South African Journal of Botany 2004, 70(2): 227–234
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF BOTANY
ISSN 0254–6299

Proline content, water retention capability and cell membrane integrity as parameters for drought tolerance in two peanut cultivars

OA Quilambo

Department of Biological Sciences, Faculty of Sciences, Eduardo Mondlane University, PO Box 257, Maputo, Mozambique
e-mail: oquilambo@rei.uem.mz, quilambo@zebra.uem.mz

Received 6 March 2003, accepted in revised form 30 September 2003

Two peanut cultivars were grown for 13 weeks under water controlled conditions in ceramic pots, lined with plastic bags. The cultivar Falcon (F) showed characteristics of drought tolerance, while cultivar Local (L) showed those of drought susceptibility. The peanut cultivar Falcon showed an osmotic adjustment mechanism that enables it to withstand short-term drought stress. A measurement of the cell-membrane integrity, with the polyethylene glycol (PEG) test, showed that membranes of the cultivar Falcon were less injured, compared to those of the cultivar Local, under drought stress. The same cultivar maintained a higher relative water content RWC (water saturation deficit, WSD) and relatively low relative saturation deficit (RSD) as compared with the cultivar Local, when both cultivars were subjected to drought stress. Additionally, proline was substantially more accumulated in this cultivar. Therefore, cultivar Falcon was classified as drought tolerator and cultivar Local as drought avoider. The relative water content (RWC), relative saturation deficit (RSD), cell membrane integrity (CMI) and proline content were effective criteria for detecting drought tolerance strategies taking into account the growth stage and duration of the stress period, while the water retention capacity (WRC) did not show any significant relation with drought tolerance.

Introduction

Plant survival and production under environmental stress is conditioned by a complex of mechanisms. Many studies point to the cell membrane as an initial site of stress injury, i.e. the function and structure of plant cell membranes are drastically damaged by environmental stress (Agarie *et al.* 1995). Thus, evaluation of cellular membrane integrity as a measure of environmental stress tolerance appears to be a relevant criterion (Sullivan 1972). The polyethylene glycol (PEG) test for measuring cell membrane stability (CMS) has been claimed as an efficient method to determine drought sensitivity (Premachandra *et al*. 1990).

Most commonly changes in the electrical impedance and leakage of intact plant cells or tissue have been measured to detect stress injury of plasma membrane. Leakage will vary in relation to the membrane's ability to take up and retain solutes and, therefore, will reflect drought stress-induced changes in both membrane potentials and membrane permeability (Agarie *et al.* 1995). Sullivan and Ross (1979) found for sorghum that membrane integrity and stability to stress, as evaluated by electrical leakage, correlated well with drought tolerance of other plant processes to stress.

Some authors referred to genetic variability and heritability of CMS and then concluded that the technique could be used as an efficient means for selection of drought tolerant genotypes in wheat (Premachandra and Shimada 1987). The same authors (1988) measured the CMS in naturally dehydrated excised leaves and found that drought tolerance was highly correlated with CMS, as measured by the PEG test.

Natural dehydration of plants exposed to drought can be measured as excised-leaf water retention capability, which is mainly affected by cuticular and stomatal resistances.

A comparison of these characteristics and other physiological measurements with the CMS, measured by the PEG test, may increase our understanding of the physiological processes involved in the differential ion leakage (Premachandra *et al*. 1989).

Premachandra and Shimada (1988) indicated that CMS, measured by the PEG test, was significantly and positively correlated with leaf water potential, osmotic potential of leaf tissues, excised leaf water retention, degree of leaf rolling, total plant weight and total root length under varied soil moisture levels. Worku (1995) reported a close relationship between high water retention capability, drought hardiness and high yield in wheat.

In peanut Venkateswarlu and Ramesh (1993) reported that cell membranes of cultured cells, originating from a drought-tolerant cultivar, had suffered much less injury than those from a drought-sensitive one. Levels of organic

osmotic solutes as sugars and proline in the cell sap of sensitive peanut accessions with a low CMS were much lower than those of tolerant ones with high CMS (Deb *et al*. 1996).

Relative water content (RWC) has been successfully used to monitor water content and drought status in peanut (Bennett *et al*. 1984). Sinclair and Ludlow (1985) argued that RWC is a more useful parameter of a plant's water balance than the leaf water potential and it should provide a universal relationship between physiological traits and level of drought stress. RWC values in well watered plants were typically in the range of 85–98% (Prabowo *et al*. 1990).

Osmotic adjustment (OA) has been suggested as a mechanism that leads to smaller changes in RWC per unit decrease of water potential (Steudle *et al*. 1977) and consequently it should help to maintain a positive and high turgor potential during water stress.

