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Morpho-anatomical and Micromorphometrical Evaluations in Soybean Genotypes during Water Stress

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ABSTRACT

In a greenhouse experiment, morpho-anatomical and micromorphometrical analyses of two soybean cultivars, MG/BR46 (Conquista) and BR16-tolerant and sensitive to drought, respectively—were used to study their water-deficit-tolerance strategies. Drought treatments were applied at reproductive stages from R_2 to R_7, where evaluations were conducted at 30 days and 45 days after stress started, respectively. The total length of Conquista plants (shoot + root) was greater than of BR16 plants. Pod dry weight was adversely affected due to the lack of moisture, decreasing productivity even of Conquista plants. Both the cultivars had normal development of root hairs; however, it was observed a decrease in the cortex:central cylinder ratio in BR16 stressed for 30 days, and they also showed similar leaflet thickness and stomata distribution. Differences in drought tolerance observed between the two cultivars seemed to be related to factors other than morphological traits since this species has a short lifecycle.

Key words: Anatomy, leguminous, drought and root.

INTRODUCTION

Plants respond to variations in water levels in the soil through the morphological, anatomical, physiological, biochemical and molecular adjustments (*Sant´Anna-Santos* et al., 2006 and *Wang* et al., 2003). Water deficit commonly occurs in commercial production of many crops. It can cause substantial negative effects in plant development, reducing the productivity (Lecoeur and Sinclair, 1996). The level of damage depends on genotype, duration and severity of stress and the developmental stage at which the drought occurs (Santos and Carlesso, 1998).

According to Grant (1992), the development of adaptation mechanisms in plants is influenced by many factors, including atmospheric CO_2 level, solar radiation, soil humidity, temperature and atmospheric relative humidity. Plants respond to water deficit in many ways such as decreased leaf area, reduced stomata conductivity, increased leaf senescence, and premature abscission of flowers

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and fruit. Furthermore, loss of cell turgidity causes reduction in leaf expansion, consequently reducing the transpiration and assimilates synthesis and translocation (McCree and Fernández 1989; Santos and Carlesso, 1998 and Taiz and Zeiger, 1991).

Morpho-anatomical aspects such as cuticle thickness, number of layers of palisade parenchyma, pubescence and leaf rugosity can decrease the surface area contribute to reduced transpiration and photoinhibition caused by stress (*Dias* et al., 2007). The total leaf thickness is partially determined by the lenght of the palisade parenchyma cells, and thicker leaves can be more efficient in the use of water (Boeger and Wisniewski, 2002). Thus, leaf thickness can affect the tolerance to water stress (Groom and Lamont, 1997 and Turner, 1994).

Water deficit may promote the expansion of root systems to deeper, more-moist zones in the soil profile. During plant development, root length increases until anthesis, decreasing subsequently; decreased efficiency of water absorption may then be apparent (Pimentel and Rossielo, 1995).

Drought tolerance is one of the most important traits and it has a very complex system to be regulated. However, in soybean, only little is known including molecular works. On the other hand, some works (Casagrade et al., 2001 and Oya et al., 2004) revealed BR16 as a very sensitive and Conquista was tolerant. Hence, the present study aimed to analyze the strategies for tolerating water loss using qualitative and quantitative analyses of plant morpho-anatomical characteristics, comparing cultivars and to verify if only morpho-anatomical strategies were efficient in soybean to tolerate moderate water stress.

MATERIAL AND METHODS

Two soybean genotypes were utilized: Conquista (MG/BR46) and BR16, known to be tolerant and sensitive to drought, respectively, on the basis of studies carried out in the field by Embrapa Soybean, Londrina-PR, Brazil (Casagrande et al., 2001 and Oya et al., 2004). In field conditions, these cultivars have different lifecycle length, whereas light supplementation during the experiment decreased flowering-time difference to two days only.

The plants were divided into two groups: a control group at 15% gravimetric humidity (GH) (near field capacity) and a stressed group at 5% of GH (Casagrande et al., 2001). Each group comprised ten plants of each genotype, sown in 10-L pots with sand and nutrient solution under greenhouse conditions (day $30^oC\pm2^oC$; night 22 ± 2^oC; RH 40%±5%) in a completely randomized design. All plants were allowed to develop for 45 days in normal conditions (15% GH). Moisture stress was initiated by withholding the irrigation, until sand humidity reached 5%. It took approximately five days, and the control group was kept at 15% GH until the conclusion of the experiment (90 days later). Samples for morpho-anatomical analysis were collected at 30 and 45 days after stress initiation, corresponding to R_2 and R_7 developmental stage (Fehr and Carviness, 1981), respectively, resulting in four samples: control 30 days (C30), stress treatment at 30 days (T30), control 45 days (C45) and stress treatment at 45 days (T45). The pots contained sand and plants were weighed daily in semi-analytical balance to monitoring sand humidity.

