

Stomatal changes induced by intermittent drought in four umbu tree genotypes

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Transpiration (E), diffusive resistance (r_s) and anatomical parameters were measured in plants of four grafted umbu tree genotypes (GBUs 44, 48, 50 and 68) in order to evaluate alterations induced by intermittent drought and possible genotypic variations. Transpiration measurements were taken daily until stomatal closure by withholding water. Measurements were also taken, when the plants were re-watered and the watering was interrupted again. This cycle was repeated for a period of 31 days (stress period). The control plants were also irrigated daily. A regular pattern in the stomatal opening/closing mechanism was observed throughout the watering period, exhibiting intra-specific differences. Stomatal behavior of GBU 44 and GBU 68 had correlation with air temperature (T_{ar}), relative humidity (RH) and vapor pressure deficit (VPD), whereas GBU 50 had correlation with photosynthetically active radiation (PAR). GBU 48 had no correlation with these environmental factors. Anatomical alterations in response to drought were observed in stomatal density (SD), reductions in the stomatal index (SI) and stomatal aperture size (SA). The anatomical features of the GBU 48 genotype remained unaltered. There was an inversion in tissue proportion in GBU 44 under stress conditions, reducing the spongy parenchyma and increasing palisade parenchyma thickness. The inverse occurred with GBU 68, while the remaining genotypes were unaltered. The results found in this study suggest that GBU 68 is the most drought-sensitive genotype and anatomical changes induced by intermittent drought are not enough to explain the physiological differences between genotypes.

Key words: diffusive resistance, *Spondias tuberosa*, stomatal density, transpiration, water deficit

Mudanças estomáticas e anatômicas induzidas pela seca intermitente em quatro genótipos de umbuzeiro: A transpiração (E), a resistência difusiva (r_s) e parâmetros anatômicos foram medidos em mudas enxertadas de quatro genótipos de umbuzeiro (BGU s 44, 48, 50 and 68) com o objetivo de avaliar alterações e possíveis variações genotípicas induzidas pela seca intermitente. As medidas de transpiração foram tomadas diariamente até ocorrer o fechamento estomático das plantas sob suspensão da irrigação, e após a reirrigação e suspensão da rega novamente, repetindo-se este ciclo por um período de 31 dias (período de estresse). As plantas controle foram irrigadas diariamente. Foi observado um padrão regular no mecanismo de abertura e fechamento estomático ao longo dos períodos de rega, exibindo diferenças entre os genótipos. O comportamento estomático dos genótipos BGU 44 e BGU 68 se correlacionaram com a temperatura do ar (T_{ar}), umidade relativa (RH) e déficit de pressão de vapor (VPD), enquanto que o BGU 50 apresentou correlação com a radiação fotossinteticamente ativa (PAR). BGU 48 não apresentou correlação com os fatores ambientais. Foram observadas alterações anatômicas em resposta à seca na densidade estomática (SD), reduções no índice

estomático (SI) e abertura do ostíolo (SA). As características anatômicas do genótipo BGU 48 permaneceram inalteradas. Houve uma inversão na proporção dos tecidos do BGU 44 sob condições de estresse, reduzindo a espessura do parênquima esponjoso e aumentando a do parênquima paliçádico. O inverso ocorreu com o BGU 68, enquanto que os demais genótipos permaneceram inalterados. Os resultados encontrados neste estudo sugerem que o BGU 68 é o genótipo mais sensível à seca e que as mudanças anatômicas induzidas pela seca intermitente não são suficientes para explicar as diferenças fisiológicas entre os genótipos,

Palavras-chave: déficit hídrico, densidade estomática, resistência difusiva, *Spondias tuberosa*, transpiração.

INTRODUCTION

Water deficit is known to alter physiological processes as well as induce morphological and anatomical changes in many plant species. These changes mainly occur in gas exchange, which influences the photosynthetic process and synthesis of organic solutes (Chartzoulakis et al., 2002). Changes due to water deficit also occur in all histological components of the leaf (Bosabalidis and Kofidis, 2002).

Plants grown in arid and semi-arid environments are exposed to long periods of water deficit and have developed adaptations in order to tolerate drought. The reduction in photosynthetic rate associated with stomatal closure due to changes in leaf water status is commonly observed in plants grown under water deficit conditions (Chartzoulakis et al., 1999; Nogueira et al., 2001; Silva et al., 2003). As water availability in the soil decreases, the transpiration rate also decreases as a result of stomatal closure. The instantaneous control of the transpiration stream by the stomata is an important defense mechanism used by many species in arid environments in order to avoid excessive water loss by transpiration (Gucci et al., 1996; Nogueira et al., 1998; Silva et al., 2004) and eventual death by desiccation (Silva et al., 2000).

