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Eucalyptus urocan drought tolerance mechanisms

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The present study was designed to identify strategies for tolerance to hydric deficit in *Eucalyptus urocan* seedlings. The experiment was conducted in a green house with 100% solar radiation capture. The completely randomized block design was used with five treatments (plants irrigated daily with water corresponding to 25, 50, 75, 100 and 125% daily evapotranspiration) and five replications. 120-day-old *E. urocan* seedlings (hybrid result from the crossing between *Eucalyptus Urophylla* x *Eucalyptus camaldulensis*) were planted in pots containing 8 L of substrate composed by oxisol, sand and muck at 3:1:0.5 ratio, respectively. The seedlings were irrigated with different volumes of water for 13 days and then analyzed. Under hydric deficit condition, *E. urocan* plants showed significant investment in the root system, reduced the breathing rate and kept enough turgor for growth. *E. urocan* plants at initial growth stage are tolerant to hydric deficit and show dehydration delay as a strategy to tolerate drought.

Key words: Hydric deficit, silviculture, initial growth.

INTRODUCTION

Brazilian forestry sector accounts for 24% of the agricultural GDP and 1.2% of all the wealth generated in the country. Brazil holds the most advanced technologies for the exploration of eucalypt and achieves yields higher than the species origin pole, Australia. Despite the successful performance in the sector, planted forests take less than 1% of the country's productive area and show great growth potential from commercial exploration of new areas (Ibge, 2014).

The Eucalyptus is the most cultivated tree genre in Brazil, which represents about 60% of commercial plantations. The Eucalyptus genus species found in Brazil suitable soil and climate conditions for rapid and appropriate growth in a short period of time. Its large-scale use is fostered by multiple applications, as "from eucalypt everything is usable". Essential oil extracted from the leaves is used in food, cleaning products, perfume and even in medicine. The bark provides tannin, and the trunk, posts, poles, mine props, boat masts, packaging boards and furniture. The fiber is used as raw material for papermaking and cellulose (Abraf, 2013). Despite the success of planted forests, the sector may

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generate even more wealth if it explores areas unsuitable for several other crops less tolerant or sensitive to abiotic stresses (Lopes et al., 2015).

Valuation of cropping areas and reduction of available ones have pushed eucalypt away to marginal areas, such as regions with low water availability and low fertility soils. Amongst all environmental resources required for agricultural productivity, water is the most abundant and also the most limiting one. Water shortage is the greatest barrier to agricultural productivity. Approximately 35% of the Earth's land is arid or semi-arid, and does not get adequate rainfall water supply for most species grown (Diaz-López et al., 2012). Current forecasts point to continuing global warming and increasing periods of drought in many regions of the planet. During drought, these plants are subjected to water shortage, which significantly hinders the accumulation of biomass. Water deficit reduces metabolic capacity and alters exchange activity with significant damage to wood formation and anatomy (Sette Jr. et al., 2010).

During the initial development phase, water deficit decisively affects growth and increases tree mortality in the field (Cabral et al., 2010). The intensity of the symptoms and the disorders observed during the drought are important variables for identification of promising materials for semi-arid regions (Reis, 2011). Drought tolerance is a result of several features that express themselves in different manners and at the same time, depending on the severity and frequency of the water deficit, the plant age and nutritional conditions, type and depth of soil, and atmospheric evaporative demand. Therefore, the adoption of a single strategy for drought adaptation is certainly not enough in any kind of environment (Sambatti and Caylor, 2007).

The conducting of research to identify drought tolerance mechanisms in eucalypt plants can increase the species productivity in traditional growing regions that have started to experience sporadic droughts and maximize commercial exploitation through the introduction of the species in areas previously considered unsuitable. This study aimed to identify the strategies for water deficit tolerance in *Eucalyptus urocan* plants.

MATERIALS AND METHODS

Experimental design

The work was carried out on a bench with transparent covering at Goiás State University, Ipameri Campus (17°43'19''S, 48°09'35''W, 773m alt.), Ipameri, Goiás. This area has AW weather according to Köppen classification. During the experiment the average relative humidity was 46%, and maximum and minimum average temperatures were 31 and 14°C, respectively.