For the morphological studies, the variables analyzed were: root and shoot length, plant-component dry weights, relative growth rate (RGR), calculated using the formula lnfDW-lniDW/time, where fDW is final dry weight, iDW is initial dry weight and time is the stress time treatment (Ferri, 1985), and leaf area. The anatomical studies were conducted on the segments of the apex of the main root and on median leaflets of the third leaf from the apex. Transverse cuts, 12-μm thick, were colored by a combination of aster-blue and basic fuchsine according to Johansen (1940) and Sass (1951) with minor modifications. The histological measurements were conducted using the Image Pro-Plus software, v.4.1 (Media Cybernetics) in an optical microscope (Leica Microsystems) coupled with a digital camera.

Before electronic scanning, leaflet segments of 0.5 cm^2 were placed in FAA 50% (formol: acetic acid: alcohol) and dehydrated in an alcohol series. After the samples were critical-point dried with CO_2, they were mounted on a metallic support, fixed with a mix of colorless enamel and graphite, covered with gold under vacuum in Balzers Union FL9496 SCD-030 equipment. Observations were made and images were collected using a scanning electron microscope (JEOL JSM–6360LV) at the Electronic Microscopy Laboratory of the UFPR, Curitiba-PR, Brazil. ANOVA were performed using the SAS program comparing the treatments and genotypes. Means were compared using Tukey's test (p≤0.05).

RESULTS

The root systems of both the cultivars and treatments presented variations in the numbers of protoxylem bundles in terms of triarc, tetrarc and pentarc structures, indicating that it was a feature of the species and not a feature probably induced by the drought. These variations were observed either in control plants or in plants submitted to water deficit (Fig. 1). No cultivar showed root-length responses to water deficit. However, shoot length was reduced by 18% and 22% in Conquista plants exposed to water deficit for 30 days (T30) and for 45 days (T45), respectively (Table 1).

In contrast, BR16 plants did not present such differences in shoot length. Leaf area of Conquista plants, submitted to water deficit for 30 days (T30), was reduced by 28%. Similarly, leaf area of BR16 was reduced by 38% by T30 and by 34% by T45 (Fig. 2).

Conquista plants exposed to T30 had a 30% decrease in root dry weight; however, at 45 days of treatment, neither cultivar showed a response in root dry weight. Shoot dry weights of Conquista and BR16 plants exposed to water deficit for 45 days (T45) decreased by 37 and 31%, respectively, relative to their controls (C45). Leaf dry weight was reduced by 28% in Conquista plants at T30, and at 45 days it was reduced by 50% in Conquista and by 39% in BR16. Plant dry weight was reduced only in BR16, by 31%, when exposed to T45 (Table 2).