Although water is the determinant factor in the stomatal aperture mechanism in plants under water deficit, several authors have demonstrated the influence of environmental factors on stomatal behavior in a number of species, e.g. air temperature (Silva et al., 2003), light and vapor pressure deficit (Gucci et al., 1996; Thomas and Eamus, 2002; Gomes et al., 2004). Environmental stress can result in both, physiological and anatomical changes in the leaf (Mott and Michaelson, 1991). Changes in the anatomical characteristics of the leaf are known to alter the diffusion of CO₂ conductance from the substomatal cavities to carboxylation sites and thus contribute toward the maintenance of photosynthetic rates despite the low stomatal conductance (Evans et al., 1994, cited by Chartzoulakis et al., 1999). Under water deficit conditions, an

increase in stomata density and the number of smaller-sized mesophyll cells of all histological components of the leaf have been observed, which result in improved control of water loss (Bosabalidis and Kofidis, 2002; Chartzoulakis et al., 2002).

The umbu tree (*Spondias tuberosa* Arruda) is a xerophytic tree belonging to the Anacardiaceae that produces edible fruit for humans and animals. It is from the dry lands of northeastern Brazil known as the Caatinga and represents a source of income for small farmers. The significant fruit production and use can contribute greatly to regional development in semi-arid areas (Cavalcanti et al., 1999). Due to the considerable variability in shape, canopy architecture, productivity, and physiochemical characteristics of the fruit, the Brazilian Institute of Agricultural Research for the Semi-arid Tropics (Embrapa-CPATSA) has implanted an active germplasm bank of umbu trees (GBU) formed by 78 genotypes (Oliveira et al., 2004). The GBU 44, 48, 50 and 68 genotypes are characterized as giant umbu, as mean weights of their fruit are 86.7g, 75.3 g, 85.0 g and 96.7 g, respectively (Santos et al., 1999). More than 40,000 GBU 48 seedlings have been distributed to small farmers in the semi-arid region. Four Observation Units have been implanted using this genotype. Two are located in the city of Floresta, Pernambuco, Brazil and another two are located in Caicó, Rio Grande do Norte, Brazil.

Only a few numbers of studies have addressed the physiological and anatomical alterations in response to water deficit in umbu tree genotypes. According to Lima Filho (2004), field observations have demonstrated that umbu trees limit water loss by transpiration through strict stomatal control, thereby assuring adequate water economy. The present study was carried out to test the hypothesis that the ability to overcome drought differs between the genotypes and physiological alterations may be explained by anatomical changes. Thus, the objective was to assess alterations in water vapor gas exchange and anatomical changes induced by intermittent drought in four umbu tree genotypes.

MATERIAL AND METHODS

Plant material, growth and experimental design:

Research was carried out in greenhouse conditions at the Laboratory of Plant Physiology of the Universidade Federal Rural de Pernambuco (UFRPE), Brazil, from November to December 2005. Six-month-old grafted umbu tree seedlings (*Spondias tuberosa* Arr. Cam.) were provided by Brazilian Institute to the Semi-Arid Tropic (Embrapa-CPATSA), from Petrolina, Pernambuco, Brazil. The plants were grown in containers with 8 kg of acrisols soil (FAO) provided by the same company, with loam-sandy texture, 71% sand, 17% clay, 12% silt and a global density of 1.51 g cm⁻³; 9.97% humidity in field capacity (0.3 atm) and 4.01% at the permanent wilting point (15 atm). The soil chemical analysis was performed at the Laboratory of Soil Fertility (UFRPE). The soil contained 41 mg dm⁻³ of P, 0.20 cmol_c dm⁻³ of Na⁺, 0.33 cmol_c dm⁻³ of K⁺; 7.15 cmol_c dm⁻¹ of Ca²⁺ + Mg²⁺; 5.15 cmol_c dm⁻³ of Ca²⁺ and 0.05 cmol_c dm⁻³ of Al³⁺. Soil correction was not performed. A randomized 4X2 factorial experimental design was used, corresponding to four umbu tree genotypes (GBU 44, GBU 48, GBU 50 and GBU 68) and two water treatments (control – with daily watering until free drainage; and stressed – withholding water until plants exhibited stomatal closure, then watering again), with six replications.

