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Classification of *Eucalyptus urograndis* hybrids under different water availability based on biometric traits

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Abstract

Aim of study: The eucalyptus grows rapidly and is well suitable to edaphic and bioclimatic conditions in several regions of of the world. The aim of this study was to assess the performance of Eucalyptus urograndis hybrids grown under different water availability conditions.

Area of study: The study was performed in south-eastern of Brazil

Material and methods: We evaluated five commercial hybrids cultivated in pots with the substrate maintained at 65, 50, 35 and 20% maximum water retention capacity. The evaluation was based on the following characteristics: total height (cm), diameter (mm), number of leaves, leaf area (dm^2), and dry weight (g $plant^{-1}$) of leaf, stem + branches, root, shoot and total and root/shoot ratio.

Main results: All the characteristics evaluated were adversely affected by reduced availability of water in the substrate. The hybrids assessed performed differently in terms of biometric characteristics, irrespective of water availability. Water deficit resulted in a greater reduction in the dry weight production compared to number of leaves, diameter and height. Hybrids H2 and H5 have favorable traits for tolerating drought. The hybrid H2 shows a stronger slowdown in growth as soil moisture levels drop, although its growth rate is low, and H5 increases the root/shoot ratio but maintains growth in terms of height, even under drought conditions.

Research highlights: The results obtained in our experiment show that productive hybrids sensitive to drought could also perform better under water deficit conditions, maintaining satisfactory growth despite significant drops in these characteristics.

Key words: *Eucalyptus urograndis;* water deficit; drought tolerance.

Introduction

In several countries, particularly in Brazil, the used area with forest species for commercial purposes is gradually increasing. These forests have been established mainly with *Eucalyptus* species, which have high productivity and good adaptation to different environmental conditions. Much of this expansion has been provided in areas with major limitation to the plant growth, such as low water availability and/or high local temperatures.

The current projections for climate change predict that water shortages and high temperatures will occur more frequently in various regions of the globe. It is therefore important to bring together different research fields in order to identify and overcome the genetic and agronomic limitations of crops in terms of growth and productivity in unfavorable environments, particularly those subject to drought (Chaves and Davies, 2010).

Whether permanent or temporary, drought affects the growth and development of plants more than any other environmental factor. The initial and most sensitive response to drought is a reduction in cell turgidity and consequent slowdown in plant growth (Larcher, 2000; Anjum *et al.*, 2011).

Drought tolerance can be achieved by balancing root water absorption and water loss, mainly through the stomata. Water absorption is maximized by maintai-

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ning growth and deepening the root system, which usually involves boosting the root/shoot ratio (Pinheiro and Chaves, 2011). On the other hand, the reduction in leaf area is the first line of defense against drought, reducing the water loss by plants (Chaves *et al.*, 2003; Taiz and Zeiger, 2010).

In perennial species like eucalyptus, it has been found that drought-tolerant genotypes are not very productive, since the tolerance mechanisms involve metabolic costs, such as stomatal closure, producing osmoprotective compounds and deepening the root system at the expense of aerial growth (Chaves *et al.*, 2009; Paula *et al.*, 2012), and genotypes that exhibit higher growth are usually more drought-sensitive, with a higher reduction in growth in response to water deficit (Pereira *et al.*, 2010). However, the ideal would be to obtain genotypes with satisfactory growth even under poor water availability conditions.

Tolerance/sensitivity to water deficit can be evaluated based on biometric parameters and accumulation of dry weight in the plant's organs during the initial development phase, such as the accumulation of total dry weightwhich has proved to be adequate for comparing genotypes under different water availability conditions (Tatagiba *et al.*, 2007).

The aim of this study was to evaluate the growth of *Eucalyptus urograndis* hybrids under different water availability conditions with a view to classifying the materials in terms of their response to soil water deficit.

Material and methods

Treatments

The performance of five commercial hybrids of *Eucalyptus urograndis*, designated H1, H2, H3, H4 and H5, was evaluated under five different soil moisture levels. The seedlings were produced using the *mini-cutting* process and at 70 days were planted in black plastic pots containing 10 kg of soil samples taken from eucalyptus cropping areas. The substrate was limed and fertilized according to the soil analysis and the technical recommendations for the crop. The experiment was conducted in a greenhouse covered with a transparent plastic film 150 μm thick, with lateral screening to intercept 30% of the light, between June and September 2011, with the following average temperature and relative humidity figures - June: 17.9°C and 68.8%; July: 20.1°C and 62.5%; August: 21.7°C and 56.3%; September: 23.0°C and 48.9%.

