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The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.)

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Abstract

Ber (*Ziziphus mauritiana* Lamk.) is a major fruit tree crop of the north-west Indian arid zone. In a study of the physiological basis of drought tolerance in this species, two glasshouse experiments were conducted in which trees were droughted during single stress-cycles. In the first experiment, during a 13 d drying cycle, pre-dawn leaf water (Ψ_{leaf}) and osmotic (Ψ_{π}) potentials in droughted trees declined from −0.5 and −1.4 MPa to −1.7 and −2.2 MPa, respectively, for a decrease in relative water content (θ) of 14%. During drought stress, changes in sugar metabolism were associated with significant increases in concentrations of hexose sugars (3.8-fold), cyclitol (scyllo-inositol; 1.5-fold), and proline (35-fold; expressed per unit dry weight), suggesting that altered solute partitioning may be an important factor in drought tolerance of *Ziziphus*. On rewatering, pre-dawn Ψ_{leaf} and θ recovered fully, but Ψ_{π} remained depressed by 0.4 MPa relative to control values, indicating that solute concentration per unit water content had changed during the drought cycle.

Evidence for osmotic adjustment was provided from a second study during which a gradual drought was imposed. Pressure–volume analysis revealed a 0.7 MPa reduction in osmotic potential at full turgor, with Ψ_{leaf} at turgor loss depressed by ∼1 MPa in drought-stressed leaves. Coupled with osmotic adjustment, during gradual drought, was a 65% increase in bulk tissue elastic modulus (wall rigidity) which resulted in turgor loss at the same θ in both stressed and unstressed leaves. The possible ecological significance of maintenance of turgor potential and cell volume at low water potentials for drought tolerance in *Ziziphus* is discussed.

Key words: *Ziziphus mauritiana*, drought, solute accumulation, osmotic adjustment, proline.

Introduction

Drought is probably the most important factor limiting crop productivity world-wide (Jones and Corlett, 1992). In semi-arid regions, where the amounts and patterns of seasonal rainfall are often erratic and unpredictable, deep-rooting perennial species may exploit deep soil water reserves. This facilitates production of yields in situations of high light coupled with drought, where shallow-rooted annual crops would normally fail. The use of perennials may help buffer farmers' production against year-to-year fluctuations in yields from annual crop species. Under such conditions, an ability to maintain cell water status (e.g. through osmotic adjustment and/or leaf area adjustment) and cell integrity (by protecting cells against photo- and chemical oxidation) may be an advantage. There are many cases where plants growing in hostile environments exhibit increased oxy-stress enzyme activities to combat lipid peroxidation by free radicals, hydrogen peroxide

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and the potentially damaging products of photosynthesis in high-light environments (reviewed by Smirnoff, 1995).

Ber (*Ziziphus mauritiana* (Lamk.), Rhamnaceae) is an important fruit crop of arid and semi-arid regions of India (Chandra *et al*., 1994). It is widely grown in the plains of Punjab, Haryana, Uttar Pradesh, Gujrat, and Rajasthan. The fruits are favoured locally for their flavour, and relatively high sugar and vitamin C content. In the north-west Indian arid zone, with annual rainfall as low as 200 mm, and day temperatures reaching more than 40 °C, some cultivars (including Seb, Gola, Tikri, and Mundi) have proven ideal for commercial propagation (Pareek, 1977). In this region, improved cultivars are generally propagated by budding on to the native drought-tolerant *Z. rotundifolia* or *Z. nummularia* rootstocks. In Africa, both wild species of *Ziziphus* (*Z. abyssinica* and *Z. mucronata*) and unimproved *Z. mauritiana* (believed to have been introduced from the Middle-East) have become 'indigenized' and are planted around homesteads (Coates-Palgrave, 1993).

Previous work has cited *Ziziphus mauritiana* as being both drought- and salt-tolerant (Chovatia *et al*., 1993), but its performance has been expressed in terms of growth and yield, with no detailed examination of how water relations and osmotic factors may contribute towards drought tolerance in this species. As part of a programme to assess the value of utilizing improved Indian cultivars of *Ziziphus* in African environments, the material has been transferred from India into Zimbabwe for field trials in a range of different environments ranging from cool highland to arid lowland climates.

Drought tolerance mechanisms have been summarized by Jones (1992) as: (i) avoidance of damaging plant water deficits; (ii) stress tolerance—adaptations that enable plants to continue functioning in spite of plant water deficits; and (iii) efficiency mechanisms that enable the plant to optimize the utilization of resources, especially water. Drought avoidance involves completion of the life/reproductive cycle during favourable conditions and would include perennial/deciduous plants that remain dormant during drought. Drought tolerance is prevalent in plants which exist in climates where drought occurs at random, and encompasses all physiological adaptations that extend the period of active growth by controlling water loss and turgor, and which enable cells to sustain water loss without damage to the metabolic systems. Effective control of water loss through stomatal closure, leaf drop or water uptake by enhanced root growth can all improve plant water status. Osmotic adjustment through the active accumulation of solutes in the cell sap, rather than through passive solute accumulation resulting from reduced cell volume (reviewed by Morgan, 1984) can also contribute to turgor maintenance, and this is a prerequisite for continued growth during drought (Hsiao *et al*., 1976). Changes in either or both of tissue elastic properties and solute concentrations may promote maintenance of turgor and cell volume despite low water potentials (Ayoub *et al*., 1992). Increased elasticity during drought has been reported (Fan *et al*., 1994), but in these cases osmotic adjustment is generally not expressed to any significant degree. This lack of osmotic adjustment may relate to the fact that, if elastic cells contain high concentrations of solutes, there would be a risk of cell rupture/tissue damage on rehydration following a period of drought. Throughout the last decade, there has been considerable debate concerning which are the most critical factors to which plants respond during drought; whether to absolute water potential, or to changing turgor and cell volume. Several reports suggest that plant metabolic processes are in fact more sensitive to turgor and cell volume than to absolute water potential, with maintenance of inter-molecular distances critical for continued metabolic activity (reviewed by Jones and Corlett, 1992).

Some workers argue that osmotic adjustment is rather peripheral to drought tolerance (Munns, 1988), whilst others have targeted early increases in sugars and amino acids as indicators of drought tolerance, with a view to using these criteria as the basis of selection for breeding programmes (Van Heerden and De Villiers, 1996; Stajner *et al*., 1995; Sabry *et al*., 1995).

