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Drought tolerance, traditional crops and biotechnology: breeding towards sustainable development

R Slabbert[1]*, M Spreeth[2] and GHJ Krüger[3]

[1] *Department of Agricultural Management, Tshwane University of Technology, P Bag X680, Pretoria 0001, South Africa*
[2] *ARC Infruitec–Nietvoorbij, Helshoogte Road, Stellenbosch, P Bag X5026, Stellenbosch 7599, South Africa*
[3] *School of Environmental Sciences and Development: Botany, Potchefstroom University for Christian Higher Education, Potchefstroom 2520, South Africa*
* *Corresponding author, e-mail: slabbertr@techpta.ac.za*

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Germplasm of traditional vegetable crops were screened for drought tolerance. Different physiological, morphological and biochemical traits of drought tolerance were investigated, including enzymes of the antioxidative pathway (SOD, AP and GR), turgor maintenance (LWP, RWC), membrane stability (CMS, TTC), osmoprotection (proline), productivity (photosynthesis), rooting (root architecture), early drought tolerance and leaf area. Different *in vitro* screening techniques were carried out. Useful traits of drought tolerance were identified in *Amaranthus tricolor, A. hybridus, A. hypochondriacus, Vigna unguiculata* and *V. subterranea*, and are currently being applied in a breeding programme in an attempt to develop tolerant genotypes of neglected vegetable and seed crops that might contribute to secure food production in rural areas in Africa.

Introduction

Drought is a permanent constraint to agricultural development in some developing countries where inhabitants are dependent on agriculture for their subsistence. It is probably the single most common and severe stress that subsistence farmers face. Chronic or sporadic periods of water deficit lead to substantial crop losses, and because access to irrigation is scarce, farmers are almost completely dependent on rainfall. Arid lands are rapidly increasing and already 35% of the world's area is classed as arid or semi-arid (Grove 1985). The rate of increase in the population of the Third World lends added urgency to the selection and breeding of crops that display tolerance to drought while maintaining their productivity.

While relatively few commercial crops dominate the world's food supply, many traditional crops are important in the daily nutrition of millions of people in resource-poor countries. These crops are an indispensable part of daily nutrition, particularly in the rural areas where it is impossible to grow exotic crops. Many of these crops have been naturally and traditionally selected and are able to survive and grow under conditions of low or frequently changing water supply. Diverse drought tolerance mechanisms exist at the molecular, metabolic and physiological levels, and under harsh African conditions crop yields are often much higher than those of exotic commercial crops. These indigenous crops are maintained and produced through traditional use and socio-cultural preferences, but until very recently have been almost completely ignored in agricultural research programmes. Significant potential exists to develop and improve these crops, as well as to increase genetic diversity, which in turn would promote production and agricultural use.

Screening Methods for Drought Tolerance in Traditional Vegetable Crops

As even traditional vegetable plants can only survive a limited period of water deficit, it is essential to understand the effect of drought on their growth, metabolism, development and yield. Because of the inherent diversity in the different traditional crops, grown for a variety of purposes, water requirements have to be determined for each individual crop.

A project to develop genotypes that might contribute to food production in developing countries was initiated at the Agricultural Research Council (ARC) at Roodeplaat, Pretoria. Physiological and biochemical mechanisms were studied in conjunction with drought survival traits. This allowed development of effective screening methods for drought tolerance and which in turn could be applied to a range of regionally-adapted vegetable crops for practical breeding purposes.

Selection of drought tolerant traits

Plants display various strategies of drought survival. We assessed the value of a large set of parameters as selection tools, targeting possible mechanisms used during stress.

