23.42 ± 2.44	b	2
IT90K59	-28.51 ± 4.73	c	3
Chappy	-35.71 ± 7.97	d	5
TVu7778	-30.51 ± 5.32	cd	4

Name	Third season	Statistical significance	Ranking
IT96D-602	-27.59 ± 14.74	ab	1
Okhalweni area	-48.09 ± 11.92	d	4
Ghana black eyed bean	-39.33 ± 19.23	acd	3
Encore	-31.03 ± 4.33	abc	2
Nigeria brown drum bean	-34.82 ± 4.54	abc	2
Zimbabwe black eyed bean	-48.46 ± 2.67	d	4
Hluhluwa area	-47.01 ± 7.01	d	4
Mpenbeni ward	-31.08 ± 7.10	abc	2
Okhaluleni area	-29.75 ± 16.37	ab	1
Fourth season			
IT96D-602	-16.62 ± 5.26	bc	2
Manguzi 1 BS	-11.46 ± 7.15	ab	1
Manguzi 2 Red	-13.23 ± 1.19	ab	1
Phelandaba	-11.53 ± 6.58	ab	1
IT97K209-4	-17.91 ± 4.52	bc	2
IT97K-338-7	-22.22 ± 8.49	c	3
IT97U-819-118	-16.73 ± 9.09	bc	2
IT93K-452-1	-10.22 ± 9.44	b	1
IT97K-608-14	-10.74 ± 5.32	a	1
Fifth season			
IT96D-602	-6.63 ± 3.66	a	1
Phelandaba	-15.87 ± 7.16	cd	4
Okhaluleni	-12.38 ± 4.41	bc	2
IT97K-608-14	-13.96 ± 6.32	bcd	3
IT97K-499-38	-11.42 ± 6.46	bc	2
IT93K129-4	-6.09 ± 8.07	ab	1
M 217	-15.10 ± 9.76	cd	4
00-11-161	-23.65 ± 9.95	d	5
00-11-145	-22.11 ± 11.19	cd	5

1 = most tolerant; 5 = least tolerant

The drought tolerant line IT96D-602 performed well according to the RWC measurements through the experiments. It was ranked within the top two lines in each of the experiments, which is a good indication of its tolerance. Other lines that did well over the seasons were IT93K129-4, Phelandaba, IT97K-608-14 and IT97K-499-38. The two

American lines (00-11-145 & 00-11-161) had the lowest RWC. The ability to keep the RWC high can be a big advantage, especially to a plant that relies on a good water balance for survival.

3.1.1.7 Leaf Water Potential (LWP)

The pressure chamber technique for measuring the LWP is based on the assumption that the tension in the xylem is equilibrated with the LWP of the cells in the leaf (Nilsen & Orcutt, 1996). Cowpeas seem to be able to maintain a higher LWP than other crops under drought stress conditions. This is a very valuable trait in terms of survival and recovery after stress conditions. This screening method correlated well with the other screening methods (Table 3.1.6) but it does have the disadvantage that the data has to be collected pre-dawn which can be a limiting factor in the number of plants that can be screened. This screening method was therefore not conducted in the subsequent growth seasons.

Table 3.1.6 Ranking of cowpea lines for drought resistance according to leaf water potential measurements taken at the height of the stress.

Name	Stress values – Control values	Statistical significance	Ranking
First growth season			
IT96D-602	8.68 ± 0.79	a	1
IT92K258-9	9.35 ± 0.70	a	1
IT90K59	12.20 ± 1.29	b	3
Chappy	12.03 ± 1.19	b	3
TVu7778	11.50 ± 2.28	ab	2
Second growth season			
IT96D-602	3.38 ± 1.85	a	1
IT92K258-9	9.95 ± 1.54	bc	3
IT90K59	11.80 ± 2.47	c	4
Chappy	11.25 ± 2.25	c	4
TVu7778	7.35 ± 2.63	ab	2
IT93K129-4	3.65 ± 1.79	a	1

3.1.1.8 Cell Membrane Stability (CMS)

The cell membranes of the drought-stressed cowpea plants were still in a good condition even after 17 day of drought stress. There were no statistical differences between the CMS of the different lines (Table 3.1.7) and this test is therefore not recommended as a screening method for drought resistance in cowpea plants. It does however give an indication of the resilience of the crop against adverse conditions.

Table 3.1.7 Ranking of cowpea lines for drought resistance according to cell membrane stability measurements

Name	Ranking	
	First and second season	
IT96D-602	95.39 ± 2.06	ns *
IT92K258-9	93.83 ± 2.12	ns
IT90K59	84.95 ± 17.05	ns
Chappy	94.77 ± 9.37	ns
TVu7778	93.01 ± 9.46	ns

* ns = not significant

3.1.1.9 Leaf area

The plants with the biggest leaves (IT92K258-9) underwent the largest reduction in the leaf area when the plants were subjected to drought stress. Chappy on the other hand showed little reduction in leaf size. IT96D-602 has the smallest leaves of all the selected cowpea lines. The leaf area of the stressed plants of IT96D-602 stayed constant over the stress period. Leaf area on its own proved not to be a reliable screening method for drought resistance, but the results obtained with this technique can make a contribution towards other screening tests if the drought resistance strategy of the crop is well understood. Smaller sized leaves were regarded as better adapted against drought stress as the transpiration surface is reduced. The plants were therefore ranked as shown in Table 3.1.8.

Table 3.1.8 Ranking of cowpea lines for drought resistance according to the leaf area. Smaller leaves are regarded as being more adapted for drought tolerant.

Name	Total stress - total control values	Statistical significance	Ranking
IT96D-602	-21.565 ± 10.62133	b	2
IT92K258-9	-44.8075 ± 17.65988	a	1
IT90K59	-4.8575 ± 22.57072	cd	4
Chappy	1.855 ± 15.89014	d	5
TVu7778	-18.04 ± 16.67856	bc	3

3.1.1.10 Tissue Culture techniques

The cowpea plants that were cultured *in vitro* initially grew well but with subsequent subculturing the condition of the explants deteriorated. This made the explants unfit for *in vitro* screening, seeing that the explants were already stressed. This method was therefore not further investigated.

3.1.1.11 Woodenbox Screening at the Seedling Stage

It is not only the ability of a plant to withstand adverse conditions which is important, but also the recovery of the plants once the stress condition is over. The seedlings that were planted in the woodenbox were therefore not only ranked at the end of the stress period but also 1 week after rewatering. The two cowpea lines that were still looking good at the end of the stress period and that also recovered well after rewatering were ITR96D-602 and IT96D-711 (Table 3.1.9). Chappy and TVu7778 did not look good at the end of the stress period and did not recover. Some lines like CH 14 have the ability to recover very good from the harshest stress. It was ranked 9th at the end of the stress period but after rewatering moved to the 3rd place. IT90K59 on the other hand went from the 4th to the 10th position on the ranking list after rewatering, as it failed to recover properly.

Table 3.1.9 Ranking of twelve cowpea lines that were drought stressed in woodenboxes. Results were taken after 10 days without water as well as one week after rewatering.

	10 days without water	One week after rewatering
IT96D-711	1	1
IT96D-602	2	2
CH14	9	3
IT92K258-9	5	4
TVU11986	6	5
IT93K596-9-12	8	6
IT93K734	3	7
IT82D849	11	8
IT93K129-4	7	9
IT90K59	4	10
Chappy	12	11
Tvu7778	10	12

The results prove that the woodenbox screening of seedlings is a valid screening technique for drought resistance. The type of drought resistance as described by Mai-Kodomi *et al.* (1999) also did not seem to make a difference to the survival of the plants. IT96D-602 exhibits the type 1 resistance (the whole plant wilts at the same time) while the other four tolerant lines are more prone to the type 2 behaviour (plants translocate all the available moisture from the lower parts of the plant to the growth tip and discard the leaves). According to Mai-Kodomi *et al.* (1999) the type 2 drought tolerant plants were more tolerant than the type 1 plants but this difference is most probably only evident when plants with similar levels of drought resistance are compared. The plants were ranked according to their appearance as it is listed in Table 3.1.10.

Table 3.1.10. Plants that were subjected to drought stress in the wooden boxes were evaluated according to their appearance.

Evaluation during stress period	Evaluation after rewatering	Value
Plants are still looking good	Unifoliate and trifoliate leaves recover	5
Whole plants starts to wilt	Only trifoliate leaves recover	4a
Only unifoliate leaves wilted		4b
50% Wilted	Stem recovered, leaves still wilted	3
75% Wilted	Stem bent but turgid	2
100% Wilted	Nearly dead	1
Dead	Dead	0

In the ranking of the plants in Table 3.1.11 it is interesting to note that the line Okhaluleni, which is normally quite drought tolerant, is ranked last. This goes to show that plants do not always have the same levels of drought tolerance. Some plants are more tolerant in the mature phase and others more tolerant in the juvenile phase. The ideal plant carries this characteristic throughout its lifetime like IT96D-602.

Table 3.1.11 Ranking of the recovery of seedlings, 7 days after rewatering, of different lines exposed to a severe drought stress by withholding water.

Cowpea lines	Woodenbox ranking	Ranking
Brown honey	3.14	1
Ghana	2.92	2
Zimbabwe	2.72	3
Nigeria white	2.40	4
Nigeria Drum	2.30	5
IT96D602	2.28	6
Encore	2.20	7
Hluhluwe	2.11	8
TVu7778	1.84	9
Oklalweni	1.75	10
Mpenbeni	1.14	11
Okhaluleni	0.58	12

3.1.1.12 Root Architecture Determination

The root architecture of the plants may provide one reason for the very good performance of IT96D-602 (Figure 3). This line covered the surface of the root architecture box with an excellent root distribution.

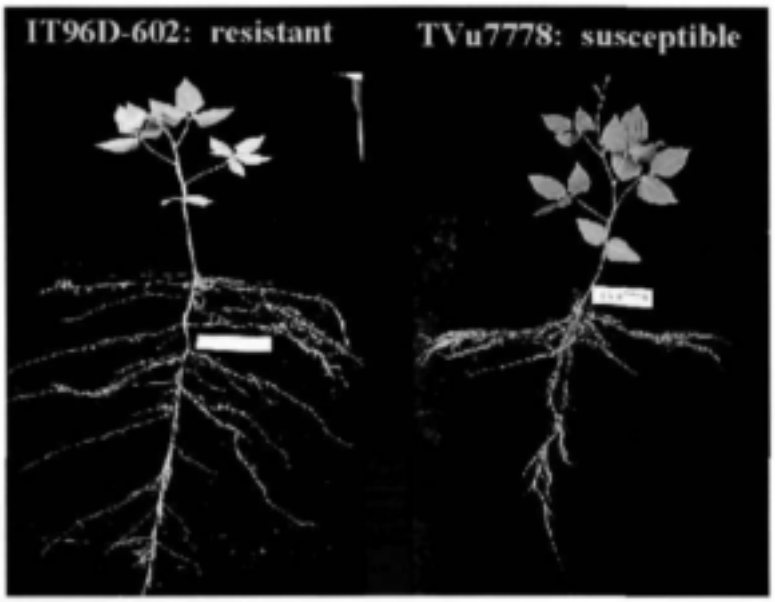

Figure 3 Root distribution of IT96D-602 compared to TVu7778

This characteristic may not make a huge difference in a pot or the woodenbox where the space is limited but in a field trial this would definitely give the plant a big advantage. The root systems of more drought susceptible plants like TVu7778 on the other hand produced much more roots close to the surface of the box and only one tap root that grew down to the bottom of the root architecture box..

3.1.1.13 Yield of greenhouse plants

Determining the yield of the stressed plants is very important because at the end of the day providing some sort of a yield is the most significant characteristic of a drought tolerant plant. It is of no use to have plants that can survive unfavourable conditions but without producing any yield. The fact that the most tolerant line that was used for this study (IT96D-602) also produced the highest yield indicates that this is a line that should

be cultivated in areas with low or variable rainfall to ensure the possibility of harvesting a crop. The lines TVu7778 and IT90K59 both produced high yields during favourable conditions, but showed a large reduction in yield under moisture stress. When selecting a plant line, it might sometimes be a safer option to select a line that gives a moderate yield under favourable conditions but will still produce a satisfactory crop under unfavourable conditions. The cowpea lines were ranked according to the yield produced in the greenhouse over the different growth seasons (Table 3.1.13).

Table 3.1.13 Ranking of cowpea lines over 5 growth seasons according to the total yield produced.

Name	No. of seeds		Seed weight (g)		Ranking according to total yield
	Control plants	*Stressed* plants	*Control* plants	*Stressed* plants	
First and second season					
IT96D-602	606	468	125.54	95.05	3
IT92K258-9	194	28	29.9	4.65	4
IT90K59	1098	337	142.76	44.85	1
Chappy	157	29	24.38	3.81	5
TVu7778	900	330	115.26	43.88	2
Third season					
IT96D-602	483	165	80.58	22.53	5
Okhalweni area	336	53	82.06	10.4	6
Ghana black eyed bean	485	203	107.27	38.61	4
Encore	974	348	121.5	44.08	2
Nigeria brown drum bean	130	29	19.93	3.56	9
Zimbabwe black eyed bean	530	310	107.15	60.83	3
Hluhluwa area	126	35	36.61	10.01	8
Mpenbeni ward	93	82	29.39	18.69	7
Okhaluleni area	1303	980	177.09	128.23	1

Name	No. of seeds		Seed weight (g)		Ranking according to total yield
	Control plants	Stressed plants	Control plants	Stressed plants	
Fourth season					
IT96D-602	n.r.	n.r.	931.44		1
Manguzi 1 BS	n.r.	n.r.	162.18		7
Manguzi 2 Red	n.r.	n.r.	216.05		4
Phelandaba	n.r.	n.r.	104.8		8
IT97K209-4	n.r.	n.r.	248.38		3
IT97K-338-7	n.r.	n.r.	171.31		6
IT97U-819-118	n.r.	n.r.	249.18		2
IT93K-452-1	n.r.	n.r.	87.4		9
IT97K-608-14	n.r.	n.r.	209.4		5
Fifth season					
IT96D-602	1001	1191	217.2	247.93	5
Phelandaba	305	450	90.72	145.63	9
Okhaluleni	1988	1836	378.37	340.63	1
IT97K-608-14	1507	1059	308.78	219.42	2
IT97K-499-38	970	1224	203.52	256.86	4
IT93K129-4	1227	593	238.07	101.49	6
M 217	1650	846	221.44	108.21	3
00-11-161	541	431	148.22	110.34	8
00-11-145	682	493	238.26	142.69	7

1 = most tolerant; 9 = most susceptible. n.r. = not recorded

3.2 Bambara groundnuts (*Vigna subterranea*)

3.2.1 Screening methods

3.2.1.1 Chlorophyll fluorescence

The measurement of the chlorophyll fluorescence is a very sensitive screening method seeing that it gives an indication of how well the photosynthetic processes (PSII function) in the plant are functioning. As a result of this sensitivity, it was possible to ranked the

bambara groundnut from most tolerant to least tolerant. Another advantage of this method is that it is not destructive and the same leaf can be measured over a stress period.

In the first seasons the line SB1-1 was ranked as number 2, when only the measurements for the individual reaction centres were evaluated. When the values for the measured cross section were also evaluated (fourth season) it became evident that the RC's of this line was working very hard but fewer RC's were activated. The low number of activated RC's might be the reason for the low ranking of SB1-1 in the fourth season, opposed to the ranking of Swazi V5B.

Table 3.2.1 Ranking of bambara groundnut plants according to chlorophyll fluorescence measurements over the test period.

Name	Ranking for each growth season
First season	
SB1-1	2
SB7-1	1
SB9-1	5
SB20-1	2
MAD-1	2
Second season	
SB1-1	2
SB7-1	5
SB9-1	3
SB20-1	3
MAD-1	1
Third season	
SB1-1	2
SB8-1 (4)	5
Swazi V5B (6)	5
SB20-2A (9)	3
AS 18 (11)	5
AS 17 (13)	4
SB2-1C (15)	8
SB9-1 (16)	1

Name	Ranking for each growth season
Fourth season	
SB1-1	8
Swazi V5B (6)	1
AS 17 (13)	7
SB9-1 (16)	6
KwaHgnase	3
NB	4
NLB	2
NR	5

1 = most tolerant; 8 = least tolerant

3.2.1.2 Changes in the Free Proline Concentration

The proline levels of most of the bambara groundnut lines changed very little over the stress period. It was only after 14 days without water that there was an increase in the proline production of SB1-1, SB7-1, SB20-1 and MAD-1 (Table 3.2.2). In the line SB9-1 the proline levels started to rise after 12 days without water and there was a further sharp increase after 14 days. SB7-1 produced the lowest levels of proline and SB9-1 produced the highest levels of proline in the first season.

Table 3.2.2 Ranking of bambara groundnut lines according to the levels of free proline (µmole proline/g dry weight) as it was registered over four growth seasons.

Name	Free Proline levels	Statistical significance	Ranking for each growth season
First season			
SB1-1	15.96 ± 8.58	bc	3
SB7-1	11.18 ± 2.84	a	1
SB9-1	87.02 ± 30.24	d	5
SB20-1	13.22 ± 3.31	ab	2
MAD-1	25.44 ± 12.25	c	4

Name	Free Proline levels	Statistical significance	Ranking for each growth season
Second season			
SB1-1	18.06 ± 7.39	b	2
SB7-1	31.09 ± 10.53	c	4
SB9-1	22.14 ± 10.26	bc	3
SB20-1	42.01 ± 17.48	d	5
MAD-1	14.54 ± 2.58	a	1
Third season			
SB1-1	14.50 ± 6.41	d	6
SB8-1 (4)	8.87 ± 4.94	bcd	4
Swazi V5B (6)	9.30 ± 1.04	cd	5
SB20-2A (9)	8.22 ± 0.79	bd	2
AS 18 (11)	14.54 ± 4.93	d	7
AS 17 (13)	25.03 ± 20.52	e	8
SB2-1C (15)	6.81 ± 0.18	a	1
SB9-1 (16)	8.38 ± 0.36	bd	3
Fourth season			
SB1-1	18.20 ± 13.04	d	7
Swazi V5B (6)	7.61 ± 0.24	c	3
AS 17 (13)	18.61 ± 14.45	d	8
SB9-1 (16)	6.75 ± 0.63	b	2
KwaHgnase	6.40 ± 0.31	a	1
NB	13.65 ± 6.67	d	4
NLB	15.31 ± 10.95	d	6
NR	14.54 ± 11.29	d	5

() SRL units code number. 1 = most tolerant; 8 = most susceptible

3.2.1.3 2.3.5-Triphenyltetrazolium Chloride Reduction assay

According to the hypothesis of the TTC reduction assay, leaf disks of tolerant plants, exposed to a stress treatment will have a higher formazan production than the control treatment over the 150 min experimental period. This will be because the leaf disks of tolerant plants will have the ability to adapt to an otherwise lethal stress when pre-treated with a moderate stress. This will result in higher formazan production in the stress

treatment compared to the control. Sensitive plants on the other hand experience the moderate stress pre-treatment as a severe stress and cannot adapt to the stress condition. These leaf disks suffered when the severe stress was applied which resulted in a lower formazan production. The vitality of the stressed plants was slightly lower than the values for the control plants. The plants could be ranked according to their vitality under drought stress with SB20-1 most tolerant and SB7-1 least tolerant. MAD-1 proved to be the most heat tolerant compared to SB1-1 the least heat tolerant (Table 3.2.3).