The experiment was carried out following the completely randomized design, with five treatments (plants irrigated daily with water volumes corresponding to 25, 50, 75, 100 and 125% of daily evapotranspiration), five replicates and parcel of a plant in a pot. The water volume referring to daily evapotranspiration was calculated according to recommendations by Allen et al. (1998). 120-day-old *E. urocan* seedlings (originated from a hybrid cross between *Eucalyptus Urophylla* x *Eucalyptus Camaldulensis*) were planted in 8-L pots with substrate composed of oxisol, sand and manure at 3:1:0.5 ratio. Chemical analysis of the mixture showed the following values: pH 6.4; 19 g dm^{-3} OM; 2.4 mg dm^{-3} P; 109 $cmol_C$ dm^{-3} K; 1.5 $cmol_C$ dm^{-3} H+Al; 3.2 $cmol_C$ dm^{-3} Ca; 1.6 $cmol_C$ dm^{-3} Mg; 27.7mg dm^{-3} Zn; 77.20% SB and 6.58 CTC. After the composition analysis, decision was made to not do fertilization, nor correct the substrate pH. Seedlings were irrigated with different water volumes for 13 days, and then the following variables were analyzed: number of leaves, leaf length and width, plant height, stem diameter, relative water content (RWC), chlorophylls and carotenoids, root mass ratio (RMR), stem mass ratio (SMR), leaf mass ratio (LMR), transpiration and total biomass.

Growth variables

The plant height, leaf length and width and stem diameter were measured by using a graduated ruler and a digital caliper. The number of leaves was obtained by counting all the plant leaves. The destructive analysis was then performed, when leaves, roots and stems were separated and oven-dried at 72°C to reach constant dry matter, and then weighed. The leaf mass ratio (LMR), root mass ratio (RMR), stem mass ratio (SMC) and total biomass were calculated based on the dry mass data.

Relative water content in the leaf (RWC)

In order to obtain the relative water content, five leaf discs were extracted each repetition of 6 mm diameter, weighed and saturated in Petri dishes with distilled water for 24 h. Then the discs were weighed again and dried at 70°C for 72 h for subsequent recording of the dry weight in grams.

Transpiration

Daily transpiration was estimated gravimetrically, by determining the difference in weight of the pots as described by Cavatte et al. (2012). However, a 24 h time interval was used with weighing at 6 pm.

Photosynthetic pigments

To determine the total chlorophyll and carotenoid concentration, foliar discs were extracted from a well-known area and put into glass containers with dimethyl sulfoxide (DMSO). Later, extraction was performed in water bath at 65°C for one hour. Aliquots were taken for spectrophotometric reading at 480, 649.1 and 665 nm. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid contents were determined according to the equation proposed by Wellburn (1994).

Data analysis

The data were submitted to variance analysis and then to regression analysis using the R software (R Core Team, 2015).

RESULTS

Analysis of variance showed no significant change in mass ratio stem, number of leaves, plant height, leaf concentrations of carotenoids and chlorophylls (a + b) by

Table 1. Analysis of variance and regression equations for relative water content (RWC), biomass (BIO), root mass ratio (RMR), height, mass ratio stem (RMS), leaf mass ratio (RMF), transpiration (*E*) in plant eucalypt under water deficit.

Variables	Mean squares		
	Regression	**Residual**	**Equations**
GF	1	23	--
RWC	5873.9**	117.8	$Y = 83.1(1\text{--}e^{-0,03x})$; $R^2 = 0,70$
BIO	70.7*	11.7	$Y = 10.93+0.05x$; $R^2 = 0.95$
RMR	0,02**	0,01	$Y = 0.39-0.001x$; $R^2 = 0.97$
Height	28.8 ns	39.05	There was no adjustment equation
RMS	0,005 ns	0,003	There was no adjustment equation
RMF	0,10**	0,008	$Y = 0.32+0.002x$; $R^2 = 0.97$
E	202884**	865.3	$Y = -44.5+2.548x$; $R^2 = 0.96$

*significant at 5% probability; **significant at 1% probability; ns = non-significant by F test.