Drought acts as a multi-dimensional stress, thus necessitating a multidisciplinary study of drought tolerance mechanisms. An integrated approach to cellular water relations, rooting characteristics, and biochemical and morphological changes was taken. The different screening techniques that were tested included: the antioxidative response in the form of superoxide reductase (SOD), glutathione reductase (GR), ascorbate peroxidase (AP), proline accumulation, 2,3,5-triphenyltetrazolium chloride (TTC) assays, early drought screening at the seedling stage (wooden box technique), cell membrane stability (CMS), relative water content (RWC), leaf water potential (LWP), leaf area, chlorophyll *a* and *b* and carotenoid content and chlorophyll fluorescence (JIP test). Greenhouse trials were performed and where practicable also evaluation using cultured tissues. The crops selected were three species of *Amaranthus* and two of *Vigna.*

Obtaining uniform greenhouse plants

Plants were grown in a greenhouse in 25cm diameter pots containing a peat:vermiculite:sand (2:1:1) mixture. Osmocote™(5–6) was added at 4g l^{-1}. Day and night temperatures were 25–30°C and 18–20°C, respectively. The plants were watered every second day, until they reached the 4- to 6-leaf stage at an age of 5–6 weeks, after which water was withheld from the treatments. Watering of the control plantlets continued every second day. Leaves of both control and stressed plants were sampled every 2–3 days from the 3rd leaf from the bottom to the top. Four control and four stressed plants were tested for each genotype, one sample taken from each plant for each different screening technique. As most of the measurements were destructive, different sets of plants were used for the different treatments. To screen for root performance, plants were grown under controlled greenhouse conditions. Water was withheld after seven weeks. Both greenhouse and *in vitro* evaluations were performed.

Specific mechanisms of drought avoidance and tolerance in amaranth

Amaranthus — marog; spinach; common pigweed; misbredie (Afrikaans); marogo; tepe (Sotho); umfino (Zulu).

Amaranthus is a crop that has been utilised by ancient civilisations as long as 7 000 years ago (NAS 1985). Vegetable and seed amaranths are still being used daily as a staple food or delicacy by both rural and urban populations world-wide. Vegetable amaranth forms a major part of the daily food intake for millions of people, and the seed, stalks and leaves are an excellent source of low-cost protein, minerals (iron, magnesium, calcium), amino acids (thiamine, riboflavin and nicotinic acid) and vitamins A and C (Van der Heever and Coertze 1996, Van Wyk and Gericke 2000). Improved varieties have been developed, and the crop is grown commercially in the USA and Mexico (Brenner *et al*. 2000). It has also been used in a variety of processed foods (Myers 1996). Amaranth can yield up to 4.5 tons dry matter hectare^{-1} after four weeks (Grubben and Van Sloten 1981). Because it is a leafy vegetable that is not cultivated commercially in Africa, but rather gathered from the wild or cultivated on small plots in home gardens, yields and consumption data are difficult to estimate. It is common in Peru, Bolivia and Mexico, but the largest producer is China, with 87 000 tons per year. In the United States, about 2 500ha have been planted in the Great Plains and Midwest states, primarily the Cruentus variety, which grows to 2m. Production trials in North Dakota have yielded as much as 1 200kg ha^{-1}, but 600kg ha^{-1} to 800kg ha^{-1} is typical (AG Innovation News, 2003).

Three amaranth species were studied. Two, *A. hybridus* and *A. hypochondriacus* are known as grain types, but are widely used as vegetable amaranths; one, *A. tricolor*, is a vegetable type.

Anti-oxidative reaction

Decreased stomatal conductance during severe moisture stress results in a decrease of CO_2, as a result of which a high proportion of the intercepted light energy remains photochemically inactive. This leads to the re-direction of electrons normally used in photosynthesis, and as oxygen may be the final electron acceptor, to the formation of toxic superoxide. Drought-stressed amaranth plants show a clear correlation between the time of formation of free oxygen radicals (that is, when chlorophyll fluorescence measurements indicate a decrease in PSII activity) and the time of scavenging these radicals (when activities of the SOD, GR and AP enzymes commence), after 17 days of drought stress. Ascorbate peroxidase displays the most rapid response, as it was active from the onset of the water deficit. Moisture stress is first experienced by *A. hybridus* and *A. hypochondriacus*, followed two days later by *A. tricolor*. Significant species differences occur in respect of the oxygen-scavenging activities of SOD, AP and GR. The combined effect of these enzymes' action may have promoted greater drought tolerance in *Amaranthus* by ensuring higher levels of regulation of H_2O_2, the toxic effects of which are associated with cellular damage.