Table 3.2.3 Ranking of five bambara groundnut lines according to their vitality as determined by the TTC reduction assay

Name	Drought			Heat		
	Total stress – total control values	Statistical significance	Ranking	Total stress – total control values	Statistical significance	Ranking
SB1-1	-0.026 ± 0.038	bc	4	-0.033 ± 0.036	b	3
SB7-1	-0.034 ± 0.027	c	5	-0.032 ± 0.031	ab	2
SB9-1	-0.017 ± 0.023	abc	3	-0.029 ± 0.032	a	1
SB20-1	-0.006 ± 0.015	ab	1	- 0.008 ± 0.024	a	1
MAD-1	-0.010 ± 0.016	abc	2	-0.008 ± 0.023	a	1

3.2.1.4 Protein Synthesis

The protein content of bambara groundnut lines over the stress period gave very similar patterns as the cowpea lines. Like the cowpeas there was no statistical difference between the protein content of the stressed plants and the control plants. The individual lines also registered very similar protein values over the stress period and in all the lines except MAD-1 the protein content decreased towards the end of the stress period. The line MAD-1 registered lower protein values throughout the experiment but the values did not decrease towards the end of the stress period. Measuring the total protein content of bambara groundnut plants is also not recommended as a screening method for drought tolerance.

3.2.1.5 Enzymes of the Antioxidative System

The SOD activity decreases in the control and in the stressed plants over the time span of the experiment. The only exception to this rule is SB1-1 where there is a slight increase after which the value stays the same. With all the lines that were used for this experiment there was no difference between the treatments and small differences between the lines. This is therefore not a good screening method for the selection for drought tolerance.

The AP levels of the stressed plants of four of the bambara groundnut lines showed little or no difference to the control pants. In all four of these lines the AP activity of both the stress treatment and the control treatment stayed constant over the stress period. The only line that did show a difference is SB1-1 where the AP levels rose steadily over the two and a half-week period. This rise occurred in the stress treatment as well as in the control plants. At the end of the stress period (after 17 days without water), there was a decline in the AP activity of the stressed plants compared to the control plants where the AP activity was still rising. It is not possible to ascribe these higher levels of AP as an indication of drought tolerance without further experiments. The AP levels are thus far not a good screening test for drought tolerance.

3.2.1.6 Relative Water Content

The reduction in the RWC in the first and second seasons gave a very similar pattern in all five of the bambara groundnut lines. They all start with a value of close to 100% and ends up with a value of between 55 and 65 % at the end of the stress period. After rewatering the RWC of the plants all recovered to ±90% within four days. The values of some of the lines were very close to each other. This can affect the ranking of the lines between seasons. The RWC should not be used as a selection method on its own, but can be of great value if it is used in conjunction with other tests.

Table 3.2.6 Ranking of bambara groundnut lines according to the relative water content as it was registered over four growth seasons

Name	RWC at end of stress period	Statistical significance	Ranking for each growth season
First season			
SB1-1	39.52 ± 7.37	d	5
SB7-1	72.62 ± 15.98	a	1
SB9-1	43.95 ± 4.30	cd	4
SB20-1	60.35 ± 13.90	ab	2
MAD-1	47.60 ± 2.79	bc	3
Second season			
SB1-1	61.40 ± 3.60	a	1
SB7-1	60.19 ± 5.43	a	1
SB9-1	56.55 ± 7.22	ab	2
SB20-1	54.00 ± 1.59	b	3
MAD-1	58.56 ± 4.64	ab	2
Third season			
SB1-1	58.74 ± 18.32	c	4
SB8-1 (4)	92.02 ± 3.81	abc	2
Swazi V5B (6)	91.27 ± 0.80	bc	3
SB20-2A (9)	93.92 ± 0.98	a	1
AS 18 (11)	74.54 ± 17.54	c	4
AS 17 (13)	64.13 ± 18.55	c	4
SB2-1C (15)	90.60 ± 2.50	bc	3
SB9-1 (16)	92.09 ± 3.01	ab	2
Fourth season			
SB1-1	68.08 ± 20.44	cd	5
Swazi V5B (6)	85.68 ± 6.21	b	3
AS 17 (13)	65.36 ± 15.91	cd	5
SB9-1 (16)	57.37 ± 11.77	d	6
KwaHgnase	77.60 ± 20.21	bc	4
NB	92.07 ± 1.85	a	1
NLB	90.15 ± 4.21	ab	2
NR	63.52 ± 9.29	cd	5

1 = most tolerant; 6 = least tolerant

3.2.1.7 Leaf Water Potential

The pattern of the increase in the LWP is very similar in all the bambara groundnut lines. The LWP stay relatively low until the 11th day without water after which it rises sharply.

The LWP of MAD-1 is slightly higher than the rest but this difference is not significant. Ranking of the plants according to this screening method was not possible as a result of high standard errors. It is thus not a good method to use to distinguish between bambara groundnut lines (Table 3.2.7).

Table 3.2.7 Ranking of bambara groundnut lines according to the leaf water potential

Name	Total stress – total control	Statistical significance	Ranking
SB1-1	11.3 ± 7.19	ns *	1
SB7-1	12.9 ± 5.62	ns	1
SB9-1	13.15 ± 2.98	ns	1
SB20-1	11.92 ± 3.63	ns	1
MAD-1	13.22 ± 2.07	ns	1

* *ns = not significant*

3.2.1.8 Cell Membrane Stability

The CMS did not change very much for any of the five selected bambara groundnut lines. This was true for the leaves that were exposed to a drought stress as well as those that were exposed to a heat stress. The CMS is therefore not a good test to distinguish between tolerant and sensitive lines.

3.2.1.9 Leaf area

The reduction of the leaf area does not seem to be a method used by bambara groundnuts to survive unfavourable conditions. One explanation for this might be that the leaves were already formed before the stress took effect. The stress period of approximately 20 days is also too short for the production of new leaves. If a moderate stress was applied over a longer period it might be possible that a reduction in the leaf area will be observed.

3.2.1.10 Tissue Culture techniques

The leaves of plants that are grown *in vitro* was still a bit small and therefore the medium was changed to ½ strength MS medium with 2% sucrose. The bambaras seem to grow

well on this medium but further investigation is still needed. The *in vitro* plants were not subjected to any stress treatment.

3.2.1.11 Woodenbox Screening at the Seedling Stage

Seven bambara groundnut lines was planted in wooden boxes and evaluated over 10 days without water. The appearance of the plants in the woodenboxes was noted just before rewatering, three day after rewatering and also 8 days after rewatering. Of these three, the appearance of the plants 8 days after rewatering is the most important, because that gives an indication of the plants that will survive and will be able to produce seeds. A good example of this is the plant SB9-1 that was still looking good after 10 days without water (ranked 2nd) (Table 3.2.8), but whose ranking dropped to the 4th place after rewatering. SB7-1 on the other hand was ranked 5th after 10 days without water and this ranking improved to the 2nd place after rewatering.

Table 3.2 .8 The ranking of seven bambara groundnut lines that was planted in wooden boxes at 10 days without water and 3 and 8 days after rewatering

	10 days without water	3 days after rewatering	8 days after rewatering
SB1-1	1	1	1
SB9-1	2	4	4
MAD-1	3	3	3
S20-1	4	5	7
SB7-1	5	2	2
M4	6	6	5
SB10-1	7	7	7

Twelve seeds each of seven bambara groundnut selections and two lines were planted in a woodenbox. The plants were allowed to grow until the first trifoliate leaf was fully extended after which the plants did not receive any water. The woodenbox was allowed to dry out until the plants were severely wilted and then rewatered (Figure 4). Only 35 of the plants survived the stress period (Table 3.2.9). SB1-1, Empangeni red and Komatipoort red produced the most seeds.

Figure 4 Bambara groundnut plants subjected to a woodenbox experiment, 1 week after rewatering

Table 3.2.9 Number of bambara groundnut plants that survived the screening for drought tolerance in a woodenbox and number of seeds produced.

Plant	Number of plants	Seeds harvested in May 2001
SB1-1	9	41
Komatipoort black	2	24
Komatipoort red	4	26
Komatipoort brown	3	16
Empangeni red	4	27
KwaHgnase black	2	4
KwaHgnase red	1	2
SB19-3	2	24
Bosbokrand, light brown	8	No seeds

Screening in pot trial

Bambara groundnut plants that were panted in pots and that were kept in the green house were allowed to grow until the 5th trifoliate leaf was fully extended. These plants were then watered one last time after which the plants received no water until the plants were severely wilted. The plants were rewatered and the recovery was noted.

Not all of the plants recovered from the stress treatment (Table 3.2.10). The line SB9-1 for instance did not survive the stress treatment. The line SB7-1 was the most successful line and 12 from the 20 plants survived and recovered. The severe stress had a detrimental effect on the seed production and very few seeds were harvested from these plants.

Table 3.2.10 The number of bambara groundnut plants still alive after 20 days without water, as well as the number of plants that recovered one week after rewatering. The stress treatment initially consisted of 20 plants.

Cultivars	Plants still alive after 20 days without water	Plants still alive one week after rewatering
SB1-1	2	3
SB7-1	12	12
SB9-1	0	0
SB20-1	10	5
MAD-1	4	6

3.2.1.12 Root Architecture Determination

Bambara seeds were planted in root architecture boxes and were allowed to grow undisturbed for four weeks. The boxes were opened and the root architecture examined. The roots of SB1-1 had a good distribution with 4 long roots that reached the bottom of the box. Another SB1-1 plant also formed a taproot as well as 5 or 6 strong roots while there were not a lot of roots formed on the surface. The SB7-1 plant formed fewer roots on the surface but had 10 long roots that grew right down. The SB9-1 plant formed only two long roots but there were more roots that formed near the soil surface. The SB20-1

plant formed a taproot that grew right tot the bottom of the box and other root that grew down. There was a good distribution of roots close to the surface of the box but very few tertiary roots were formed. The examining the root systems of these plans might provide an answer to the high levels of drought tolerance of these plants.

The seed size can also make a difference in the distribution and quantity of the roots as demonstrated in Figure 5.

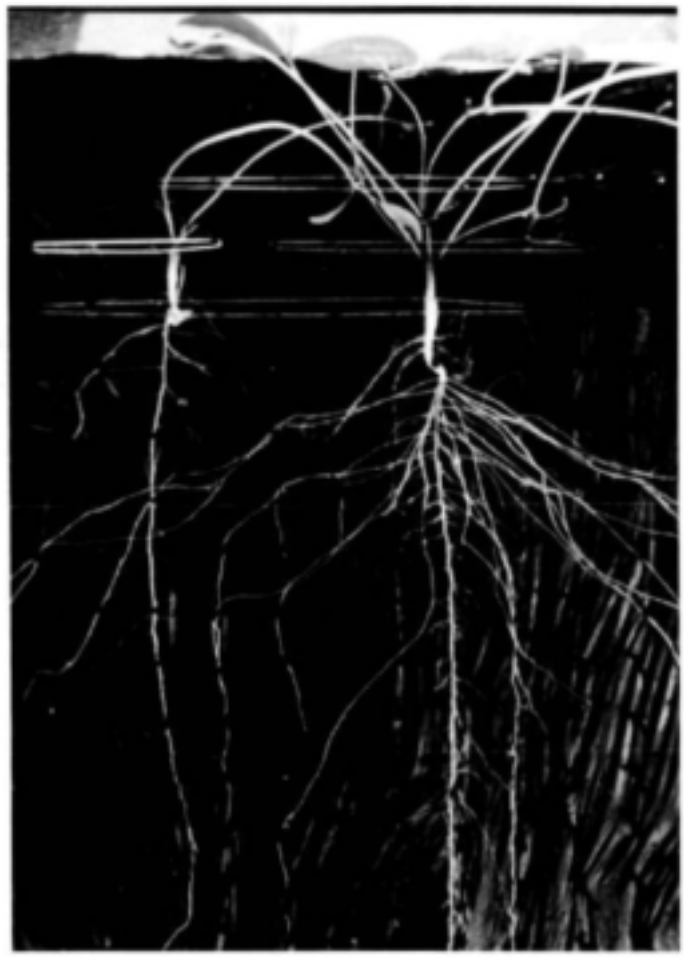

Figure 5. Two bambara groundnut plants that were planted in a root architecture box. The plant on the left originated from a small seed (6 mm diameter) and the plant on the right originated from a big seed (12 mm diameter)

3.2.1.13 Yield of greenhouse plants

All the seeds that were produced by the stress and control plants that were kept in the greenhouse were harvested and the weight of the seeds was determined. During the first and second season the line SB1-1 produced the highest and second highest yield (Table 3.2.11). This was true for the stressed and control plants. Although the number of seeds that were produced by the stressed and control plants was the same the seeds of the control plants were heavier. The differences between the stress and control treatments were not as large as in SB7-1 and SB20-1 where the weight and number of seeds of the stressed plants were only half of that of the control plants. The line SB9-1 produced hardly any seeds in the greenhouse in both the control and stress treatments, while the yield of MAD-1 was not much better.

In the last growth season the line SB9-1 produced the highest yield and the line SB1-1 the 2nd highest. The line Swazi V5B, which did well in most of the other screening tests, was ranked last (Table 3.2.11). This shows that although the plants of this line were coping well with the adverse conditions it did not produce a good yield. This makes the line unsuitable for cultivation in unfavourable growing conditions.

Table 3.2.11 Ranking of bambara groundnut lines according to the yield as it was measured over four growth seasons.

Name	No of seeds		Seed weight		Ranking for each growth season
	Control plants	Stressed plants	Control plants	Stressed plants	
First season					
SB1-1	252	36	128.55	15.55	2
SB7-1	214	108	70.61	38.15	1
SB9-1	184	0	78.13	0	4
SB20-1	160	13	83.08	7365	3
MAD-1	260	26	88.6	6.6	5

Name	No of seeds		Seed weight		Ranking for each growth season
	Control plants	Control plants	Control plants	Control plants	
Second season					
SB1-1	104	105	48.29	39.1	1
SB7-1	93	41	30.63	13.12	2
SB9-1	6	0	1.53	0	5
SB20-1	88	46	40.75	15.45	2
MAD-1	34	11	13.55	3.65	4
Third season					
SB1-1	27	22	18.68	14.16	1
SB8-1 (4)*	14	9	3.96	3.33	7
Swazi V5B (6)	13	23	4.68	4.01	4
SB20-2A (9)	5	14	1.14	9.66	5
AS 18 (11)	42	28	31.11	11.43	1
AS 17 (13)	13	7	14.98	7.63	3
SB2-1C (15)	6	7	1.43	0.82	8
SB9-1 (16)	8	6	6.77	2.96	5
Fourth season					
SB1-1	171	27	78.28	11.28	2
Swazi V5B (6)	43	21	9.47	3.97	8
AS 17 (13)	34	37	15.38	15.5	5
SB9-1 (16)	40	85	18.61	30.26	1
KwaHgnase	48	87	12.91	15.90	5
NB	68	42	24.13	11.40	3
NLB	97	34	35.66	7.72	5
NR	52	57	20.82	11.70	3

3.3 Amaranthus

3.3.1 Screening methods

3.3.1.1 Chlorophyll fluorescence

Information obtained from the fast fluorescence rise and technical fluorescence parameters gives us information on the antenna size, primary photochemistry, trapping flux, and the electron transport flux beyond Q_A^-. It was clear from the results obtained in the 2002/3 growth season that the response of PSII differs between the different amaranth species tested. Data in Table 3.3.1 shows the effect of maximum stress at day 9, just before rewatering. Clear differences were observed in the response of PSII during this prolonged water stress. *A. candatus* (US) and *A. lividus* (India) experienced the highest increase in DI_o/CS_o, TR_o/CS_o, ABS/CS_o, and F_o/F_m. Whereas *A. hypochondriacus* (Nepal), *A. cruentus* (Mexico) and *A. candatus* (India) experienced the highest increase in ET_o/CS, F_v/F_o, $PI_{(cso)}$, and $PI_{(abs)}$.

Changes in F_o and F_v are a reflection of effects on the photosystem and primary photochemical events (Corlett & Choudhary, 1993). Chronic photoinhibition is often recognised by a sustained reduction in photochemical efficiency of PSII φ_{PO} (F_v/F_m) and an increased F_o, associated with a decline in the intrinsic quantum yield of CO_2 assimilation (Murchie *et al.*, 1999). A low F_o/F_m thus indicates possible photoinhibition for *A. hypochondriacus* (Nepal). Changes in quenching of variable fluorescence also includes effects on carbon assimilation (Jefferies, 1992). A decrease in the ratio F_v/F_o could indicate the participation of the antenna apparatus in the down regulation of photosynthesis for the former species (Mohanty & Yamamoto, 1996). The ET_o/CS increased for all the species under severe drought stress, indicating stability under water stress. The ET_o/CS increased the most for *A. candatus* (India), while *A. candatus* (US) experienced the lowest electron transport during severe water deficit. The efficiency with which a trapped exciton can move an electron into the electron transport chain (PSI_o) was the lowest for *A. candatus* (US), followed by *A. hibridus* (USA), *A. hypochondriacus*

(Mexico) and *A. lividus* (India). This is compensated by a high ABS/CS. The highest PSI_o was experienced for *A. candatus* (India) and *A. cruentus* (Mexico).

The $PI_{(abs)}$ was lowest for *A. candatus* (US) during severe moisture stress conditions after 9 days of water stress. The performance index (PI) is a multiparametric expression or index of three independent parameters: density of reaction centers, quantum yield of trapping and probability that trapped exciton will move an e^- into the ET chain beyond Q_A. The highest driving force was experienced in *A. hypochondriacus* (Nepal), *A. cruentus* (Mexico) and *A. candatus* (India). The plants with the lowest driving forces are the species that stressed most during water deficit conditions, and these plants had to work harder in order to survive these unfavourable conditions.

The phenomenological flux of dissipated excitation energy (DI_o/CS_o) decreased the most for *A. hypochondriacus* (Nepal), *A. cruentus* (Mexico) and *A. candatus* (India) and increased only for *A. candatus* (US). The higher DI_o/CS_o could reflect a waist of energy and a less effective e^- transport system, which usually means a poor efficiency. It could also show a lower utilization of energy during drought. This lower utilization of energy could mean a lower accumulation of redox, which could lead to photoinhibition, as well as lower production of ATP and NADPH, which could give rise to irreparable cell damage. By wasting energy the plant could try to prevent cell damage.

During the different growth seasons a number of species were evaluated using similar parameters and hypothesis. The results of these evaluations were summarised in Table 3.3.2.

Table 3.3.1 Multi-parametric presentation of the change in some technical fluorescence parameters and energy fluxes for *Amaranthus* over a prolonged period of drought stress.