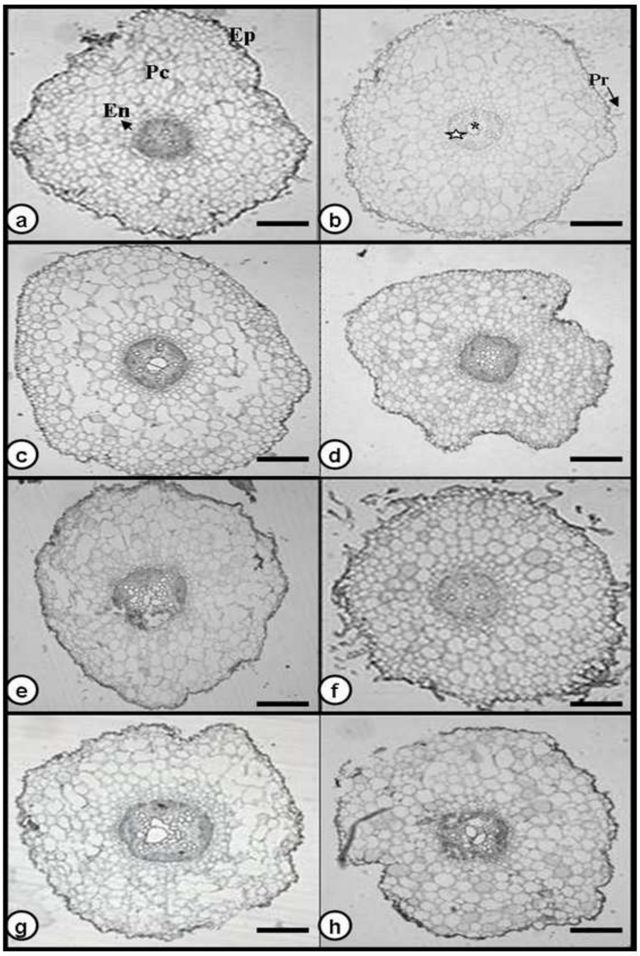

Figure 1 - Transverse sections of the main root of *Glycine max*: Conquista (C30) (a), Conquista (T30) (b), BR16 (C30) (c), BR16 (T30) (d), Conquista (C45) (e), Conquista (T45) (f), BR16 (C45) (g), and BR16 (T45) (h). En: endodermis, Ep: epidermis, ☆ : protoxylem, *: xylem. Bars = 100 µm.

Table 1 - Length of the root, shoot and total length of *Glycine max* under control condition (C) and subjected to moderate water deficit (T) for 30 and 45 days, of two cultivars: Conquista (drought-tolerant) and BR16 (sensitive). Different letters in a column denote statistical difference between means (Tukey **P≤0.05).

Treatments	**Lenght (cm)**		
	Root	**Shoot**	**Total**
Conquista (C30)	35.7ns	179.4A	215.1A
Conquista (T30)	38.3	147.7B	186B
Conquista (C45)	41.2ns	168.1a	199.3a
Conquista (T45)	36.4	131.1b	167.5b
BR 16 (C30)	36.9ns	131.7 ns	168.6ns
BR 16 (T30)	38	118.6	156.6
BR 16 (C45)	38.8ns	111,6ns	150.4ns
BR 16 (T45)	35.2	112.8	148.0

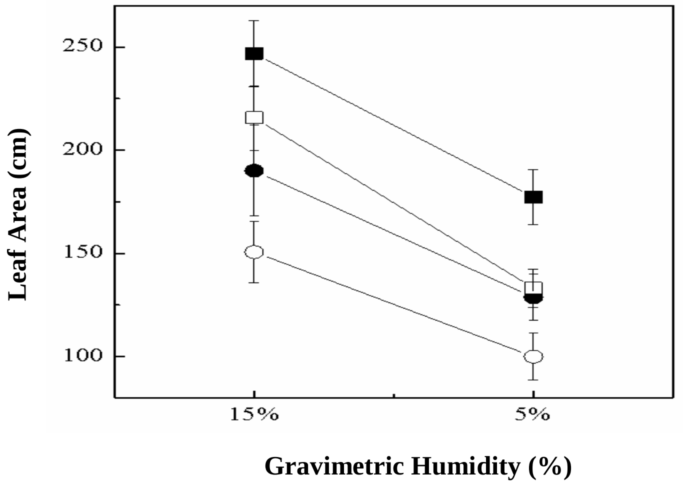

Figure 2 - Leaf area of *Glycine max* under control condition and moderate water deficit for 30 and 45 days, of two cultivars: Conquista (drought-tolerant) and BR16 (sensitive). Coincident standard error bars denote no difference between means (Tukey, p≤0.05).

Table 2 - Dry weight of roots and shoots of two *Glycine max* cultivars: Conquista (drought-tolerant) and BR16 (sensitive) under control (C) and moderate water deficit (T) for 30 and 45 days of treatment. Different letters in a column denote statistical difference between means (Tukey **P≤0.05).

Treatments	**Dry Weight (g)**					
	Root	**Shoot**	**Leaf**	**Pod**	**Total**	**R/S**
Conquista (C30)	7.11A	6.66ns*	9.89A	3.5ns	27.2ns	0.45ns
Conquista (T30)	5.01B	5.83	7.10B	3.65	21.6	0.40
Conquista (C45)	4.23ns	9.08a	12.1a	8.38ns	33.7a	0.20ns
Conquista (T45)	3.26	5.76b	6.14b	6.71	21.9b	0.28
BR 16 (C30)	5.34ns	4.74ns	7.38ns	4.64ns	22.1ns	.43ns
BR 16 (T30	8.11	4.75	6.45	4.9	24.2	0.72
BR 16 (C45)	4.36ns	5.15a	6.63a	10.1a	26.3a	0.36ns
BR 16 (T45)	2.39	3.59b	4.02b	6.96b	16.8b	0.32

Leaf relative growth rate (LRGR) was reduced by 37 and 60% in Conquista subjected to water deficit for 30 days (T30) and for 45 days (T45), respectively. BR16 plants had an LRGR reduction only after 45 days, by 83% reduction. Total relative growth rate (TRGR) was reduced only in Conquista, by 17% with T30 and by 28% with T45 (data computed from Table 2) (Table 3). For root: shoot ratio, no differences were observed in terms of treatment or cultivar.