OA has received increasing attention in the last decades and refers to active accumulation of solutes in cells beyond the increase in concentration caused by loss of water. OA provides certain advantages: lowering of the leaf osmotic potential permits turgor to remain more positive under stress conditions. As a result, cell growth can continue, root cells can penetrate a greater soil volume, stomata will remain open longer and therefore photosynthesis can continue at greater drought levels (Parsons and Howe 1984). OA reduces sensitivity of turgor-dependent processes, such as leaf expansion, stomatal conductance and leaf rolling, to declining leaf water potentials (Jones *et al*. 1980, Morgan 1984). However, Munns (1988) argued that OA is not the only factor for maintaining leaf turgor, since reduction in stomatal aperture can also accomplish maintenance of leaf turgor.

Proline is believed to act as an (i) osmotic solute in plant cells (Hu *et al*. 1992, Delauney and Verma 1993); (ii) as a stabilising agent for membranes, through an effect on the hydration layer surrounding phospholipids (Rudolph *et al*. 1986); and (iii) as a source of nitrogen and carbon during recovery from stress. Proline content correlated positively with membrane integrity, measured as ion leakage, in tobacco leaves (Van Rensburg *et al*. 1993), suggesting its use as a selection criterion for drought tolerance in *Nicotiana tabacum*. While some authors associate proline with drought tolerance, Hanson *et al.* (1977) and Andrade *et al*. (1995) found accumulation of proline in drought-sensitive cultivars of barley and beans, associating this change with a more rapid decline in water potential or as a symptom of severe stress. Andrade *et al*. (1995) confirmed the suggestion that proline was synthesised in the leaves and translocated to the roots and other organs and that it may act as a mechanism for drought tolerance.

The peanut is a legume that under many conditions fixes N_2 through symbiotic relations, to avoid N deficiency. However, factors such as peanut cultivar, variety, presence of inoculum, crop rotation, soil type, moisture and temperature, all can affect N_2-fixation (Gascho and Davis 1994).

Peanut is grown on P deficient soils in Mozambique. Phosphorus is the most deficient element, although this deficiency is limited to areas which have never been fertilised with P, where fertilisers are not available or where their cost is prohibitive.

The objective of the present study is to determine and compare the leaf water relations' responses of two peanut cultivars to water stress. Differences that might be observed may partially explain the observed differences in growth of the cultivars response to imposed drought stress (Quilambo 2000). A further objective is to evaluate how proline levels differ among the cultivars (drought-avoider and tolerator) and how its contribution changes with increasing drought stress. For this reason cultivar Falcon, a drought-tolerator, and cultivar Local, a drought-avoider, were selected for this study.

Material and Methods

Plant material

Two peanut cultivars (*Arachis hypogaea* L.), Local (L) and Falcon (F), were grown for 13 weeks in 12l ceramic pots, lined with plastic bags, filled with soil collected from the experimental farm of the Faculty of Agronomy and Forestry Engineering of the Eduardo Mondlane University in Maputo, Mozambique. The soil is classified as arenosol and its characteristics are given in the Table 1.

Growth conditions

The plants were grown in a plant nursery in Maputo, Mozambique (25°28'S, 32°36'E), from November 1998 to February 1999, under water-controlled conditions.

The mean air temperature during the growth period was 28.3 ± 3.0°C in the morning, 31.5 ± 2.5°C at midday and 29.1 ± 3°C in the afternoon.

The mean relative humidity ranged from 57 ± 12% in the morning, 47.8 ± 10% at midday and 55 ± 9% in the afternoon. The illumination was screened natural light, resulting in an average photon flux density at canopy level of 285 ±

Table 1: Physical and chemical characteristics of the soil used in the experiment

Parameters (Units)	Value
Sand (%)	85.6
Silt (%)	13.4
Clay (%)	1.0
pH	6.8
Bulk density (g cm^{-3})	2.37
Electrical conductivity (ms cm^{-1})*	0.06
Ca^{2+}	26
Mg^{2+}	9.1
Cation exchange capacity (meq kg^{-1})	
Na^{+}	0.6
K^{+}	0.9
Carbon (%)	0.07
Organic matter (%)	0.12
Total Nitrogen (%)	0.08
Total P (mg kg^{-1})	
P-Bray (II) mg $100g^{-1}$)	30.7

* Electrical conductivity (EC) was determined by diluting soil in distilled water at a rate of (1:2.5 v/v) and measuring the EC of the solution

18µmol m^{-2} s^{-1} in the morning, 436 ± 24µmol m^{-2} s^{-1} in midday and 577 ± 26µmol m^{-2} s^{-1} in the afternoon, measured with a quantum sensor (SK P215, Skye Llandrindod Wells, UK).

During the first week the plants were irrigated to field capacity with normal tap water. The salt level of the tap water was 0.31ppm and 2.54g l^{-1} measured as $CaCO_3$ and NaCl content, respectively. The plants were water-stressed by soil drying (in preference to other methods), since this procedure would more accurately reflect the field characteristics.

Water deficit was created by withholding irrigation water from week two onwards until the moisture content of the soil reached 3% (near the wilting point, according to preliminary experiments), measured with the use of a Thermal Domain Reflectometer (TDR, Eijkelkamp, Giesbeck, The Netherlands).