Amaranthus species	**RC/CS_m**		**DIo/CS_o**		**ETo/CS_o**		**TRo/CS_o**		**ABS/CS_o**		**PSI_o**		**Fv/F_o**		**F_o/F_m**		**PI(abs)**		**Final ranking**
Control	1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		
A. candatus (US)	0.73	7	1.31	7	1.19	6	1.04	1	1.13	1	1.14	7	0.80	7	1.15	7	0.71	1	7
A. hypochondriacus (Mexico)	0.84	5	0.86	3	1.44	4	0.93	3	0.91	3	1.55	5	1.73	1	0.72	1	1.89	3	3
A. hibridus (USA)	0.87	4	0.81	4	1.33	5	0.89	5	0.86	4	1.49	6	1.08	5	0.95	5	1.90	4	6
A. cruentus (Mexico)	0.97	2	0.63	6	1.85	2	0.80	6	0.74	6	2.33	2	1.10	4	0.94	4	4.90	6	4
A. candatus (India)	0.97	2	0.78	5	2.55	1	0.93	3	0.86	4	2.75	1	1.26	2	0.85	2	4.08	5	1
A. lividus (India)	0.82	6	0.99	2	1.64	3	0.95	2	0.97	2	1.72	4	1.19	3	0.90	3	1.82	2	2
A. hypochondriacus (Nepal)	0.98	1	0.45	1	1.64	3	0.78	7	0.62	7	2.11	3	0.96	6	1.02	6	5.27	7	5

Table represents fluorescence parameter values (in black) and ranking (in red)

Table 3.3.2 Summary of chlorophyll fluorescence data obtained through project

First season		PSI_o	ABS/RC	TR_o/RC	ET_o/CS	RC/CS_o	TR_o/CS_o	K_n	DI_o/CS_o	Final ranking
A.albus		→	→	→	→	→	→	→	↑	1
A.hypochondriacus		↑	↑	↑	↑	↓	→	→	↓	3
A.tricolor		→	→	→	→	→	→	↑	↓	2
A.hybridus		↑	↑	↑	→	↓	→	↓	↑	4
Second season	PI_{abs}		ABS/CS		ET_o/CS	RC/CS_o	TR_o/CS_o		DI_o/CS_o	Final ranking
Community 1	↓		↑		↓	→	→		↑	3
Community 2	↑		→		→	↓	↓		↑	12
Community 3, 4	↓		↑		↓	↑	↑		↑	4
Amaranthus sp. "MacDonald"	↓				↓	↓	↓		↑	11
A. cruentus	↑		↓			↓	↓		↓	4
A. hypochondriacus 1	↑		↓		↑	↓	↓		↓	1
A. hypochondriacus 2	↑		↓		↑	↓	↓		↓	9
A. hypochondriacus 3	↑		↓		↑	↓	↓		↓	2
A. hypochondriacus 4	↑		↓		↑	↓	↓		↓	10
A. tricolor	↓		→		↓	↓	↓		↑	8
A. hybridus	↑		↓		↑	↓	↓		↓	4
Third season		PHI_o	ABS/RS	TR_o/RC	ET_o/CS	RC/CS_o	TR_o/CS_o		DI_o/CS_m	Final ranking
A. tricolor I		↓	↑	→	↓	↓	↓		→	5
Indigenous I		↓	↑	↑	↓	↓	→		↑	2
Indigenous II		→	↑	↑	↓	↓	↓		↓	9
A. palmeri		→	→	→	↑	↓	↓		↓	8
A. tricolor II		↓	↑	→	↓	→	→		↑	3
Imbuya (red stem)		↓	↑	↑	↓	↓	↓		↓	6
Imbuya (green stem)		↓	↑	→	↓	↑	→		↑	4

Third season continue		PHI_o	ABS/RS	TR_o/RC	ET_o/CS	RC/CS_o	TR_o/CS_o		DI_o/CS_m	Final ranking
A. dubius		→	→	→	↓	↓	↓		↓	9
A. tricolor III		↓	↑	→	↓	→	→		↑	1
A. cruentus		↓	↓	↑	↑	↓	↓		↓	7
Fourth season	PI_{abs}	PSI_o	ABS/RC	TR_o/RC	ET_o/CS	RC/CS_o	TR_o/CS_o	K_n	DI_o/CS_m	Final ranking
A. cruentus (Anna)	↓	↓	↑	↑	↓	↓	↓	→	↓	8
Ficksburg sel.	↑	↑	↑	↑	↑	↓	↓	→	↓	6
Krugersdorp sel.	↓	↓	↑	↑	↓	→	↑	↓	↑	3
A. cruentus (Amar)	↑	↑	→	↑	↑	↓	↓	↑	↓	7
A. hybridus (Krugersdorp)	↑	↑	↓	↓	↑	→	↓	↑	↓	5
Local sel.	→	↓	↓	↓	↓	↑	↑	↓	↑	1
Callaloo	↑	→	→	→	↑	→	→	→	→	4
A. tricolor	↓	↓	↑	↓	↓	↑	↑	↓	↑	2
Fifth season	PI_{abs}	PSI_o	ABS/CS	TR_o/RC	ET_o/CS	RC/CS_o	TR_o/CS_o	K_n	DI_o/CS_m	Final ranking
A. candatus (US)	↓	↑	↑		↑	↓	→		↑	7
A. hypochondriacus (Mexico)	↑	↑	↓		↑	↓	↓		↓	3
A. hibridus (USA)	↑	↑	↓		↑	↓	↓		↓	6
A. cruentus (Mexico)	↑	↑	↓		↑	↓	↓		↓	4
A. candatus (India)	↑	↑	↓		↑	↓	↓		↓	1
A. lividus (India)	↑	↑	↓		↑	↓	↓		→	2
A. hypochondriacus (Nepal)	↑	↑	↓		↑	→	↓		↓	5

↑ stress value higher than control (at last day of stress)

↓ stress value lower than control (at last day of stress)

→ stress value equal to control (at last day of stress)

3.3.1.2 Changes in the Free Proline Concentration

Accumulation of proline was the highest after 9 days without water for all the species tested in the greenhouse during the growth season of 2002/2003. *A. hypochondriacus* (Nepal) (68.83 µmol/g dry weight) and *A. hibridus* (USA) (41.46 µmol/g dry weight) (Figure 6) encountered the highest accumulation. The highest overall concentration of proline (calculated as stress-control over time) was formed in *A. hypochondriacus* (Nepal) (82.9) and *A. cruentus* (Mexico) (32.6) compared to the lowest in A. hypochondriacus (Mexico) (8.14).

The species that formed the highest concentration of proline, were also proved to be the more drought sensitive plants (compared with RWC). The production of proline in amaranth during drought stress could be used as an indication of plant water status, indicating that the species with low RWC and thus water deficit sensitive have a high proline production. Proline does not seem to play a protective role during water stress in amaranth, as also documented by Lazcano-Ferrat & Lovatt (1999) for *Phaseolus vulgaris*.

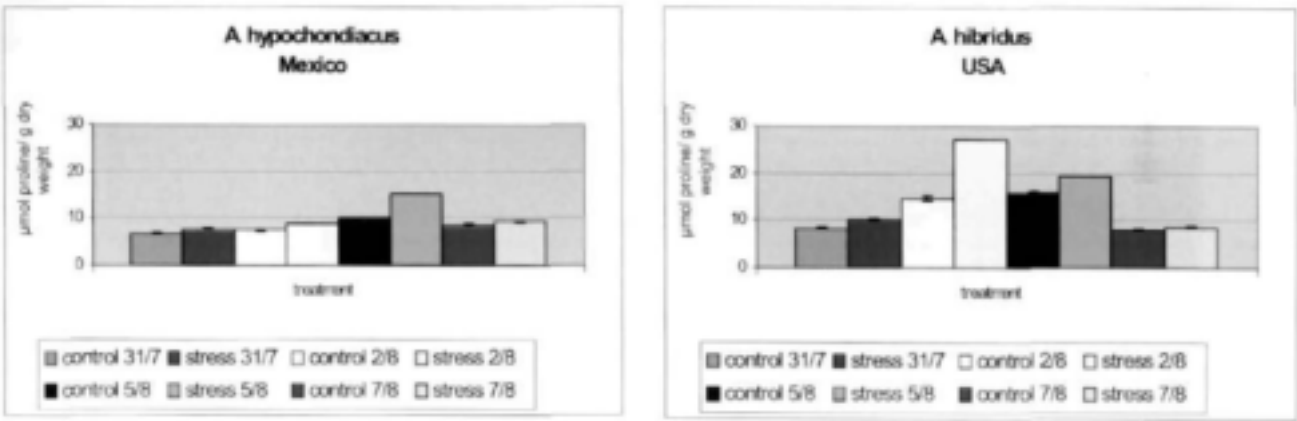

Figure 6: Proline accumulation for *A. hypochondriacus* & *A. hybridus* expressed as µmol proline/gram dry weight.

After rewatering the enhanced level of proline decreases rapidly to control levels in all species except *A. cruentus* and *A. lividus* (Table 3.3.3). Recovery is very important for the survival of plants after drought stress, and should be recognised as a selection criterion for drought tolerance in breeding programmes. It seems that amaranth has a high recovery factor

even after severe drought stress, which makes it a very good choice for growing as a vegetable crop in water restricted areas. The species with the higher proline production on average had a slower recovery from high proline levels to normal proline levels.

There exists a lot of controversy around the role of proline during abiotic stress conditions, and research reports such as that done by Ibarra-Caballero *et al.* (1988), propose that proline accumulation is merely symptomatic and fulfils no protective function during drought stress. There is still no satisfactory model available to explain the role of proline accumulation, as evidence for adaptive functions for proline accumulation are often indirect (Larher *et al.*, 1993).

There is evidence that selection for high proline-accumulating potential in many instances could be used as a tool in breeding for drought resistance (Al-Sulaiti *et al.*, 1990), whether it indicates a protective role of proline, or indicates the water status of the plant, as was found for amaranth. According to this, proline accumulation could be used as an indicator of drought injury in amaranth, indicating plant water status during water deficit conditions, and indicating to us that the higher proline production is associated with lower RWC, and thus sensitivity.

Table 3.3.3 Summary of the Stress - Control values of the tested species during the trial.

	5 dww	**7 dww**	**9 dww**	**Rating at 9 dww**	**Rewater**	**Proline ranking after rewatering**
A. candatus (USA)	0.37±0.26 a	1.53±0.37 c	9.74±0.65 c	3	0.98±0.29 ab	1
A. hypochondriacus (Mexico)	0.90±0.26 b	1.38±0.54 c	5.09±0.17 b	2	0.78±0.18 a	1
A. hibridus (USA)	1.71±0.47 bc	12.42±1.47 e	3.44±0.76 a	1	0.56±0.22 a	1
A. cruentus (Mexico)	1.10±0.28 b	0.44±0.28 b	27.00±0.71 e	5	4.14±0.21 d	3
A. candatus (India)	1.76±0.28 c	-0.09±0.09 a	8.20±1.36 c	3	1.66±0.09 c	2
A. lividus (India)	0.59±0.24 a	2.23±0.41 d	12.09±0.56 d	4	10.31±0.76 e	4
A. hypochondriacus (Nepal)	1.13±0.26 b	20.56±0.83 f	59.96±1.51 f	6	1.25±0.41 bc	2

1 = most tolerant; 4 = most susceptible

a - f indicate significant differences

During the different growth seasons a number of species were evaluated using proline as indicator. The results of these evaluations were summarised in Table 3.3.4

Table 3.3.4 Ranking of various *Amaranthus* species and cultivars according to the free proline concentration

First season	**Total stress – total control values**	**Statistical significance**	**Proline ranking for each growth season**
A.hypochondriacus	1599.38 ±148.12	b	2
A.tricolor	233.53±213.32	a	1
A.hybridus	250.05±146.94	a	1
Second season	**Total stress – total control values**	**Statistical significance**	**Proline ranking for each growth season**
Community 1	648.23±50.72	gh	8
Community 2	831.23±70.29	i	9
Community 3	406.73±102.78	ef	6
Community 4	188.24±20.7	bc	3
"MacDonald"	102.76±34.78	a	1
A. cruentus	551.84±98.99	fg	7
A. hypochondriacus 1	286.80±79.92	cd	4
A. hypochondriacus 2	360.65±79.92	de	5
A. hypochondriacus 3	159.80±39.92	ab	2
A. hypochondriacus 4	704.63±45.37	h	10
A. tricolor	153.88±29.38	ab	2
A. hybridus	997.87±180.41	j	11
Third season	**Total stress – total control values**	**Statistical significance**	**Proline ranking for each growth season**
A. cruentus (Anna)	307.36±158.9	e	6
Ficksburg selection	107.96±80.80	de	5
Krugersdorp selection	-12.34±14.00	a	1
A. cruentus (Amar)	191.58±51.32	e	6
A. hybridus (Krugersdorp)	21.23±21.23	d	4
Local selection	18.82±20.59	d	4
Callaloo	3±0.40	b	2
A. tricolor	8.46±0.20	c	3

Fourth season	Total stress – total control values	Statistical significance	Proline ranking for each growth season
A. tricolor I	52.26±25.55	d	5
Indigenous I	1.73±1	b	2
Indigenous II	173.68±60.90	e	7
A. palmeri	55.09±31.51	d	5
A. tricolor II	13.2±6.79	c	3
Imbuya (red stem)	34.53±30.12	cd	4
Imbuya (green stem)	-0.42±0.4	a	1
A. dubius	146.62±76.81	e	7
A. tricolor III	14.21±8.62	c	3
A. cruentus	110.47±55.26	de	6
Fifth season	**Total stress – total control values**	**Statistical significance**	**Proline ranking for each growth season**
A. candatus (US)	12.63±0.92	b	2
A. hypochondriacus (Mexico)	8.15±0.57	a	1
A. hibridus (USA)	18.13±1.90	c	3
A. cruentus (Mexico)	32.68±0.90	e	5
A. candatus (India)	11.53±1.22	b	2
A. lividus (India)	25.22±1.20	d	4
A. hypochondriacus (Nepal)	82.90±1.91	f	6

1 = most tolerant; 12 = most susceptible

a - h indicate significant differences

3.3.1.3 2.3.5- Triphenyltetrazolium Chloride Reduction

The cell viability was measured for *Amaranthus hypochondriacus, A. tricolor and A. hybridus* that was grown in the greenhouse. *A. hybridus* was the most tolerant for drought, with the smallest negative value, while *A. hypochondriacus* was the most sensitive, *A. tricolor* was intermediate. The absorbency values were non significant for heat stress. The difference between the stress and control values of the different treatments was calculated over time, and is presented in Table 3.3.5a & b.

Table 3.3.5a Summary for the TTC drought assays performed during this study:

	A. hybridus		*A. tricolor*		*A. hypochondriacus*	
	Mean S-C values	**Statistical significance**	**Mean S-C values**	**Statistical significance**	**Mean S-C values**	**Statistical significance**
Glasshouse TTC, first season	-0.020 ±0.0088	1 a	-0.040 ±0.0095	2 b	-0.100 ±0.0084	3 c

1 = most tolerant; 3 = most susceptible

Table 3.3.5b Summary for the TTC heat assays performed during this study

	A. hybridus		*A. tricolor*		*A. hypochondriacus*	
	Mean S-C values	**Statistical significance**	**Mean S-C values**	**Statistical significance**	**Mean S-C values**	**Statistical significance**
Glasshouse TTC, first season	0.0275 ±0.005	a	0.030 ±0.008	a	0.0250 ±0.006	a

3.3.1.4 Protein Synthesis

The effect of prolonged drought stress on protein synthesis was evaluated during the 1998/1999 growth season. The protein synthesis did not increase significantly for the stressed amaranth plants over the period of 21 days, although there was a slight tendency for increased protein synthesis for *A. tricolor* and *A. hybridus*. Although the overall productions of proteins are normally reduced during water-deficit stress conditions (Mason *et al.*, 1988), plants and other organisms are known to induce genotype-specific, stress-responsive proteins in several cases (Creelman *et al.*, 1990; Krishnan *et al.*, 1989; Jorgensen *et al.*, 1992). The stressed amaranth plants seemed to produce proteins equal to the control plants. The maintenance of the relative stable protein production during water deficit could be due to the fact that, although normal protein synthesis was decreased during water stress, specific stress protein synthesis was increased, as was also found by Van der Mescht & De Ronde (1993) on cotton, therefore the seemingly constant protein synthesis for the amaranths. Further protein studies could clarify the specific production of possible drought stress proteins.

3.3.1.5 Enzymes of the Anti-oxidative System

Greenhouse plants were evaluated for the changes in the anti-oxidative enzyme system over a period of 20 days without water (Table 3.3.6). There was a tendency towards the increase in SOD activity over time for three of the species tested, indicating an age effect on SOD activity. SOD activity increased noticeably after 11 days water stress for *A. hypochondriacus* and *A. hybridus*, while an increase in activity was only noticed after 13 days in *A. tricolor*. After rewatering SOD production dropped in *A. hypochondriacus* to a level below that of the control even after tree days recovery, indicating a slower recovery tempo after the shock treatment. SOD production in *A. tricolor* decreased to the same level as the control treatment within three days after rewatering, indicating the best recovery of the species tested. GR activity increased after 9 days for *A. hypochondriacus* and *A. hybridus*, but only after 11 days for *A. tricolor*. *A. tricolor* had the highest overall GR production, followed by *A. hybridus* and the lowest activity in *A. hypochondriacus*. Recovery of the GR antioxidative activity was also the best in *A. tricolor*, where the activity was stable one day after rewatering. The tendency in both *A. hypochondriacus* and *A. hybridus* was an increase in AP activity over time (ageing effect) but it was the opposite for *A. tricolor* where a decrease in AP was noticed over time. For *A. hypochondriacus* AP activity started to increase noticeable after 6 days already, while that of *A. hybridus* only slightly increased after nine days water stress, and continued to be low for the stress treatment over the whole period of time. AP activity in *A. tricolor* was lower in the stress treatment until day sixteen, when only then the AP activity increased. After rewatering AP activity *A. tricolor* recovered to normal within three days, while both *A. hypochondriacus* and *A. hybridus* had a complete collapse of the AP system after rewatering, and did not yet recover three days after rewatering.

Malan *et al.* (1990) reported that the increased activity of only SOD or only GR are not sufficient to confer tolerance in maize. The capacity to increase AP and GR activity is a possible drought tolerance selection criteria for tobacco (Van Rensburg & Kruger, 1993). This could also be true for amaranth. As drought and other physiological stresses cause oxidative injury, the ability to increase the levels of antioxidant capacity or increased levels of antioxidants during water stress can limit membrane damage and may correlate with stress tolerance.

Table 3.3.6 Summary for the enzyme assays performed during this study:

	SOD		GR		AP	
Greenhouse	**Difference between stress and control**	**Reaction/ ranking**	**Difference between stress and control**	**Reaction/ ranking**	**Difference between stress and control**	**Reaction/ ranking**
A. hypochondriacus	Non significant	Sensitive 3	Significant	Sensitive 3	Non significant	Sensitive 2
A. tricolor	Non significant	Tolerant 2	Non significant	Sensitive 1	Non significant	Sensitive 2
A. hybridus	Significant	Tolerant 1	Non significant	Sensitive 2	Significant	Tolerant 1

3.3.1.6 Relative Water Content

The relative water status of the drought stressed plants is a direct indication of the drought tolerance or susceptibility of the plant. Decrease in turgor pressure below 80% was noticed after 7 days without water for all the species tested during the growth season of 2002/2003 except for *A. candatus* (US) (Table 3.3.7). On the 9th days without water the RWC of *A. candatus* (US) also decreased below 80%, while RWC of *A. hibridus* (USA) was 54.5% and the RWC of *A. hypochondriacus* (Nepal) was only 42.4%.