Table 3 - Histological measurements of *Glycine max* root, under control condition (C) and moderate water deficit (T) for 30 and 45 days of treatment, of two cultivars: Conquista (drought-tolerant) and BR16 (sensitive). Different letters in a column denote statistical difference between means (Tukey **P≤0.05).

Treatments	Cortex/Central Cylinder Ratio	
	Conquista	**BR 16**
C30	15.4ns	15.6A
T30	16.3	13.2B
C45	9.61ns	11.1ns
T45	11.7	11.7

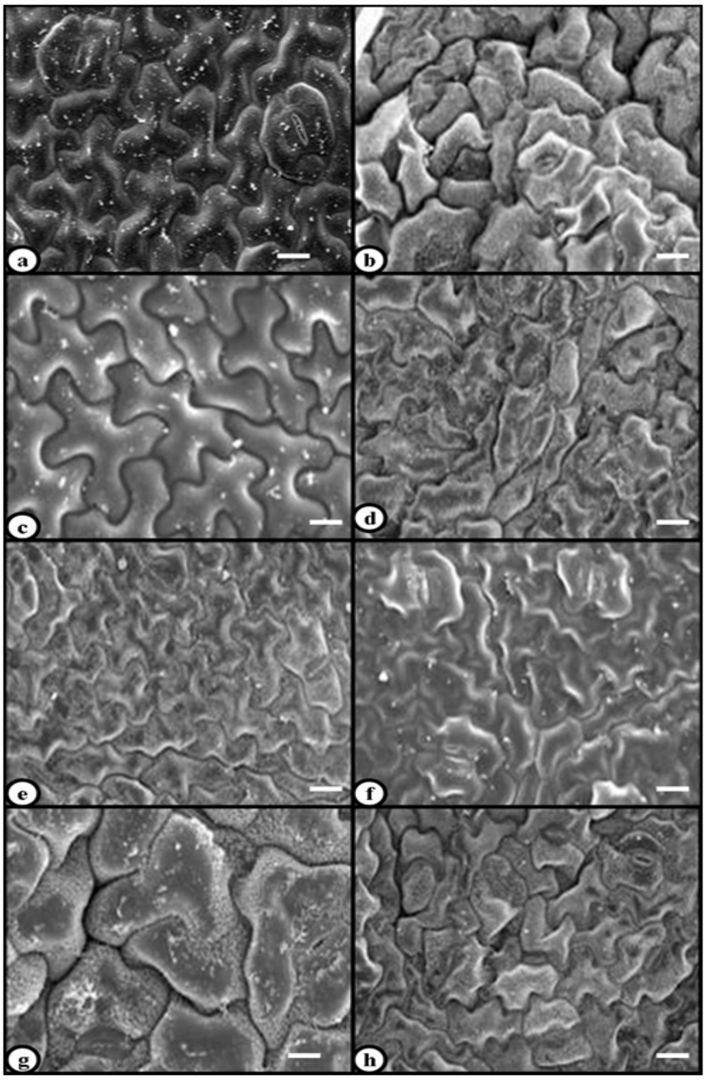

Figure 3 - Scanning electron micrographs of epidermis of *Glycine max* leaflets of cultivar Conquista: adaxial (C30) (a); abaxial (C45) (b); adaxial (T45) (c); abaxial (T45) (d). Cultivar BR 16: adaxial (C45) (e); abaxial (C45) (f), Bars=20 µm; adaxial (T45) (g), Bar = 10 µm; adaxial (T45) (h), Bar = 20 µm. Ce: epidermal cells. Es: stomata

The cortex:central cylinder ratio showed no effects of drought stress in Conquista (T30 or T45). In contrast, BR16 plants, subjected to water deficit for 30 days presented a 16% smaller ratio. However, after 45 days of water deficit, this cultivar did not show a difference in this variable (Table 4). The epidermis of leaflets in both the cultivars was constituted by a unique layer of cells with periclines and anticlines. The stomata were predominantly paracytic (Figs. 3 and 4). Occasionally, Conquista plants submitted to water deficit showed closed ostioles whereas moisture-stressed BR16 plants invariably had open ostioles.