The control plants were regularly watered, according to the evaporative condition and transpiration demand to a soil moisture content of above 20%, using TDR, a moisture level near to field capacity. Whenever the soil had dried out beyond this limit, water was added in the morning to restore the soil moisture content back to the pre-determined level.

The plants were harvested at week four after initiation of drought stress (vegetative stage) and at ten and thirteen weeks after drought stress initiation (pod-setting and maturity stages).

At each harvest the following determinations were made:

a) Water retention capacity of the leaves
b) Leaf water relations
c) Cell membrane integrity of the leaves
d) Proline content of leaves and roots

Water retention capacity of the leaves

The water retention capacity (WRC) of the leaves was determined according to Worku (1995). Pots were covered in the night, preceding the measurement with a black plastic sheet to avoid water loss due to pot evaporation.

One leaf per branch (8 replicates) was detached and weighed immediately. The leaves were kept at room temperature (20–25°C) for free transpiration.

The weight of these excised leaves was recorded every hour for a period of 8h and once again after 24h.

The WRC was calculated as the relative decrease of weight in percent per hour, using the formula: (fresh weight of the excised leaf x 100) / fresh weight of the leaf after 8h and 24h of free transpiration.

Leaf water relations

The leaf water relations were determined as relative water content (RWC), water saturation deficit (WSD) and relative saturation deficit (RSD) as described by Turner (1986) and Ashraf *et al*. (1996). The second fully expanded tetrafoliate leaf of the main stem was used for this determination (8 replicates). The leaves were excised in the morning and immediately weighed (fresh weight = FW).

Leaves were then kept in a humid chamber in test tubes, containing 10ml of distilled water, for at least 12h at room temperature.

The leaves were then taken out, the water was removed from the surface and weighed again [turgid (or saturated) weight = TW]. The dry weight (DW) was obtained by weighing after placing the leaves in an oven at 70°C for 48h.

The RWC was determined as follows:

$[(FW – DW) / (TW – DW)] \times 100$

The WSD was computed as follows:

$WSD = 100 – RWC$

and the RSD as

$[(TW – FW) / TW] \times 100$

Cell membrane integrity of the leaves

For measurements of the cell membrane integrity the PEG test was used, as adapted from Agarie *et al*. (1995) and Ashraf *et al*. (1996). Thirty leaf discs, obtained from the uppermost fully expanded leaves, were washed three times with deionised water in a test tube. The leaf discs were then submerged in 30ml of 40% PEG 600-solution (T_1) or deionised water as a control (C_1) and both were left for 24h at 10°C. The leaf discs were then quickly washed with deionised water and allowed to remain in 30ml deionised water for another 24h at 10°C. The electrical conductivity (EC) of the liquid was measured afterwards. The leaf discs, still in the same solution, were then killed by autoclaving for 20min to release all ions from the tissue, cooled to 25°C (T_2 and C_2) and the EC was again determined.

The cell membrane integrity was evaluated as percentage of injury (PI), using the formula:

$PI = [(1 – T_1/T_2) / (1 – C_1/C_2)] \times 100$

Proline content of the leaves

The free proline was determined according to Bates *et al*. (1973). The ninhydrine derivate was extracted with toluene and analysed spectrophotometrically at 520nm.

The possible interference of other free aminoacids has been reported as minimal in stressed plants, due to the high levels of proline under these conditions and to the fact that the colour of these aminoacids is also very low.

The proline concentration was determined from a standard curve and expressed as µmol proline g^{-1} FW.

Data analysis

Differences in the parameters measured between the treatments were analysed using the Student t-test.

Trends in RWC, proline content and cell membrane integrity were analysed by a linear regression, using the GraphPad Prism package, Version 2.01.

Results

Water retention capability (WRC) of the leaves

No differences between the two cultivars were found under well-watered conditions. Under water-stressed conditions, the cultivar Local had a slightly, not significantly, lower WRC than cultivar Falcon (Figure 1).

Water-stressed plants showed a slightly, not significantly, higher WRC, compared to the well watered control plants.

The cultivar Local showed under drought stress a lower WRC in the first 6h of free dehydration, but it retained more water after 8h and 24h.

No significant differences were found in WRC in the later growth stages: pod-setting and maturity. Both cultivars increased WRC during growth and during drought stress, up to 97%.

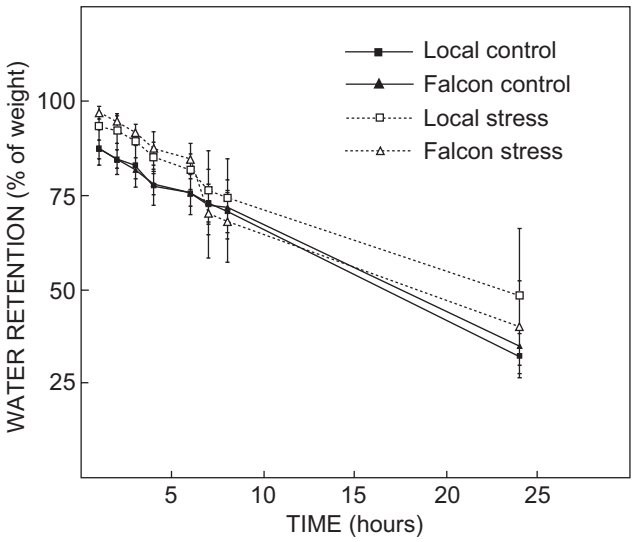

Figure 1: Water retention capability of two peanut cultivars under well watered and water stressed conditions. Data represent mean of eight plants (±SD)

Under well watered conditions the cultivar Falcon showed the lowest water retention capability 24h after free dehydration, both in the pod-setting and maturity stage as shown in Table 2.