Drought response indexes have strong associations with water loss during water deficit conditions, indicating cultivars showing low water loss to be more drought tolerant (Dhanda *et al.*, 1999). The ability of some of the species to maintain a high RWC during severe drought stress conditions must be ascribed to cellular or whole plant functions. Factors such as stomatal conductance, maintenance of leaf area, and all other biochemical functions are linked to these factors, such as enzyme function, photosynthesis and respiration. Restoration and repair upon rehydration is a most critical component of desiccation tolerance, and one that is often ignored (Blum, 1996). Restoration of RWC two days after rewatering was high in all the species tested.

Table 3.3.7 Summary for the RWC assays performed during this study:

Second season	**Mean RWC values over time (S-C)**	**Statistical significance**	**Ranking according to mean**	**RWC % at max stress 17 dww**
Community 1	-33.39±3.11	d	11	38
Community 2	-30.16±3.07	cd	10	40
Community 3	-20.94±2.37	b	2	74
Community 4	-18.89±1.34	b	2	87
"MacDonald"	-21.14±2.39	b	2	57
A. cruentus	-26.77±2.46	c	6	53
A. hypochondriacus 1	-24.85±5.42	bc	5	58
A. hypochondriacus 2	-28.46±1.29	c	6	50
A. hypochondriacus 3	-28.10±4.03	c	6	50
A. hypochondriacus 4	-42.74±5.63	d	11	33
A. tricolor	-16.42±0.29	a	1	77
A. hybridus	-25.40±5.51	bcd	9	48
Third season	**Mean RWC values over time (S-C)**	**Statistical significance**	**Ranking according to mean**	**RWC % at max stress 10dww**
A. cruentus (Anna)	-26.67±3.76	c	8	44
Ficksburg selection	-19.12±3.23	b	3	46
Krugersdorp selection	+1.60±2.62	a	1	88
A. cruentus (Amar)	-20.55±4.67	bc	7	54
A. hybridus (Krugersdorp)	-15.34±6.02	b	3	53
Local selection	-13.21±7.95	b	3	56
Callaloo	-14.52±3.77	b	3	51
A. tricolor	-0.23±1.00	a	1	74
Fourth season	**Mean RWC values over time (S-C)**	**Statistical significance**	**Ranking according to mean**	**RWC % at max stress 13 dww**
A. tricolor I	-14.20±2.86	c	6	60
Indigenous I	-5.60±2.04	ab	1	79
Indigenous II	-26.60±2.98	d	8	43
A. palmeri	-23.60±4.90	d	8	43
A.tricolor II	-8.60±2.08	b	3	76
Imbuya (red stem)	-10.00±5.54	abc	4	66
Imbuya (green stem)	-5.00±1	a	1	87
A. dubius	-18.40±4.22	c	6	48
A. tricolor III	-10.40+2.44	bc	4	68
A. cruentus	-24.60±7.80	d	8	46

Fifth season	Mean RWC values over time (S-C)	Statistical significance	Ranking according to mean	RWC % at max stress 9 dww
A. candatus (US)	-0.74±0.62	a	1	76
A.hypochondriacus (Mexico)	-13.14±0.41	b	2	53
A. hibridus (USA)	-13.28±1.73	b	2	55
A. cruentus (Mexico)	-14.27±0.98	b	2	45
A. candatus (India)	-13.88±1.33	b	2	45
A. lividus (India)	-14.63±2.31	b	2	49
A.hypochondriacus (Nepal)	-16.26±3.44	b	2	43

1 = most tolerant; 12= most susceptible

3.3.1.7 Leaf Water Potential

Plant water status, and the ability to maintain it under drought conditions, is very important for the well being of a crop. Plant growth, function, productivity and water use is related to its water status. It is possible to measure the response of a given plant to soil water stress, and the relationship of growth or yield to the internal water status. The aim of this work was thus to determine the relationship between available soil moisture and amaranth water status expressed through the leaf water potential (LWP) and the relative water content (RWC) during the drought trial in the growth season of 1999/2000. *A. tricolor* was able to maintain a very high RWC and LWP, even under severe water deficit conditions (Figure 7). The RWC dropped from 93% at a LWP of –0.5MPa, to RWC of 77% after 16 days. The other two species showed a much more severe drop in RWC. The RWC of *A. hypochondriacus* already started to decline at –1.0 MPa and –2.0 MPa and reached the lowest RWC of 33% after 16 days without water. A. *hybridus* had the ability to maintain the RWC at a relatively high level even at LWP of –1.0 MPa and –2.0 MPa, and reached a RWC of 48% after 16 days. Although the RWC of *A. tricolor* and *A. hybridus* recovered 90-96% within 48 hours after rewatering, the RWC of *A. hypochondriacus* recovered only 70%. It is clear from the results that *A. tricolor* was in a good cellular water condition, and had the ability to maintain both a high RWC and LWP during severe stress conditions for 16 days.

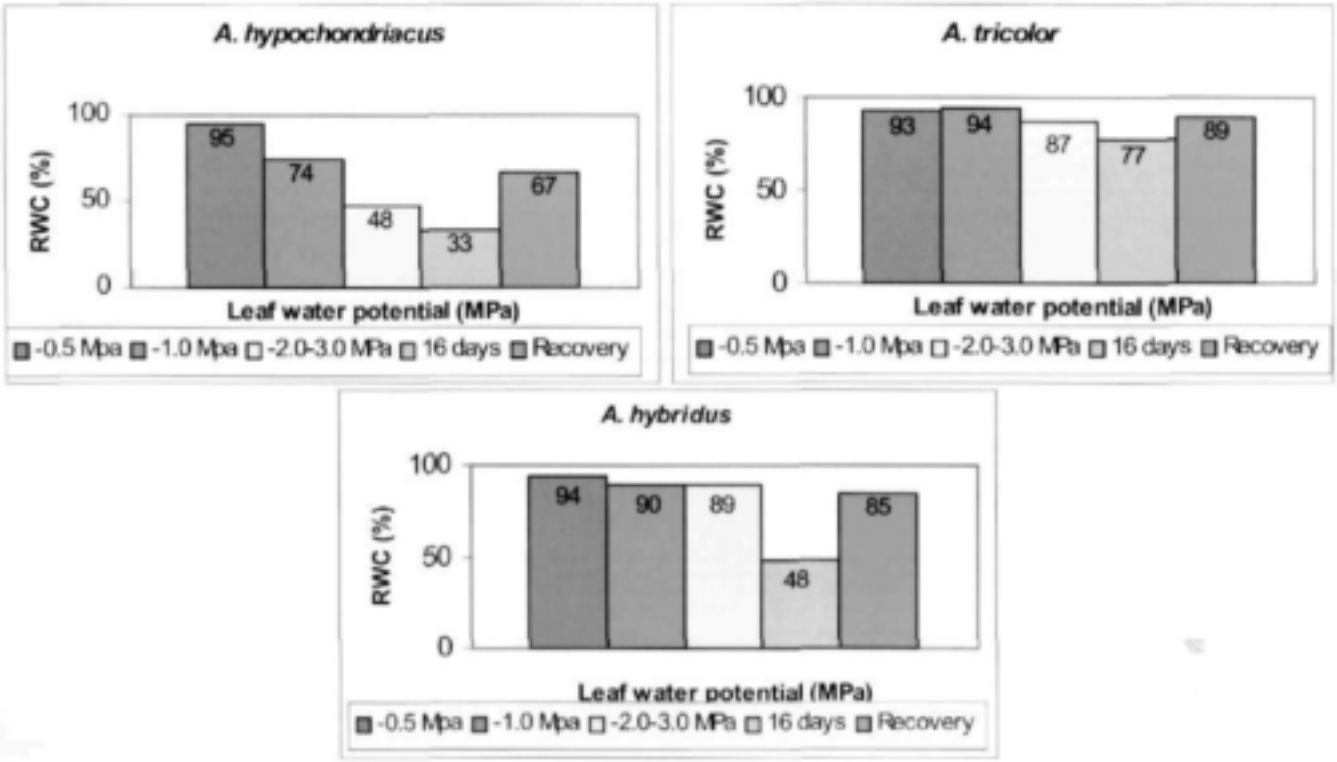

Figure 7: The changes in RWC values at specific LWP (ψ) for *Amaranthus* during water deficit and recovery

The ability to maintain leaf turgor even under high water deficit shows an adaptation mechanism for both *A. tricolor* and *A. hybridus* plants (Table 3.3.8). *A. tricolor* and *A. hybridus* had the ability to maintain a high RWC and LWP at 13 days of stress, compared to the RWC of *A. hypochondriacus* that dropped at lower LWP. Adaptation to water stress in seed plants involves the ability to either tolerate cellular dehydration, or to minimize water loss and hence maintain turgor pressure.

Table 3.3.8 LWP observed at 13 dww compared to the RWC:

	LWP at RWC values	**Ranking**
Second season		
A. hypochondriacus 4	-2.375mPa @ 48%	3
A. tricolor	-1.175mPa @ 94%	1
A. hybridus	-2.875mPa @ 89%	2

1 = most tolerant; 3 = most susceptible

3.3.1.8 Cell Membrane Stability

Distinct differences were noticed between the cell membrane stability (CMS) of the different species tested in growth season 1999/2000. The CMS declined statistically meaningfully over the 17 days drought testing period and were the lowest for *A. hypochondriacus* 4 (32%) and the highest for Community 4 (117%) and *A. hybridus* (108%). The higher CMS correlated with the wilting tempos in the glasshouse. *A. hypochondriacus* 4 wilted first, followed by the other species in order of increasing CMS. The overall picture showed a decline in CMS during drought stress for amaranth over the test period, indicated a possible increased sensitivity to water deficit with increased age. Care was taken to sample amaranth leaves of the same developmental stage and position on the stem, using young plants (between 6-8 weeks of age) during this experiment. It was also found by Blum & Ebercon (1976; 1981) that younger leaf tissues are more tolerant to drought than older tissues. Therefore careful account should be taken of sampling procedures and growth stage of different genotypes, as results of Blum & Ebercon (1981) on wheat also indicates that for a given growth stage, leaf position may also contribute some variations into the comparisons.

The CMS for the heat treatment showed a definite declined over the period of 19 days only for *A. hybridus*. The other species all seemed to have a slight increase in CMS for heat stress. The lowest CMS for heat was for A. *hybridus* (12%) and the highest *A. hypochondriacus* 4 (50%) (Table 3.3.9).

Blum (1998) compared CMS for heat stress at different stages of wheat development after imposing controlled heat hardening of either seedlings or plants, and found that thermotolerance tended to increase from seedling to flowering stages, and thermotolerance for CMS was well correlated between growth stages of the eight cultivars tested. Care was taken again to sample leaves within the same stages of growth during this experiment with CMS determination in amaranth. The increase in heat tolerance over time for amaranth compared very well with the study of Blum (1998). Reynolds *et al.* (1994) have shown that heat tolerance in wheat in terms of CMS was well associated with yield and its components under dryland conditions across diverse genetic materials.

Table 3.3.9 Summary for the CMS assays performed during this study:

CMS rating Second season	Drought % after 17 days	Drought ranking	Statistical significance	Heat % after 19 days	Heat ranking	Statistical significance
Community 1	73.42±7.78	3	b	34.41±5.60	2	b
Community 2	86.01±15.02	2	ab	22.01±4.16	3	c
Community 3	88.29±17.37	2	ab	16.55±2.34	4	cd
Community 4	117.66±17.70	1	a	25.66±8.41	2	b
"MacDonald"	84.99±10.32	3	b	25.38±8.03	2	b
A. cruentus	87.96±10.33	3	b	22.89±2.27	3	c
A. hypochondriacus 1	86.48±18.34	2	ab	21.07±3.58	3	c
A. hypochondriacus 2	86.54±10.63	3	b	29.52±21.97	2	ab
A. hypochondriacus 3	88.75±9.65	3	b	21.29±3.56	3	c
A. hypochondriacus 4	31.57±14.33	4	c	50.40±10.23	1	a
A. tricolor	97.19±3.42	1	ab	16.48±5.32	4	cd
A. hybridus	108.02±8.01	1	a	12.54±4.67	5	d

1 = most tolerant; 5 = most susceptible

3.3.1.9 Tissue Culture techniques

3.3.1.9.1 Establishment of an *in vitro* multiplication technique

The possibility to multiply amaranthus plantlets via tissue culture techniques was evaluated as the establishment of callus cultures and plant regeneration have successfully been established from callus of *Amaranthus paniculatus* (Prasda *et al.*, 1992).

The *A. hybridus, A. hypochondriacus* and *A. tricolor* plantlets grew slowly on MS-medium. Various growth regulator combinations for rapid multiplication were tested. This did not improve multiplication rate, and undesirable calluses were formed in some instances. Elongation was much slower on the media containing growth regulators, than on plain MS-medium. Plantlets only reached a high of approximately 40 mm after 6 weeks, compared to 80-100 mm after 6 weeks on MS-medium. After subculturing every 4 weeks onto MS-medium (internodes for *A. hybridus* and *A. hypochondriacus*, whole/large plantlets for *A. tricolor)* nutrient deficit symptoms (yellowing, necroses and leaf drop) were observed on the leaves. By doubling the nitrogen (NH_4) concentration of MS-medium (AMS-medium), better

results were obtained concerning the vigour and proliferation of the explants. Plantlets can be subcultured every 4 weeks, obtaining 6-10 internodes from each explant for *A. hybridus* and *A. hypochondriacus*. The AMS medium is thus recommended for amaranth *in vitro* cultures.

A.tricolor was initially difficult to multiply *in vitro*, as the plantlets grew very slowly. Internodes did not grow sufficiently, and mostly remained stunted, while some internodes died, the rest grew so slowly that it could only be subcultured every 8-12 weeks. It was discovered that by using either whole seedlings (5-8 leaf stage) or larger segments of explant (not less than 5 leaves) as explant material, *A. tricolor* could be successfully be propagated. Plantlets could be subcultured every 6-8 weeks, with a multiplication rate of 3-4 (large segments of explant) or 1 (undivided explant). It was also noticed that amaranth plants *in vitro* (being short day plants) changed from vegetative growth to reproductive growth when the illumination period was shorter than 16 hours. Plantlets could thus successfully be incubated at long days of 16 hours light.

Using this technique, *in vitro* plantlets can be used in transformation studies of amaranth, to incorporate specific genetic material into selected lines to enhance drought tolerance as well as multiplication of the desired lines. This study can have an important impact when transformation is considered an option for increasing the drought tolerance in amaranth.

3.3.1.9.2 *In vitro* selection pressure

Changes in free proline concentration

Chemical substances, such as PEG, mannitol, salt and sorbitol, have been widely used as an osmoticum to impose water stress in plants for measuring proline accumulation during an *in vitro* trial. The osmotic shock imposed by 4% PEG 6000, is similar to drought, as the plant tries to compensate for this condition by reducing desiccation and modifying the leaf anatomy.

Higher levels of free proline were measured for PEG stressed explants *in vitro* than observed with the greenhouse plants (compare Figure 6), but extremely high variation in the data were

observed. *A. hybridus* produced *in vitro* an average of 200 µmol proline/gram dry weight for control treatments, and 1200 µmol/gram dry weight for stress plants. Proline production for *A. hypochondriacus* increased from 140µmol/gram dry weight for control treatments, to 2400 µmol/gram dry weight for stress treatments. Proline production for *A. tricolor* was similar for stress and control treatments (varies between 6000 and 6500 µmol/gram dry weight), indicating that the *in vitro* plantlets of *A. tricolor* was severely stressed, even before the PEG treatment was induced. As a result of the great variation experienced in proline production with the *in vitro* PEG induced stress, it was suggested that this test should only be applied to greenhouse plants.

Table 3.3.10 Summary for the TTC assays performed using *in vitro* plants

	Total stress – total control values	**Statistical significance**	**Proline ranking for each growth season**
In vitro *A.hypochondriacus*	1.10±0.20	b	2
In vitro *A.hybridus*	0.42±0.14	a	1

2.3.5-Triphenyltetrazolium Chloride Reduction

The 2.3.5-Triphenyltetrazolium chloride reduction test was performed on *in vitro* leaf disks of *A. tricolor*, *A. hybridus* and *A. hypochondriacus*. The *in vitro* heat stress results of *A. tricolor* show a definite tolerant reaction over time, compared to the heat stress results of *A. hybridus* and *A. hypochondriacus*, which were heat sensitive. *A. tricolor* was initially more drought tolerant, but over time showed a definite drought sensitive reaction. *A. hybridus* and *A. hypochondriacus* experienced both drought and heat sensitive reaction.

The reason for the difference in the reaction of glasshouse (Table 3.3.5) and *in vitro* results (Table 3.3.11) could be because the plantlets stressed while grown *in vitro*. The medium, growth conditions such as light and temperature, or some other unknown factors, was not optimum for *in vitro* growth. It is important to make sure that the *in vitro* plantlets are not subjected to any other kind of stress while examining possible methods for *in vitro* screening, as these results could give a false indication of traits tested. Therefore, it is important to always compare *in vitro* results with field or glasshouse trials. It is not

recommended that the TTC assay be used for screening for drought or heat tolerance in amaranth, as it could not distinguish between lines.

Table 3.3.11 Summary for the TTC assays performed using *in vitro* plants:

Drought stress						
	A. hybridus		***A. tricolor***		***A. hypochondriacus***	
	Mean S-C values	**Statistical significance**	**Mean S-C values**	**Statistical significance**	**Mean S-C values**	**Statistical significance**
In vitro TTC, first season	-0.0137 ±0.0088	a	na	na	-0.0101 ±0.0126	a
In vitro TTC, second season	-0.0094 ±0.0103	a	0.0004 ±0.045	a	-0.0101 ±0.0109	a
Heat stress						
In vitro TTC, first season	-0.0147 ±0.0103	a	na	na	-0.0299 ±0.0097	a
In vitro TTC, second season	-0.0048 ±0.0099	a	0.0304 ±0.038	a	-0.0149 ±0.0089	a

Enzymes of the Anti-oxidative System

Some enzymes in the anti-oxidative enzyme system, SOD, GR and AP, were evaluated using *in vitro* plants stressed with 4% PEG. The SOD activity for both *A. hybridus* and *A. hypochondriacus in vitro* plantlets declined over time for both control and stress treatments, showing a possible age effect. The GR activity was high in both *A. hypochondriacus* and *A. hybridus* during the induced drought stress conditions with PEG for almost the complete period of 11 days, while *A. tricolor* showed an increase in GR activity after only 11 days of PEG treatment. The AP activity was higher in the PEG stress plants than the control plants for *A. hypochondriacus, A. tricolor* and *A. hybridus*. The enzyme analysis indicating that *A. hypochondriacus* is more drought tolerant under *in vitro* conditions than the other species (Table 3.3.12).