Transpiration and diffusive resistance: Transpiration (E) and diffusive resistance (r_s) were measured using a steady state porometer, model LI-1600 (LI-COR, Inc. Lincoln, NE, USA), which set the null point near humidity in the greenhouse. As the porometer displayed the E values in units of $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$, the values were converted to $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Two mature and fully expanded leaves located in middle of each plant were sampled. Measurements were carried out daily from 9.00 to 10.00 h. Air temperature (T_{air}), photosynthetic active radiation (PAR) and air relative humidity (RH) measurements were also taken and the vapor pressure deficit was calculated (VPD). These variables exhibited variations from 28.7 °C to 34.14 °C; 179.7 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 470.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 28.7% to 43.3% and 2.46 kPa to 3.75 kPa, respectively.

Soil moisture: Soil humidity was determined from three soil samples of each treatment and each genotype, resulting in 24 samples overall. Samples were taken when plants exhibited stomatal closure. The soil was collected after the assessment of stomatal behavior and before re-watering. Soil moisture was determined using the equation: $\theta = (\text{SWW} - \text{SDW})/\text{SDW} \times 100$,

where θ = soil humidity in the mass base; SWW= soil wet weight; SDW= soil dry weight. The soil-water characteristic curve (SWCC) was determined through a Richards's membrane pressure chamber. This curve was used to estimate the soil water potential when stomatal closure occurred.

Stomatal density, stomatal index, stomatal aperture size and proportions between tissues: Twelve leaves per treatment from each genotype were collected at the end of the experimental period for anatomical analysis. The leaves were rinsed in running water, dried on absorbent paper and fixed for 48 hours in 37 to 40% formaldehyde, 50% glacial acetic acid and ethyl alcohol (1:1:18 v/v). The leaves were then transferred to 70% ethanol (Johansen, 1940) and sent to the Anatomy Laboratory of the Universidade Federal of Minas Gerais, where the anatomical analysis was performed. Cross and paradermal freehand sections were taken from the intermediate region of the leaves, stained with Astra Blue and safranin and placed on semi-permanent glass slides. Epidermal dissociation was performed using the Jeffrey method [10% chromic acid, 10% nitric acid (1:1 v/v)], as described by Kraus and Arduin (1997). Permanent slides were mounted after dehydration of the leaves in a butanolic series (Johansen 1940), unfiltered in Paraplast® (Kraus and Arduin 1997), cut on a Jung Biocut® rotary microtome and submitted to Astra Blue and safranin staining (Kraus and Arduin 1997). The slices were performed in Entelan® following the usual plant anatomy method for light microscopy.

Leaf cell and tissue structures from each treatment and genotype were characterized. The comparison and identification of variations between treatments and genotypes were performed by measuring stomatal density (SD) and the number of cells (mm²), using a lit chamber coupled to an Olympus CH30 microscope, in 20 areas of 0.01 mm² (40x objective); area was gauged by an Olympus micrometric slide. Stomatal index (SI) was calculated according to Cutter (1986). Aperture size (AS) was calculated using the ellipse formula ($A = a \cdot b \cdot \pi$, where a = half-axis of larger diameter and b = half-axis of smaller diameter) after digital imaging of the stomata using a Motic® camera and respective program. Leaf thickness and tissue proportions were determined by linear measurements of cross-sections using the digitalized images from the Motic® system. Photomicrography was performed using an Olympus BH2 microscope with the AD photographic system.

Statistical analysis: Data were submitted to analysis of variance (ANOVA) and means compared by Tukey’s multiple range test ($P < 0.05$), using a statistical program (Statistica version 6.0.).

RESULTS

The intermittent drought induced significant reductions in transpiration rates (E) in all genotypes studied (Table 1). Highest E was observed in GBU 48 irrespective of the treatments applied, while GBU 68 had the lowest E when cultivated under adequate water availability in the soil. However, latter genotype did not differ from the GBU 44 and GBU 50 genotypes when submitted to water deficit.

The stomata of the GBU 68 genotype appeared more drought-sensitive, reducing its stomatal aperture faster than the other genotypes. GBU 68 generally exhibited stomatal closure in five-day intervals, amounting to five re-watering sessions during the experimental stress period (31 days). GBU 44, GBU 48 and GBU 50 followed this sensitivity pattern, with GBU 50 maintaining the stomata open for a higher period of time than the others (about seven days) (See arrows in Fig. 1 and Fig. 2).