The present work investigates the extent to which osmotic adjustment and changes in wall elastic properties may contribute to drought tolerance in *Ziziphus mauritiana*.

Materials and methods

Experimental material

In 1990, two cultivars of *Ziziphus mauritiana* (cvs Seb and Gola), grafted onto local Indian rootstock (*Z. rotundifolia*) were imported from India to the UK. They were grown under glasshouse conditions in 65 l pots containing equal parts of Levingtons C2, John Innes No 2 and horticultural grit. Prior to the experiment, all trees were maintained at day/night temperatures of 25/15 °C with an optimal supply of water and nutrients. During the winter months a 14 h day was maintained using supplementary lighting supplied by SON-T 400 W lamps. Environmental conditions in the glasshouse during the experimental period (June–July 1995) are shown in Table 1.

Experimental design

The current paper combines two data sets: the 'primary experiment' on the effects of drought on water relations, gas exchange, solute concentrations and oxidative enzyme activities; and a further 'complementary study' in which pressure–volume (P–V) relationships were analysed to identify the main components of osmotic change during drought.

Primary experiment (June–July 1995): 16 trees were selected (8 from each cultivar) and designated as either control (irrigated whenever soil water potential (Ψ_{soil}) reached -0.02 MPa), or droughted (trees received no irrigation through a single 13 d drying cycle until wilting). Trees were then re-irrigated to pot

Table 1. *Mean day/night temperatures, relative humidity (RH), vapour pressure deficit (*VPD*) and photosynthetically active radiation (*PAR*) in the glasshouse during the experimental period (±SE; 14 d.f.)*

	Temperature (°C)	RH (%)	*VPD* (kPa)	*PAR* (μmol m^{-2} s^{-1})
Day	24.1 (0.14)	52.3 (1.7)	2.14 (0.04)	370 (16)
Night	17.1 (0.15)	75.5 (0.8)	1.45 (0.01)	—

capacity based on gravimetric water loss (measured to 0.1 kg precision [Salter Weigh-Tronix Ltd, UK]). Trees were arranged in four randomized blocks within the glasshouse. Each block contained one tree of each cv. in each treatment.

Complementary study (September–October 1996): Using cv. Gola only, a small-scale study compared the *P–V* relationships in leaves of single irrigated and droughted trees. During this study, drought was applied over a period of 18 d through deficit irrigation (based on gravimetric moisture loss of control).

Measurements

Water relations (*primary experiment*): In control pots, Ψ_{soil} was monitored twice daily using pairs of mercury manometer tensiometers installed at 10 cm and 30 cm depths. The mean water potential was calculated from the readings and control trees were irrigated whenever Ψ_{soil} reached −0.02 MPa.

Pre-dawn and midday leaf water potentials (Ψ_{leaf}), osmotic potentials (Ψ_π) and relative water content (θ; midday only) were determined for fully expanded outer canopy leaves (three per tree) on day 0, 2, 7, 9, 13, and 16. Pre-dawn and midday turgor pressures (Ψ_P) were calculated as the difference between Ψ_{leaf} and Ψ_π at each time of day.

Ψ_{leaf} was measured using a pressure chamber (Skye SKPM 1400, Skye Instruments, Powys, Wales) after Scholander *et al.* (1965). To minimize errors due to water losses during the procedure, leaves were cut and transported to the chamber wrapped in humidified polythene bags, and the base of the chamber was lined with moistened tissue. Leaf samples for determination of Ψ_π and θ were taken from the same leaves that had been used for Ψ_{leaf}. Immediately after determination of Ψ_{leaf}, a portion of the leaf was removed and sealed into a 0.5 ml Eppendorf tube, frozen in liquid nitrogen and stored at −20 °C until required. Leaf samples were thawed at room temperature and the sap removed by centrifugation (10 000 rpm for 10 min at 0–4 °C, Desaspeed MH–2K, Sarstedt, Germany) into larger tubes, which were immediately sealed and stored on ice. Ψ_π of the extracted sap was determined using a vapour pressure osmometer (Wescor 5100 C, Chemlab Scientific Products, Hornchurch, Essex, UK).

Relative water content (θ) was determined from measurement of fresh (*FW*), turgid (*TW*) and dry (*DW*) weights of 4 discs/replicate leaf using the formula:

$$\theta(\%) = 100 \times (FW–DW)/(TW–DW)$$

text Turgid weight was determined from rehydrated discs (floated on distilled water overnight under low illumination (to avoid respiratory losses)). Dry weight was measured after drying material to constant weight at 80 °C.

Leaf expansion (*primary experiment*): Newly emerging leaves were tagged on each tree (three leaves per tree) and leaf lengths and widths were measured daily with a 0.5 mm graduated rule. Leaf areas (single plane projection) were determined from the following empirically derived relationship between length, width and area:

$$\text{Area} = 0.768 \times \text{length} \times \text{width}\ (r^2\ 0.992,\ 285\ \text{d.f.})$$

Gas exchange and chlorophyll fluorescence (*primary experiment*): On days 1, 6, 9, and 17, assimilation (*A*) and conductance (g_{leaf}) were measured in fully expanded, exposed leaves (three per tree) using a portable leaf chamber gas analyser (LCA-4, Analytical Development Company, Hoddesdon, Herts. UK). Measurements were taken between 10.00 and 13.00 h with 600 μmol m^{-2} s^{-1} *PAR* supplied from an artificial light source (PLU-2, ADC, UK).

To study the effect of drought on the efficiency of photosystem II, chlorophyll fluorescence (F_v/F_m) was measured for dark-adapted (overnight) mature leaves in full-sun positions (three leaves per tree) using a PSM Biomonitor (Sweden).

Harvest of leaf material for determination of sugars, starch, proline, and antioxidant enzyme activity (primary experiment): Harvests were made on day 0, 7, 13, and 16. At each harvest date, duplicate mature outer canopy leaf samples were harvested from each tree during the period 10.00 to 12.30 h. Samples for enzyme assay (200–300 mg fresh weight per sample) were immediately weighed, frozen in liquid nitrogen and stored at −80 °C until required for analysis. Leaf samples for determination of sugars, anions and cations were quickly weighed, then microwave-dried to 'kill' enzymes and ensure minimal change in composition (Popp *et al.*, 1996) and dried to constant weight.