Table 3.3.12 Enzyme analysis performed on *in vitro* plantlets

	SOD		GR		AP	
	Difference between stress and control	**Reaction/ ranking**	**Difference between stress and control**	**Reaction/ ranking**	**Difference between stress and control**	**Reaction/ ranking**
A. hypochondriacus	Significant	Tolerant 1	Significant	Tolerant 1	Significant	Tolerant 2
A. tricolor	Non significant	Sensitive 3	Non significant	Sensitive 3	Significant	Sensitive 3
A. hybridus	Non significant	Sensitive 2	Non significant	Sensitive 2	Significant	Tolerant 1

3.3.1.10 Woodenbox Screening at the Seedling Stage

Young amaranth plants were screened in various wooden boxes to select for early drought tolerance. Rehydration upon rewatering is fast, which means that even small amounts of rain will be sufficient to ensure survival of amaranth in the field. The initial water uptake after rewatering can take place via existing roots, followed by new roots formed after the drought treatment (Eissenstat *et al.*, 1999). According to the latter, it is possible for plants to maintain surface roots during prolonged periods of drought. This could also have contributed to the fast recovery of amaranth seedlings after severe moisture stress periods.

Early drought screening gives an indication of the ability of the seedlings to withstand water deficit conditions at an early stage of development. During the 1999/2000 growth season Community 1, Community 2, Community 3 and *A. hypochondriacus* 2 recovered the best within 6 days after the stress period, followed by *Amaranthus* sp. "MacDonald", *A. hypochondriacus* 3 and Community 4 (Figure 8). Although sprouting started, *A. tricolor*, *A. hybridus* and *A. hypochondriacus* 4 lost all their leaves and seemed not to recover that fast compared to the others.

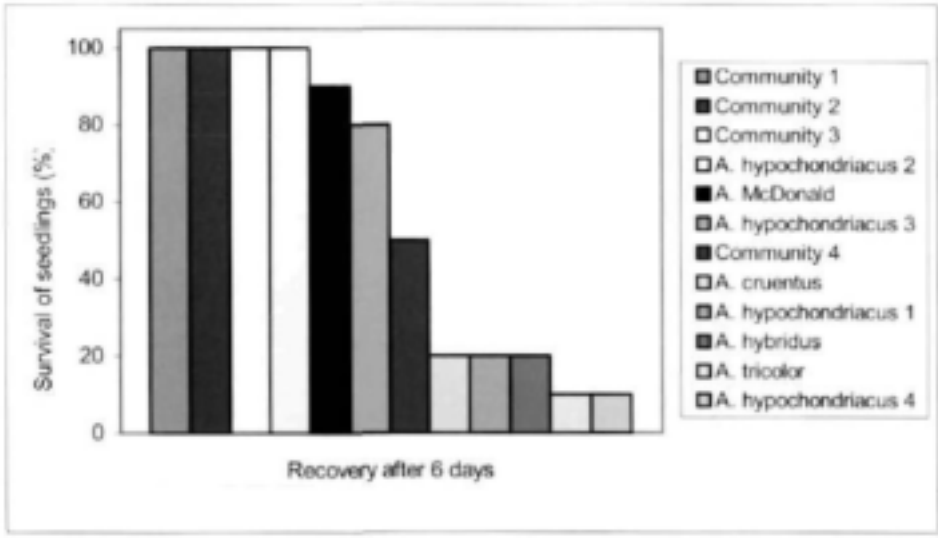

Figure 8: Recovery of seedlings after early drought screening in the woodenbox, 6 days after rewatering

There were distinct differences in the way the different species react to early drought stress over seasons (Table 3.3.13). Some of the plants that wilted most during early drought stress conditions lost all their leaves, but recovered quickly after a few days by growing new leaves. This is in comparison with others that recovered within a day or two by simply recovering turgor pressure in the existing stems and leaves. These mechanisms could be an indication of drought avoidance at an early developmental stage.

Table 3.3.13 Summary of all the woodenbox experiments performed over the years

Woodenbox	**Rating according to survival during drought stress**	**% Recovery after stress**
First season		
A. tricolor (Beijing)	4	Not recorded
A. tricolor (India)	1	Not recorded
A. tricolor (America)	3	Not recorded
A. albus (Canada).	1	Not recorded
A. cruentus (Mexico)	2	Not recorded
A. spinosus (Zimbabwe)	4	Not recorded
A. hypochondriacus (Nepal)	3	Not recorded
A. hypochondriacus (America)	3	Not recorded
A. hybridus (Greece)	3	Not recorded
A. hybridus (America)	3	Not recorded

Woodenbox	Rating according to survival during drought stress	% Recovery after stress
Second season		
Community 1	5	100
Community 2	6	100
Community 3	6	100
Community 4	3	50
Amaranthus sp. "MacDonald"	8	90
A. cruentus	9	20
A. hypochondriacus 1	9	20
A. hypochondriacus 2	1	100
A. hypochondriacus 3	1	80
A. hypochondriacus 4	9	10
A. tricolor	9	10
A. hybridus	4	20
Third season		
A. cruentus (Anna)	2	100
Ficksburg selection	4	67
Krugersdorp selection	6	33
A. cruentus (Amar)	3	100
A. hybridus (Krugersdorp)	7	33
Local selection	8	0
Callaloo	1	100
A. tricolor	5	40
Fourth season		
A. tricolor I	7	26
Indigenous I	7	34
Indigenous II	6	34
A. palmeri	5	80
A.tricolor II	3	73
Imbuya (red stem)	2	94
Imbuya (green stem)	9	0
A. dubius	1	94
A. tricolor III	4	40
A. cruentus	9	0

1 = most tolerant; 12 = most susceptible

3.3.1.11 Root Architecture Determination

Root architecture can be an indication of the efficiency of water absorption from the surrounding soil, which could mean a difference between survival and death for the plant under drought conditions (Singh, 1998). During the growth season of 1999/2000 it was found that *A. cruentus* formed the longest root system, followed by *A. hybridus*, *A. hypochondriacus* 4 and *Amaranthus* sp. "MacDonald" (Table 3.3.14). Community 2 formed the tallest plants, followed by *A. cruentus* and *A. hypochondriacus 4*. The plant with the highest leaf number was also *A. cruentus*. Although *A. tricolor* was one of the best species to withstanding the water deficit (RWC, LWP), it had the smallest root system and lowest amount of leaves, but the plant was also smaller with less leaves, therefore transpiration will not be as high as for a larger plant, preventing desiccation.

Plants with a well-developed root system will be able to absorb small amounts of water from a wider area around the root system of the plant, while weakly developed root systems will not be able to absorb more water than available in the small area surrounding the small root system. It is therefore crucial to select plants with a well-developed root system to be used in breeding programs for water restricted areas, where people do not have irrigation systems, but are completely dependant on rainfall for their crop harvest. This technique allows for a relatively easy way to select for plants with the ability to develop a well-established root system within a short period of time (4-6 weeks) to be selected for breeding programmes.

Table 3.3.14 Summary of the root architecture experiment

	Number of leaves	**Statistically significant**	**Length of roots (mm)**	**Statistically significant**	**Length of plant (mm)**	**Statistically significant**
A. hypochondriacus 4	15±0.00	b	82.5±4.95	ab	30±2.83	b
A. tricolor	9±1.41	d	66±39.60	ab	15±7.07	d
A. hybridus	15±0.00	b	90±4.24	a	22.5±0.70	d
A. cruentus	17±1.41	a	92±14.14	ab	39.5±13.43	abc
Community 2	15±0.00	b	79±3.00	b	44±0.00	a
Amaranthus sp. "MacDonald"	14±0.00	c	81±2.00	b	26±0.00	c

3.3.1.12 Yield of greenhouse plants (and multiplication)

Five amaranth species were selected on the grounds of their drought tolerance performance during previous screening tests for multiplication trials. These included Community 4, *A tricolor*, Callalloo, *A. hypochondriacus* and Krugersdorp selection. These species need to be distributed to communities for testing of general performance and acceptability, but the seed has to be bulked up first. During a small seed production trial, these species were planted at Roodeplaat in a greenhouse and seed was harvested (Table 3.3.15). Seed was send to and planted at the University of KwaZulu Natal, as the climate in KwaZulu Natal is suitable for the cultivation of amaranths during winter months.

Table 3.3.15 Seed production by five *Amaranthus* species and selections

Specie / selection	Seeds harvested (g)
Community 4	25.6
A. tricolor	200.33
Callalloo	60.92
A hypochondriacus	55.0
Krugersdorp	20.76

3.3.1.13 Leaf area

Exposure of plants to extreme conditions such as drought causes a diverse set of physiological, morphological and developmental changes (Jensen *et al.*, 1996). Regulatory mechanisms are implemented to prevent early death (Tardieu, 1996). Because this short-term regulation such as transpiration is usually not so effective, the phenotypical appearance of the plant changes i.e. the reduction of the leaf area, increased root/shoot ratio and the reduction of total leaf area by leaf senescence. Leaf area plays an important part in many functions of the plant, such as growth, photosynthesis, which in effect has a direct impact on production. It is important for a crop to be able to adjust the leaf expansion, as this is a means by which a drought-stressed crop can maintain control over water-use, and reduce water loss through transpiration (Blum, 1996). Cell expansion is one of the most sensitive processes affected by water deficit (Hsiao, 1973), resulting in decreased leaf extension and

growth. 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 leaf size and ability to maintain leaf area varied greatly between the different species tested (Table 3.3.16). Leaf size decreased in most species tested over the stress period, but increased in five of the species in the third season: *A. hibridus* (Krugersdorp), local, Callaloo, and *A. tricolor* I and II. This ability to maintain leaf expansion over a longer period during drought stress conditions would have a direct impact on photosynthesis and production. Amaranth can thus modulate leaf area and thereby adjust water loss from the canopy to the size that can be effectively supplied by the existing soil water.

Table 3.3.16 Ranking of *Amaranthus* plants according to the leaf sizes as it was measured over three growth seasons.

Second season	**Leaf area ranking for each season**	**Statistical significance**	**Mean size reduction over time**
A. hypochondriacus 4	3	b	-24.33±3.59
A. tricolor	1	a	-1.61±5.83
A. hybridus	2	b	-20.56±4.07
Third season	**Leaf area ranking for each season**	**Statistical significance**	**Mean size reduction over time**
A. cruentus (Anna)	5	b	-6.85±9.99
Ficksburg selection	7	c	-34.34±7.54
Krugersdorp selection	1	a	4.47±1.51
A. cruentus (Amar)	8	d	-63.16±11.60
A. hybridus (Krugersdorp)	1	a	4.71±0.23
Local selection	5	b	0.00±0.95
Callaloo	3	ab	5.35±8.59
A. tricolor	3	ab	7.00±10.59

Fourth season	Leaf area ranking for each season	Statistical significance	Mean size reduction over time
A. tricolor I	9	d	-100±20.00
Indigenous I	1	a	-8±1.62
Indigenous II	4	b	-30±8.56
A. palmeri	1	a	-10±3.32
A.tricolor II	9	d	-90±18.90
Imbuya (red stem)	6	bc	-30±3.54
Imbuya (green stem)	4	b	-22±5.69
A. dubius	7	c	-40±8.45
A. tricolor III	3	ab	-18±6.23
A. *cruentus*	7	c	-35±4.32

3.4 Germplasm collection

3.4.1 Seeds collected by ARC-SRL unit

Seeds were collected from various sources in the Gauteng, Mpumalanga and Northern Province. These included local markets or fairs, the National Department of Agriculture and individual contact persons (Table 3.4.1).

Four collections of *Amaranthus* were made from the Thohoyandou area in Venda. In this document they will be referred to as Community 1, Community 2, Community 3 and Community 4. Most of the samples that were collected in Venda are local South African species but there are also some collections that originally came from Nigeria. These lines were planted in the field for evaluation and seed propagation.

Table 3.4.1 A list for the plant material collected during the last season in the Pietersburg area, Bushbuckridge and Ladismith in KwaZulu Natal

Genus	Specie	Cultivar / Common Name(s)	Reference on container	Additional Reference	Comments	Source/Origin	Person / Institution
Amaranthus	spp.	Red stem imbuya				ACAT seed fair	Hugh Trollop
Amaranthus	spp.	Green stem imbuya				ACAT seed fair	Hugh Trollop
Amaranthus	spp.	Imbuya				ACAT seed fair	Hugh Trollop
Amaranthus	spp.	Thepe	Thabamoopo 1		Thin strap like leaves	Thabamoopo – 25 June 2001	
Amaranthus	spp.	Amaranthus	Roodeplaat				
Amaranthus	spp.	Appelschbos Imbuya	Appelschbos			Appelschbos	
Amaranthus	spp.	Amaranthus, Setototwane cillege				Setototwane college near Pietersburg	
Amaranthus	spp.		2025	NDA			NDA
Amaranthus	spp.		2008	NDA			NDA
Amaranthus	spp.		2002	NDA			NDA
Amaranthus	spp.		1999	NDA			NDA
Amaranthus	spp.		1986	NDA			NDA
Amaranthus	spp.		1972	NDA			NDA
Amaranthus	spp.		1964	NDA			NDA
Amaranthus	spp.		1961	NDA			NDA
Amaranthus	spp.		1826	NDA			NDA
Amaranthus	spp.		1825	NDA			NDA
Amaranthus	spp.		1824	NDA			NDA
Amaranthus	spp.	Thepe		Vukani		Vukani	
Amaranthus	spp.	Thepe		Thabamoopo	Thin leaves	Thabamoopo	
Amaranthus	spp.				Light red stem, 3m in height	Thohoyandou, Venda	
Amaranthus	spp.				Deep red stem 1.5m in height	Thohoyandou, Venda	
Amaranthus	*hypochondriacus*					Ex Nigeria	
Amaranthus	*hybridus*					Ex Nigeria	
Amaranthus	spp.	MacDonald				Pietermaritzburg	
Amaranthus	spp.				Very pale red stem, 1m in height	Thohoyandou, Venda	
Amaranthus	spp.				Red stems at maturity, 2m in height.	Thohoyandou, Venda	
Amaranthus	*hypochondriacus*	*Amaranthus hypochondriacus*				ex Arusha	
Amaranthus	*cruentus*	*Amaranthus cruenthus*				ex Arusha	

Genus	Specie	Cultivar / Common Name(s)	Reference on container	Additional Reference	Comments	Source/Origin	Person / Institution
Vigna	*unguiculata*	Vegetable Cowpea Fahari				ex Arusha	
Vigna	*unguiculata*	Vegetable Cowpea Bakawa				ex Arusha	
Vigna	*unguiculata*	Veg Cowpea Vuli				ex Arusha	
Vigna	*unguiculata*	Veg cowpea 1				ex Kenya	
Vigna	*unguiculata*	Veg cowpea 2				ex Kenya	
Vigna	*subterranea*		Jugo beans (Bambara)				

3.4.2 Seeds collected by University of Zululand

Fourteen cowpea lines were collected from different areas in KwaZulu-Natal and Mpumalanga (Empangeni, Mahlabathini, Pietermaritzburg and Nkomazi area). Seeds that originally came from Ghana, Mozambique, Nigeria and Zimbabwe were also collected. The seed varied in size, colour and texture (Table 3.4.2). Some of the seed colours that were collected were white, brown, spotted brown, light brown, dark brown and reddish brown. The size of the hilum in the white coloured seeds also varied.

Table 3.4.2 *Vigna unguiculata* seed collected by the personnel of the University of Zululand

Seed source	Seed size	Skin texture	Seed colour	Hilum size
Mozambique	Large	Very smooth	Spotted brown	-
Mozambique	Small	Very smooth	White	-
Empangeni	Medium	Very smooth	Grey	-
Empangeni	Large	Very smooth	Reddish brown	-
Mahlabathini	Large	Very smooth	Brown	-
Mahlabathini	Medium	Very smooth	Mixed (brown, grey & black)	-
McDonalds	Medium	Very smooth	Mixed (brown, grey & black)	-
Portugal	Medium	Smooth	White	Large, black
Ghana	Medium	Smooth	White	Large, black
Nigeria	Medium	Smooth	White	Large, black

Seed source	Seed size	Skin texture	Seed colour	Hilum size
Zimbabwe	Medium	Smooth	White	Large, black
Nigeria	Large	Rough	Brown	-
Ghana	Medium	Smooth	White	-
Nigeria	Small	Rough	Brown	-
Mpembeni, Hlabisa district	Medium	Smooth	Dark brown	-
Okhalulenì I, Ingwavuma ward	Small	Smooth	Brown	-
Okhalweni II, Ingwavume district	Medium	Smooth	Mixed-red, black & maroon	-
Nibela, Hluhluwe area	medium	Smooth	Mixed-light brown, black & brown	-

Twenty-four lots of bambara groundnut were collected from Ingwavuma District, KwaNgwanase (KwaMusa and Manguzi), Empangeni and Komatiport (Table 3.4.3). The predominant colours of the seeds that were collected were red and brown. Black was a rare colour in the seed collections and only available in limited quantities. The black seeds were only obtained from KwaNgwanase I & II, Komatiport and Empangeni II. The majority of the collected lines had normal hilum (eye) size, while a few that were collected in Empangeni I and KwaNgwanase II had larger hilums. One distinguishing feature in bambara groundnut is the colour around the hilum. It is a very common trait in bambara that the colour around the hilum is usually different from the colour of the other parts of the seed coat. The most common colour is black. The size or spread of the colour vary from thin to broad just as in black-eyed beans.

Table 3.4.3 *Vigna subterranea* seeds collected from various sources in KwaZulu-Natal.

Seed source	Seed colour	Seed size	Eye (Hilum) size	Colour around the eye
Empangeni I	Red	Varied	Normal	Black
	Brown	Medium	Normal	Deep brown
	Light brown	Big	Big	Black
Empangeni II	Red	Medium	Normal	Red
	Brown	Varied	Normal	Black, brown
	Light brown	Medium	Normal	Black, light brown

Seed source	Seed colour	Seed size	Eye (Hilum) size	Colour around the eye
	Black	Varied	Normal	Black
Ingwavuma II	Red	Varied	Normal	Black, red
	Brown	Varied	Big	Black, brown
	Light brown	Varied	Normal	Black
Ingwavuma I	Red	Varied	Big	Black, red
(Okhalweni area)	Brown	Varied	Normal	Black, brown
	Light brown	Varied	Normal	Black, blackish, red, deep red
KwaNgwanase I	Red	Small	Normal	Black
(KwaMusa)	Brown	Medium	Normal	Black
	Light brown	Medium	Normal	Dark brown
	Black	Varied	Normal	Black
KwaNgwanase II	Red	Varied	Big	Black & light red
(Manguzi)	Light brown	Varied	Big	Black
	Black	Varied	Normal	Black
Komatiport	Red	Varied	Normal	Red
	Brown	Big	Normal	Deep brown
	Light brown	Varied	Big	Black
	Black	Varied	Normal	Black
Nkomazi area	Light brown	Large	Normal	
	Black	Large	Normal	
	White with black spots	Large	Normal	
	Red	Large	Normal	
Kwa-Ngwanase	Light brown		Normal	
Kwa-Ngwanase	Mixed (black, brown, light brown and red)	Varied	Normal	

The personnel of University of Zululand collected three amaranth accessions from the KwaZulu Natal area and obtained four species from Nigeria (Table 3.4.4). The amaranth germplasm that was collected was mainly of grain amaranth (*A. hypochondriacus*), as one of the limitations is that the crop is collected from the wild where it is growing as a weed on cultivated and uncultivated lands. The grain amaranths vary in seed colour from cream, to light brown or a mixture of cream and black seeds

Table 3.4.4 Amaranth seed collected from various sources by University of Zululand

Seed name	Seed source	Seed colour
Amaranthus sp. var. McDonalds	McDonalds seed company Pietermaritzburg	Pink seed
Indigenous I and II	Gardens in Mtubatuba	Black seed
A. hypochondriacus (AH84/4463)	NIHORT, Nigeria	Cream
A. hypochondriacus (DKA 98 – 2)	NIHORT, Nigeria	Mixed cream & cream, black Few black seeds
A. hypochondriacus (DKA 98-2) NH84/444	NIHORT, Nigeria	Mix cream & black Cream with scanty black seeds
A. cruentus	NIHORT, Nigeria	Black seeds

3.4.3 Germplasm obtained from other genebanks

The cowpea lines Chappy, CH14 and Encore were obtained from the ARC genebanks at Roodeplaat and the cowpea line IT93K129-4 was obtained from the ARC genebank at Potchefstroom. Dr.B.B. Singh of the IITA, in Nigeria, supplied the lines that are used as drought tolerant and susceptible control lines (IT96D-602 and TVu7778) as well as other drought tolerant lines such as IT97K-608-14. Some drought tolerant lines were also obtained from Prof. T. Hall at the Dept. of Botany and Plant Sciences, University of California (00-11-161, 00-11-145) (Table 2.1).