Cultivar Local seems to better retain water after 24h dehydration, even if it retained less in the first 8h of dehydration than cultivar Falcon.

Relative water content (RWC), water saturation deficit (WSD) and relative saturation deficit (RSD)

RWC of well watered plants did not differ significantly between the two cultivars. However, under water-stressed conditions the cultivar Local showed the lowest RWC value (Table 3).

At the vegetative stage cultivar Local showed the highest values for WSD and RSD, characteristic of drought susceptible plants (Ashraf *et al.* 1996). At the pod-setting stage, no differences were found in RWC, WSD and RSD. At the maturity stage drought stressed plants showed a lower RWC value and higher WSD and RSD values.

Cultivar Falcon showed a low RWC value under drought stress conditions (68%), compared to the control treatment (91%, Table 3). This result is in contrast to RWC in the initial growth stage, where RWC was hardly decreased (Table 3).

Apparently, a long duration of the drought stress affected the cultivar Falcon more negatively than cultivar Local.

Cell membrane integrity

At the vegetative stage, the cell membrane injury as measured with the PEG test was relatively high in the drought-

Table 2: Effect of drought stress on water retention capacity after 24h of free transpiration (%) at the pod-setting and maturity stages. LC and FC are the control plants of the cultivars Local and Falcon, and LS and FS are the drought stressed plants of the cultivars Local and Falcon, respectively; dap are days after planting. Each value is the mean of eight replicates for each parameter (±SD). Per growth stage, values of the same cultivar followed by the same letter are not significantly different at $P < 0.05$ level, using the Student t-test

	Water retention capacity			
	LC	FC	LS	FS
Pod-setting stage (77dap)	56.2 ± 1.7 a	43.4 ± 9.0 b	56.3 ± 3.3 a	55.4 ± 4.9 a
Maturity stage (91dap)	61.6 ± 6.9 a	57.1 ± 8.4 a	69.3 ± 4.4 b	65.5 ± 8.9 a

Table 3: Effects of drought stress on relative water content (%), water saturation deficit (%) and relative saturation deficit (%) of two peanut cultivars, at the vegetative and maturity stages. LC and FC are the control plants of the cultivars Local and Falcon, and LS and FS are the drought stressed plants of the cultivars Local and Falcon, respectively; dap are days after planting. Each value is the mean of eight replicates for each parameter (±SD). Per growth stage, values of the same cultivar followed by the same letter are not significantly different at $P < 0.05$ level, using the Student t-test

Treatment	RWC (%)	WSD (%)	RSD(%)
Vegetative stage (0–35dap)			
LC	99.0 ± 0.6 a	0.92 ± 0.05 a	1.1 ± 0.37 a
LS	86.0 ± 7.0 b	14.0 ± 6.72 b	11.7 ± 5.31 b
FC	98.5 ± 0.4 a	1.53 ± 0.31 a	1.2 ± 0.31 a
FS	92.3 ± 3.0 b	7.71 ± 2.90 b	7.4 ± 2.35 b
Reproductive stage (42–91dap)			
LC	90.0 ± 4.0 a	10.50 ± 3.50 a	8.3 ± 2.80 a
LS	74.0 ± 9.0 b	26.30 ± 8.50 b	21.3 ± 6.70 b
FC	92.0 ± 4.1 a	9.50 ± 3.60 a	8.5 ± 3.50 a
FS	68.0 ± 6.0 b	32.10 ± 5.60 b	26.0 ± 5.40 b

susceptible cultivar Local under both well watered and water-stressed conditions (Table 4).

The two cultivars showed different responses when control and stress treatment plants were compared. Cultivar Falcon, showed a higher but not significant percentage of injury under drought stress conditions, and the cultivar Local showed a slightly higher but not significant value, under well watered conditions in the vegetative stage.

At the pod-setting stage, no significant differences were found in cultivar Local, whereas in cultivar Falcon under drought stress, a significantly lower percentage injury was observed.

At the maturity stage, the cultivar Falcon showed slightly higher values in percentage injury (data not shown). Drought tolerance of cultivar Falcon apparently decreased with increasing duration of the drought stress period.

Proline content

Roots and leaves showed a higher proline content under drought stress conditions (Figures 2a and b).

Leaves showed an increased proline content under drought stress (Figure 2a) except for cultivar Falcon at week 10. The increase varied from 700% at week 5, 100% at week 10 and 166% at week 13 for cultivar Local and 1 700% at week 5, to 270% at week 13, for cultivar Falcon.