Leaf samples (100 mg) were homogenized in 5 ml TRIS–HCl buffer (pH 7, 0–4 °C), centrifuged at 10 000 rpm for 20 min (0–4 °C) and the supernatant removed for assay. Total soluble proteins were assayed using the method of Bradford (1976). Leaf samples were prepared for assaying the activities of peroxidase and ascorbate peroxidase as described previously (Dinesh *et al.*, 1996).

Determination of sugars and starch (primary experiment): Dried leaf samples were milled to a fine powder and extracted in hot water (4% w/v). Aliquots were diluted and analysed on HPLC using an anion exchange column (Carbopac PA 100, 50 × 4 mm, Dionex). Low molecular weight carbohydrates and cyclitols were eluted by 150 mM NaOH at 32 °C and detected using PAD (Pulsed Amperometric Detection; ED40, Dionex).

For determination of starch content, 20 mg of the finely ground powder was extracted with 1 ml 50% ethanol at room temperature, centrifuged, and the pellet re-extracted twice with 1 ml of 90% ethanol at 60 °C. Dried pellets were incubated in 1ml distilled water at 85 °C for 30 min with 8 μkat heat stable α-amylase (from *Bacillus licheniformis*, Sigma). The assays were centrifuged and 100 μl aliquots of the supernatant incubated in 0.5 ml 20 mM sodium acetate (pH 4.6) at 55 °C with 160 nkat amyloglucosidase (from *Aspergillus niger*, Boehringer-Mannheim). The reaction was terminated after 30 min by addition of 0.5 ml chloroform. Glucose was quantified in aliquots of the supernatants as described for low mol. wt. carbohydrates.

Ionic osmotica (primary experiment): Aliquots of the diluted hot water extracts were analysed for inorganic anions by chemically suppressed ion-chromatography (DX 500 Dionex) on anion exchange column (AS11,250 × 4 mm i.d., Dionex). Anions were separated on a NaOH gradient (2.5 min at 0.05 mM, then linear increase to 37.5 mM over the subsequent 15.5 min) at 32 °C at a flow rate of 2 ml min^{-1}. Cations (Na^+ and K^+) were assayed in diluted aliquots of the hot water extract using Flame Emission Spectrometry, and calcium and

magnesium concentrations were determined by Atomic Absorption Spectrometry (Unicam 939 AA Spectrometer).

Proline determination (primary experiment): Proline was quantified according to the method of Troll and Lindsey (1955).

Pressure–volume (P–V) relationship: (complementary study): The pressure bomb was used to obtain pressure–volume relationships for leaves from irrigated and drought-stressed trees (three leaves per tree). Fully expanded, mature outer canopy leaves were removed by cutting the petiole under distilled water, and they were rehydrated overnight in a darkened humid chamber. Pressure–volume curves were generated using the repeat pressurization technique, with leaves drying on the laboratory bench between measurements (Tyree and Hammel, 1972). Turgid weight was estimated graphically by extrapolation of the relationship between fresh weight and Ψ_{leaf}. Osmotic potential at full turgor ($\Psi_{\pi 100}$) and apoplastic water fraction (R_a) were estimated for each treatment by extrapolation of the regression of the linear portion of the curve (when $\Psi_P = 0$) to the y and x axes, respectively. Symplast volume was estimated according to Meinzer *et al.* (1990). Höfler diagrams were constructed of change in potential with θ and the turgor loss point estimated graphically. Normalized bulk modulus of elasticity near full hydration ($\epsilon_B = \delta P/(\delta V/V)$ was calculated according to Jones (1992)). The data were fitted using a modified quadratic function, which took the value zero for θ below some level. The function was constrained to go through $-\Psi_{\pi 100}$ at full turgor, with the additional constraint that the curve be differentiable, that is: that the quadratic be at its minimum of zero at the θ value below which $\Psi_1 = \Psi_\pi$. This gives one degree of freedom for the function. The lack of fit from further constraining the turgor loss point to be the same for both trees, was compared to that when it was allowed to differ.

Statistical analyses

Primary experiment: Data trends which met criteria for treatment as split plots (after Box, 1950) were analysed using analysis of variance. Most of the time-series data could not be treated as split plots, and were subjected to analysis using a method based on Gabriel's ante-dependence covariance structure (Gabriel, 1962), with the ante-dependence order assigned according to Kenward (1987).

Complementary study: As this was based on single tree comparisons, it was not possible to compare treatments directly. Based on the assumption that water relations parameters in both trees were equivalent before the study (when both trees were unstressed), within-tree variability was analysed using the Student t-test and inferences between trees made on these data.

Results

Primary experiment

Statistical analyses showed no significant effect of cultivar on any variable measured during the experiment. Data

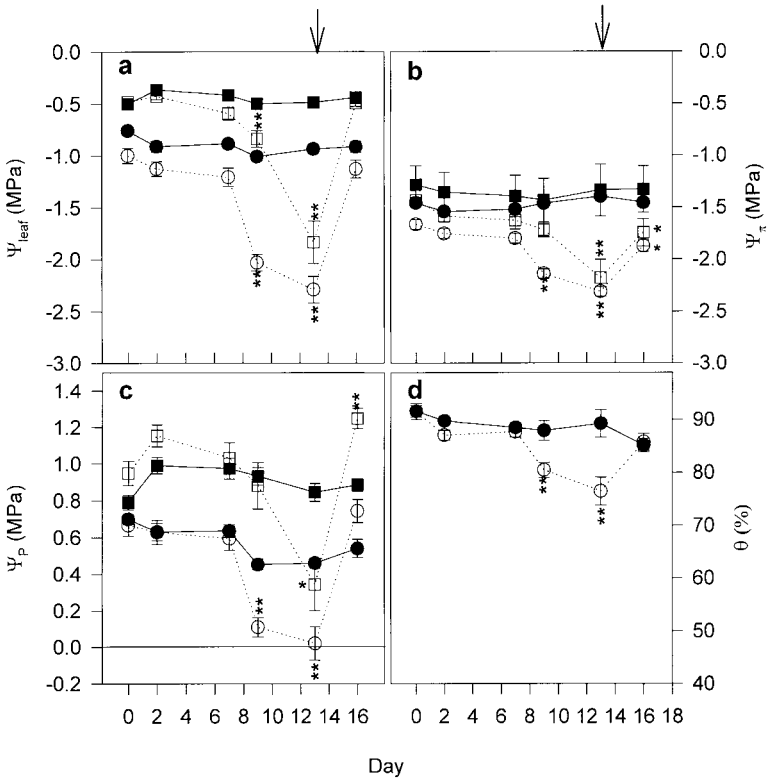

Fig. 1. Changes in pre-dawn (■/□, control/droughted) and midday (●/○, control/droughted): (a) leaf water potential (Ψ_{leaf}); (b) osmotic potential of freeze-thawed cell sap (Ψ_π); (c) turgor potential (Ψ_P), and (d) relative water content (θ) in *Ziziphus mauritiana* during a single drying cycle and following rewatering (arrow); ±1 SE; $n = 24$; *, **, significant $P < 0.05$ and 0.001, respectively. Where no error bars are apparent, they are smaller than the size of the symbol.

for both cultivars were therefore combined for further analysis.