The majority of the amaranth species were obtained from NIHORT in Nigeria (Table 3.4.5).

Table 3.4.5 *Amaranthus* seeds obtained from other genebanks

Cultivar	Seed Colour	Source
A . hypochondriacus	Cream	ARC
A. hypochondriacus (JM 94/18)	Cream	NIHORT, Nigeria
A. hypochondriacus (NH84/441-2)	Cream	NIHORT, Nigeria
A. hypochondriacus (NH84/190-1)	Cream	NIHORT, Nigeria
A. hypochondriacus (NH8445/190)	Light brown	NIHORT, Nigeria

3.5 On-farm Trials and Community Evaluation

3.5.1 Trials conducted by SRL unit at ARC-Roodeplaat

Thirteen lines of upright cowpeas were planted at the ARC-Roodeplaat and harvesting of the pods took place during March 1999. An international IITA line IT85F2687 produced the highest pod and seed yield (Table 3.5.1) and one of the local lines PAN 311 produced the second highest yield. The lowest pod yield was produced by an IITA line, IT93K452-1 and the lowest seed yield was produced by the IITA line IT88D876-11. The other two local lines evaluated in this trial, PAN 326 and Glenda respectively obtained 9th and 11th place out of 13 lines. Overall the international IITA lines produced higher yields than the local lines. The line IT93K129-4 obtained a 6th place out of 13 lines. These results indicate that this line produced relatively high yields under the local conditions.

Table 3.5.1 Total pod and seed production of 13 erect cowpea lines evaluated at ARC-Roodeplaat (Kg/plot).

	Line	Total pod mass	Total seed mass	Position
1	IT 85F2687	9.69	6.87	1
2	IT 88D876-11	3.58	2.24	12
3	Glenda	3.28	2.41	11
4	IT 93K2046-1	7.79	5.49	4
5	IT 93K637-1	5.61	4.23	8
6	IT 93K2046-2	6.12	4.72	5
7	IT 93K129-4	6.41	4.70	6
8	PAN 326	5.60	4.12	9
9	PAN 311	8.63	6.19	2
10	IT 93K370	4.43	3.01	10
11	IT 90K59	8.58	5.76	3
12	IT 93K 513-2	6.64	4.58	7
13	IT93K452-1	2.92	2.30	13

The following diseases were observed in the field during the 1998/1999 growing season: *Alternaria cassiae* on leaves and stems, *Colletotrichum* sp. on stems and *Pseudomonas* sp. and *Xanthamonas* sp. on leaves

Bambara

Bambara groundnut seed were propagated on the experimental farm at Roodeplaat to alleviate the problem of seed availability. From the plants that were planted in the field at Roodeplaat approximately 2.25 kg seeds were harvested. These seeds were used for all the subsequent trials and also for planting in the communities.

Amaranthus

The communities in rural areas are used to harvest *Amaranthus* from the fields. It was therefore necessary to convince the farmers that amaranth can also be cultivated as a crop. The framers needed to be trained in the cultivation practices and had to establish a market for the crops. The result of the trials looks promising seeing that most of the farmers accepted the crop. They identified potential markets in other members of their community, hawkers and in restaurants. The crop was sold either as fresh leaves, dried leaves or as cookies.

Usage of amaranthus

Throughout Africa, the tender leaves or young shoots, and often the flowers of amaranth are eaten boiled as a potherb, tasty relish, stew or side dish. The picked leaves are normally washed and cooked in water with salt and sometimes bicarbonate of soda. Cooking times vary from a few minutes to up to two hours, depending on specie or cultivar. The cooked leaves are eaten on their own or are mixed into a relish with fried tomatoes and onions, minced peanuts or peanut butter. The cooked leaves, "morogo or imfino", are then eaten with a stiff porridge or "styfpap". Fresh leaves are used as ingredients in other mashed foods, and dried leaves are ground and incorporated in weaning foods. The leaves of some species are rather bitter, and for this reason are cooked with other leafy vegetables such as cowpea and nightshade (*Solanum nigrum* L.). To reduce the bitterness, milk is added to the boiled leaves, and the mixture is normally left overnight in a cooking pot, or pounded groundnuts are added to dishes to enhance the flavour. The high fibre contends of the leaves enables them to be dried and stored. The leaves are cooked, blanched and made into smaller balls and dried in the sun. These balls can be stored for more than 6 months, and are reconstituted by soaking in water before being used in cooking. Leaves are not always blanched; however the blanching process plays a roll in the retention of nutrients. Steam blanching followed by

dehydration will be the most effective to retain ascorbic acid (vitamin C). Whole leaves are also dried in sun to semi-shade and these dried leaves are stored in a well-ventilated place and used in seasons when the leafy vegetables are not available.

Economic value of leafy amaranth

Amaranthus plants, both as cultivated crop and harvested from the field, have the potential to play an important role in the maintenance of food security in households in rural areas. The main aim of promoting indigenous crops is to demonstrate to rural people and policy makers the positive contributions of woodland resources to rural livelihoods and the local economy. Understanding the importance of the protection and promotion our indigenous crops may in a way minimize the huge destruction of the natural vegetation for agricultural activities, industries and other land uses.

3.5.2 On-farm trials conducted by University of Zululand

Cowpeas, bambara groundnuts and amaranth were planted in the experimental plots at the University of Zululand as well as in pots in the greenhouse. Some woodenbox trials were also conducted.

Cowpea plants in the field

Despite the relatively low temperature in spring, good seed germination and plant growth was observed. The five cultivars used however differ in their vegetative growth patern. The Nigeria honey bean was a relatively tall variety but produced fewer leaves (Table 3.5.2). The Ghana white cultivar which had relatively long and big pods, but it produced the lowest number of pods per plant. These plants also matured late. At 13 weeks after planting, 50 percent of pods produced by Nigeria white and Zimbabwe white were brown indicating maturity whereas pods produced by Ghana white were still green and growing actively. The Nigeria honey bean showed intermediate maturity and the pods were carried in a relatively more erect position, well above the leaves compared to other varieties. At 13 weeks after planting, the crop pods were already light green and a few were turning brown. The strategic location of pods in Nigeria honey bean could allow for mechanical harvesting of the crop.

Table 3.5.2 Growth performance of five selected cowpea lines at 13 weeks after planting.

Cultivar	Mean plant height (cm)	Mean leaf number	Mean pod number	Mean pod length (cm)	Seed mean per pod	Mean 1000 seed weight (g)
Ghana white	86.83	30.18	9.28	17.96	13.30	242.01
Nigeria drum	77.67	29.75	9.83	16.08	10.00	176.71
Nigeria honey bean	116.70	12.58	14.58	12.59	10.30	126.43
Nigeria white	69.83	30.00	17.67	16.22	10.30	202.73
Zimbabwe white	75.42	34.33	15.67	15.83	10.30	202.79

Woodenbox

When the drought tolerance of the cowpea seedlings in the woodenboxes was compared the linesTVu7778, Pan311 and IT93K734 showed the first signs of stress. After 13 days without water these plants started to wilt while CH14, Mahlabathini brown, Empangeni brown and IT96D-711 were still looking fine. When the most susceptible lines were either dead or severely wilted the boxes were rewatered and the recovery was noted. The lines that recovered well were: CH 14, Mahlabathini brown, Empangeni brown, IT96D-711, IT93K129-4, Nigeria brown, Portugal, Zimbabwe and Ghana. The lines from which only a few plants or no plants recovered at all, were: TVu7778, IT90K59, IT92K258-9, PAN311, IT93K734, IT93K596-9-12, IT82D849 and Renoster.

Taste evaluation of cowpea

Cowpea seeds of two lines (Nigeria brown and Zimbabwe) were prepared by cooking them with maize. Local farmers who are familiar with the taste of the indigenous Zulu cowpeas and also dry beans were asked to evaluate the crop. The participants preferred the taste of the two above mentioned cowpea lines to that of the indigenous cowpea and also the taste of the popular sugar bean (*Phaseolus vulgaris*).

Bambara groundnuts

Intercropping with maize

Weed intensity and frequency of weeding was reduced in bambara groundnut plants that were intercropped with maize plants opposed to the sole crops. Moreover, the maize crop could be harvested in January, while the bambara groundnuts were still in the field. Hence, intercropping had the advantage over the mono cropping that the income as well as the food supply could be spread over time. This method also helped to compensate for the long waiting period required for the bambara groundnut to mature.

Due to heavy rainfall the yield of the crops was limited. One of the problems experienced with bambara groundnuts is the length of time between flowering and the ripening of the seeds. If the soil stays too moist due to heavy rainfall or dew the older seeds will either germinate or start to rot before the younger seeds had ripened. This problem has also been encountered with the bambara groundnuts that were planted at Stellenbosch.

The problem with the production of enough seeds will be solved if more farmers grow the crop. Our responsibility will be to provide the market with drought tolerant cultivars that can produce a good crop even under adverse conditions.

Although no culinary experiments were conducted with bambara groundnuts, some seeds were prepared and farmers that attended a farmers day at the University were able to taste the prepared food.

Amaranthus

Organic fertiliser

The number of leaves were counted and the height of the plants was measured for each plant per block and the average was calculated. The application of poultry manure resulted in significantly higher number of leaves as compared to the plants that were fertilised with kraal manure and the plant that did not receive any fertiliser. After four to five weeks there was an increase in the number of leaves of the control plants and the plants that were fertilized with kraal manure but it was still lower than the plants that received the poultry manure. The

plants that were fertilised with the poultry manure also produced the tallest plants, followed by the plants treated with kraal manure and the control treatment. As expected, those plants fertilised with manure grew far better than the control. In the light of these results, poultry manure could be a very attractive fertiliser for small-scale farmers who grow indigenous crops like *Amaranthus*.

Chemical fertilizers

The Amaranthus plants that were treated with NPK produced significantly taller plants with bigger leaves than the plants treated with Superphosphate or the control plants. The colour of the leaves was also darker green than those of the Superphosphate treatment and the control. Some of the leaves of the plants treated with Superphosphate showed a pale green colour, which might to be caused by a lack of nitrogen.

The results obtained in this trial indicated that the application of NPK in Amaranthus would contribute to a good yield. The green colour of the leaves of plants treated with NPK would also lead to a better product for the market. The pale colour in plants treated with Superphosphate and the control treatment indicates an imbalance of the nutrients in the soil, thus it is important to have well-balanced fertilization for the Amaranthus plants. The results showed clearly that the quality and yield of the Amaranthus could be improved by using NPK fertilizer.

Taste evaluation of amaranth

The *Amaranthus* plants that were cultivated for the taste panel were *A. hypochondriacus*, *A. tricolor* and the cultivar Callaloo. The farmers preferred to grow the specie *A. hypochondriacus* because these plants produced more leaves than the other two.

During the culinary trials leaves of these plants were prepared as a basic recipe by boiling the leaves or a modified recipe where cheese, onions or nuts were added. The members of the tasting panel all preferred the modified versions of the cooked *Amaranthus* and said the basic recipes were bland. The modified version of Callaloo was ranked first by 90% of the panellists. Some of the panellists said it was the best relish they ever tasted and they could

even prepare it at their parties. The *A. hypochondriacus* was ranked second and most people liked it because of the cheese that was added. The panellists agreed that the three modified versions of the *Amaranthus* tasted very differently from their basics recipes.

3.5.3 Trials conducted by SRL unit in various Communities

The project started with two demonstration trials in Soshanguve and Bronkhorstspruit in 1999 and was extended in 2002 to twenty new farmers plots all around Gauteng. Previously the farmers used to plant various types of vegetables. Three farmers opted to plant sugar beans rather than cowpeas. The reason for this was that the suitable cowpea seeds were not available and that sugar beans are also popular on the markets. Due to the drought and unstable economic situation in Zimbabwe the local markets are also experiencing a shortage of seeds. One farmer elected to plant soybeans on a large scale. His yield was more than one ton per hectare and the soybean price is still better than the maize price. Soybeans might be a good choice under normal rainfall conditions but this crop cannot compare to cowpeas in terms of drought tolerance.

Cowpeas are one of the most popular crops in the communities and most of the rural farmers plant the crop for their own use as well as for marketing purposes. The rural farmers make a living out of mixed cropping, but the urban farmers cannot make a living purely out of vegetable production. The land available for farming is too small and the farmers must either use more intensive farming methods or form consortiums. This will enable them to concentrate on only one crop per season but the different members of the consortium can offer the hawkers a whole range of vegetables.

Table 3.5.3 gives some information on the feedback received from six farmers that took part in trials performed in 2001. The biggest limitation for most of the farmers was the absence of a reliable market.

Table 3.5.3 Feedback on the three crops included in this study by small-scale farmers in the Gauteng, Mpumalanga area.

Farmer	Crops	Advantage	Constrains
Mr. Mosala	*Amaranthus*, bambara groundnut, cowpea plus other vegetables	Good market for cowpea and bambara groundnut	Limited market for *Amaranthus*
Mrs. Chisale	*Amaranthus*, bambara groundnut, cowpea plus other vegetables	Owns a tuck shop, also sells at produce church market. Sold all the material that was produced	Limited market for cowpeas
Mrs. Chabalala	*Amaranthus*, bambara groundnut, cowpea	Sold crops from the field – might benefit from value adding	Small market – needs to be extended. Lack of co-operation between farmers
Mr. Mpoza	*Amaranthus*, bambara groundnut, cowpea	Obtained a good harvest from *Amaranthus*	Inconsistent market – needs more stable marketing channels.
Mr. Cele	*Amaranthus*, bambara groundnut, cowpea	Sold all the cowpeas and bambaras that were produced	Clients prefer *A. hybridus* not the red *Amaranthus* and fibrous types.
Mr. Mahlangu Tweefontein, Pta.	*Amaranthus*, bambara groundnut, cowpea	Does not know the crops but is willing to try	Needs training in crop cultivation

The cowpea cultivar "Betsuana White" is currently still the preferred cultivar. Although there are better-yielding cultivars the farmers have trust in Betsuana White and prefer to plant it. It is a runner type and the leaves can be consumed as well as the seeds. Most of the farmers prefer the runner types as they covers the planting area and weeding is not necessary, which can be costly because it must be done by hand. The upright types are more viable for the smaller areas but the disadvantage is that there are too few leaves to consume while the farmer is waiting for the beans to mature. Otherwise leaves were harvested until the onset of flowering. The young leaves are picked in the morning and left to wilt until the next morning and then cooked, left to cool down and then pressed into cookies to dry in the sun for winter consumption. On the cowpea cultivar "Renoster" three diseases were identified, namely *Alternaria cassiae*, *Pseudomonas* spp and *Xantomonas* spp.

The *Amaranthus* plants cultivated in the Gauteng, Mpumalanga and Northern Province areas were especially well received by customers from Indian origin. This might be the case because these people are familiar with the crop and they know how to prepare it. The

indigenous crops on the other hand were sometimes not as well received by the market because these crops are not as well known. This is an area that must be exploited seeing that these crops once were well known and cowpeas were even regarded as staple food approximately 60 years ago.

Bambara groundnuts are a highly sought after as a crop but the availability of seeds still is a limiting factor. Seed availability is a big problem. Many dealers import their stock from Zimbabwe and Mozambique, with a result that the local market is having problems to provide enough seeds, because of the drought and political conditions experienced in above mentioned countries.

The other constraint is the seed size. The rural and urban farmers first fulfil the needs of the household and then look after the market. The farmers select the big seeds for household consumption, keep some seed for next season's plantings, and the rest will be sold. This can be detrimental to the development of the crop since it has been shown that the plants that develop from the bigger seeds develop much faster and have a better chance to produce a good crop.

Another problem for bambara groundnut cultivation is the harvesting and shelling of the crop. It can be very expensive to harvest by hand because of high labour cost. To harvest the crop by hand is also very time consuming and the tendency is now to sell the crop still in the shell. This lowers the price of the product seeing that the consumer still have to do the shelling before it can be consumed. A low cost harvester will provide the farmers with a method to add value to the crop that will increase the household income.

The long growth season of the crop also makes it unpopular for cultivation by the urban farmers. They can grow more profitable crops in the same area and it will provide them with at least 2 - 3 harvest in the same time span. One farmer who planted bambara groundnut in the Gauteng project covered about 4000m^2 under dry land conditions and he managed to sell the seeds unshelled for R30 per 10 litre bowl. He also planted some ordinary groundnuts and

the price obtained for this product was slightly higher per 10 litre bowl than for the bambara groundnuts.

Bambara groundnuts were well received in some areas. Not all consumers knew the crop and this aspect would need attention if the market were to be extended. The marketing potential was bigger in areas where more people from countries outside S.A. live. The availability of enough seeds still suppresses the development of this crop.

3.5.4 Trials conducted by Zululand University in various Communities

The results obtained by the 5 families that grew the experimental crops are listed in table 3.5.4. Each crop will be discussed separately.

Table 3.5.4 Feedback on the cultivation of the three crops included in this study by small-scale farmers in the KwaZulu-Natal area

Households	Crops	Advantage	Constrains
Mdletshe	*Amaranthus*, bambara groundnut, cowpea	Good *Amaranthus* harvest	Low yield from cowpeas; no seeds from bambara groundnuts
Mthembu	*Amaranthus*, bambara groundnut, cowpea	Good *Amaranthus* and cowpea harvest	No seeds from bambara groundnuts
Khumalo	*Amaranthus*, bambara groundnut, cowpea	Good *Amaranthus* harvest	Low yield from cowpeas; no seeds from bambara groundnuts
Mngomezulu	*Amaranthus*, bambara groundnut, cowpea	Good *Amaranthus* harvest	Low yield from cowpeas; no seeds from bambara groundnuts
Mkhwanazi	*Amaranthus*, bambara groundnut, cowpea	Good *Amaranthus* harvest Bambara groundnuts did produce some seeds	Low yield from cowpeas

Cowpea

The cowpea line, Phelandaba needed a longer time than the other two varieties to reach maturity. Its creeping characteristic shows that it is a good competitor for nutrients, water and space and can overpower the weeds, which meant that weeding was not necessary. Phelandaba took eleven weeks from planting to flowering.