Soybean mesophyll is dorsiventral, with biseriate palisade parenchyma and extended cells in many sizes, disposed perpendicularly to the limbo surface. The spongy parenchyma had two or three layers of irregular cells, with prevalent intercellular spaces (Fig. 5). Comparative analysis of leaflet tissue thickness did not reveal differences between the treatments in either of the cultivars analyzed (Table 5). The leaflet central nervure presented xylem in the central portion with external phloem, confirming the organization of the vascular system of dicotyledonous, and a thin layer of sclerenchyma under the phloem. No significant differences were observed in the relative amounts or quantities of these tissues between the treatments or cultivars (Fig. 6). Trichomes were observed on leaves of both soybean genotypes. However no visual differences among the treatments were observed (Fig. 7).

Table 4 - Histological measurements of leaflets of *Glycine max* in the control condition (C) and with moderate water deficit (T) for 30 and 45 days of treatment, of two cultivars: Conquista (drought-tolerant) and BR16 (sensitive). Different letters in a column denote statistical difference between means (Tukey **P≤0.05).

	Thickness (μm)				
Treatments	Adaxial surface of epidermis	Palisade Parenchyma	Spongy Parenchyma	Abaxial surface of epidermis	Leaflet
Conquista (C30)	14.7ns	65.0ns	41.8ns	13.3ns	135.0ns
Conquista (T30)	14.6	64.3	52.1	14.6	145.7
Conquista (C45)	13.2ns	51.2ns	40.5ns	14.2s	118.7ns
Conquista (T45)	12.7	57.7	43.2	12.3	126.0
BR 16 (C30)	14.1ns	63.4ns	41.2ns	15.8ns	134.5ns
BR 16 (T30)	15.0	62.7	38.0	16.1	131.8
BR 16 (C45)	13.2ns	55.8ns	39.7ns	13.7ns	122.5ns
BR 16 (T45)	12.8	61.1	33.1	14.5	121.6

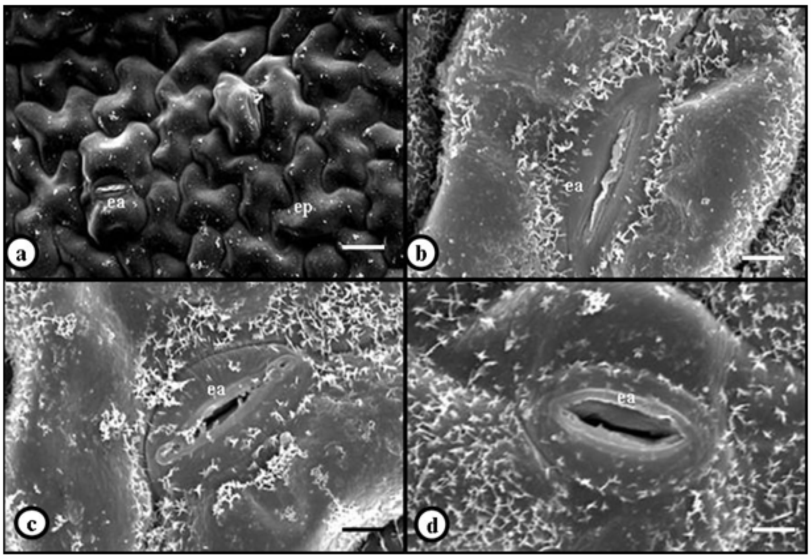

Figure 4 - Scanning electron micrographs of leaflet epidermis of *Glycine max* with stomata in the cultivars: Conquista (C30) (a), Conquista (T45) (b); BR16 (C45) (c), BR16 (T45) (d). Ea: stomata antechamber. Ep: epidermis. Bars = 20 μm (a) and 5 μm (b-d).

Table 5 - Relative growth rates of root, shoot, leaf and total and root: shoot ratio of *Glycine max* in the control condition (C) and to moderate water deficit (T) for 30 and 45 days, of two cultivars: Conquista (drought-tolerant) and BR16 (sensible). Different letters in the column denote statistical difference between means (Tukey **P≤0.05).