Table 2. Analysis of variance and regression equations for leaf width, leaf lengh, number of leaves, stem diameter, carotenoids (CAR), chlorophyll *a*, total chlorophyll Chl (*a*+*b*) and ratio of chlorophyll *a* chlorophyll *b* (Chl *a* / Chl *b*) in plant Eucalypt under water deficit.

Variables	Mean squares		
	Regression	**Residual**	**Equations**
GF	1	23	--
Leaf width	2.46*	0.51	$Y = 4.88+0.009x$; $R^2 = 0,41$
Leaf length	13.4*	2.40	$Y = 12.95+0.02x$; $R^2 = 0.44$
Number leaf	180.5 ns	209.5	There was no adjustment equation
Stem diameter	7.61**	0.25	$Y = 4.85+0.02x$; $R^2 = 0.99$
CAR	0,03ns	0,08	There was no adjustment equation
Chl *a*	0.66*	0.12	$Y = 1.31+0.005x$; $R^2 = 0.48$
Chl *a* /Chl *b*	0.94 **	0,05	$Y = 1.87(1\text{--}e^{-0,05x})$; $R^2 = 0.50$
Chl (*a*+*b*)	1.93 ns	1.67	There was no adjustment equation

*significant at 5% probability; **significant at 1% probability; ns = non-significant by F test. The biomass, stem diameter, leaf mass ratio showed increasing values with increasing availability of water, however, the root mass ratio values decreased with increasing water availability (Figure 1).

F test and the data did not fit in any regression model significant at 5% probability, and significant variations in relative water content, biomass ratio of root and leaf mass, sweating, stem diameter, leaf length and width (Tables 1 and 2).

The biomass, stem diameter, leaf mass ratio showed increasing values with increasing availability of water, however, the root mass ratio values decreased with increasing water availability (Figure 1).

The leaf chlorophyll a, ratio chlorophylls a/b, length and width of the fully expanded leaf showed increasing amounts with increasing water availability (Figure 2).

The transpiration and relative water content showed increasing values with increasing availability of water (Figure 3).

DISCUSSION

Tolerance to water deficit is critical for the expansion of the agricultural frontier through exploration of previously unsuitable areas. Maintaining growth and preventing metabolic damage under low water availability in the soil and/or in the atmosphere is important indication of drought tolerance (Matos et al., 2014). Eucalyptus showed a significant decrease in growth variables when subjected to water deficit as discussed subsequently.

Water shortage interfered significantly with initial growth of eucalypt plants. The reduced values of biomass, stem diameter, leaf mass ratio, length and width leaf combined with the high root mass ratio values in plants irrigated with little water indicates the high sensitivity of eucalypt seedlings to variation in soil water level. Growing in low water volume (25 and 50%) is indicative of drought tolerance, because under this condition the eucalyptus plant allocated high percentage of biomass to the root system, exploring larger volume of soil and possibly absorbing more water to maintain the necessary turgor for shoot growth. The largest biomass