The drought tolerant control line IT96D–602 was favoured above Phelandaba because it can withstand drought and can compete with other crops. It is very good for inter-cropping with maize and other summer growing grains. It is a fast growing crop and its upright growth reduces unnecessary competition with other crops. It took six weeks to flower after planting. The seeds were harvested after ten weeks. IT93K129-4 is not such a fierce competitor as Phelandaba and therefore weeds should be controlled. It took six weeks to flower after planting. The plants were harvested after twelve weeks.

Bambara Groundnuts

Bambaras should be grown in dry areas with lower humidity especially if water is available to establish the crop. The farmers believe it must be planted on red Hutton soils and on sandy soil. The plants took seven weeks to flower after planting. Bambara germinated and grew very well but no seeds were obtained except for Mkhwanazi plot. 125g seeds were harvested from the plants of the Mkhwanazi household. These plants were harvested sixteen weeks after planting.

Amaranthus

The three varieties were identified according to colour, leaves and size of the leaves. *A. tricolour* has large yellowish, brownish and green leaves. It is very palatable and grows very well. *A. hypochondriacus* have smaller green leaves, very palatable and good smell. It is the variety that grows well. Callaloo have brownish intermediate leaves that grow very well.

A. hypochondriacus produced almost double the number of leaves as compared to Callaloo and *A. tricolour*. The community preferred hypochondriacus because of the large number of leaves produced as well as the good taste of the leaves. Callaloo produced the tallest plants though the difference with other two varieties was not significant.

The farmers were really enthusiastic about the crops and are willing to grow it as it would constitute more to the family food security The major limiting factor voiced by farmers was that of seed scarcity.

Trails conducted in the community garden at Empangeni

Seeds of five cultivars of amaranth *A. cruentus*, *A. hybridus*, *A. hypochoricus*, *Amaranthus* sp. "McDonalds" *and A. tricolour* were sown in the community garden at Empangeni in September, 1999. The planted cultivars of amaranth were observed to be well adapted to the Northern KwaZulu-Natal environment as good seed germination was observed in all the cultivars in spring except for *A tricolor*. Hence the latter had fewer stands per replicate compared to the other cultivars. It was also observed that *A. tricolor* was less adaptable to the field conditions and was highly susceptible to insect damage.

The five cultivars differ in height, leaf colour and the shape of the inflorescence. *A. cruentus* is a relatively tall cultivar with red (showy) inflorescence. Other tall cultivars are *A. hybridus* and *A. hypochondriacus*. Although *Amaranthus* sp. "McDonalds" is a tall variety, it has slender stems. Consequently, the stems bend over when the plant grows taller than 30 cm. The leaves are pale green and the pendulous inflorescence is cream coloured. The fifth cultivar *A. tricolor* is comparatively shorter but has a sturdy stem that promotes upright plant growth (table 3.5.5).

The differences observed in the five cultivars with regard to the leaf production were not significant even though *Amaranthus* sp. "McDonalds" had comparatively fewer leaves than other species. With regard to the harvestable weight of the amaranth, the highest mean shoot fresh weight per plot at 6 weeks after planting (2.56 kg) was observed in *A. cruentus*, compared to 1.79 kg observed in *Amaranthus* sp. "McDonalds". In case of *A. tricolor* the leaves were not harvested because of the uneven seed germination.

Table 3.5.5 Plant height and shoot production of five amaranth lines planted in the community garden at Empangeni.

Name	Plant height 10 weeks after planting (cm)	Mean shoot weight (Kg)
A. tricolor	32.58	Not harvested
A. hybridus	91.95	2.50
A. cruentus	91.88	2.56
A. hypochondriacus	69.08	2.37
A. sp. "McDonalds"	78.95	1.79

Community Evaluation

Problems: The major problem associated with the evaluation of the crop by farmers was organizational problems. The seeds that were sown germinated and had good vegetative growth but later the crops were abandoned due to the fact that the cooperative disintegrated for some time.

The experimental plot was flooded during heavy rainfall and the crop plants became water logged. The high humidity prevented the seeds from drying and a lot of seeds were lost due to fungal infection.

Uses: Vegetable amaranth is highly sought after by the Zululand community. It is popularly used for soup and goes along with stiff pap, and 'uphuthu'. Apart from the above-mentioned, vegetable amaranth can be used in salad, and as protein supplement in rice, potato porridge (sweet or Irish) dishes.

Acceptability: Through communication with the community farmers, women at the grocery section of supermarkets and colleagues at work, both men and women regard amaranth 'imifino' or 'imbuya' as a delicacy. It is more preferred than Swiss chard.

Farmers' Perception of amaranth:

The farmers preferred the amaranths to the cowpeas. The explanation for this trend might lie in the fact that *Amaranthus* has long been a favourite vegetable. The availability of seeds as well as the germination of the seeds is still a point of concern.

Chapter 4

Conclusion

4.1 Cowpea

Cowpeas have long been regarded as staple food all over Africa, especially in the North Western part of the continent. This crop is normally cultivated in summer rainfall areas where the pods are harvested at the onset of the dry season. The aim of this project was to determine if it was possible to make a distinction between the levels of drought tolerance in different cowpea lines and compare the levels of drought tolerance of the cowpea lines from the IITA with the indigenous lines planted in the communities in South Africa. Some cowpea lines have also been obtained from the University of California for the same purpose.

Twenty six cowpea lines were subjected to different physiological screening methods as well as phenotypical screening in the greenhouse. Field trials were also conducted with some of these lines on the experimental farms of the University of Zululand and at ARC-Roodeplaat. A few of the selected lines were also planted in the communities in KwaZulu-Natal, Gauteng, Northern Province and Mpumalanga.

The reliability of the screening tests was determined during the first two seasons of screening, by comparing selected drought tolerant and drought susceptible cowpea lines that were obtained from the IITA. Lines from other African countries as well as seeds that were collected from the communities in KwaZulu-Natal, Gauteng and Mpumalanga were also included in the drought screening from the third season onward (Table 4.1). In the first two seasons the drought tolerant control line IT96D-602 proved to be the most tolerant. In the third season the line, Okhaluleni proved to be most tolerant but not in juvenile phase. IT96D-602 was ranked 3rd in this season, with the Ghana black eyed bean 2nd. In the fourth season the line from the IITA, IT97K608-14 was the most drought tolerant, followed by IT96D-602 and Manguzi 1 BS and Manguzi 2 Red. In the fifth season when some the toughest lines were compared, Okhaluleni again proved to be even more tolerant than IT97K-608-14 and IT96D-602 which were ranked 2nd and 3rd.

Table 4.1 Cowpea lines ranked according to different physiological screening methods as well as greenhouse measurements.

	Chlorophyll Fluorescence	Proline	RWC	Total yield	Wooden box recovery	Total rating
First and second season						
IT96D-602	1	1	1	3	1	1
IT92K258-9	3	2	2	4	2	2
IT90K59	3	2	3	1	3	3
Chappy	2	2	5	5	4	5
TVu7778	5	2	4	2	5	4
Third season						
IT96D-602	6	2	1	5	4	3
Okhalweni area	3	6	4	6	7	6
Ghana black eyed bean	8	1	3	4	1	2
Encore	5	5	2	2	5	3
Nigeria brown drum bean	9	5	2	9	3	7
Zimbabwe black eyed bean	7	6	4	3	2	4
Hluhluwa area	4	3	4	8	6	5
Mpenbeni ward	1	4	2	7	8	4
Okhaluleni area	2	1	1	1	9	1
Fourth season						
IT96D-602	5	3	2	1	n.r.	2
Manguzi 1 BS	2	1	1	7	n.r.	2
Manguzi 2 Red	5	1	1	4	n.r.	2
Phelandaba	7	2	1	8	n.r.	6
IT97K209-4	3	4	2	3	n.r.	3
IT97K-338-7	4	4	3	6	n.r.	5
IT97U-819-118	7	5	2	2	n.r.	4
IT93K-452-1	8	4	1	9	n.r.	7
IT97K-608-14	1	1	1	5	n.r.	1

	Chlorophyll Fluorescence	Proline	RWC	Yield	Wooden box	Total rating
Fifth season						
IT96D-602	8	1	1	5	n.r.	3
Phelandaba	1	1	4	9	n.r.	3
Okhaluleni	2	1	2	1	n.r.	1
IT97K-608-14	7	2	3	2	n.r.	2
IT97K-499-38	4	1	2	4	n.r.	5
IT93K129-4	5	4	1	6	n.r.	4
M 217	6	5	4	3	n.r.	6
00-11-161	3	5	5	8	n.r.	7
00-11-145	9	3	5	7	n.r.	8

1 = most tolerant; 9 = less tolerant; n.r. = not recorded

The exceptional performance of the community lines Okhaluleni, Phelandaba, Manguzi and Ghana black eyed against the drought selected lines of IITA and California University shows the enormous potential of the indigenous South African germplasm. Seed of these lines must be multiplied and tested under different climatic conditions and in different communities.

The feedback received from the farming communities in KwaZulu-Natal was that the farmers preferred the upright cowpea line IT96D-602 to the local, twining type Phelandaba. The advantage of the Nigerian line was the shorter growth cycle as well as high levels of drought tolerance. Phelandaba on the other hand has a more twining growth form, which means that weeding was not necessary. The leaves of these plants could also be harvested over a longer period. The farmers did however complain about the low yield of the cowpeas as only one of the farmers that took part in the trial produced a good yield. If cowpeas are to be planted in KwaZulu-Natal the timing of the planting seems to be crucial and the crop has to be planted in such a way that the seeds will ripen and mature in the driest time of the year. Alternatively, other methods to dry the pods should be investigated but this technology is normally not available to the small scale farmers. New methods must be developed and the

technology must be transferred to the communities. Follow-up visits will be necessary to see if the implementation was successful.

The farmers in the Gauteng and Mpumalanga areas on the other hand produced good yields and found the crop well suited for the local markets. They did however identify the inconsistent market situation as a constraining factor in the building of a stable income. The availability of suitable cultivars that are selected for the farmers' needs and the environmental conditions were also identified as constraining factors. The SRL unit of ARC-Roodeplaat can play a role in fulfilling this need.

The results of the trials planted on the experimental farms confirm the suitability of these crops for the summer rainfall areas. It also became apparent that IITA has produced a lot of high yielding cowpea lines, which seem to be well adapted to our climate. The introduction of these plants in our communities might prove to be beneficial to the small scale farmers. Some of the lines like IT93K129-4 and Nigerian honey bean, which bears the pods above the plant canopy, are also suitable for mechanical harvesting and these plants can be recommended to farmers that have access to mechanical harvesters.

The development of cowpeas as a cash crop instead of a crop for food security might fill a niche for small scale farmers especially seeing that the industry is looking at alternative beans for the canning industry.

4.2 Bambara groundnut

Bambara groundnuts are still being regarded as a neglected crop and although a lot of people in rural areas of Africa rely on this crop for food security the potential of this crop is still largely unexploited. The aim of this study was to determine the levels of drought tolerance in this specie and to compare the known bambara groundnut lines with the plant material that was collected from the communities is South Africa.

The status of the bambara groundnuts lines in terms of drought tolerance has not been determined before this study and no reference lines were available. The lines that performed best in the first season were SB7-1 and SB1-1, with SB1-1 and MAD1 leading in the second season. In the third season, SB9-1 and SB20-2A had the highest ranking with SB1-1 in the second place, while NB and KwaHgnase were ranked first and second in the fourth season. In the fourth season SB9-1 produced the highest yield, although its drought ranking was 3rd. This is in comparison to the 3rd season when the drought ranking of SB9-1 was 1st, but the yield was low (5th). The line SB1-1 consistently produced good yields throughout the drought experiments, which shows stability under drought conditions (Table 4.2). This is an extremely important characteristic seeing that bambara groundnuts normally experience a dramatic drop in productivity under stress conditions.

Just like cowpeas, bambara groundnuts also use good water management practice to survive adverse conditions. The price for this strategy is measured in terms yield loss. The selection of high yielding plants under stress conditions can make a huge contribution to the development of this crop. In bambara groundnuts, proline accumulation is an indication of the levels of stress experienced by the plants and the plants that starts to accumulate proline at an earlier stage is regarded as susceptible to drought. The advantage of this screening method is that large numbers of plants can be screened in a relative short period and because freeze dried plant material is used to determine the proline content, the experiment is not dependent on the survival of the plants or the growth season.

The chlorophyll fluorescence measurements give an indication of how well the photosynthetic systems of a plant is still functioning under stress conditions. This however does not give any indication of the yield of the plants. In the fourth year, for instance the line Swazi V5B had the highest ranking in terms of the chlorophyll fluorescence and was looking very good at the end of the stress period. Its yield was however very low and this line was overall ranked third. It might therefore be useful when ranking these plants to put more emphasis on a criterion like yield, which is the most important reason for planting this crop.

Table 4.2 Ranking of the bambara groundnut lines according to different physiological screening tests, woodenbox screening at the seedling stage as well as the yield.

	Chlorophyll Fluorescence	Proline	RWC	Yield	Wooden box	Total rating
First season						
SB1-1	2	3	5	2	1	2
SB7-1	1	1	1	1	2	1
SB9-1	5	5	4	4	4	5
SB20-1	2	2	2	3	5	3
MAD-1	2	4	3	5	3	4
Second seasons						
SB1-1	2	2	1	1	n.r.	1
SB7-1	5	4	1	3	n.r.	3
SB9-1	3	3	2	5	n.r.	4
SB20-1	3	5	3	2	n.r.	4
MAD-1	1	1	2	4	n.r.	2
Third season						
SB1-1	2	6	4	1	n.r.	2
SB8-1 (4)	5	4	2	7	n.r.	5
Swazi V5B (6)	5	5	3	4	n.r.	3
SB20-2A (9)	3	2	1	5	n.r.	1
AS 18 (11)	5	7	4	1	n.r.	4
AS 17 (13)	4	8	4	3	n.r.	6
SB2-1C (15)	8	1	3	8	n.r.	7
SB9-1 (16)	1	3	2	5	n.r.	1
Fourth season						
SB1-1	8	7	5	2	n.r.	5
Swazi V5B (6)	1	3	3	8	n.r.	3
AS 17 (13)	7	8	5	5	n.r.	6
SB9-1 (16)	6	2	6	1	n.r.	3
KwaHgnase	3	1	4	5	n.r.	2
NB	4	4	1	3	n.r.	1
NLB	2	6	2	5	n.r.	3
NR	5	5	5	3	n.r.	4

1 = most tolerant; 9 = most susceptible; n.r. = not recorded

Bambara groundnuts are normally cultivated in summer rainfall areas. These plants should not be exposed to too much water at the end of the growth season seeing that the seeds are borne underground. High moisture levels in the soil caused the seeds to rot or to germinate in the wrong growth season all of which leads to crop reduction. This problem was encountered on the experimental farm at the University of Zululand, in the communities in Zululand and also at the experimental farm in Stellenbosch. In all these instances, the moisture content of the soil was too high and this affected the yield negatively. The farmers in KwaZulu-Natal experienced problems with low yield and this might attributed to the high humidity. Only one farmer managed to produce a harvest.

Bambara groundnuts were however well accepted by the farmers in the Mpumalanga, Northern Province and Gauteng areas and although some of the farmers did not know the crop they were still willing to plant it and obtained good yields. Some of the farmers did experience problems to market the seeds but the majority sold all the material produced. Although some of the farmers were not familiar with the crop they were very keen to obtain seeds and also information on the cultivation of this crop. This indicates a willingness of the farmers to return to more traditional crops that are better adapted to our climatic conditions. Methods for adding value to the crop should still be investigated. Some of the possibilities include the development of inexpensive harvesters that can help with the shelling of the crop and also the development of marketing channels like the people who are selling warm cooked bambara seeds in cups on cold winter mornings.

One of the biggest problems with bambara groundnuts is still the availability of seeds. This situation was somewhat alleviated by the production of seeds on the experimental farm at Roodeplaat. This is a role that might be extended in future. Another important role of the personnel of the SRL unit is also to educate the farmers in the cultivation practices of these crops and to continue to provide support throughout the growth season.

The lines performed best during the test period; SB7-1, SB1-1, MAD1, SB9-1, SB20-2A, NB and KwaHgnase, need to be tested against each other in a rain shelter trial, in order to quantify their tolerance rating. The best lines must then be multiplied and distributed to the

community members for testing in the field. In order for bambara groundnuts to become more than just a subsistence crop there is a desperate need for agronomical and physiological research to be done. Although the ARC can provide such a service the funding of such a project is still a problem.

4.3 *Amaranthus*

One of the main reasons for the interest in this genus is the high nutritional value of this crop. Apart from the high fibre content this crop also has a very high vitamin A content. Along with these good qualities these plants also have a remarkable resilience against drought conditions. This is illustrated by the fact that some of the *Amaranthus* species are regarded as weeds. The indigenous people from Africa have long ago realised the nutritional value of this crop and even today, these plants are harvested from the field and used as an additional vegetable dish in traditional cooking. The aim of this project was to determine if *Amaranthus* species or selections with good culinary qualities, as well as high levels of drought tolerance, could be identified.

Amaranthus plants from various sources were subjected to different physiological screening methods as well as phenotypical screening under drought conditions in the greenhouse. At the same time, some of these lines were planted in the communities in Kwa-Zulu Natal and Mpumalanga and on the experimental farms of the University of Zululand and ARC-Roodeplaat.

In the end more than 20 *Amaranthus* species, selections and lines were included in the physiological screening tests. In the first year it was the species *A. tricolor* and *A. hybridus* that gave the best results. This was followed by Community 4 and *A. hypochondriacus* in the second season and Krugersdorp and Callaloo in the third. One of the locally collected lines (Indigenous I) and *A. tricolor* were ranked first and second in the fourth year and *A. hypochondriacus* and *A. candatus* in the fifth year (Table 4.3).

The material that was collected locally compared exceptional favourable with the material that is available in the rest of the world. Each season render a community selected line under the top four performers. As a result of different entrees every season, it was not possible to identify one specie or selection as most tolerant during this study. Thus, the collections that performed the best under drought conditions; Community 3 and 4, Krugersdorp, Callaloo, Imbuya and Indigenous I, must be tested against each other as well as the best species in a field trial. The best lines and species must then be multiplied and distributed to the communities for further evaluation.

Table 4.3 *Amaranthus* species and selections ranked according to different physiological screening methods as well as greenhouse measurements.