Roots also showed a high proline content under drought stress (Figure 2b) except for cultivar Local at week 10.

The increase varied from 400% at week 5 to 125% in week 13, while in week 10 no differences were found in cultivar Local. In cultivar Falcon an increase of 300% at week 5, 300% at week 10 and 250% at week 13 was observed (Figure 2b).

Table 4: Effects of drought stress on percentage cell membrane injury with polyethylene glycol (PEG) test in two peanut cultivars at the vegetative and pod-setting stages. LC and FC are the control plants of the cultivars Local and Falcon, and LS and FS are the drought stressed plants of the cultivars Local and Falcon, respectively; dap are days after planting. Each value is the mean of eight replicates for each parameter (±SD). Per growth stage, values of each cultivar followed by the same letter, are not significantly different at P < 0.05 level, using the Student t-test

Treatment	Injury in PEG test (%)
Vegetative stage (28dap)	
LC	23.7 ± 10.0a
LS	21.2 ± 7.6a
FC	15.0 ± 3.5a
FS	17.2 ± 4.2a
Pod-setting stage (77dap)	
LC	48.4 ± 9.4a
LS	33.7 ± 10.8a
FC	33.7 ± 10.8a
FS	16.0 ± 4.0b

Discussion

Drought stress and water relations

Water retention capability (WRC)

WRC, which could be strongly related to drought hardiness, did not show any significant trend in both growth stages, in contrast to the results of Worku (1995), working with wheat under well watered conditions. However, drought stressed plants, at least in the early stages of growth showed a higher WRC than well watered plants (Figure 1).

In the first 6h under drought stress cultivar Local showed a slightly lower WRC than cultivar Falcon, but Local retained relatively more water after 8h and 24h of dehydration. Few experiments have reported results on WRC after 8h and 24h, but assuming that a low WRC in the first 6h of natural

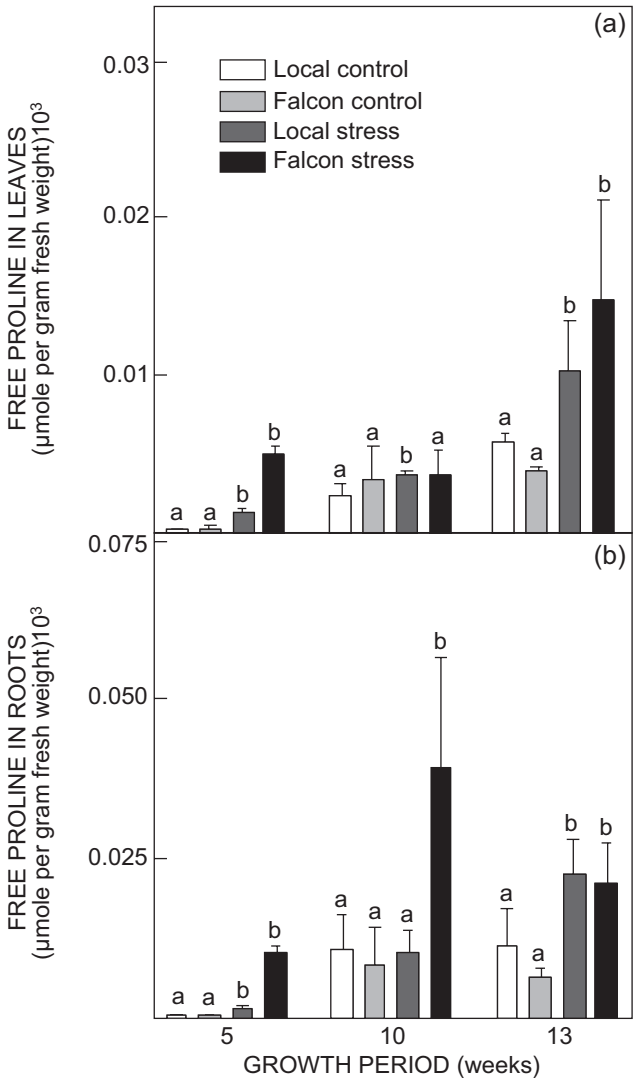

Figure 2: Proline content in two peanut cultivars, leaves (a) and roots (b) as affected by drought stress. Data represent mean of 8 plants (±SD). Different letters at a particular growth period denote values that are significantly different at P < 0.05 using the Student t-test

dehydration is associated with drought susceptibility, as in wheat (Worku 1995), it may be speculated that a high WRC after 8h and 24h may also have a relation with drought sensitivity, in this case drought susceptibility. In fact, Rascio (1985) showed that water retention capability of durum wheat leaves 24h after excision was higher in the cultivars susceptible to a shortage of water than in those resistant to drought. Thus, under stressed conditions the cultivar Local was slightly more susceptible to drought than the cultivar Falcon. The relation between WRC and drought resistance after 8h and 24h needs further testing.