Treatments	**RGR(mg g-1 day -1)***				
	Root	**Shoot**	**Leaf**	**Total Plant**	**R/S**
Conquista (C30)	0.06A	0.04ns**	0.03A	0.04A	0.45ns
Conquista (T30)	0.04B	0.04	0.02B	0.04B	0.39
Conquista (C45)	0.03ns	0.03a	0.02a	0.03A	0.20ns
Conquista (T45)	0.02	0.02b	0.01b	0.02B	0.28
BR 16 (C30)	0.04ns	0.03ns	0.02ns	0.04ns	0.43ns
BR 16 (T30)	0.05	0.03	0.02	0.04	0.71
BR 16 (C45)	0.02ns	0.02a	0.01a	0.03ns	0.36ns
BR 16 (T45)	0.01	0.02b	0.002b	0.02	0.32

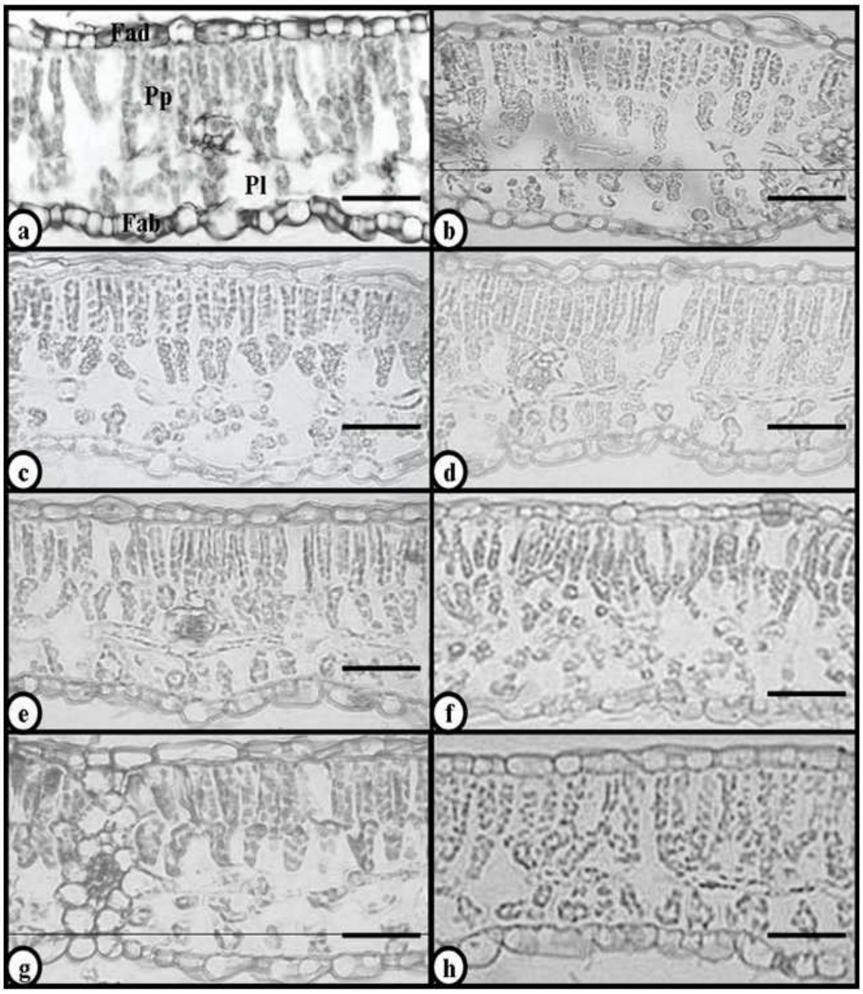

Figure 5 - Transverse section of *Glycine max* leaflets: Conquista (C30) (a), Conquista (T30) (b), BR16 (C30) (c), BR16 (T30) (d), Conquista (C45) (e), Conquista (T45) (f); BR16 (C45) (g), BR16 (T45) (h). Es: Stomata. Fab: abaxial face of epidermis. Fad: adaxial face of epidermis. Pl: spongy parenchyma. Pp: palisade parenchyma. Bars = 100 μm.