Maintenance of turgor

Cell desiccation, characterised by lower turgor or RWC and LWP, disrupts metabolism and growth. Lower RWC and LWP values coincide with lower photosynthetic activity and decreases in leaf area during the enforced drought stress. Mechanisms at the whole plant level such as decreased leaf growth, leaf area or leaf number and increased root/shoot area, which all contribute to osmotic adjustment, enable the avoidance of cell desiccation and damage. Modulation of both LWP and RWC was evident in the maintenance of turgor. Reduction in leaf area is probably important in regulating LWP in amaranth, and is probably also linked to the maintenance of RWC. Amaranth modulates leaf area, thereby adjusting water loss from the canopy to amounts that can effectively be supplied from the existing soil water. Adjustment of leaf size enables more effective control over water-use and may help to avoid desiccation under severe drought conditions. Recovery of leaf area, RWC, CMS and LWP 15h after re-watering is rapid in all three species.

Repair and restoration upon rehydration is a critical but often neglected component of desiccation tolerance. All three species show very rapid recovery rates when re-watered after having been subjected to severe drought stress. This is a physiological advantage in areas of intermittent or sporadic rainfall.

Membrane stability

The inability of membranes to retain and selectively transport cellular solutes is a parameter of cellular membrane function. CMS and TTC reduction assays are used to indicate the extent of leakage of cellular electrolytes and thus of membrane damage. *A. tricolor* maintains a higher CMS during severe water deficit while *A. hypochondriacus* shows the highest percentage of membrane damage during water deficit. However, the latter recovers most rapidly after re-watering, and also displays the greatest membrane stability during heat stress, which possibly explains its good performance under conditions of drought. Although the TTC assay of greenhouse plants indicated that *Amaranthus* species are both drought and heat sensitive, the standard deviation between the treatments was high, and therefore the differences in drought and heat tolerance/sensitivity between *A. tricolor* and *A. hypochondriacus* may not always be statistically significant. Because of the high standard deviation between treatments obtained, this test is not recommended for testing drought and heat tolerance in amaranth greenhouse plants.

Osmoprotection

Screening for proline accumulation is a method for assessing genotype variation in drought tolerance, since distinct differences in time of onset of proline accumulation, as well as the amount accumulated as a result of severe water deficit, may be observed. Some authors claim that high proline production is an indication of tolerance to moisture stress. However, we interpret the higher concentrations of proline production in amaranth during drought as a sign of low RWC and LWP, and hence to sensitivity rather than tolerance to water deficit.

Productivity

Analysis of chlorophyll fluorescence transients and changes in pigment content enable identification of a number of possible limitations to photosynthesis and biomass production during drought stress. Reduced levels of chlorophylls *a* and *b*, and carotenoids coincide with a down-regulation of PSII, and which may also be correlated with a reduction in LWP during severe water deficit. The energy fluxes of absorption, electron trapping and transport were assessed by measuring chlorophyll fluorescence (JIP test)(Krüger *et al.* 1997). This allowed detection of plants with a higher photosynthetic efficiency during moisture stress. Concerning antenna size (ABS/RC), maximum trapping flux (TR_o/RC), density of reaction centres per cross section (RC/CS) and trapping flux per cross section. (TR_o/CS), *A. hybridus* and *A. tricolor* remained the most constant during water stress. *Amaranthus hypochondriacus* and *A. hybridus* maintain high photosynthetic electron transport rates under water stress by increasing the conversion efficiency of trapped excitation energy to electron transport beyond Q_A. No significant changes in either ABS/RC or TR_o/RC suggested that water stress had little effect on the oxidising side of PSII, the antenna and the efficiency of electron transport per PSII reaction centre. Amaranth clearly adjusts the photochemical and non-photochemical deactivation constants of PSII (k_p and k_n) by means of photoregulation, which forms the basis of the quenching of chlorophyll *a* fluorescence.