Table 1. Transpiration rates (E) and diffusive resistance (r_s) of four grafted umbu tree genotypes grown in greenhouse conditions under intermittent drought. Means of 145 assessment made along the stress period (31 days) are shown.

E (mmol.m ⁻² .s ⁻¹)		
Genotypes	Control	Stressed
GBU 44	4.86 aB	1.96 bB
GBU 48	6.52 aA	2.71 bA
GBU 50	4.75 aB	1.66 bB
GBU 68	3.60 aC	1.68 bB

r_s (s.cm ⁻¹)		
Genotypes	Control	Stressed
GBU 44	1.84 bA	19.30 aA
GBU 48	1.70 aA	10.69 aB
GBU 50	2.38 bA	15.38 aAB
GBU 68	3.37 bA	25.19 aA

Values followed by different letters, lower case among treatments and capital letters among genotypes, differ by Tukey’s test ($P < 0.05$).

Figure 1 clearly shows that GBU 44 and GBU 68 recovered their transpiration rate more quickly than GBU 48 and GBU 50, reaching equal to or near control plant values after 24 h of re-watering. GBU 48 and GBU 50 generally maintained lower

E values after re-watering in comparison to control plants. Recovery was observed in these genotypes only after 21 and 26 days, respectively, under intermittent drought conditions. When plants exhibited stomatal closure, soil moisture was near the permanent wilting point (-15 atm) (data not shown).

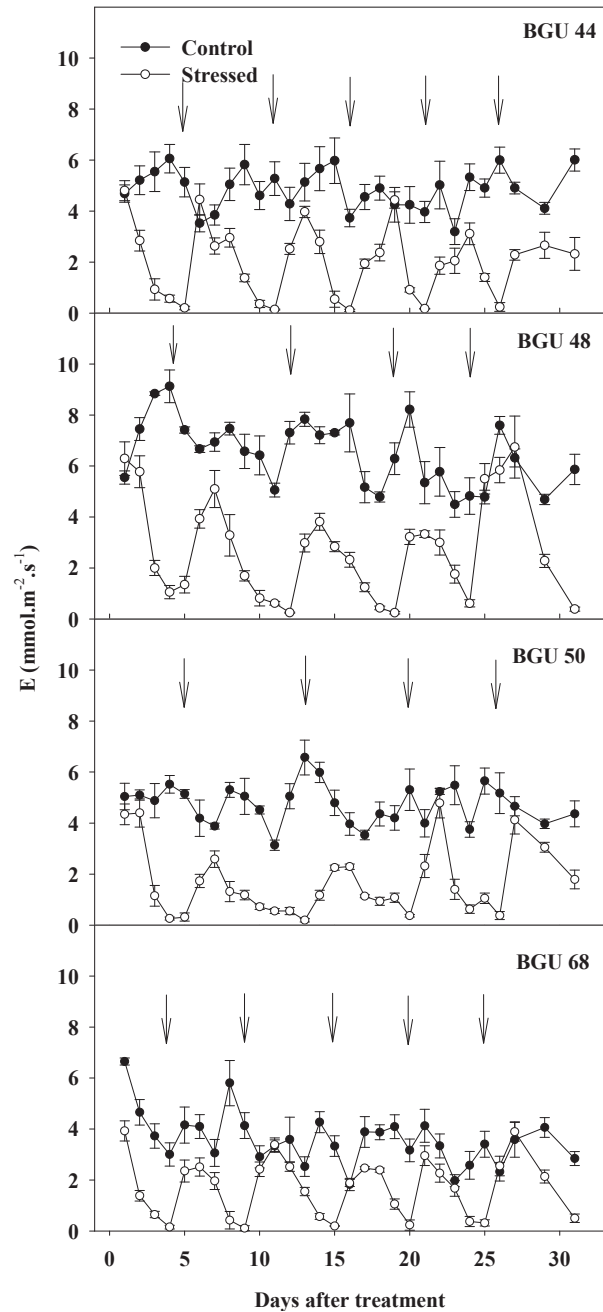


Figure 1. Transpiration (E) in four grafted umbu trees genotypes grown in greenhouse conditions under intermittent drought and re-watered when presented stomatal closure. Arrows indicate the re-watering days. Means ± Stand-deviation of six replicates are shown.