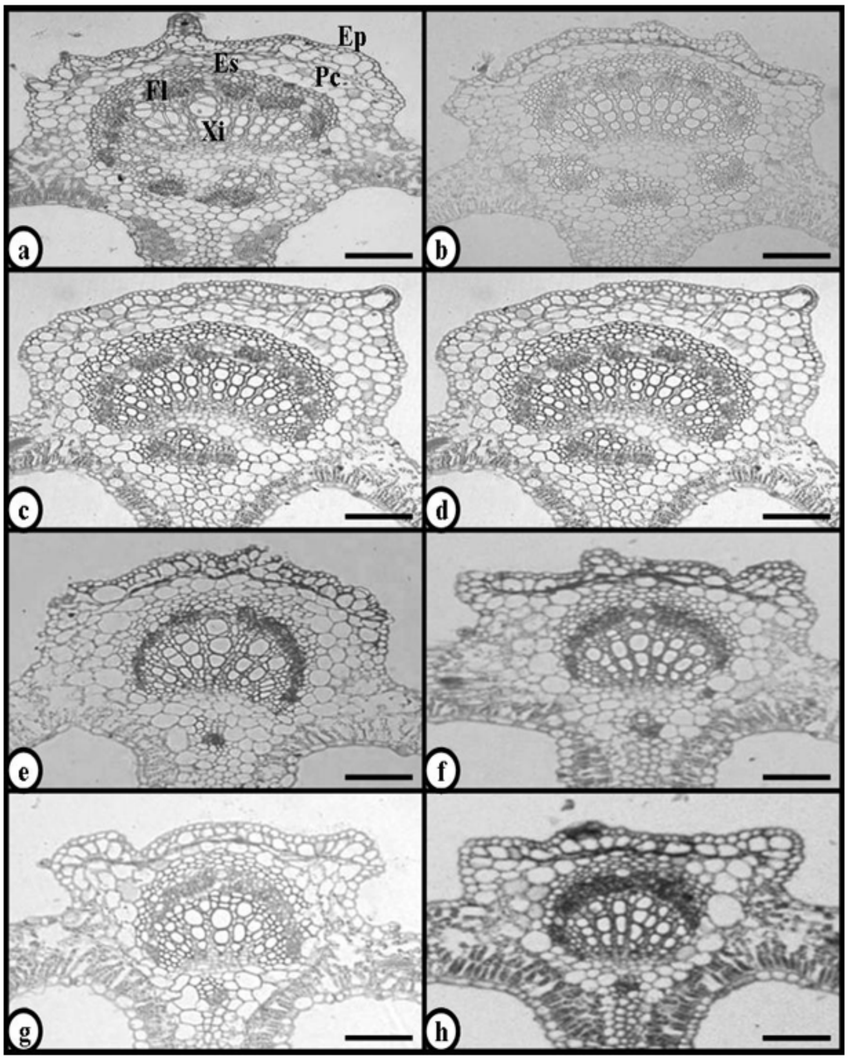

Figure 6 - Transversal section of *Glycine max* leaflet nervure of Conquista (C30) (a), Conquista (T30) (b), BR16 (C30) (c), BR16 (T30) (d), Conquista (C45) (e), Conquista (T45) (f), BR16 (C45) (g) ad BR16 (T45) (h). Pc: cortical parenchyma, Ep: Epidermis, Es: Sclerenchyma, Fl: Phloem e Xi: Xylem. Bars = 100 μm.

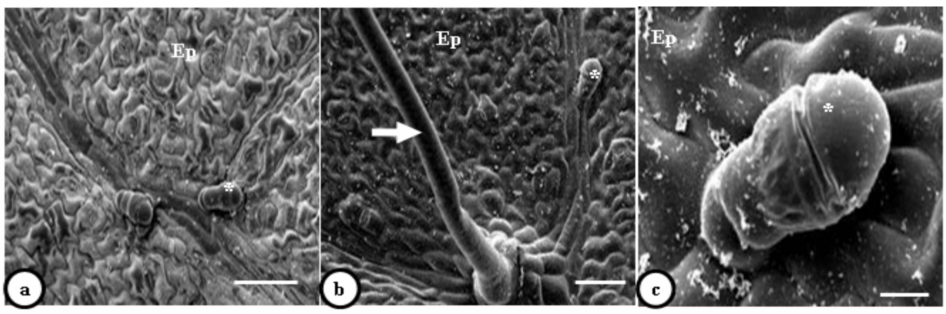

Figure 7 - Scanning electron micrographs of leaflet epidermis of *Glycine max* with glandular trichomes in detail, in the cultivars: BR16 (T30) (a), Conquista (C30) (b), Bars = 50 μm; Conquista (T30) (c), Bar = 10 μm. Ep: epidermis, *: glandular trichomes and arrow: trichome.

DISCUSSION

The experiment was conducted with sand-cultured plants in the pots, which explained why no significant root-length differences were observed as a result of moisture stress. In field experiments, soybean cultivars characterized as drought tolerant usually showed large quantities of diageotropic roots in the superficial layers of soil (Hudak and Patterson, 1996). Hirasawa et al. (1994) also observed that drought tolerant soybean subjected to moisture stress before flowering developed more-extensive root networks that correlated with higher yields when compared with sensitive genotypes.