Rooting ability and early drought tolerance

The wooden box technique is an easy and inexpensive screening method for large numbers of plants. All three species tested at the seedling stage showed early drought avoidance characteristics. In spite of leaf abscission during severe moisture stress (a form of drought avoidance), the plants recovered well after re-watering to form shoots again. Amaranth seedling roots exposed to extended periods of moisture stress show rapid recovery and survival upon re-watering. Two-dimensional root screening allows comparison between well-developed and weak root systems, but the results should also be analysed in conjunction with other characteristics. In amaranth, the root/shoot area ratio appears to be a more important criterion of drought tolerance than simply a large root system. Drought-tolerant plants are usually associated with a well-developed root system, but both deep and superficial roots have roles as edaphic and climatic conditions can vary. Changes in water resistance in the plant probably also play a part in maintaining RWC and LWP, rather than rooting characteristics alone.

Selection in vitro

Screening of plant material *in vitro* was exploited with the aim of reducing large and expensive greenhouse and field trials. Polethylene glycol (PEG)-induced osmotic stress *in vitro* was used to simulate drought stress *ex vitro*. It was also used as an alternative to dryland trials to measure antioxidative stress, TTC reduction and proline production in amaranth. Both GR and AP had elevated activities during imposed water stress conditions. Statistically significant differences were observed between PEG treatments (moisture stress) and controls in respect of GR and AP, but the physiological significance of these differences for individual enzymes as they relate to the *in vivo* oxidative stress was more difficult to assess. Due to the high incidence of statistical insignificance and difficulty in relating *in vivo* results of TTC and proline to *in vitro* assays, it is suggested that results of *in vitro* screening should first be correlated with field trial results. This is to ensure credibility of the deductions and conclusions drawn, since *in vitro* stress factors such as irradiation, culture medium, growth regulators, etc., could have additional stressful effects on growth, which means that the stress results measured could be false indicators of sensitivity or tolerance. Data of our greenhouse and *in vitro* assays of the three amaranth species, resulted in the rankings shown in Table 1.

Table 1: The amaranth species, namely A*maranthus hybridus, A. tricolor* and *A. hypochondriacus*, ranked with respect to drought/heat tolerance, according to different physiological and morphological screening criteria

Cvs.	Screening criteria for drought tolerance												Heat tolerance		
	Chlorophyll	CMS	TTC	Proline	RWC	LWP	SOD	GR	AP	Leaf area	Wooden box	Total rating	CMS	TTC	Total rating
A. hypochondriacus	3	2	2	2	3	3	1	2	2	3	2	2	1	1	1
A. tricolor	1	1	1	1	1	1	2	1	1	1	2	1	2	1	2
A. hybridus	2	1	3	3	2	2	3	3	3	2	1	2	3	1	3

1 = best performance (tolerant)
3 = poor performance (sensitive)

Specific mechanisms of drought avoidance and tolerance in Vigna unguiculata

Vigna unguiculata — cowpea; southern pea, black-eyed pea, crowder pea (English); frijol or judia (traditional English); akkerboon (Afrikaans); dinawa (Sotho, Tswana); munawa (Venda); caupies (Sp.) (Cubero 1994); faseol (Gr.); faseolus (L.); fasulia (Arabic). Cowpea is also referred to by other names such as augenbohne, lubia, niebe, coupe, pois a vaches, rabiza, morogo wa dinawa or njugu bean (Duke 1981, Madisa and Tshamekang 1997, Wiersema 1999, Davis *et al.* 2000).