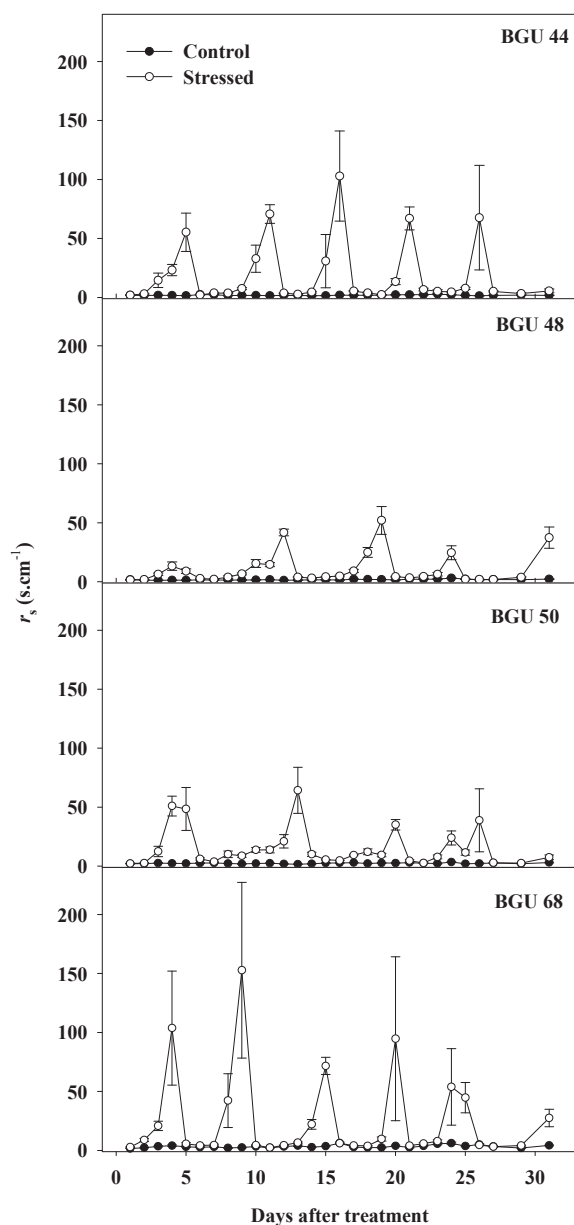


Figure 2. Diffusive resistance (r_s) in four grafted umbu trees genotypes grown in greenhouse conditions under intermittent drought and re-watered when presented stomatal closure. Arrows indicate the re-watering days. Means \pm Stand-deviation of six replicates are shown.

Although significant differences relative to transpiration were observed between genotypes ($P < 0.01$), diffusive resistance (r_s) did not differ significantly in control plants, suggesting that E can exhibit variations for the same r_s value (Table 1). However, intermittent drought induced increases in r_s for most of the genotypes studied, with a greater increase in BGU 68. This genotype exhibited the highest r_s recorded as well as the greatest variation in r_s values (Figure 2). The GBUs 44

and 68 genotypes exhibited a similar pattern of behavior, with high r_s and great variation between values, whereas the BGU 48 and 50 genotypes exhibited lower r_s and little variation.

Stomatal closure occurred with lower r_s values in BGU 48, whereas the BGU 68 and 44 genotypes considerably increased r_s values to close their stomata. For some genotypes, values of r_s around 20 $s.cm^{-1}$ has limited transpiration, as observed in BGU 48 (Figs. 1 and 2), while other genotypes have considerably increased r_s values to close stomata (BGU 44 and 68). In general, E values less than 0.5 $mmol.m^{-2}.s^{-1}$ were associated with high r_s values.

Photosynthetic active radiation (PAR) had a positive correlation with transpiration in BGU 50 alone, while relative humidity (RH), air temperature (T_{air}) and vapor pressure deficit (VPD) had a correlation with E in BGU 44 (Table 2). This genotype seems to be more influenced from climatic parameters than the others. In the BGU 68 genotype, there was a negative correlation between RH and r_s and a positive correlation between r_s and T_{air} as well as VPD. In BGU 48, however, there were no correlations with the environmental parameters assessed. This demonstrates that water was the sole determinant factor to stomatal closure in BGU 48.

Table 2 – Simple correlation between environmental (PAR, T_{air} , RU and VPD) and physiological (E and r_s) parameters of four grafted umbu tree genotypes grown in greenhouse conditions under intermittent drought.