Trends in water relations during drought: Figure 1 shows changes in pre-dawn and midday Ψ_{leaf} (Fig. 1a), Ψ_{π} (Fig. 1b), Ψ_{P} (Fig. 1c), and midday θ (Fig. 1d) during a 13 d drought cycle and on day 16, 3 d after re-irrigation of the stressed trees. Pre-dawn Ψ_{leaf} started to diverge from control values 7 d into the stress cycle, and significant differences were first observed on day 9 ($P<0.001$, Fig. 1a), with Ψ_{leaf} reaching a minimum of -1.7 MPa on d 13. By this time, leaves were starting to show signs of wilting during the day (Fig. 1c), and following rewatering on day 13, Ψ_{leaf} recovered to control values within 3 d. In both treatments, midday Ψ_{leaf} were more negative than pre-dawn values due to the effect of transpiration (Fig. 1a). In control trees, there was no significant diurnal variation in Ψ_{π} (~ -1.4 MPa, Fig. 1b), and the 0.4–0.5 MPa difference in pre-dawn Ψ_{P} (Fig. 1c) compared to midday could be largely attributed to the diurnal depression in Ψ_{leaf}. By 9 d of drought, there were highly significant ($P<0.001$) reductions in midday plant water status (Figs 1a–d), with Ψ_{leaf} and Ψ_{π} reaching -2.3 MPa with θ reduced to 76%.

During the drying cycle, a 14% reduction in θ (relative to control values; Fig. 1d) corresponded to a 0.8 MPa reduction in pre-dawn Ψ_{π} (from -1.4 to -2.2 MPa, Fig. 1b). Following irrigation on day 13, when pre-dawn Ψ_{leaf} and θ had recovered to control values (Fig. 1a, d), pre-dawn Ψ_{π} was still 0.30–0.40 MPa more negative than the control. This resulted in pre-dawn Ψ_{P} 0.3–0.4 MPa higher than control values (Fig. 1d).

Leaf expansion: When compared to control leaves, rate of leaf expansion in stressed trees appeared to be lower during the first 7–8 d of the drying cycle. This reduction in leaf expansion rate was observed before significant decreases in water status were measured (Figs 1, 2). By the time drought stress had resulted in significant reductions in leaf water status (day 9), leaves had stopped expanding (Fig. 2). For values of $\Psi_{P}<0.2$ MPa leaf expansion was inhibited and leaf area actually decreased (Figs 1c, 3).

Gas exchange and chlorophyll fluorescence: In line with the water relations data, A and g_{leaf} were unaffected by drought until day 9 (Fig. 3). Gas exchange rates declined from about the time that midday Ψ_{leaf} dropped below -1.3 MPa. By the time midday Ψ_{leaf} reached -2 MPa (pre-dawn $\Psi_{leaf}=0.75$ MPa), A was reduced by 30% (from 17.3 ± 0.44 to 12.0 ± 0.91 μmol m^{-2} s^{-1}), while g_{leaf} was reduced by 50% (from 0.35 ± 0.01 to 0.17 ± 0.02 mol m^{-2} s^{-1}) compared to control values. No gas exchange data are available for day 12, so it is not possible to determine whether A and g_{leaf} declined further

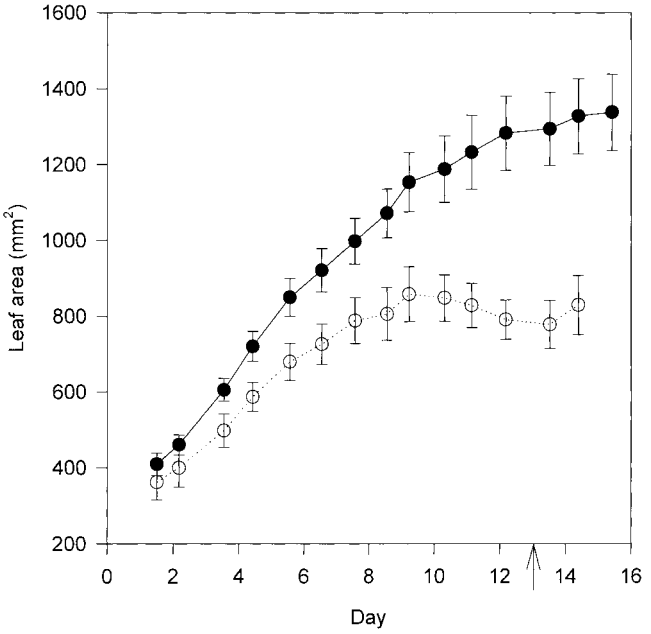

Fig. 2. Leaf area expansion in *Ziziphus mauritiana* with time after withholding irrigation, and after rewatering on day 13 (arrow). (●/○, control and droughted treatments respectively; ±1 SE; $n=6$.