In the selection of crops, in order to secure food production, it is important to use plants that are well-adapted to the climatic and growth conditions. A crop that can fulfil this niche is cowpea (*Vigna unguiculata*). This crop originated in Africa and is well adapted to a variety of growth conditions. As a legume, it is high in protein, in addition to its ability to fix nitrogen. Cowpea also possesses an extensive root system that helps combat soil erosion.

Protein content may be as high at 36% and is influenced by genotype and environmental factors (Coertze and Venter 1996). Lysine content is relatively high but cowpea is deficient in sulphur-containing amino acids. Total carbohydrate can be as high as 68%, with starch contributing 32–48%. The carbohydrate fraction is relatively rich in total sugars. The amount of amylose in the starch influences starch solubility, lipid binding and many functional properties such as swelling and solubility, water absorption, gelatinisation and pasting (Coertze and Venter 1996).

Subsistence farmers in the semi-arid parts of Africa are the main producers of cowpeas. Cowpea is not only planted for seed, but the leaves are also consumed by humans and also used as animal fodder. In 2000 the production of cowpeas amounted to more than 3 million tonnes in developing countries (Quin 1997, Fowler 2000) as compared to about 52 000 tonnes in other countries. Nigeria, Niger, Mali, Myanmar and Malawi were the main producers, while 23 other countries also cultivated this versatile crop (Fowler 2000). Outside Africa, the major production areas are Asia and Central and South America (Guazzelli 1988). According to the South African National Seed Organization (SANSOR) 30 000ha of cowpeas were planted in South Africa in 2002/2003.

Although cowpeas is regarded as a drought resistant crop, a lot of variation occurs within the genotype. The problem that needed to be solved by our study was to find selection methods that could be used to screen large numbers of plants for drought tolerance. The research project was premised on the belief that intra-species differences in response to drought could be used to enhance the understanding of the physiological and phenotypical basis of drought resistance and that this knowledge could be used to identify practical screening parameters. This knowledge would then help with the expansion of the crop and thus be of economic importance. The selection of the right genotype is of value not only for commercial farmers but for subsistence farmers in areas with low precipitation or varied rainfall patterns.

For this study various cowpea lines were selected. Two of the lines obtained from the International Institute of Tropical Agriculture (IITA) in Nigeria are known to be drought resistant (IT96D-602) and drought susceptible (TVu7778), respectively. These two lines were used as reference lines. The other lines were selected on the grounds of their high or low yield obtained under field conditions in South Africa. The selected lines included some more of the IITA's lines, locally bred lines, and a few lines that were collected from the communities in KwaZulu-Natal.

The selected cowpea lines were planted in pots and kept in a green-house where the mature plants were subjected to drought stress by withholding water, after which various screening methods were used to determine the levels of drought resistance. These included phenotypical observations in the greenhouse as well as biochemical and physiological screening in the laboratory. The experiments conducted in the green-house were mainly to determine the root architecture of the different lines as well as screening of seedlings for drought resistance using the wooden box technique (Singh *et al*. 1999).

Other screening methods used were assessment of photosystem II (PSII) function by chlorophyll fluorescence (JIP test), chlorophyll *a* and *b* concentrations, CMS, RWC, LWP and free proline levels. The TTC for determining viability under drought stress conditions was also included, as well as the determination of the activity of the enzymes of the antioxidative system (SOD, GR and AP). The size of the leaves and the yield of stressed and control plants in the greenhouse were also determined. The plants were monitored over a 17-day drought stress period and the recovery of the plants after rewatering was also recorded.