During 26 days after planting, the substrate was watered daily to ensure that the cuttings survived. The treatments corresponding to the different soil moisture levels (SML) were determined based on maximum water retention capacity, so as to maintain the substrate at 65, 50, 35 and 20% of this capacity until the end of the experiment, which lasted 120 days as from planting. The gravimetric method was used to replenish the water daily and maintain the required moisture levels.

Plant growth

Plant growth was evaluated based on height (HGT); collar diameter (DIAM); number of leaves (NL); leaf area (LA) using a leaf area meter (Li-Cor 3100); dry weight of leaf (LDW), stem + branches (SBDW) and root (RDW). The dry weight data were used to obtain the shoot (SDW = SBDW + LDW) and total dry weight (TDW = SDW + RDW) and the root/shoot ratio (R/S = RDW/SDW). Evaluations were made at the beginning and end of the experiment. To determine the initial dry weight, when the experiment was set up we dried six plants representative of each hybrid.

Data analysis

On analyzing the data, the effects of the water regimes on each hybrid were compared to the treatment at 65% SML in terms of alteration in growth (%), and to eliminate the effects of differences in the development of the cuttings on setting up the experiment, the data were also analyzed in terms of relative increment (RI) using the following formula: $RI = [(Ae - Ab)/ Ab] * 100$, where Ae is the value of characteristic A (height, diameter, etc.) at the end of the experiment and Ab is the value of characteristic A (height, diameter, etc.) at the beginning of the experiment.

The experimental design was fully randomized in a 5 × 4 factorial arrangement (five hybrids and four soil moisture levels) with six single-plant replications, involving a total of 120 plants. Analysis of variance (ANOVA) followed by *F* test was carried out and the means of treatments were compared using the Tukey test at 5% probability, using the SISVAR program (Ferreira, 2011).

Table 1. Alteration (%) in height (HGT), diameter (DIAM), number of leaves (NL), leaf area (LA), dry weight of stem + branches (SBDW), leaf (LDW), shoot (SDW), root (RDW) and total (TDW) of the five eucalyptus hybrids under soil moisture levels of 50, 35 and 20% compared to the control (65%). (–) indicates a drop and (+) an increase compared to the control

Hybrid	SML relation	HGT	DIAM	NL	LA	SBDW	LDW	SDW	RDW	TDW
H 1	65 → 50%	–7.7	–3.1	–10.4	–15.2	–17.3	–26.0	–22.4	–28.1	–24.0
	65 → 35%	–15.2	–27.4	–36.1	–43.8	–50.0	–52.0	–51.2	–39.6	–47.8
	65 → 20%	–28.5	–37.7	–66.5	–77.3	–80.6	–79.5	–80.0	–81.1	–80.3
H 2	65 → 50%	–12.0	–15.8	–24.7	–26.0	–27.3	–25.3	–26.3	–12.7	–22.4
	65 → 35%	–16.7	–25.5	–45.6	–55.1	–55.8	–53.2	–54.5	–49.8	–53.1
	65 → 20%	–33.4	–44.8	–77.1	–83.0	–82.0	–81.6	–81.8	–81.9	–81.8
H 3	65 → 50%	–2.1	–6.9	–6.6	–14.5	–16.9	–19.9	–18.7	–12.3	–17.1
	65 → 35%	–12.3	–19.0	–31.9	–49.6	–51.0	–56.0	–53.9	–41.7	–51.0
	65 → 20%	–27.0	–46.5	–69.0	–81.6	–79.5	–82.4	–81.2	–79.4	–80.7
H 4	65 → 50%	+1.6	–10.3	–9.0	–20.0	–20.7	–25.1	–23.4	–10.0	–20.0
	65 → 35%	–19.9	–28.2	–37.6	–47.4	–41.5	–52.7	–48.3	–39.7	–46.1
	65 → 20%	–29.8	–41.1	–60.0	–78.9	–80.9	–80.3	–80.6	–78.7	–80.1
H 5	65 → 50%	–2.1	–5.00	–1.9	–16.0	–23.4	–22.7	–23.0	–27.5	–24.0
	65 → 35%	–18.5	–21.5	–24.8	–46.9	–51.9	–56.6	–54.5	–41.3	–51.4
	65 → 20%	–41.5	–48.6	–50.4	–80.1	–83.2	–84.2	–83.8	–78.4	–82.5

To classify the hybrids in terms of water use efficiency and response to water deficit, we used an adaptation of the method proposed by Fageria and Kluthcouski (1980), with a Cartesian graph representation, the x-axis representing water use efficiency defined by the mean of the characteristic for each hybrid at the lowest SML (20%), and the y-axis representing the drought response obtained by the difference between the characteristic at 65% and 20% SML. The origin of the graph is the mean efficiency and mean response of the hybrids. Thus, hybrids appearing at the top right are classified as efficient and sensitive (ES), at the top left as not efficient and sensitive (NES), at the bottom left as not efficient and tolerant (NET) and at the bottom right as efficient and tolerant (ET).