Parameters	Genotypes			
	GBU 44	GBU 48	GBU 50	GBU 68
E X r_s	-0.690 **	-0.681 **	-0.691 **	-0.638 **
E X PAR	0.208 NS	0.151 NS	0.463 **	0.203 NS
E X RH	0.390 **	-0.049 NS	-0.131 NS	0.138 NS
E X T_{air}	-0.380 **	0.032 NS	0.229 NS	-0.197 NS
E X VPD	-0.417 **	0.032 NS	0.196 NS	-0.190 NS
r_s X PAR	-0.043 NS	-0.0003 NS	-0.122 NS	0.024 NS
r_s X RH	-0.171 NS	-0.066 NS	-0.170 NS	-0.290 *
r_s X T_{air}	0.259 *	0.0642 NS	0.054 NS	0.266 *
r_s X VPD	0.223 NS	0.078 NS	0.141 NS	0.306 **

NS Non significant ; * Significant ($P < 0.05$) ; ** Significant ($P < 0.01$)

The umbu plants exhibited anomocytic stomata located on the lower surface of the leaves (Figure 3). We rarely found isolated stomata on the upper surface, which does not characterize an amphistomatous leaf. There

were significant differences ($P < 0.01$) between genotypes regarding some anatomical parameters. GBU 50 had the highest stomatal density (SD) under control conditions, whereas GBU 44 had higher SD under stress conditions (Table 3). Short-term intermittent drought (31 days) induced an increase in SD in GBU 44 alone. Unexpectedly, there was a significant reduction in SD in GBU 50. The remaining genotypes were unaltered (Table 3). Significant differences between genotypes ($P < 0.01$) were also observed regarding the stomatal index (SI). Under control conditions, GBU 50 had a higher SI in comparison to the other genotypes. Under stress conditions, the SI was reduced in both GBU 50 and 44. The remaining genotypes were unchanged (Table 3). There was significant difference in stomatal aperture (SA) ($P < 0.01$). GBU 68 had the highest SA values under both control and stress conditions (Table 3). Reductions in SA were observed in GBU 44 and 68 under stress conditions. This reduction was approximately 50% in GBU 44 and 28.6% in GBU 68.

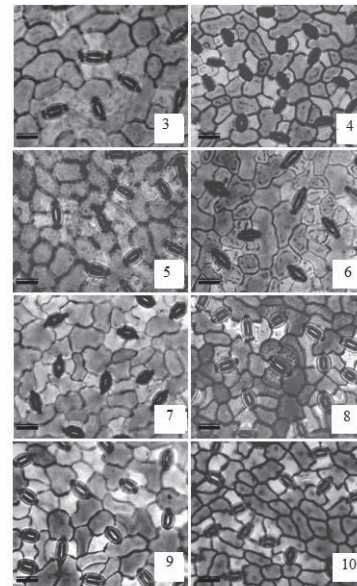


Figure 3. Abaxial epidermis of four grafted umbu trees genotypes (*Spondias tuberosa* Arr. Cam.). Genotypes: 3–4. GBU 44; 5–6. GBU 48; 7–8. GBU 50; 9–10. GBU 68. Treatments: Control (3, 5, 7, 9) and stressed (4, 6, 10, 12). Bars = 20 μm .

Table 3. Stomatal density (SD), stomatal index (SI), and stomatal aperture size (SA) in four umbu tree genotypes after 31 days under intermittent drought.

Genotypes	Stomatal density (mm^2)		Stomatal index (%)		Stomata aperture size (mm)	
	Control	Stressed	Control	Stressed	Control	Stressed
GBU 44	340 bB	535 aA	20.84 aAB	18.00 bA	41.63 aB	20.34 bC
GBU 48	300 aB	340 aB	17.58 aC	15.41 aA	39.94 aB	33.71 aB
GBU 50	497.5 aA	215 bC	21.19 aA	12.62 bB	38.15 aB	35.62 aB
GBU 68	385 aB	330 aB	18.36 aBC	17.33 aA	74.73 aA	53.33 bA

Values followed by different letters, lower case among treatments and capital letters among genotypes differ by Tukey's test ($P < 0.05$).

Differences between GBU 44 and GBU 68 were observed regarding tissue proportion as a consequence of the inversion in both spongy and palisade parenchyma thickness that occurred in these genotypes (Table 4). The same was not observed in GBU 48 and GBU 50. Drought induced a reduction in spongy parenchyma and an increase in palisade parenchyma thickness in GBU 44, which is a classic plant response to water deficit (Larcher, 2003), whereas GBU 68 increased spongy parenchyma and reduced palisade parenchyma. Comparing

control treatments between genotypes, GBU 68 was different from the others, exhibiting the largest lower surface thickness, least spongy parenchyma and largest palisade parenchyma thickness. GBU 44 had the lowest surface thickness (Table 4). Comparing genotypes of the stressed plants, GBU 44 was the most different genotype, with the lowest lower proportion of spongy parenchyma and highest proportion of palisade parenchyma. Total leaf thickness was not altered by any treatment.