	Chlorophyll fluorescence	Proline	RWC	Leaf area	Wooden box	Total rating	Four most tolerant plants
First season							
A. albus	1	n.r.	n.r.	n.r.	1	1	*
A. hypochondriacus	3	2	n.r.	3	3	4	3
A. tricolor	2	1	n.r.	1	2	2	1
A. hybridus	4	1	n.r.	2	3	3	2
Second season							
Community 1	3	8	11	n.r.	5	9	
Community 2	12	9	10	n.r.	6	11	
Community 3	4	6	2	n.r.	6	3	3
Community 4	4	3	2	n.r.	3	2	2
Amaranthus sp. "MacDonald"	11	1	2	n.r.	8	7	
A. cruentus	4	7	6	n.r.	9	8	
A. hypochondriacus 1	1	4	5	n.r.	9	4	4
A. hypochondriacus 2	9	5	6	n.r.	1	6	
A. hypochondriacus 3	2	2	6	n.r.	1	1	1
A. hypochondriacus 4	10	10	11	n.r.	9	12	
A. tricolor	8	2	1	n.r.	9	5	
A. hybridus	4	11	9	n.r.	4	10	

	Chlorophyll fluorescence	Proline	RWC	Leaf area	Wooden box	Total rating	Four most tolerant plants
Third season							
A. cruentus (Anna)	8	6	8	5	2	7	
Ficksburg selection	6	5	3	7	4	6	
Krugersdorp selection	3	1	1	1	6	1	1
A. cruentus (Amar)	7	6	7	8	3	8	
A. hybridus (Krugersdorp)	5	4	3	1	7	4	4
Local selection	1	4	3	5	8	5	
Callaloo	4	2	3	3	1	2	2
A. tricolor	2	3	1	3	5	3	3
Fourth season							
A. tricolor I	5	5	6	9	7	8	
Indigenous I	2	2	1	1	7	1	1
Indigenous II	9	7	8	4	6	9	
A. palmeri	8	5	8	1	5	6	
A .tricolor II	3	3	3	9	3	4	4
Imbuya (red stem)	6	4	4	6	2	5	
Imbuya (green stem)	4	1	1	4	9	3	3
A. dubius	9	7	6	7	1	7	
A. tricolor III	1	3	4	3	4	2	2
A. cruentus	7	6	8	7	9	10	
Fifth season							
A. candatus (US)	7	2	1	n.r.	n.r.	4	4
A. hypochondriacus (Mexico)	3	1	2	n.r.	n.r.	2	2
A. hibridus (USA)	6	3	2	n.r.	n.r.	5	
A. cruentus (Mexico)	4	5	2	n.r.	n.r.	5	
A. candatus (India)	1	2	2	n.r.	n.r.	1	1
A. lividus (India)	2	4	2	n.r.	n.r.	3	3
A. hypochondriacus (Nepal)	5	6	2	n.r.	n.r.	7	

1 = most tolerant; 12 = most susceptible; n.r. = not recorded

* This specie was only used in two of the screening trials and was therefore not ranked with the other lines

Since the yield of the leafy amaranth is determined in terms of the number and size of the leaves as well as the condition of the leaves, measuring the chlorophyll fluorescence, RWC and the free proline concentration are very important selection criteria. All three these screening methods give an indication of how well the plants are still functioning under stress conditions.

The farmers in the in the Mpumalanga, Northern Province and Gauteng areas preferred *A. hybridus* instead of the red and fibrous types. Amaranthus was well accepted by the farmers in KwaZulu-Natal area and most of the farmers included in the study preferred planting amaranths to cowpeas or bambara groundnuts. The plants grew very well in the tropical climate of Kwa-Zulu Natal, especially in the Empangeni and Richardsbay areas. The plants are however sensitive to the day length and short days induce flowering and stop all vegetative growth. This aspect will still need further research.

The crop was also well received by the tasting panel, especially when the leaves were mixed with cheese or nuts. The marketing of *Amaranthus* as a household as well as a commercial crop will be crucial in the development of this crop. Extension officers, researchers as well as staff members at the Universities should fulfill this function.

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Appendix

Capacity building

(a) Community trials

KwaZulu-Natal community trials

Families involved in the community-farming programme

Mrs. Anastasia Khumalo, husband, 2 children and 4 grandchildren

Mrs. Ntombi Mdletshe, husband, 7 children, two grandchildren and one family assistant.

Mrs. Miriam Mkhwanazi, husband and 9 grown children

Mrs. Rota Mngomezulu, 6 children, 1 grandchild, brother in-law and his wife and their child

Mrs. Thembekile Mnqayi, husband, 4 children and 6 grandchildren

Mrs. Enedicta Mthembu, husband, 10 children and 2 grandchildren

Students that were involved in the research on-farm and at communities

Mr. N.F. Gumede	BSc (Agriculture) (Honours)
Mr. M.E. Mdamba	BSc (Agriculture) (Honours)
Mr. S.L. Ngcobo	BSc (Agriculture) (Agronomy) at third year level
Ms. K.Z. Shoyisa	BSc (Agriculture) at fourth year level
Mr. M.M. Mkhwanazi	Dip Agriculture (Intern)
Mr. M.I. Siyaya	BSc (Agriculture) (Honours)
Mr. E.S. Mthethwa	BSc (Agriculture) at fourth year level
Ms. F.N. Luhlanga	BSc (Agriculture) at fourth year level
Ms. L.E. Nxumalo	BSc (Agriculture) (Honours)

Gauteng area community trials

People involved in the community-farming programme

Mr. Mosala and the Doornkop studygroup

Mrs. Chisale, Mrs. Chabalala and the Zierbekom studygroup

Mr. Mpoza and Mr. Cele, and the Poortjie studygroup

Mr. and Mrs. Mahlangu from Tweefontein

The study groups meet once a month and consist of between ten and twenty people

(b) Degrees obtained from the results of the research

Honours degrees

Ms. L.E. Nxumalo obtained an Honours degree in Agronomy. Her topic was entitled: The effect of planting date on growth and yield of *Amaranthus hybridus* in Northern KwaZulu-Natal. University of Zululand.

Ms. I.B. Khusi obtained an Honours degree in Consumer Science. Her topic was entitled: Consumer Acceptability of the wild green *Amaranthus* varieties. University of Zululand.

PhD degrees

M.M. Slabbert. 2001. Drought tolerance in *Amaranthus* species: A study of some physiological and biochemical adaptation mechanisms. Potchefstroom University for CHE.

M.H. Spreeth. 2001. Assessing drought resistance in selected *Vigna unguiculata* lines using phenotypical and physiological criteria under controlled conditions. Potchefstroom University for CHE.

(c) Drought courses

The following courses were presented utilising the information, techniques and crops used in this study.

ICRO-UNESCO course on screening methods for drought tolerance in food crops, 2000, ARC-Roodeplaat, Pretoria.

AFRA Regional Training Course: Improved mutation, *in vitro* culture and drought screening techniques for the improvement of African crops, 15 October 2001, ARC-Roodeplaat, Pretoria.

AFRA RAF/5/050 Project Co-ordination Meeting: Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology, 10-14 March 2003, Rietfontein, Pretoria.

Third FAO/IAEA research coordination workshop: Genetic improvement of under utilized and neglected crops in LIFDCS through irradiation and related techniques, 19 – 23 May, 2003, Rietfontein, Pretoria.

(d) Technology transfer

Conference Proceedings

Papers presented 1998

Slabbert, M.M., Van der Mescht, A., Spreeth, M.H., De Ronde, K. and Van der Merwe, T. 1998. The use of drought screening methods in *Vigna* spp. *In vivo* and *in vitro* mutation techniques for improvement of seed-propagated crops. Cairo, Egypt.

Slabbert, M.M., Spreeth, M.H. and Van der Mescht, A. 1998. Development of drought tolerant *Amaranthus* (pigweed) plants using mutation technology. Soils and crops towards 2000, combined congress. Alpine Heath, Drakensberg, Natal.

Slabbert, M.M., Spreeth, M.H. and Van der Mescht, A. 1998. The use of mutation technology for the induction of drought tolerance. Regional (AFRA) training course on selection methods for drought tolerance in cereals and legumes. ARC-Roodeplaat, Pretoria.

Slabbert, M.M., Van der Mescht, A. and Spreeth, M.H. 1998. 2,3,5-Triphenyltetrazolium chloride as a method of determining drought tolerance in *Amaranthus*. South African Plant Breeders Association (SAPBA) Plant Breeding Symposium, Golden Gate Highland National Park, Natal. 17-19 March 1998.

Slabbert, M.M. and Krüger, G.H.J. 1998. Development of a physiological screening method for drought tolerance in *Amaranthus* spp. using *in vitro* techniques and glasshouse evaluation. Postgraduate symposium: University of Potchefstroom, Potchefstroom.

Slabbert, M.M., Van den Heever, E., Van Zijl, J. and Venter, S. 1998. Development of *Amaranthus* as a leafy vegetable. First FAO/IAEA research co-ordinating meeting on "Genetic improvement of under-utilised and neglected crops in LIFDC's through irradiation and related techniques". Vienna, Austria.

Slabbert, M.M., van den Heever, E., van Zijl, J. and Venter, S. 1998. . A five-year research plan for mutation breeding in *Amaranthus*. First FAO/IAEA research co-ordinating meeting on "Genetic improvement of under-utilised and neglected crops in LIFDC's through irradiation and related techniques". Vienna, Austria.

Slabbert, M.M., Van Den Heever, E., Van Zijl, J.J.B., Hancke, F., Venter, S.L. and Spreeth, M.H. 1998. Induced mutation technology for the improvement of leafy *Amaranthus tricolor*. Southern African New Crop Research Association mini-symposium and workshop, Pretoria.

Spreeth, M.H., Slabbert, M.M. and Van der Mescht, A. 1998. A strategy for the development and evaluation of drought tolerant mutant germplasm of *Vigna*. Plant Breeding Symposium, Golden Gate.

Spreeth, M.H., Slabbert, M.M. and Van der Mescht, A. 1998. Screening of *Vigna* germplasm for drought tolerance. Southern African New Crop Research Association mini-symposium and workshop, Pretoria.

Spreeth, M.H. and Krüger, G.H.J. 1998. Screening of *Vigna* germplasm for drought tolerance by tissue culture techniques as well as physiological tests on greenhouse plants. Postgraduate symposium: Department of Plant and Soil Sciences, PU for CHE, Potchefstroom.

Van der Mescht, A., De Ronde, K., Spreeth, M.H., Slabbert, M.M., Laurie, R. and Van der Merwe, T. 1998. Physiological approach to drought tolerance. Department of Botany, University of Pretoria.

Posters and Papers presented during 1999

Ayodele, V.I. 1999. Influence of soil water stress at different physiological stages on growth and seed yield of *Amaranthus*. Lisbon, Portugal.

Ayodele, V. I. and Fawusi, M.O.A. 1999. Utilisation of organic waste material nursery plant production. Chania-Crete, Greece.

Slabbert, M.M. 1999. Development of a physiological screening method for drought tolerance in *Amaranthus*. The 3rd International Symposium on novel and non-conventional plants: prospects of their practical use. Puschino, Russia.

Slabbert, M. M. 1999. Development of *Amaranthus* as a leafy vegetable. University of St. Petersburg, St. Petersburg, Russia.

Van den Berg, N., Malemela, L. and Venter, S.L. 1999. Uses and nutritional value of *Vigna subterranea*. Poster. SASAT, October 1999, Drakensberg. Combined congress 2000, January, Bloemfontein.

Papers presented during 2000

Caetano, T. and Spreeth, M.H. 2000. Develpoment and evaluation of drought tolerant mutant germplasm of *Vigna*. Regional IAEA / AFRA workshop at Kano Nigeria.

De Ronde, J.A. 2000. Introduction to drought stress. ICRO-UNESCO course on screening methods for drought tolerance in food crops, Pretoria.

De Ronde, J.A. 2000. Free proline accumulation during drought stress. ICRO-UNESCO course on screening methods for drought tolerance in food crops, Pretoria.

Laurie R.N. and De Ronde, J.A. 2000. Various methods available to screen for drought tolerance. Hungarian Research Institute, Martonvasar, Hungary.

Slabbert, M.M. and Krüger, G.H.J. 2000. Proline accumulation and enzyme activity in water stressed *Amaranthus* leaves: a comparison between glasshouse and *in vitro* plants. SAGP Potchefstroom.

Spreeth, M.H. and Krüger, G.H.J. 2000. The use of the woodenbox technique to determine differences in root architecture of *Vigna sp.* with different levels of drought tolerance. SAAB Congress, Potchefstroom.

Spreeth, M.H., Slabbert, M.M. and Caetano, T. 2000. Mutation analysis of root characteristics in *Vigna sp.* related to plant performance in regard to drought tolerance. the First Research Co-ordination Meeting of the Co-ordinated Research Project on "Mutational analysis of root characters in annual food plants related to plant performance".

Spreeth, M.H., Caetano, T and Krüger,G.H.J. 2000. Drought resistance in cowpeas: How do they do it? Symposium at PU for CHE, Potchefstroom.

Papers presented during 2001

Brink, J.A., Slabbert, M.M. and Spreeth, M.H. 2001. AFRA Annual Report 2000. Development of improved crop varieties (AFRA III-3), AFRA workshop Arusha, Tanzania.

De Ronde, J.A. Introduction to drought tolerance, Regional (AFRA) Training Course, Improved mutation, *in vitro* culture and drought screening techniques for the improvement of African crops, 15 October 2001, ARC-Roodeplaat.

De Ronde, J.A. Free proline accumulation, Regional (AFRA) Training Course, Improved mutation, *in vitro* culture and drought screening techniques for the improvement of African crops, 16 October 2001, ARC-Roodeplaat.

De Ronde, J.A., Slabbert, R., Spreeth, M., Caetano, T. and Laurie, R., 2001. Abiotic stress in South Africa. UFZ, Leipzig, Germany.

De Ronde, J.A., Slabbert, R., Spreeth, M., Caetano, T. and Laurie, R., 2001. Abiotic research for a better life. Aventis, Lyon, France.

De Ronde, J.A, Slabbert, M.M., Spreeth, M.H., 2001. Towards a better understanding of drought tolerance, IAEA working group meeting on drought and salinity, Vienna, Austria.

De Ronde, JA, Slabbert, M.M., Spreeth, M.H., 2001 Development and evaluation of mutant germplasm for drought tolerance. AFRA Regional co-ordinators meeting October, Pretoria.

Spreeth, M.H., Caetano, T and Krüger, G.H.J., 2001. Drought resistance in cowpeas: How do they do it? Joint conference UP, Pretoria.

Papers presented during 2002

De Ronde, JA, Slabbert, M.M., Spreeth, M.H., 2002. Development of improved underutilized crop varieties, AFRA regional co-ordinators workshop, Pretoria, 8 November.

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Nxumalo, L.E. and Ndou, A.M. 2002. The effect of planting date on growth and yield of *Amaranthus* in Northern KZN. University of Zululand.

Mkhwanazi, F.H. and Ndou, A.M. 2002. Effect of fertilizers on chemical composition of *Amaranthus hypochondriacus*. University of Zululand.

Xulu, S.S. and Ndou, A.M. 2002. Effect of fertilizers on growth of *Amaranthus*. University of Zululand.

Luhlanga, F.N. and Ndou, A.M. 2002. Effect of fertlizers on *Amaranthus* yield. University of Zululand.

Mkhize, B.P. and Ndou, A.M. 2002. Effect of organic manure on growth of *Amaranthus*. University of Zululand.

Ndou, A.M. 2002. The importance of indigenous vegetable (*Amaranthus*, cowpea and bambara groundnuts). Presentation at Farmers day held in KwaMbonambi on the 26th of June.

Papers presented during 2003

De Ronde, JA and Spreeth M.H, 2003. Development of improved crop varieties in South Africa, AFRA Project III-3, Project Co-ordination Meeting: Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology, Pretoria, 11 March.

De Ronde, JA, 2003. Abiotic stress in plants. Hydroponic course ARC Roodeplaat, Pretoria, 15 May.

Slabbert, M.M. Breeding Methods for the Improvement of Drought Tolerance, Regional (AFRA) Training Workshop on Standardization of Crop Lusaka, Zambia. 10 -14 November 2003.

Papers presented during 2004

Spreeth, M. Bambara groundnut research in South Africa. AFRA workshop on techniques to shorten generation cycles in Bambara groundnut, Stellenbosch, March 2004.

Publications

Ayodele, VI. 1999. Influence of soil water stress at different physiological stages on growth and seed yield of Amarathus species. Acta Horticulturae, 537: 767-772.

In press

Jansen van Rensburg, W.S., Venter, S.L., Netshiluvhi, T.R., Van den Heever, E., Vorster, H.J. and De Ronde, J.A., 2004. The role of indigenous leavy vegetables in combating hunger and malnutrition. South African Journal of Botany.

Slabbert R and Spreeth M, 2004. Drought tolerance, traditional crops and biotechnology: breeding towards sustainable development. South African Journal of Botany.

In preparation

Spreeth, M.H. *et. al.* Determination of the levels of free proline in drought stressed cowpea plants.

Spreeth, M.H. *et. al.* Drought resistance in cowpeas as determined by the 2, 3,5-triphenyltetra- zolium chloride viability assay.

Spreeth, M.H. *et. al.* The chlorophyll fluorescence transient as a tool to distinguish between plants with different levels of drought resistance.

Slabbert, M.M., Krüger G.H.J. and Caetano, T. Proline accumulation in water stressed Amaranthus leaves: a comparison of glasshouse and *in vitro* plants.

Slabbert, M.M. and Krüger G.H.J. Antioxidant enzyme activity of amaranthus in response to drought stress – a comparison of greenhouse and *in vitro* plants.

Slabbert, M.M., De Ronde, J.A. and Caetano T. Improvement and rehabilitation of traditional and neglected food crops through mutation techniques: Development and evaluation of mutant germplasm of *Amaranthus tricolor.*

Venter, S.L *et. al.* Indigenous crops with potential but underutilised in South Africa.

Other related WRC reports available:

Molecular and physiological approach to drought and heat tolerance for selected crops.

De Ronde JA; van der Mescht A; Laurie RN; Spreeth MH; Cress WA

In South Africa, where drought is a severe problem, the value of drought tolerance in economically important crops cannot be underestimated. Since most plants can only survive limited drought, an understanding of how water stress affects their growth, metabolism, development and yield is of practical value. Little attention has been paid to plants with a high degree of drought tolerance. For economic reasons, it is important to explore the mechanisms of drought tolerance. The general aim of the project was to identify and characterise the genes, which are involved in drought tolerance in plants, and to transfer such genes to drought-sensitive plants. Different approaches were followed with different crops, namely tobacco, potatoes, cotton and maize. It was found that there are no genes for drought tolerance as such, only genes for traits that contribute to drought tolerance. Thus, the traits involved in drought tolerance offered the opportunity to develop a screening method. From the results it can be deduced that the mechanisms of drought and heat tolerance involved a series of anatomical and physiological traits, but that the importance of these traits differs between species and stresses. In tobacco, the proline pathway is crucial in sustaining drought tolerance. A balanced antioxidative enzyme ratio was found to be important in sustaining drought tolerance in potatoes. The levels of the enzymes involved in the antioxidative pathway, Cu/Zn superoxide dismutase, glutathione reductase and ascorbate peroxidase, must all be high to achieve the maximum advantage for the cultivar during drought tolerance. With cotton it was observed that the dehydrogenases involved in the triphenyltetrazolium chloride reduction assay play a vital role in the drought- and heat-tolerance mechanisms. It was also observed that proline metabolism cannot be used as an indicator of heat tolerance. The maize study established that the tolerant cultivar responded by growing a greater amount of roots in the deeper, wetter soil and was thus able to maintain a higher transpiration rate for longer than the sensitive cultivar.

It is thus evident that physiological and anatomical screening methods can be used in distinguishing between sensitive and tolerant cultivars. Screening for different traits involved in tolerance has enhanced the knowledge of genes contributing to the tolerance of the cultivars tested and is of great economic importance in terms of benefits to the breeding programme and predicting the optimum locality for a specific cultivar.

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