Results

All the characteristics evaluated were adversely affected as the soil moisture level (SML) dropped, irrespective of the hybrid. However, there was a greater reduction in the accumulation of dry weight than in height (HGT), diameter (DIAM) and number of leaves (NL). For instance, leaf dry weight (LDW) under water deficit dropped 84.2% for hybrid H5, whereas NL dropped by only 77.1% for H2 (Table 1).

At the first indication of water shortage, when the soil moisture level dropped from 65% to 50%, H2 exhibited the steepest drops in HGT (12%), DIAM (15,8%), NL (24,7%) and leaf area (LA; 26%), whereas under the same conditions, the H1 exhibited decrease of 7.7% in HGT and 10.4% in NL and the H4 dropped 10.3% in DIAM and 20% in LA. At other soil moisture levels, the drops in the values of these characteristics were similar across all hybrids (Table 1).

Hybrids H2, H3 and H4 did not change significantly ($p > 0,05$) the root/shoot ratio (R/S) as the soil moisture level dropped. However, for H1, R/S was highest at 35% SML and for H5 at 20% SML and for each soil moisture level, H5 usually exhibited the lowest R/S, except at 20% SML, the level at which there were no differences among the hybrids (Table 2).

The relative increment (RI) in biometric traits and accumulation of biomass resulted similar classifica-

Table 2. Root/shoot ratio (R/S) of the five eucalyptus hybrids at four soil moisture levels

Hybrid	65%	50%	35%	20%
H 1	0.41^{aB}	0.38^{abB}	0.51^{aA}	0.40^{aB}
H 2	0.39^{abA}	0.46^{aA}	0.43^{abA}	0.39^{aA}
H 3	0.31^{abA}	0.34^{bA}	0.39^{bA}	0.34^{aA}
H 4	0.33^{abA}	0.39^{abA}	0.39^{bA}	0.36^{aA}
H 5	0.30^{bBC}	0.29^{bC}	0.39^{bAB}	0.41^{aA}

Means followed by the same letter (lowercase in columns, uppercase on rows) did not differ in the Tukey test ($p > 0.05$).