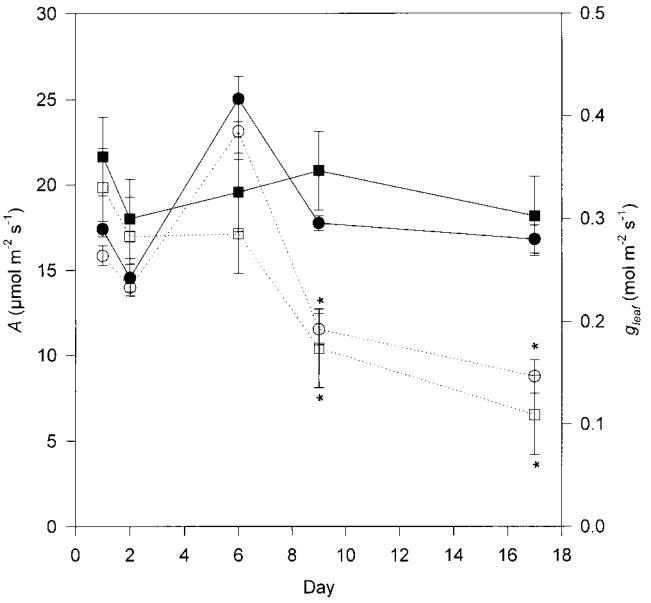

Fig. 3. Assimilation (A; ●/○) and leaf conductance (g_{leaf}, ■/□) in *Ziziphus mauritiana* during drought, Trees were rewatered on day 13; closed/open symbols, control/droughted; ±1 SE; $n=24$; *, significant $P<0.005$.

before rewatering. Following irrigation on day 13, both g_{leaf} and A remained depressed despite a full recovery in leaf water status. Fluctuations in gas exchange in control leaves between sample dates were probably due to day-to-day variation in the ambient irradiance to which the leaves had become acclimated.

Fluorescence induction of dark-adapted leaves showed

no significant effect of treatment on PSII efficiency (the ratio of F_v/F_m; mean = 0.729 ± 0.08 for both treatments).

Solute accumulation and oxidative enzyme activity during drought stress: *Sugars:* There was an 85% increase in hexose concentration in droughted trees by day 7 (glucose and fructose; significant at $P < 0.001$), and a 19% increase in the cyclitol scyllo-inositol ($P < 0.001$; Fig. 4a). These increases occurred before drought had significant effects on water status (Tables 2, 3; Fig. 1). By day 13, total hexose and cyclitol concentrations had increased 3.8-fold and 53%, respectively, compared with control values. Glucose accounted for 60% of hexoses at this time, and when compared to increases in hexoses, scyllo-inositol accounted for 8% of the overall increase. High hexose concentrations were maintained beyond the time of stress-relief (Fig. 4a) and there was no significant effect of drought on either sucrose or myo-inositol concentrations (Table 2).

The accumulation of hexose sugars during drought stress corresponded to reductions in starch concentration (expressed as glucose equivalents, Fig. 4a). Decreased starch content could account for the observed increases in hexose concentration in the absence of external factors. At the end of the drying cycle, reduction in starch concentration outweighed the increase in hexoses by 64% (127 μmol g^{-1} dry matter), indicating that a net export of sugars from the leaf may have occurred during the day.

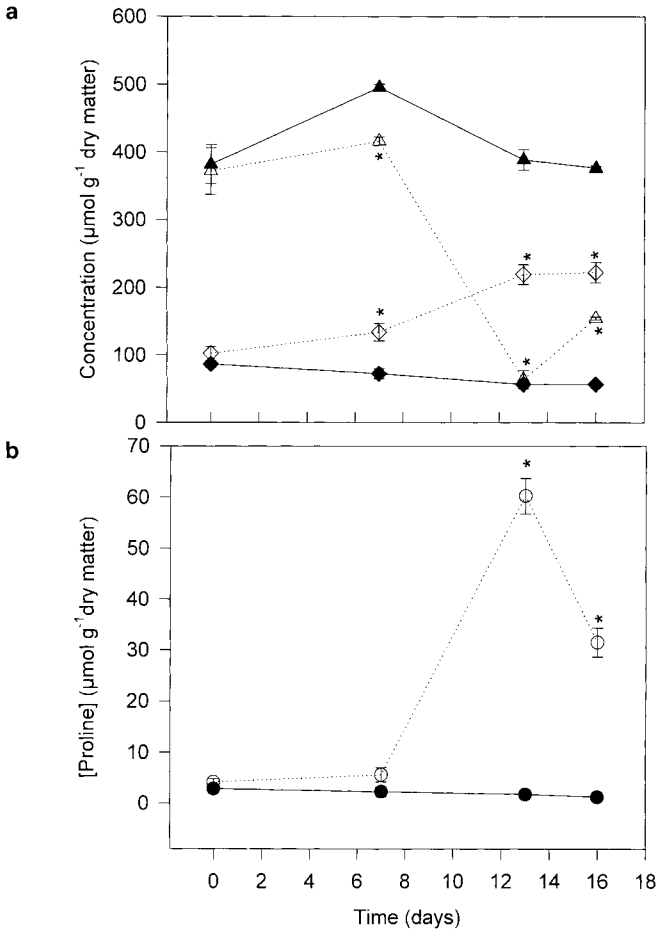

Fig. 4. Changes in concentrations of (a) hexose sugars (◆/◇), starch (expressed as hexose equivalents; ▲/◿); and (b) proline (●/○) in *Ziziphus mauritiana* during a drying cycle and after rewatering on day 13; solid/open symbols = control/droughted; ± 1 SE; $n = 16$. *, significant $P < 0.005$. Where no error bars are apparent, they are smaller than the size of the symbol.

Table 2. *Changes in concentration of cyclitols and sucrose in* Ziziphus mauritiana *with time after witholding irrigation*

Day	Concentration (μmol g^{-1} dry matter)					
	Scyllo-inositol^{sp}		Myo-inositol^{sp}		Sucrose^{sp}	
	C	D	C	D	C	D
0	29.2	27.7	6.6	7.2	214	205
7	33.7	39.4***	4.4	5.9	295	328
13	24.9	37.9***	4.6	7.2	227	247
16	33.0	49.1***	2.7	4.8	265	268
SED ($n = 16$)	1.84		0.27		9.8	

X^{sp}, analysed as a split plot; C, control treatment; D, drought treatment; ***, significant drought effect at $P < 0.001$.

Table 3. *Changes in concentration of monosaccharide sugars in* Ziziphus mauritiana *with time after witholding irrigation*

Day	Concentration (μmol g^{-1} dry matter)					
	Glucose^{1}			Fructose^{2}		
	C	D	SED ($n = 16$)	C	D	SED ($n = 16$)
0	54.4	61.6	11.14	32.3	40.7	7.09
7	46.2	81.6***	3.67	26.3	52.5*	5.76
13	34.9	129.5***	14.57	21.8	89.5***	6.09
16	34.5	106.8***	21.42	22.3	115.0***	17.43

X^{1,2}, ante dependence order for analysis of variance; C, control treatment; D, drought treatment; */***, significant drought effect at $P < 0.05/0.001$.