Plants that have the ability to endure extremely dry periods use mechanisms that maintain the internal water balance at a high level. This can be either through the accumulation of organic compounds like proline or betaine (Paleg *et al*. 1984) or by implementing increased diffusion resistance (Larcher 1980). The latter can be achieved through sensitive stomatal control or effective cuticular protection (Larcher 1980, Gwathmey and Hall 1992). Increased water conductivity and a reduced transport distance (shorter internodes) are two more behavioural measures that enable plants to avoid desiccation (Larcher 1980). In some plants the transpiration surface is reduced, helping to conserve water. This can be done by folding or rolling of leaves, cessation of new leaf expansion, or shedding of leaves (Larcher 1980). The reduction of leaf area by leaf senescence and abscission is an extreme avoidance response, which has a detrimental effect on photosynthetic capacity and yield potential (Gwathmey and Hall 1992). Under stress conditions more than one system may be in use.

In most cases the results of the different screening methods showed very good correlation and also confirmed the drought ranking reported by the IITA. The screening methods for which the data showed the best correlation were the JIP-test (chlorophyll fluorescence measurements), chlorophyll *a* and *b* levels, RWC, the wooden box technique, yield of the greenhouse plants, and the root architecture (Table 2). The determination of the free proline levels and LWP showed relatively good correlation with the above mentioned screening methods. The TTC reduction assay and leaf area measurements, as well as the AP activity led to the ranking of IT96D-602 as the most tolerant line; the ranking of the other lines varied according to these three tests. The other two screening tests, regarding activity of the enzymes of the antioxidative system, SOD and GR, and the CMS did not show differences in the drought resistance levels of the cowpea lines.

The information gathered in this study contributes towards a better understanding of the physiological and morphological basis of drought resistance in cowpeas. Although all the plants had similar CSM, the plants of IT96D-602 maintained a higher RWC and water potential than plants of all the other lines. This improved the viability of the plants, was evident from the TTC reduction assay and the chlorophyll fluorescence data. The fact that IT96D-602 experienced lower stress levels than the other lines was also evident from the lower free proline levels and the preservation of more chlorophyll *a* and *b* molecules. A higher ranking was given to a line with a lower proline concentration, because it was established that the level of free proline in cowpeas give an indication of the level of stress. The drought resistant line IT96D-602 started to produce proline at a later stage and at lower concentrations than the susceptible line TVu7778. This is contrary to the widespread assumption that higher proline levels indicate drought resistance in plants. The same argument was also used for the ranking of the plants according to the AP levels. Another criterion that can be interpreted in different ways is leaf size. Larger leaves may result in higher production, but smaller leaves reduce the transpiring surface and help to conserve water. A higher ranking was therefore given to lines with smaller leaves, e.g. IT96D-602.

Other qualities that were detected in the line IT96D-602, which might have contributed to water retention, were an upright growth form and a more extensive root system than the other lines. This root system plays an important role in the uptake of ground water, providing that there is still water left in the soil. For cowpeas the principle of maximum uptake and minimum loss can be applied. Under prolonged drought conditions the drought resistant lines probably use selective moisture mobilisation. Water is transferred from the lower leaves to the growth tip and young leaves and the old leaves are discarded. This is in accordance with the findings of Mai-Kodomi *et al*. (1999).

According to our results, methods suitable for screening large numbers of cowpea plants for drought resistance include determination of chlorophyll fluorescence free proline levels and wooden box screening for drought resistance at the seedling stage. The relative water content has also proven to be a good measure of water stress.

***Specific mechanisms of drought avoidance and tolerance in* Vigna subterranea**

Vigna subterranea — bambara goundnut, African or Madagascar groundnut, or the njugo bean (English); dopboontjie or jugoboon (Afrikaans); izidlubu (Zulu); nduhumvenda or phonda (Venda); tindhluwa or ndhluwa (Shangaan); ditloo-marapo (Sotho); njugu-mawe (Swahili); kacang bogor (Indonesia). Other names that have also been

Table 2: Five cowpea (*Vigna unguiculata*) lines ranked for drought and heat tolerance according to different physiological screening methods as well as greenhouse measurements