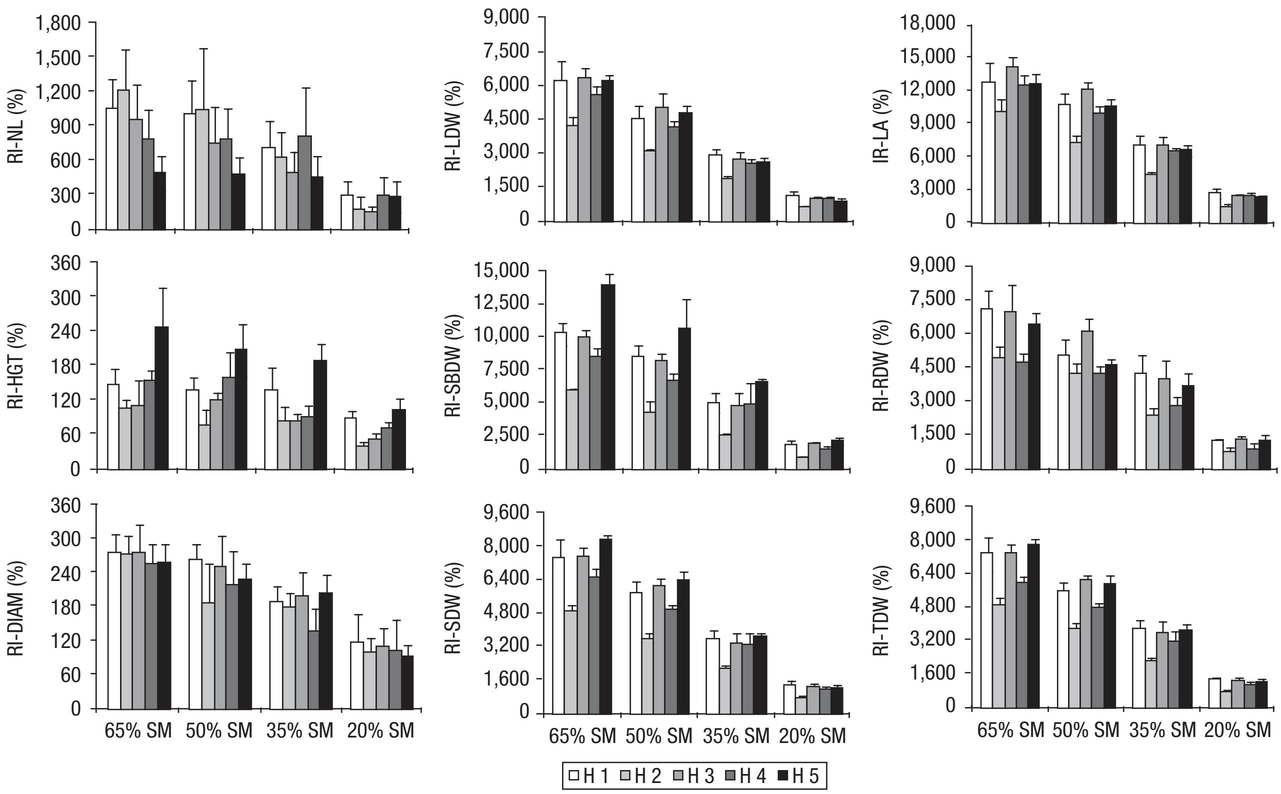

Figure 1. Means and standard deviation (n = 6) of relative increment in number of leaves (RI-NL), height (RI-HGT), diameter (RI-DIAM), leaf area (RI-LA), dry weight of leaf (RI-LDW), shoot (RI-SDW), stem + branches (RI-SBDW), root (RI-RDW) and total (RI-TDW) of the five eucalyptus hybrids grown at four soil moisture levels.

tion of hybrids at the highest levels of water availability, 65% and 50% soil moisture level (Fig. 1). In terms of NL, the most contrasting genotypes were H2 and H5, with H5 exhibiting the lowest relative increment. The highest RI-HGT was obtained for H5 at 65% SML. At 50% SML, the differences among the hybrids in terms of height were greater, with the highest value for H5 and H2 exhibited the lowest increment. The highest RI-LA was obtained for H3 and H2 exhibited the lowest increment. In general, the relative increment for dry weight was higher in H1, H3 and H5, the H2 exhibited the lowest increment at 65 and 50% SML. Significant differences ($p < 0.05$) in RI-DIAM were found between the hybrids only at 50% SML: H1 exhibited a higher RI-DIAM than H2 (Fig. 2).

In the treatment at 35% SML, no significant differences were detected among the hybrids for RI-NL and RI-DIAM. However, the RI-HGT was higher for H1 and H5, which, together with H3, also exhibited a higher RI-RDW. H2 exhibited a lower relative increment in terms of leaf area and leaf, stem + branches and total dry weight (Fig. 2).

At the lowest water availability (20% SML), significant differences ($p < 0.05$) between the hybrids were detected only in terms of RI-HGT where H5 exhibited the highest value and H2 and H3 the lowest ones (Fig. 2).

In classifying the hybrids in terms of water use efficiency and response to water deficit based on the increment in NL, LA, HGT and DIAM, we noted that each of these characteristics resulted in a different classification for the hybrids, whereas based on the dry weight increments (except for LDW), the classification was similar (Fig. 2).

Efficient and tolerant (ET) hybrids were identified based on the relative increment in NL (H4 and H5), LA (H4 and H1), HGT (H1), DIAM (H1) and LDW (H4). In respect of the additional dry weight parameters, efficient hybrids H1, H3 and H5 were also classified as sensitive, *i.e.* they exhibited a severe drop in the accumulation of dry weight as water availability was reduced (Fig. 2). This analysis method could provide a useful tool for identifying the most productive and drought-tolerant genotypes in breeding projects.