Amino acids: In the drought treatment, there was a 35-fold increase in proline concentration between days 7 and 13 relative to the control (sig. $P < 0.0001$) and the concentration declined rapidly following irrigation on day 13 (Fig. 4b).

In contrast to the above data, imposition of water stress did not significantly affect (tested at $P = 0.05$) the levels of the ionic solutes and the activities of peroxidase and ascorbate peroxidase (data not presented).

Complementary study: pressure–volume (P–V) analyses

Data from the main experiment demostrated that there were significant increases in the concentration of hexose sugars (expressed on a dry weight basis) during drought stress, and that this was coupled with a lowering of Ψ_π relative to the control after stress relief in the droughted

treatment. These observations suggest that changes in sugar metabolism may have a role in the drought tolerance of this species. By analysing pressure–volume curves for stressed and unstressed trees, the complementary study addressed the question of whether the observed changes in sugar concentrations could be involved in osmotic adjustment. In the second study, Ψ_{leaf} prior to treatment was about the same as that in the unstressed trees in the primary experiment. Ψ_{leaf} declined gradually during drought, reaching a minimum of -2.4 ± 0.3 MPa compared to -0.8 ± 0.1 MPa in the control.

Data from *P–V* analyses (Fig. 5a; Table 4) indicate that in drought-stressed leaves, osmotic potential at full turgor ($\Psi_{\pi 100}$) was -2.24 MPa, 0.72 MPa lower than in unstressed leaves, with low intra-tree variability. Apoplastic water fraction (R_a) decreased 2.2-fold, from

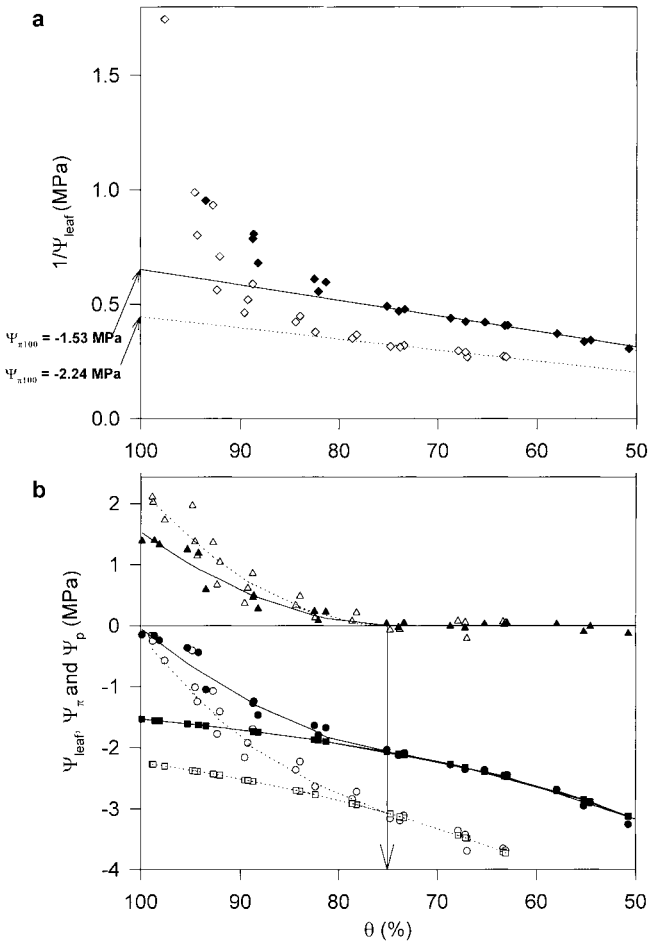

Fig. 5. (a) Pressure–volume relationship for control and drought-stressed (◆/◇, respectively) leaves of *Ziziphus mauritiana* after imposition of a gradual drying cycle (each curve = data for three representative leaves). Regression of linear portion of the curves (where $\Psi_P = 0$) and extrapolation to axes, yield osmotic potential at full turgor ($\Psi_{\pi 100}$) in control and droughted leaves (solid/dotted lines). (b) Höfler diagram showing changes in leaf water potential (Ψ_{leaf}, ●/○), osmotic potential (Ψ_π, ■/□) and turgor potential (Ψ_P, ▲/△) with relative water content (θ). Data modelled using a quadratic function (solid/dotted lines and solid/open symbols = control/droughted).

Table 4. *Water relations parameters of control and drought-stressed* Ziziphus *leaves determined from pressure-volume analysis*

Variable	Control		Droughted	
	Mean	SE	Mean	SE
V_{100} ($m^3 \times 10^{-9}$)	664	145	611	63
R_a (%)	7.7	0.35	3.4	0.21
DW/TW ratio	0.30	0.004	0.36	0.007
ϵ_B (MPa)	6.2	0.82	10.2	1.79
$\Psi_{\pi 100}$ (MPa)	−1.52	0.08	−2.24	0.13

7.7% to 3.4%. In both control and droughted leaves, there was great variability in symplastic water volume at full turgor (V_{100}) indicating that there was no consistent effect of drought on this variable. There was little variation in the estimated ratio of dry : turgid weight, and a marked increase in this ratio in drought-stressed leaves (Table 4). The increase in the ratio of dry : turgid weight was accompanied by a 65% increase in the bulk modulus of elasticity (ϵ_B) or wall rigidity. The model for change in Ψ_P with θ showed that there was no significant ($P = 0.05$) increase in lack of fit from constraining the point of turgor loss to be equal in the two trees.

Discussion

Gas exchange during stress

In *Ziziphus*, reduced *A* during drought was probably due to stomatal closure (reduced g_{leaf}) occurring when predawn and midday Ψ_{leaf} were below -0.75 and -1.3 MPa, respectively, rather than non-stomatal limitations (Jones, 1992). Chlorophyll fluorescence data show that the ratio of F_v/F_m was unaffected by drought over the range of θ experienced during the experiment, indicating that PSII efficiency was not affected as *A* declined over the range of leaf water contents experienced during drought. This confirms reports that PSII efficiency as measured by F_v/F_m is affected only when drought stress becomes severe and is not a sensitive indicator of drought stress (Corlett and Choudhary, 1993).