Lines	Screening criteria for drought tolerance											Heat tolerance	
	Chlorophyll fluorescence	LWP	CMS	RWC	Proline	SOD	GR	AP	Yield	Wooden box	Final ranking	TTC	Final ranking
IT96D-602	1	1	1	1	1	1	1	1	1	1	1	1	1
IT92K258-9	4	3	2	3	4	1	1	1	2	2	2	2	2
IT90K59	2	3	3	3	3	1	1	1	4	3	3	3	3
Chappy	3	3	3	2	2	1	1	1	4	4	3	3	3
TVu7778	5	3	3	3	5	1	1	1	2	5	5	3	3

1 = best performance (tolerant)
5 = poor performance (susceptible)

recorded include: mandubi d'Angola, voanjo, voandzou (Goli 1997), ditloo, njugu mawe which means hard stone (Howell 1994), jugo, njugo and round beans (Swanevelder 1998).

Another member of the *Vigna* genus that is well-equipped to the often harsh conditions in Africa is the bambara groundnut. Bambara groundnuts originated on the African continent, most probably in the north-western part. Bambara plants form much branched, leafy lateral stems that grow just above ground level (Linneman and Azam-Ali 1993). Like cowpeas, bambara groundnuts also possesses nitrogen fixation properties and a well-developed root system. It is a versatile crop in that both leaves and seeds can be consumed. The biggest draw back for this crop is the time it takes for the seeds to mature and also that most plants produce very little seeds especially under stress conditions. Both these traits can be improved through selection and breeding.

The ripe beans are very nutritious, and can be used as a substitute for meat (Venter and Coertze 1997). It contains about 16% protein, 6% fat and relatively high levels of amino acids (Van Wyk and Gericke 2000).

Bambara groundnuts are cultivated mainly for their seeds. The bulk of bambara groundnuts is produced in Nigeria, Niger and Ghana, where it is third in importance only to cowpea and peanuts (Howell 1994). The introduction of the common groundnut has lead to a decline in the production of bambara groundnuts, but in recent years there has been renewed interest in this crop. The reason for this interest is probably the high degree of drought resistance as well as the ability to produce a reasonable crop when grown on poor soils (Swanevelder 1998). According to Rachie and Roberts (1974) 330 000 tonnes of dry seed are produced annually on 400 000ha. However, it is difficult to verify this figure since bambara groundnuts are mainly cultivated by small-scale farmers or in home gardens in remote areas. In recent years this crop has received more attention from breeders and scientists and it might in future prove to be one of the staple crops that will help sustain food security in Africa.

Seeds of various bambara groundnut lines were subjected to the same drought screening tests as the cowpeas lines. The difference was that there were no reference lines present and that the lines had to be compared with each other (Table 3). The selected lines included landraces like SB1-1 and AS17 but also seeds that were collected from the communities in KwaZulu-Natal that were clumped together purely on the basis of testa colour and the locality from where the seeds were collected.

During the course of this investigation it became evident that bambara groundnuts react in a similar way to drought stress as cowpeas. An exception is that the bambara plants seldom lose their leaves even under severe stress. This might be attributed to their growth form. As opposed to cowpea, the bambara grows horizontally and the leaves protect the growth tips and also provide shading which results in lower soil temperatures.

There were no differences between the enzyme levels of the stressed plants and the control plants, and the free proline levels also remained remarkably stable over the stress period. The free proline levels only increased when the plants showed severe signs of drought stress, a result similar to that in cowpeas. The CMS was retained through the stress period and the LWP only started to increase after the plants had been without water for 15 days. The bambara lines that produce larger leaves seemed to give higher yields and better results and had a higher harvest in light energy, but were unable to maintain RWC as high as the plants with smaller leaves; they also displayed higher levels of free proline than smaller-leaved plants. Bambara plants do not produce root systems with features as distinct as drought resistant cowpea plants. Most of the root systems displayed good distribution through the available area and normally formed some prominent lateral roots.