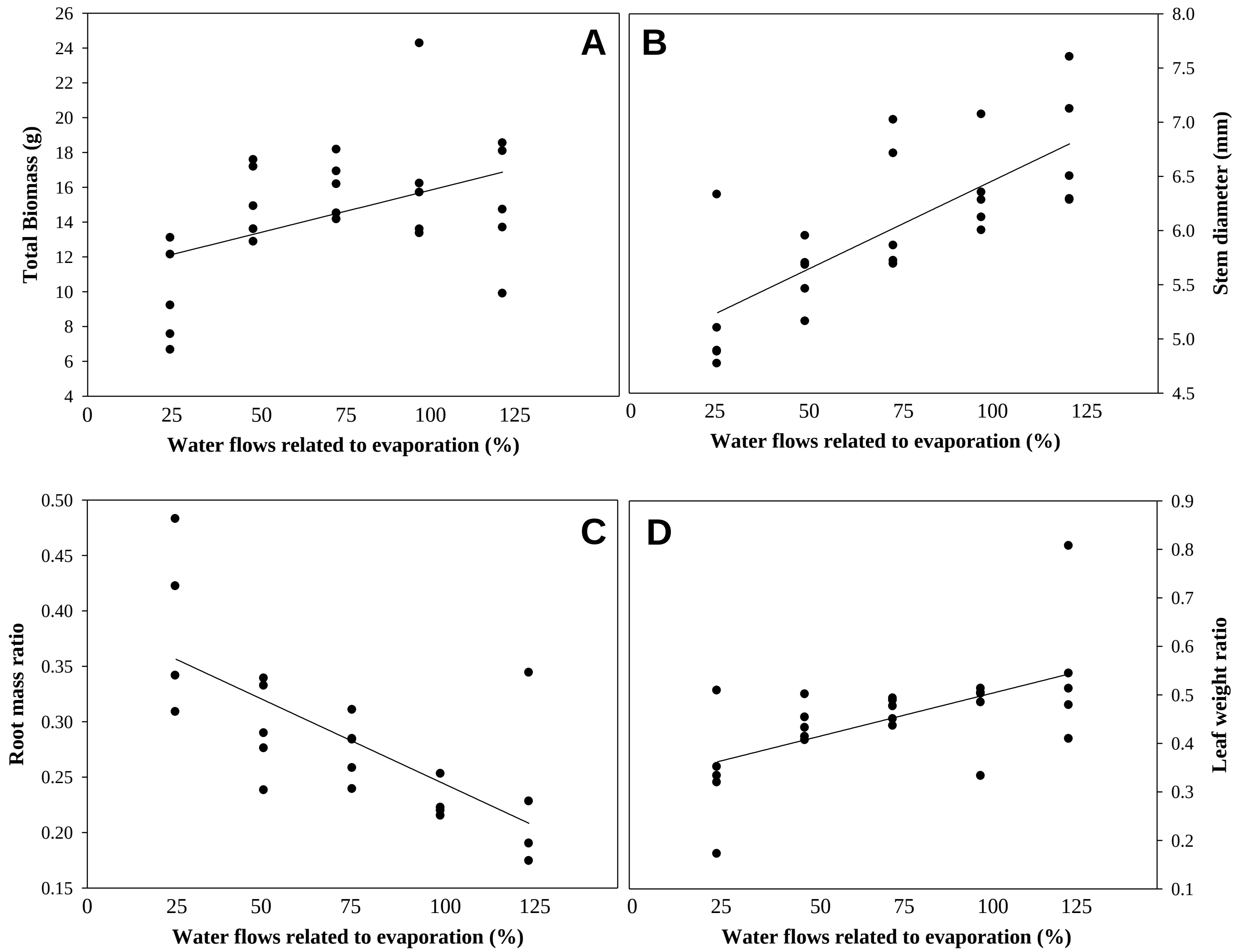

Figure 1. Regression equations for total biomass "A" [Y = 10.93+0.05x; R^2 = 0.95], stem diameter "B" [Y = 4.85 + 0.02x; R^2 = 0.99], root mass ratio "C" (Y = 0.39-0.001X; R^2 = 0.97) and leaf mass ratio "D" [Y = 0.32 + 0.002X; R^2 = 0.96] of *Eucalyptus urocan* seedlings irrigated with water volumes corresponding to 25, 50, 75, 100 and 125% evapotranspiration.

partitioning to the root system under reduced osmotic and water potential is a common response in several species (Góes et al., 2009; Matos et al., 2013; Souza et al., 2015.). Possibly, the eucalyptus plant has an efficient ground water extraction mechanism, for even under low water availability the plant draws water from the soil in sufficient volume to sustain growth.

The initiation and development of leaf primordia are dependent on the plant water status. According Taiz and Zaiger (2013), all of the absorbed water by the plant, 97% is transpired, 2% used in cell expansion and 1% in plant metabolism. Thus, low water availability reduced the leaf mass ratio. The lower biomass partitioning for leaves contributes to reducing perspiration and turgor maintenance. Among the morphological changes in water deficit condition, the reduced number and size of the leaves is the most significant (Santana et al., 2003; Souza et al., 2015; Diaz-López et al., 2012). Reduced transpiration in plants under water deficit may be associated with high stomatal sensitivity and lower "investment" in leaves. Low transpiration enabled maintenance or slight decrease in the relative water content in leaves and reasonable hydration sufficient for metabolism and growth. According to Souza et al. (2015), the reduced number of leaves, small change in relative water content and reduced plant transpiration in *E. urophylla* under low water availability is a sign of drought tolerance.