Table 4. Abaxial epidermis, adaxial epidermis, spongy parenchyma, and palisade parenchyma thickness (μm) of four umbu tree genotypes after 31 days grown under intermittent drought.

Genotypes	Abaxial epidermis (μm)		Adaxial epidermis (μm)	
	Control	Stressed	Control	Stressed
GBU 44	4.57 aB	4.53 aB	7.3 aB	8.2 aA
GBU 48	4.13 aB	4.57 aB	8.57 aA	9.27 aA
GBU 50	4.53 aB	4.30 aB	8.00 aAB	8.33 aA
GBU 68	5.50 aA	5.87 aA	9.13 aA	9.40 aA
Genotypes	Palisade parenchyma (μm)		Spongy parenchyma (μm)	
	Control	Stressed	Control	Stressed
GBU 44	47.57 aA	40.03 bB	40.70 bAB	47.23 aA
GBU 48	48.93 aA	49.73 aA	38.40 aB	36.50 aBC
GBU 50	47.47 aA	47.87 aA	40.03 aAB	39.73 aB
GBU 68	43.07 bB	49.27 aA	42.37 aA	35.70 bC

Values followed by different letters, lower case among treatments and capital letters among genotypes differ by Tukey's test ($P < 0.05$).

DISCUSSION

There were significant differences between genotypes with regard to water vapor gas exchange ($P < 0.01$). Intra-specific differences were observed in transpiration rates, time intervals and recovery time (Table 1, Figs. 1 and 2). These results have also been observed in another species. Gomes et al (2004) found significant reductions on transpiration and stomatal conductance in orange trees after seven days of withholding water. Nogueira and Silva (2002) observed similar results for *Schinopsis brasiliensis*, with reductions in E as soil drought was increased after seven days of withholding water.

Maize plants under water deficit recovered stomatal conductance after just three days of re-watering (Bergonci and Pereira, 2002). The same behavior was observed in grafted orange plants cv. 'Valência' (Medina and Machado, 1998), in which recovery occurred after just two days of re-watering. These facts suggest that there is a chemical communication between the roots and shoots, inducing stomatal movement (Schurr et al., 1992; Tardieu et al., 1992), which must be triggered by a growth substance, most likely ABA produced in the roots (Tardieu et al., 1992; Davies et al., 1994, Bergonci and Pereira, 2002). Thomas and Eamus (1999) found that ABA accumulation in the leaves of *Eucalyptus tetrodonta* contributed toward a decrease in stomatal conductance. When plants face periods of water deficit, ABA synthesis increases in the roots and is transported to the shoot through the xylem, causing stomatal closure (Taiz and Zeiger, 2002; Gomes et al.,

2004). This statement should explain the behavior of the GBU 48 and 50 umbu genotypes. Further research on this topic should be pursued.

In the present study, the umbu genotypes decreased transpiration in intervals ranging from 5 to 7 days when soil humidity was near to the permanent wilting point. GBU 68 was more drought-sensitive than the other genotypes. As the plants did not exhibit reduced leaf water potential at that time (data not shown), reductions in E were a result of the reduction in soil water content. Similar results were found in grafted orange plants var. 'Valência', with a decrease in stomatal conductance between the 4th and 5th days after withholding water. This result was caused more in response to the low soil moisture rather than the leaf water potential (Medina and Machado, 1998). Sasaki and Machado (1999) found that a reduction in soil moisture from 16% to 12% induced significant E reductions in two wheat cultivars, although it did not significantly affect photosynthesis. The authors suggest that the stomatal aperture responds more quickly to soil water content variations than to leaf water potential and must be a response to a signal received and emitted by the roots.

How the stomata respond to environmental changes is related to interactions between relative humidity, transpiration and leaf water potential. A high difference in the vapor pressure deficit between the leaf and air results in stomatal closure (Thomas and Eamus, 1999; Larcher 2000). However, Thomas et al. (2000) found increases in E in five woody species from Australia when VPD increased during the rainy season, whereas E remained

unchanged in the dry season despite increases in VPD. Nogueira et al. (2001) observed that the stomatal behavior in Surinam cherry plants throughout the water stress period was more dependent on the water potential than of other environmental factors such as light and relative humidity. In orange plants, Gomes et al. (2004) observed that decreases in photosynthesis rates, transpiration and stomatal conductance in both watered and non-watered plants were the result of alterations in climatic changes such as T_{air} , VPD and PAR. Thus, plants respond differently under stress conditions, as observed in the present study on umbu plants, with the soil water availability certainly the most important environmental factor.