During the main experiment, diurnal fluctuations in atmospheric humidity in the glasshouse were constant, and leaf-to-air temperature differences were not significantly different between treatments (due to the low ambient irradiance). It is therefore assumed that trees in both treatments experienced the same effective *VPD*, with the main difference due to soil dryness. The data suggest that stomatal closure was in response to soil dryness, with stomatal sensitivity to *VPD* altered as a result of a root–shoot signal (Zhang and Davies, 1989), rather than by direct responses of stomata and subsidiary cells to changing *VPD*. The observation that there was a delay of several days before stomata recovered after rewatering also indicates the existence of a residual signal resulting

from drought (Fischer, 1970). This is supported by work by Correira and Pereira (1994), who showed that following drought stress in lupin, residual ABA in the cell apoplast continued to inhibit stomatal opening for 2 d after rewatering. In addition to the effects of ABA on limiting assimilation through reducing stomatal aperture and therefore CO_2 availability, reduced photosynthetic capacity through down-regulation of photosynthetic gene activity has been reported as resulting directly from sugar accumulation (reviewed by Van Oosten and Besford, 1996). The current data show increased concentrations of hexoses, cyclitols and proline in response to drought stress in *Ziziphus*, but the relative contributions of sugars and ABA to the observed reduction in assimilation cannot be determined from the available data.

In semi-arid environments, where rainfall patterns are highly variable, it may be ecologically advantageous for trees to dampen stomatal responses to drought in this way, thereby maximizing water use by avoiding rapid cycling in stomatal aperture between erratic rainfall events. Unpublished work from this laboratory indicates that in the field, drought-stressed *Ziziphus* effectively control water loss both through reduction in leaf area and by stomatal closure resulting in higher intrinsic water use efficiency (ratio of A/g_{leaf} (Jones, 1992)) than in unstressed trees. Further work will be required to identify and quantify ABA and sugar concentrations in relation to moderating g_{leaf} and A in *Ziziphus* during drought stress.

Increased solute concentrations during drought

The hexose sugars, glucose and fructose, accounted for the vast majority of change in sugars, with the cyclitol, scyllo-inositol contributing to a lesser extent. The current data are consistent with the increased hexoses resulting from starch hydrolysis. Effects of drought on the balance of sugars and starch have been known for many years (Morgan, 1984). Hexose sugars (particularly glucose) have been reported as contributing directly to osmotic adjustment in several species including cottonwood (Tschaplinski and Tuskan, 1994), poplar (Gebre *et al.*, 1994), oak (Epron and Dreyer, 1996), pine (Meier *et al.*, 1992) and *Fragaria chiloensis* (Zhang and Archbold, 1993), whereas the role of cyclitols is thought to be mainly protective through stabilization of DNA structure during dehydration (reviewed by Popp and Smirnoff, 1995).

In addition to increased sugar concentrations, drought stimulated a 35-fold increase in the concentration of the amino acid proline, which is also a common cytoplasmic compatible solute, which is thought to have several roles including the stabilization of membranes and proteins, protection against temperature extremes and salt and oxidative damage (review by Samaras *et al.*, 1995). The observed increase in hexoses prior to a build-up in proline confirms the data of Kameli and Losel (1993), who also showed that hexoses accumulated before proline in winter wheat, suggesting that these were a more sensitive indicator of drought than proline. Proline accumulation under moisture stress was previously also reported in *Z. rotundifolia* seedlings (Choudhary *et al.*, 1996) and the authors suggested that this solute may contribute to osmotic adjustment, although detailed data relating to water relations were not presented. Proline synthesis and degradation has been shown to be highly correlated with osmotic environment (Rhodes and Handa, 1989), and the ability to accumulate proline has been used as a basis for selection for drought tolerance in several species (Van Rensburg and Kruger, 1994; Stajner *et al.*, 1995; Van Heerden and De Villiers, 1996). However, it must be noted that although proline accumulation has been shown to occur in conjunction with osmotic adjustment in drought-tolerant species, it may also accumulate in drought-susceptible cultivars as a symptom of stress as in cassava (Sundaresan and Sudhakaran, 1996) and *Phaseolus* (Andrade *et al.*, 1995). In the latter case, high proline concentrations may help to protect cell metabolism and facilitate recovery after stress.

In *Ziziphus*, cytoplasmic accumulation of hexose sugars, scyllo-inositol and proline during drought stress may confer an increased ability to lower osmotic potential, stabilize DNA and membranes, and also ameliorate the deleterious effects of free radicals produced in response to the combination of high irradiance and water deficit normally experienced under field conditions.

Recent work by Choudhary *et al.* (1996), demonstrated that drought increased oxidative enzyme activities in *Ziziphus rotundifolia* grown in India. The non-significant effect of drought on oxidative enzyme activities in glasshouse-grown *Ziziphus* in the current experiment may be due to the development of a less severe drought (min. $\theta =$ 76% compared to 46% in the Indian experiment) which, together with relatively low irradiance may result in little or no oxidative damage.

Water relations during drought stress

The diurnal changes in Ψ_{leaf} and Ψ_{π} observed in the main experiment were smaller than values measured in the field (Clifford and Arndt, unpublished data), with photosynthesis in glasshouse-grown trees declining at much higher Ψ_{leaf} than in the field. A component of the difference relates to the rate of development of stress, with rapid stress inhibiting processes such as osmotic adjustment (Radin, 1983). This study's data support work by Jones and Rawson (1979), which showed that when stress was imposed slowly, the range of Ψ_{leaf} over which stomata close was increased. In both sorghum (Jones and Rawson, 1979) and yellow cedar (Grossnickle and Russell, 1996),

the degree of osmotic adjustment decreased when stress developed quickly, and in the latter, changes in wall elasticity dominated the response to drought in fast drying cycles. Despite the relatively fast development of stress in the first glasshouse experiment (0.09 MPa d^{-1} compared to 0.008 MPa d^{-1} in field-grown sorghum; Hsiao *et al.*, 1976), considerable solute accumulation occurred. The observation that after rewatering droughted *Ziziphus*, lowered Ψ_π resulted in significantly higher pre-dawn turgor than in controls (whilst both Ψ_{leaf} and θ had recovered to control values) indicates that active osmotic adjustment had occurred.