The length (shoot+root) of plants submitted to water deficit was greater for Conquista, which were 10cm higher than BR16 plants, confirming Casagrande et al. (2001) results. It probably happened because Conquista was more tolerant in relation to BR16 plants having a better response to low available water. Leaf abscission occurred during the period of water deficit, which contributed to the reduction of leaf area in both the cultivars. The drought effects on leaf area and abscission could be a reflection of leaf-nutrient deficiencies. Reduction in leaf area constituted a defense strategy to minimize the water loss (Begg and Turner, 1976; Lawlor, 1993; Mansfield and Davies, 1985) and could be viewed as a xeromorphic characteristic (Brüning, 1973); Lleras, 1977 and Turner, 1994). According to Boeger and Wisniewski (2002), leaf area is regulated by the balance between the carbon gain and water lost.

Dry weight was significantly affected in BR16 plants submitted to water deficit for 45 days, although no difference was observed at 30 days. This could be related to the growth phase in which the stress occurred. At the 30th day of stress, plants were at the R_2 developmental stage when vegetative and reproductive development occured simultaneously, and because of that, carbohydrate production and translocation usually were at their peak (Fehr and Caviness, 1981). Consequently, larger differences won show up among treatments only after this period. As these pods developed further, less carbohydrate was translocated. If a drought event occurs, translocation is reduced even more. Capacity to retain higher rates of translocation during periods of water deficit could be one of the reasons Conquista had higher dry weights at both sampling dates. Oya et al. (2004) reported lower yields of BR16 in relation to other cultivars in the field, confirming its sensitivity to drought. Reduction of root relative grown rate (RRGR) in Conquista plants submitted to water deficit for 30 days (Table 3) could be due to a decrease in the production of new roots, or due to root death. Conquista is well known to develop a relatively more-extensive root system. However, the 22% reduction observed could be caused by the limitations imposed by pot volume, inducing a plant-signaled reduction in root growth. The decrease in leaf relative growth rate (LRGR) of both the cultivars was a result of abscission and small production of new leaves. The RGR expresses plant development as a function of dry weight accumulation over time (Ferri, 1985). According to Chiariello et al. (1991), the RGR is a physiological index appropriate for comparison of agronomic traits effects, because it is relative rather than absolute.

Regarding the decrease in the cortex:central cylinder ratio observed in both the cultivars under water deficit Vasellati et al. (2001) showed that drought increased the number of root hairs and decreased the diameter of the metaxylem bundle in *Paspalum dilatatum*, a common characteristic of plants submitted to drought that could cause a reduction in the cortex:central cylinder ratio.

Trichomes observed on the leaves of both soybean genotypes, were barriers to air movement, consequently decreasing water loss from the leaf surface (Mauseth, 1988). In the present work was observed stomata in the epidermis on both surfaces of the leaf. According to Mott et al. (1982), amphistomatic leaves have a potentially higher capacity for carbon dioxide capture and could achieve elevated levels of photosynthesis, if conditions were suitable.

Glycine max and other species use many strategies to optimize the utilization of water in dry environments and during periods of moisture shortage. Some of these adaptations are anatomical and constitutive; others are transient. Differences between these two cultivars observed previously in the experimental and field conditions were not related to anatomical characteristics according to the present results. Probably, it occurred via physiological and metabolic mechanisms. Furthermore, stress was applied during reproductive growth, (R_2 to R_7), the most-sensitive phase. At this stage, all basic organs and tissues were already formed, thus adaptive morphological modifications could represent disadvantage in relation to the metabolic and physiological modifications. Certainly, molecular and physiological mechanisms are in place to differentiate these two genotypes (Bray, 2004).

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RESUMO

Análises morfo-anatômicas e micromorfométricas de duas cultivares de soja, MG/BR46 (Conquista) e BR16—tolerante e sensível à seca, respectivamente, em experimento conduzido em casa de vegetação—foram feitas para estudar as diferentes estratégias de tolerância ao déficit hídrico. Tratamentos de seca foram aplicados no estádio reprodutivo R_2 e R_7, onde avaliações foram conduzidas em 30 dias e 45 dias após o inicio do estresse, respectivamente. O comprimento total das plantas da cultivar Conquista (parte aérea e raiz) foi maior do que das plantas da cultivar BR16. A massa seca da vagem foi adversamente afetada pelo déficit hídrico, diminuindo a produtividade das plantas da cultivar Conquista. Ambas as cultivares tiveram o desenvolvimento de pêlos radiciais normais e, uma diminuição da razão córtex:cilindro central foi observada em BR16 estressada por 30 dias, além de apresentar uma espessura do folíolo e distribuição dos estômatos normais. Diferenças na tolerância à seca observada entre as duas cultivares devem estar relacionadas também a outros fatores, alm das características morfológicas, já que esta espécie possui um ciclo de vida curto.

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