The stomatal density (SD) reductions in GBU 50 (Table 3) might result in better water loss control, which should explain the longer transpiration time. However, as stomatal differentiation is a process that occurs during leaf development (Alquini et al., 2004), the reductions in SD of GBU 50 occurred soon after beginning the applied stress verified by the analyses of the SD in leaves of distinct ages (nodes) along the experimental time (when leaves were young); maturity was only reached at the end of the experimental period, precisely when gas exchange recovery was observed. This data suggest a quick accessory physiological compensation in relation to the mechanism of morphological adjustment which is slower, and demands a complex program of cell differentiation. This is in agreement with data in studies by Cominelli et al. (2005) and Inamullah and Akihiro (2005), who demonstrated the importance of the stomata in response to water stress. Thus, the initial changes observed in all the genotypes studied should be interpreted as modulative (Larcher, 2000), thereby permitting better water availability management.

The anatomical alterations in GBU 44 and 50 demonstrate that these genotypes have higher phenotypic plasticity relative to genotypic expression regarding stomatal differentiation (Table 3 and 4). Changes in leaf morphology as a response to water stress can be considerable (Rojas et al. 2005). Reductions in the stomatal index were also rather limited in the umbu genotypes, observed only in GBU 44 and 50. A reduction in the stomatal index is an expected response in plants submitted to water stress, as observed in two avocado cultivars (Chartzoulakis et al., 2002) as well as in *Trigonella foenum-graecum* L (Ranjitha-Kumari et al., 1999) and in response to water deficit and high temperature in *Leymus chinensis* (Xu and Zhou, 2005).

The variation in stomatal aperture is decisive to the capacity of adaptation of the genotypes, as the aperture size is an important factor in stress response (Zhu et al. 2005) and plays a significant role in water-loss control processes by transpiration (Cominelli et al., 2005). As the survival of plants growing in drought conditions is associated to water economy, stomata in the leaves perform an important role in restricting water loss by transpiration (Bosabalidis and Kofidis, 2002). Changes such as increase in stomata density and leaf thickness, as demonstrated in *Zygophyllum qatarense* Hadidi during the dry season (Sayed, 1996), and a reduction in stomata size (Bosabalidis and Kofidis, 2002), as observed in two olive cultivars submitted to intermittent drought, are examples of the adaptations to drought conditions. Studies on two olive cultivars demonstrate that anatomical changes in response to drought cycles occur in the long term (Chartzoulakis et al., 1999). Thus, it can be inferred that anatomical aspects of umbu trees may be altered with the prolongation drought cycles, as is classically expected in plants with phenotypic plasticity in heterogeneous environments (Bradshaw, 1965; Bradshaw and Hardwick, 1989; Bussotti et al., 1995).

The anatomical differences observed in the umbu genotypes do not consistently support the physiological differences found in the present study. The lower E values found in GBU 68 led us to expect that this genotype would exhibit lower stomatal density or a reduction in stomata size. This, however, was not observed (Table 3). Similarly, the high transpiration rates observed in GBU 48 should be explained by a higher stomatal density or aperture size, which should facilitate stomatal conductance and explain the lower r_s when exposed to adequate soil water availability. However, the genotypes that had reductions in stomata aperture size (GBU 44 and GBU 68) were those that were capable of recovering transpiration rates after re-watering. These results suggest that morphological changes have an important physiological implication, likely related to the speed of the guard cell response, as stated by Niu et al. (2005).

There were differences in tissue proportions between genotypes. Drought induced a reduction in spongy parenchyma and an increase in palisade parenchyma thickness in GBU 44. These anatomical changes may result in higher photosynthetic efficiency in GBU 44, as the palisade parenchyma contains the most photosynthetic cells. However, we cannot make this claim, as photosynthesis was not measured in the present

study, but we can affirm that drought induces different responses in different umbu tree genotypes.

Summarizing, stomatal responses to water deficit suggest that stomatal closure is the first line of defense from desiccation in umbu plant genotypes. The regularity of the stomatal closure period and the water vapor gas exchange recovery suggest that GBU 68 is the most drought-sensitive genotype. Anatomical changes induced by intermittent drought demonstrate that the genotypes exhibit markedly different responses to water deficit, but not enough to explain the physiological differences between them.

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