The non-significant variation in chlorophyll *b* concentration is indicative of absence of damage to D_1 protein of photosynthesis PSII and, thereby the variation in chlorophyll *a* / chlorophyll *b* ratio was due to changes

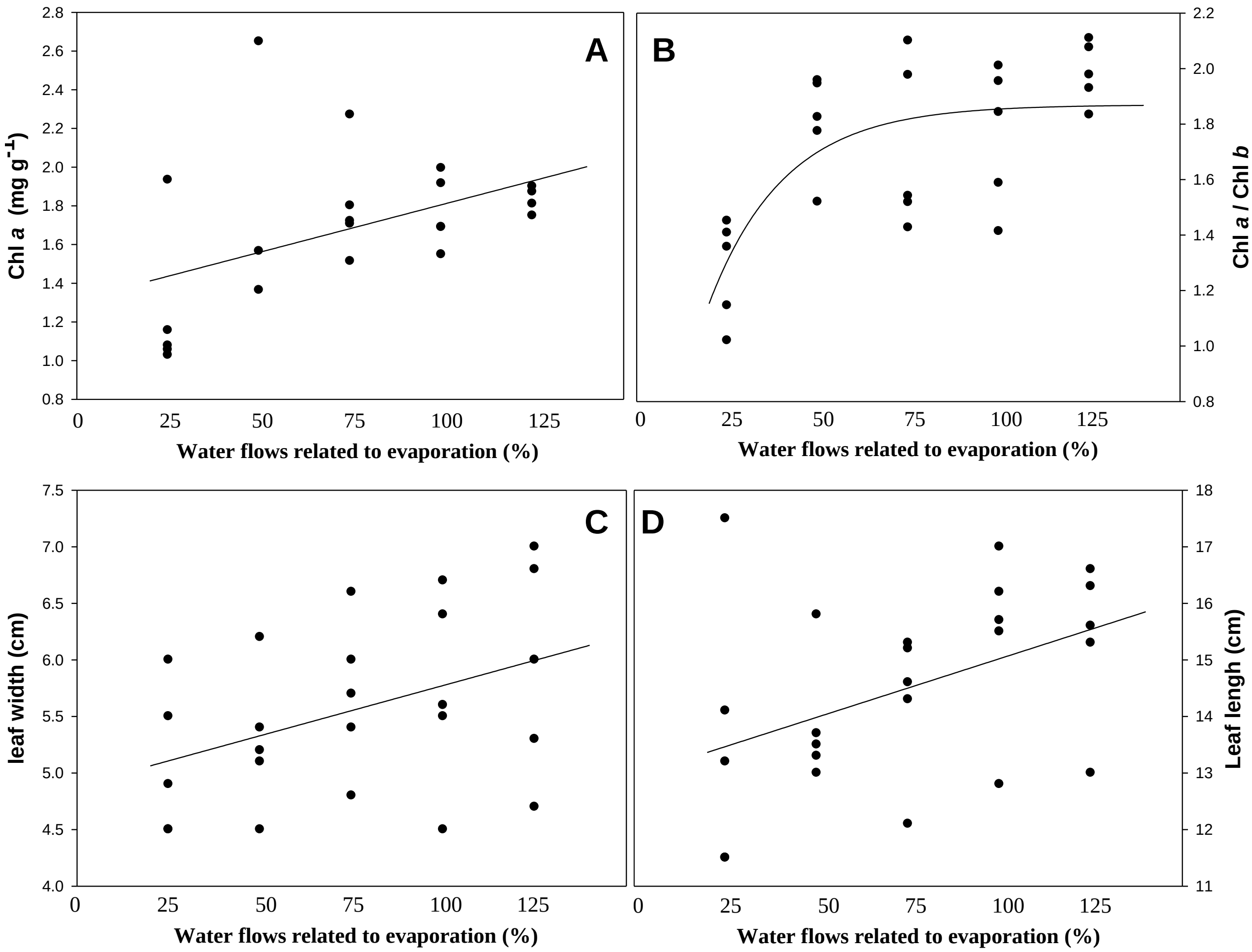

Figure 2. Regression equations for chlorophyll *a* "A" [Y = 1.31 + 0.005x; R^2 = 0.48], chlorophylls *a*/*b* ratio "B" [Y = 1.87(1-$e^{-0,05x}$); R^2 = 0.50] length leaf "C" [Y= 12.95+0.02x; R^2 = 0.44] and width leaf "D" [Y =4.88+0.009x; R^2 = 0.41] of *Eucalyptus urocan* seedlings irrigated with water volumes corresponding to 25, 50, 75, 100 and 125% evapotranspiration. Significant at *5 and **1% probabilities.

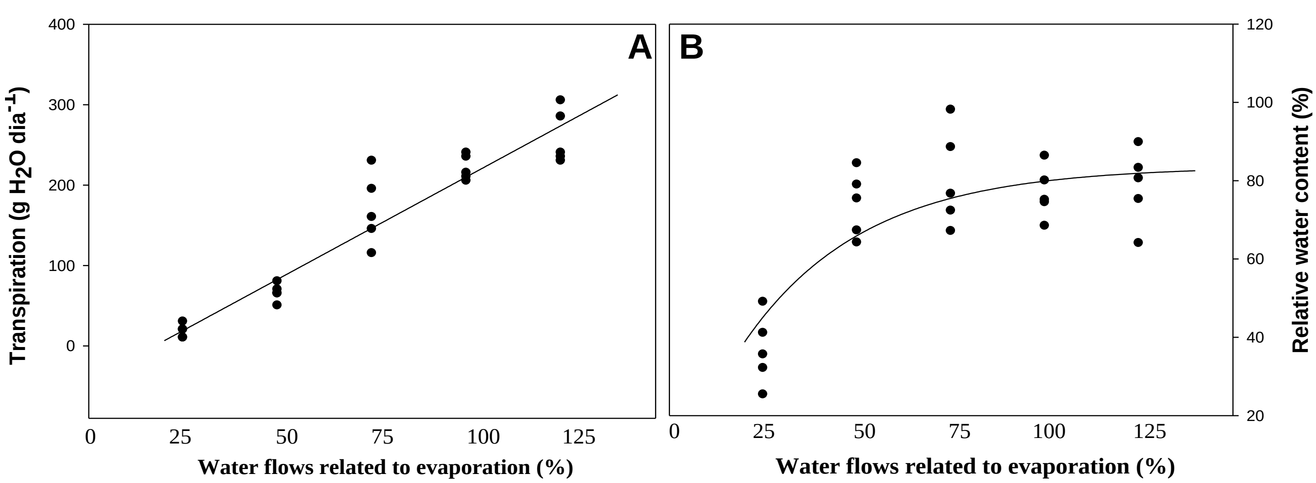

Figure 3. Regression equations for transpiration "A" [Y = -44.5 + 2.548x; R^2 = 0.96**] and relative water content (RWC) "B" [Y = 83.1(1-$e^{-0,03x}$); R^2 = 0.7**] of *Eucalyptus urocan* seedlings irrigated with water volumes corresponding to 25, 50, 75, 100 and 125% evapotranspiration. Significant at *5 and **1% probability.

in foliar concentration of chlorophyll *a* and may be associated to plant photoprotection against oxidative stress, since under low stomatal conductance, occurrence of excess excitation energy is common.

According to Matos et al. (2009), reduction in light energy absorption by lower leaf chlorophyll concentration is an important protective strategy against oxidative stress.

Under water deficit condition *E. urocan* plants showed significant investment in the root system, reduced transpiration rate and turgor maintenance sufficient to sustain growth.

Conclusions

1. *E. urocan* plants at initial growth stage are tolerant to water deficit, but shows a different biomass partition.
2. *E. urocan* plants use delayed dehydration as a strategy to tolerate drought.

Conflict of Interests

The authors have not declared any conflict of interests.

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