The bambara plants with smaller leaves can survive severe drought conditions but this results in a severe reduction in yield. It might therefore be more beneficial to select plants with larger leaves that are quite hardy and still able to produce a moderate yield under adverse conditions.

Conclusion

The ultimate goal is understanding the mechanisms of drought tolerance so as to improve traditional vegetable crops for arid-land production. Drought affects almost all aspects of plant growth, especially yield and product quality. Biotechnology is an important tool in the study of traits of drought tolerance, in identifying the most important tolerance characteristics, and finding ways of modifying and improving the genetic base of traditional African crops.

Should global warming increase, currently irrigated areas may in future face water shortage, which means that agri-

Table 3: Ranking of the five bambara groundnut (*Vigna subterranea*) lines according to different physiological screening tests as well as woodenbox screening at the seedling stage and yield

	Screening criteria for drought tolerance											
Lines	Chlorophyll fluorescence	CMS	TTC	Proline	RWC	LWP	SOD	GR	AP	Yield	Wooden box	Total rating
SB1-1	2	1	1	3	1	1	1	1	1	1	1	1
SB7-1	3	1	1	1	1	1	1	1	1	3	2	2
SB9-1	4	1	1	5	1	1	1	1	1	5	4	5
SB20-1	5	1	1	3	1	1	1	1	1	2	5	4
MAD-1	1	1	1	1	1	3	1	1	1	4	3	3

1 = best performance (tolerant)
5 = poor performance (susceptible)

cultural production will increasingly come to rely on drought tolerant crops. Drought seldom occurs in isolation, and mostly interacts with a variety of other abiotic and biotic stresses. Usually, drought is experienced in conjunction with high temperatures, and plants are then again more susceptible to biotic stresses such as disease. The challenge is to improve yield and productivity during abiotic stress conditions by investigating possible strategies for breeding in areas with limited water availability, and to suggest possible ways of breeding and selection for drought conditions.

Drought tolerance is complex and involves numerous interactions between the plant and its environment. To gain understanding of the possible escape/avoidance/tolerance mechanisms, all aspects of drought tolerance have to be investigated. By identifying practical and useful traits and parameters by which traditional crops can be screened for tolerance, not only will useful information for further breeding purposes be found, but it would allow screening of existing germplasm for tolerance.

The use of improved drought-tolerant traditional crops opens the possibility of exploiting marginal areas where arid and semiarid conditions prevail, and drought periodically occurs during the growth season. Under such conditions, it is important to understand (a) how the plant reaches and utilises available soil water (rooting ability), (b) the amounts and rate of water loss (RWC, LWP), (c) coping or prevention of excessive water loss (leaf area); and (d) the physiological and biochemical responses activated in the plant that reduce oxidative stress (certain key enzymes), adjust the osmotic regulation (proline) and control productivity (photosynthesis). The duration of the stress conditions and recovery of the plant when re-watered, also plays an important part.

The results of this study resulted in five methods being chosen for screening of 22 locally-grown amaranth species, 19 cowpea lines and 15 bambara groundnut species of which the best five were then selected for distribution to local communities in South Africa where the training of small-scale farmers is under implementation. Selection of germplasm by the Resource Poor Agriculture Division of ARC–Roodeplaat is occurring in Soshanguve Pretoria, Mpumalanga, Northern Province and in KwaZulu-Natal, as well as from entries of other African countries. Correlation of screening results with field trials will be exploited in the resource-poor communities mentioned above.

The answers obtained during this study provided insight and understanding to characteristics important in selection and breeding for arid-land conditions. This study gave insight into some of the regulatory elements that respond to environmental signals such as drought and that lead to specific gene expression in amaranth, enabling it to survive severe water deficits. The selection methods characterised here could help to enhance future breeding strategies for traditional crops. The ultimate goal of ensuring food security for the growing population in developing countries can only be achieved through the persistent and coordinated efforts of scientists in the fields of biotechnology, plant breeding, plant physiology and plant pathology.

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