The susceptibilty of the cultivar Local to drought stress confirms the results reported elsewhere by Quilambo (2000), when the cultivar Local was tentatively classified as a drought-avoider and drought-susceptible, whereas the cultivar Falcon was indicated as a drought-tolerator on the basis of changes in leaf area, specific leaf area (SLA), root weight ratio (RWR) and maximum root length to leaf area ratio (MRLAR).

In the pod-setting and maturity stage no changes in WRC were found under either well watered or stressed conditions, which may indicate that in peanut the water retention capability as a selection criterion for drought resistance depends on the growth stage.

Relative Water Content (RWC) and Relative Saturation Deficit (RSD)

From emergence until peg-initiation, the cultivar Falcon, the drought-tolerator, maintained a higher RWC (WSD) and a relatively low relative saturation deficit (RSD) as compared with cultivar Local, when both cultivars were subjected to drought. These results are consistent with several reports, indicating that drought-tolerant species exhibit significantly higher RWC and lower RSD (Premachandra *et al*. 1995, Ashraf *et al*. 1996).

At the pod-setting stage drought did not significantly change RWC and RSD in either cultivar and treatment, although slightly higher values in RSD were observed in the cultivar Local. Drought did not affect the water content of the leaves at this growth stage, in contrast to the results of Rao *et al*. (1985) in peanut cultivar. At the maturity stage significant differences among the cultivars and treatments were observed. The well watered plants showed higher RWC, while under stressed conditions the cultivar Falcon showed the lowest RWC value (Table 3). The high sensitivity of this cultivar to drought is also supported by the percentage of injury of the cell membranes, as discussed below. Although showing the lowest RWC value, the percentage of reduction in RWC in relation to control was slightly higher in the cultivar Local (82%), than in cultivar Falcon (74%). RSD, however, increased by 255% in the cultivar Local and 308% in cultivar Falcon.

The high values of RSD indicate a high sensitivity of the peanut plants at this growth stage to drought, since water is required not only to maintain the regular growth but to maintain an adequate peg turgor in order allow the peg to penetrate the soil.

Drought stress and proline content

Osmotic adjustment has been suggested as a mechanism, that leads to smaller changes in RWC per unit decrease in water potential in drought resistant species (Steudle *et al.* 1977) and consequently may help to maintain a positive turgor potential during water stress. This is a result of an increase in content of solutes such as proline. Both cultivars accumulated significantly more proline under drought stress, showing the highest values for the roots (Figure 2b) in early stages of growth.

The drought tolerant cultivar Falcon had a much greater accumulation of proline in the leaves than the drought-avoider Local. Ali Dib *et al*. 1994 found that proline accumulation in wheat explained 59% of the drought sensitivity index (DSI) in wheat suggesting that the capacity of a genotype to accumulate proline under stress with respect to the same genotype without stress, could give a good prediction of grain yield sensitivity to water stress, even if the physiological role of this amino-acid is not fully understood. No yield differences were observed in this experiment, which could be ascribed to the level of proline content, making it questionable as a selection criterion in these peanut cultivars.

In the pod-setting stage no significant changes in proline content were observed among the cultivars and treatments in accordance with the apparent insensitivity to drought stress of the cultivars at this stage, as was also shown by observations that no changes in RWC occurred.

At the maturity stage proline continued to accumulate under stress conditions (Figure 2). At this stage there was a strong and negative relationship between proline content and RWC ($r = 0.97$ at $P < 0.05$), and a strong positive relationship between proline content and RSD ($r = 0.97$ at $P < 0.05$), an indication that a low water content was associated with a high proline content. This relationship, which is contrary to the results at the vegetative stage, was used to question the use of proline content as a selection marker in durum wheat (Ali Dib *et al*. 1994). On the other hand, the continuous increase in proline content even in the well-watered plants makes clear that it is a result of several growth factors.

In summary, the cultivar Falcon accumulated substantially more proline under stress conditions than the cultivar Local (24 times more in the initial stages *versus* 9 times for the cultivar Local), in accordance with a previous supposition (Quilambo 2000) that the drought insensitivity of this cultivar, at least in the vegetative stage, may be linked to a mechanism of maintaining a positive turgor potential during prolonged water stress.

The results of the vegetative and pod-setting stages support the suggestion that a high level of proline in peanut leaves may indicate drought resistance, as proposed by Singh and Paleg (1972) in barley, Karamanos *et al.* (1983) in wheat and beans, and Ali Dib *et al*. (1994) in durum wheat; but it contradicts results of Andrade *et al*. (1995) who found that drought-susceptible *Phaseolus vulgaris* genotypes accumulated more proline than the drought-resistant ones. Premachandra *et al*. (1995) also found that in sorghum the contribution of osmotic adjustment and the rate of increase of

proline with decreasing water potential were greater in the drought-susceptible line. The physiological significance of proline accumulation in stressed plants is not clearly understood. It has been described as a symptom of injury (Hanson *et al.* 1979, Ibarra-Caballero *et al*. 1988), playing a role in osmotic adjustment (Handa *et al.* 1986, Hu *et al*. 1992, Delauney and Verma 1993) or in the storage of C and N for stressed tissues (Singh and Paleg 1972, Purvis and Yelenosky 1982), which can be used again during recovery from stress and in involvement in cell osmoregulation and protection of proteins during dehydration (Sheyakova 1984).