Pressure–volume relationships in stressed and non-stressed leaves

In *Ziziphus*, gradual drought stimulated a marked lowering of osmotic potential at full turgor ($\Psi_{\pi 100}$), together with large increases in the tissue elastic modulus (ϵ_B) and the dry/turgid weight ratio. This indicates that in addition to solute accumulation, there were changes in cell wall rigidity in stressed leaves. When plotted on a Höfler diagram (Fig. 5b), the overall effect of these changes, as well as increasing turgor at full hydration, was to maintain cell volume and turgor at lower water potentials than in unstressed leaves (Fig. 6). The increase in ϵ_B counteracted the potential extended range of θ over which turgor could be maintained purely through lowering $\Psi_{\pi 100}$, and resulted in turgor loss at the same θ in both stressed and unstressed leaves.

Turgor maintenance can be mediated either through the accumulation of solutes to lower osmotic potential actively, or by changes in wall elasticity (Radin, 1983). Drought has been shown to both increase and decrease wall elasticity (reviewed by Meier *et al.*, 1992; Schulte, 1993). For a given Ψ_π, increased elasticity facilitates turgor maintenance over a greater range of water contents by effective solute concentration through reduction in cell volume. As elasticity decreases, cell walls become more rigid, and this leads to larger reductions in both water potential and turgor per unit change in volume (Radin, 1983). Although inelastic cell walls preclude turgor maintenance to low water contents, they do have several potential advantages over elastic cell walls. (i) In species which show osmotic adjustment and accumulate significantly high solute concentrations, a rigid cell wall may be necessary to maintain cell/tissue integrity on rehydration following a period of stress. (ii) Rigid cell walls may facilitate the maintenance of lower Ψ_{leaf} at any given volume than elastic walls. This increases the gradient in water potential between the soil and plant, thereby promoting more effective water uptake from drying soils (Cheung *et al.*, 1975; Bowman and Roberts, 1985). (iii) It has been suggested that reduced inter-molecular space during drought correlates with reduced enzyme activity, and so maintenance of turgor and cell volume, rather than water potential, would be an important factor for sustained metabolic function during drought (Kaiser, 1987; Sen Gupta and Berkowitz, 1987; Meinzer *et al.*, 1990).

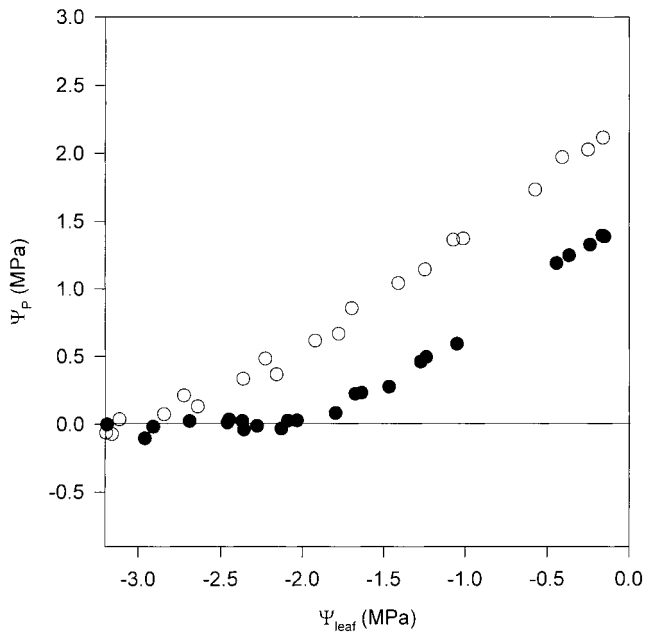

Fig. 6. Changes in turgor (Ψ_P) with leaf water potential (Ψ_{leaf}) in control (●) and drought stressed (○) leaves of *Ziziphus mauritiana* from pressure–volume analysis. Each data set represents all measurements for three leaves.

In contrast to the above argument, it has been suggested that trees with elastic cell walls have a high inherent drought tolerance (Fan *et al.*, 1994; Zimmermann and Steudle, 1978). This may be true in the absence of osmotic adjustment, and several reports indicate that in species where drought stimulates increased wall elasticity, there tends to be little or no osmotic adjustment, with Ψ_P maintained over a wider range of θ (Nunes *et al.*, 1989 [*Ceratonia siliqua*]; Evans *et al.*, 1992 [*Artemisia tridentata*]; Fan *et al.*, 1994 [jack pine, black spruce and flooded gum]). In *Artemisia tridentata*, increased proline concentrations were observed in response to drought (Evans *et al.*, 1992), and this form of drought tolerance may correlate with a necessity to protect membranes and macro-molecules against extreme reduction in cell volume during drought.

In *Acer pseudoplatanus* (Ayoub *et al.*, 1992), *Pinus taeda* (Meier *et al.*, 1992), yellow cedar (Grossnickle and Russell, 1996), coffee (Meinzer *et al.*, 1990), and sorghum (Jones and Rawson, 1979), there were significant decreases in elasticity together with osmotic adjustment in response to drought. In these cases, as with *Ziziphus*, decreased elasticity generally offset the potential increase in the range of θ over which positive turgor could be sustained, in favour of maintenance of cell volume at low water potentials. In coffee, osmotic adjustment and

decreased elasticity in response to drought are thought to be most important in sustaining gas exchange mainly through effective maintenance of symplast volume (Meinzer *et al*., 1990).

Current data indicate that a component of drought tolerance in *Ziziphus* is provided by a combination of solute accumulation and increased wall rigidity resulting in the maintenance of cell volume at low water potentials. In semi-arid environments, mechanisms such as these which enable plants to maintain physiological activity/integrity, even if only for a few days longer than would normally be expected, may provide a strong competitive advantage in an unpredictable rainfall environment.

From the current data it is not possible to determine whether the observed osmotic adjustment was a result of remobilization of sugars from senescing leaves back into the stem and roots, or from newly assimilated carbon. It has been suggested that for tropical trees in dry environments, osmotic adjustment, together with leaf shedding (to reduce xylem tension) and remobilization of sugars into the stem are prerequisites for early bud-break before the rains (Borchert, 1994). Although glasshouse data give no indication of rooting responses in this species, previous field observations (unpublished) indicate that leaf area adjustment and vigorous rooting at depth are predominant features of *Ziziphus* growth during drought. Further studies of seasonal changes in solute distribution, and redistribution of sugars between leaves, stems and roots during stress will identify how these factors combine to promote drought tolerance in this species under field conditions.

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