Drought stress and cell membrane integrity

Cell membrane integrity (CMI), measured as electrolyte leakage in the initial stages of growth, showed a higher percentage of injury in the cultivar Local under both controlled and drought stressed conditions (Table 4) than in cultivar Falcon. Few authors have reported CMI in peanut crop. Venkateswarlu and Ramesh (1993) showed that the cell membranes of a drought-tolerant peanut cultivar suffered much less injury than those of a drought-susceptible one, and that the differences were more marked in cultured cells.

Vasquez-Tello *et al*. (1990) indicated that an important strategy for drought resistance is the maintenance of membrane integrity after water stress. Cultivar Falcon appeared to be drought tolerant confirming previous findings by Quilambo (2000). In contradiction to these results Deb *et al*. (1996), working with four *Arachis* accessions, found that sensitive accessions which retained a higher proline content suffered more injury to its membranes under stress. However, the molecular mechanism, underlying this effect of proline, is not completely understood. In the present study although no significant relationship between CMI and proline content was found (Premachandra *et al.* 1992), the cultivar that accumulated more proline in the leaves, the cultivar Falcon, suffered relatively less membrane injury compared to the other cultivar (Table 4).

Many other authors have reported a direct relation between CMI and osmotic adjustment (e.g. Rudolph *et al*. 1986), but in the present study this relation was not found particularly at the maturity stage. However, as the pods had developed, the cell membrane injury did not reduce the final yield.

From the results of this study it is concluded that: (i) cultivar Falcon exhibited characteristics of drought tolerance as found in other experiments, and (ii) RWC, CMI and proline content were useful parameters to detect drought stress in peanut plants, but the pattern of change was much dependent on the growth stage.

Acknowledgements — Special thanks to Dr Ineke Stulem, H Doddema, I Weissenhron and PJC Kuiper for reading the manuscript. This study was financially supported by the DEIBI project (Desenvolvimento do Ensino e Investigação na área de Biologia) within the MHO programme administered by NUFFIC.

References

Agarie S, Hanaoka N, Kubota F, Agata W, Kaufman PB (1995) Measurement of cell membrane stability by electrolyte leakage as a drought and heat tolerance test in rice (*Oryza sativa* L.). Journal of the Faculty of Agriculture, Kyushu University **40**: 233–240

Ali Dib T, Monneveux P, Acevedo E, Nachiti MM (1994) Evaluation of proline and chlorophyll fluorescence quenching measurements as drought tolerance indicators in durum wheat (*Triticum turgidum* L. var. *durum*. Desf). Euphytica **79**: 65–73

Andrade JL, Larque-Savedra A, Trejo CL (1995) Proline accumulation in leaves of four cultivars of *Phaseolus vulgaris* L. with different drought resistance. Phyton Experimental Botany (Argentina) **57**: 149–157

Ashraf MY, Naqvi MH, Khan AH (1996) Evaluation of frou screening techniques for drought tolerance in wheat (*Triticum aestivum*). Acta Agronomica Hungarica **44**: 213–220

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil **39**: 205–207

Bennett JM, Boote KJ, Hammond LC (1984) Relationships among water potential components, relative water content, and stomatal resistance of field grown peanut leaves. Peanut Science **11**: 31–35

Deb N, Alam B, Guptka SD, Ghosh BC (1996) Cell membrane stability of leaf tissues and its relation with drought tolerance in *Arachis*. Indian Journal of Experimental Botany **34**: 1044–1047

Delauney AS, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. The Plant Journal **4**: 215–223

Gascho GJ, Davis JG (1994) Mineral Nutrition. In: Smartt L (ed) The Groundnut Crop: A Scientific Basis for Improvement. Chapman and Hall, London, UK, pp 214–245

Handa S, Handa AK, Hasegawa PM, Bressan RA (1986) Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiology **80**: 938–945

Hanson AD, Nelsen CE, Everson EH (1977) Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Science **17**: 720–726

Hanson AD, Nelsen CE, Pedersen AR, Everson EH (1979) Capacity of proline accumulation during water stress and its implications for breeding for drought resistance. Crop Science **19**: 489–493

Hu CA, Delauney AS, Verma DPS (1992) A bifunctional enzyme (pyrroline-5-carboxylase synthetase) catalyses the first two steps in Proline biosynthesis in plants. Proceedings of the National Academy of Sciences of the United States of America, pp 9354–9358

Ibarra-Caballero J, Villanueva-Verduzco C, Molina-Galan J, Sanchez-de-Jimenez E (1988) Proline accumulation as a symptom of drought stress in maize: A tissue differentiation requirement. Journal of Experimental Botany **39**: 889–897

Jones MM, Osmomnd CB, Turner NC (1980) Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. Australian Journal of Plant Physiology **7**: 193–205

Karamanos AJ, Drossopolus JB, Niavis CAJ (1983) Free proline accumulation during development of two wheat cultivars with water stress. Journal of Agricultural Sciences **100**: 429–434

Morgan JM (1984) Osmoregulation and water stress in higher plants. Annual Review of Plant Physiology **35**: 299–319

Munns R (1988) Why measure osmotic adjustment? Australian Journal of Plant Physiology **15**: 717

Parsons LR, Howe TK (1984) Effects of water stress on the water relations of *Phaseolus vulgaris* and the drought resistant *Phaseolus acutifolius*. Physiologia Plantarum **60**: 197–202

Prabowo A, Prastowo B, Wright GC (1990) Growth, yield and soil water extraction of irrigated and dryland peanuts in South Sulawesi. Irrigation Science **11**: 63–68

Premachandra GS, Hahn DT, Rhodes D, Joly RJ (1995) Leaf water relations and solute accumulation in two grain sorghum lines exhibiting contrasting drought tolerance. Journal of Experimental Botany **46**: 1833–1841

Premachandra GS, Saneoka H, Fujita K, Ogata S (1992) Leaf water

relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. Journal of Experimental Botany **43**: 1569–1576

Premachandra GS, Saneoka H, Ogata S (1989) Nutrio-physiological evaluation of Polyethyleneglycol test of cell membrane stability in maize. Crop Science **29**: 1287–1292

Premachandra GS, Saneoka H, Ogata S (1990) Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. Journal of Agricultural Science **115**: 63–66

Premachandra GS, Shimada T (1987) The measurement of cell membrane stability using polyethylene glycol as a drought tolerance test in wheat. Japanese Journal of Crop Science **56**: 92–98

Premachandra GS, Shimada T (1988) Evaluation of PEG test of measuring cell membrane stability as drought tolerance test in wheat. Journal of Agricultural Sciences **110**: 429–433

Purvis AC, Yelenosky G (1982) Sugar and proline accumulation in grape fruit flavedo and leaves during cold hardening of young trees. Journal of the American Society for Horticultural Sciences **107**: 222–226

Quilambo OA (2000) Functioning of Peanut (*Arachis hypogaea* L.) Under Nutrient Deficiency and Drought Stress in Relation to Symbiotic Associations. PhD Thesis, University of Groningen, The Netherlands, 168pp

Rao RCN, Singh S, Sivakumar MVK, Srivastava KL, Williams JH (1985) Effect of water deficit at different growth phases of peanut. I. Yield responses. Agronomy Journal **77**: 782–786

Rascio A (1985) Water retention capability of 2 *Triticum durum* cultivars receiving 2 different water regimes. Genetic Agriculture **39**: 409–416

Rudolph AS, Crowe JH, Crowe LM (1986) Effect of three stabilising agents — Proline, Betaine and Trehalose — on membrane phospholipids. Archives of Biochemistry and Biophysics **245**: 134–143

Sheyakova NI (1984) Metabolism and physiological role of proline in plants under conditions of water and salt stress. Soviet Journal of Plant Physiology **31**: 597–608

Singh TN, Paleg LG (1972) Proline accumulation and varietal adaptability in barley. A potential metabolic measure of drought resistance. Nature **236**: 188–190

Sinclair TR, Ludlow MM (1985) Who taught plants thermodynamics? The unfulfilled potential of plant water potential. Australian Journal of Plant Physiology **12**: 213–217

Steudle E, Zimmerman U, Luttge U (1977) Effects of turgor pressure and cell size on wall elasticity of plant cells. Plant Physiology **59**: 285–289

Sullivan CY (1972) Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. In: Rao NGP, House LR (eds) Sorghum in the Seventies. Oxford and IBH Publishing Co, New Delhi, India, pp 247–264

Sullivan CY, Ross WM (1979) Selection for drought and heat resistance in grain sorghum. In: Mussel H, Staples R (eds) Stress Physiology in Crop Plants. John Wiley and Sons, New York, USA, pp 263–281

Turner NC (1986) Crop water deficits: a decade of progress. Advances in Agronomy **39**: 2–51

Van Rensburg L, Kruger GHJ, Kruger H (1993) Proline accumulation as drought tolerance criterion its relationship to membrane integrity and chloroplast ultrastructure in *Nicotiana tabacum* L. Journal of Plant Physiology **141**: 188–194

Vasquez-Tello A, Zuily-Fodil Y, Thi ATP, Da Silva JBV (1990) Electrolyte leakage and soluble sugar content as physiological tests for screening for resistance to water stress in *Phaseolus* and *Vigna* species. Journal of Experimental Botany **41**: 827–832

Venkateswarlu B, Ramesh K (1993) Cell membrane stability and biochemical response of cultured cells of groundnut under PEG-induced water stress. Plant Science **90**: 179–185

Worku T (1995) Water-retention capability as a selection criteria for drought resistance in wheat. Journal of Plant Nutrition **18**: 2105–2109

Edited by P Scott