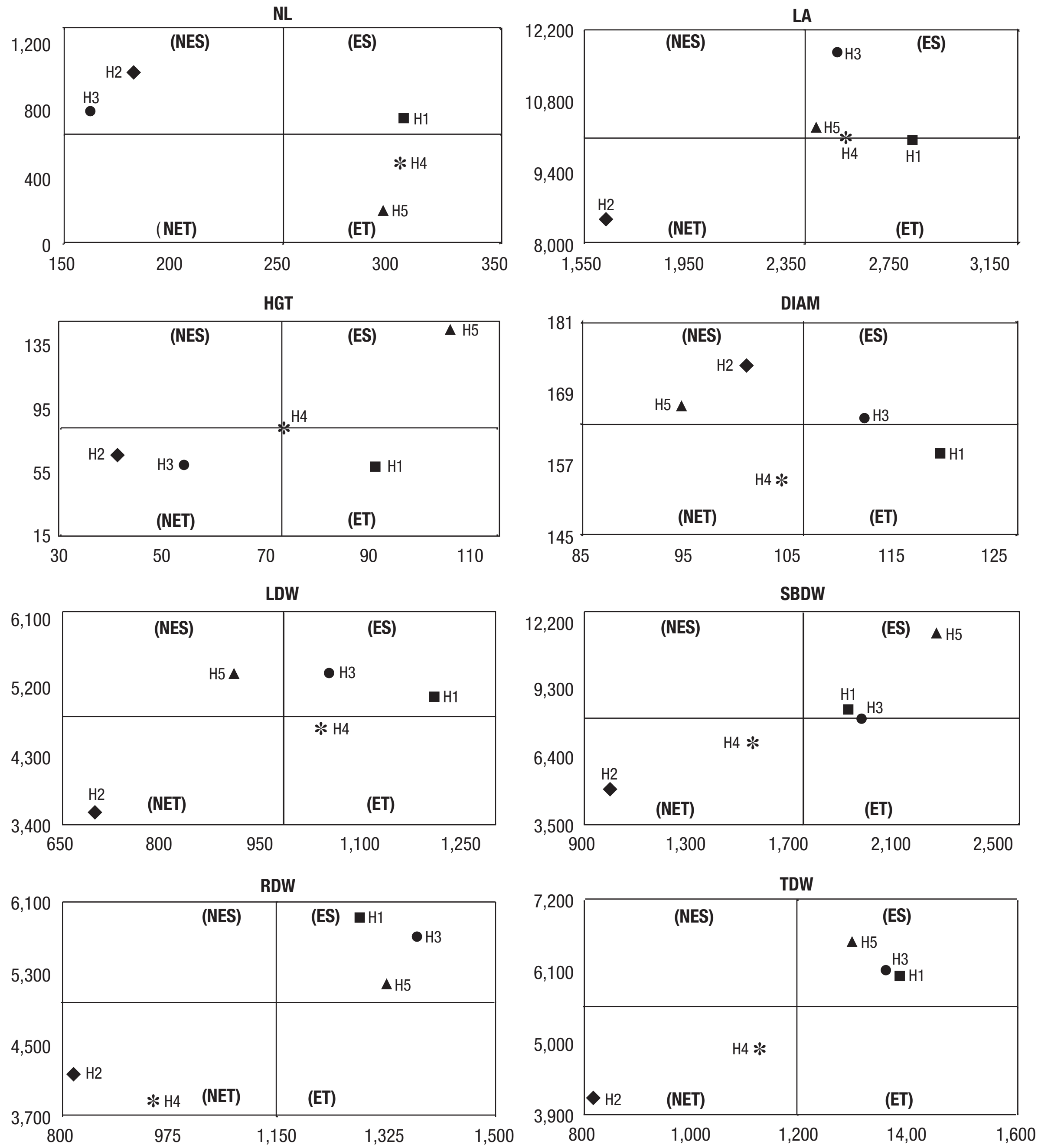

Figure 2. Eucalyptus hybrids classified as efficient and sensitive (ES), not efficient and sensitive (NES), not efficient and tolerant (NET) and efficient and tolerant (ET) based on the increment in number of leaves (NL), height (HGT), leaf area (LA), diameter (DIAM), dry weight of leaf (LDW), stem + branches (SBDW), root (RDW) and total (TDW) of the five eucalyptus hybrids grown at four soil moisture levels.

Discussion

The water restriction promoted a greater reduction in the accumulation of dry weight than in height (HGT), diameter (DIAM) and number of leaves (NL). This may be due to reduced leaf area and consequently a drop in the plant's assimilation of carbon to produce biomass. Similar results were obtained by Nascimento *et al.* (2011) studying the jatoba (*Hymenaea courbaril* L.), who showed that dry weight production was the characteristic most sensitive to water deficit.

Carbon fixation can begin to drop due to stomatal closure, even under moderate water deficit conditions. In the plant, a drop in carbon assimilation can occur simultaneously with or even before growth is inhibited (Chaves and Oliveira, 2004). In the eucalyptus hybrids evaluated, the drop in accumulation of biomass was more severe and occurred prior to the drop in growth in terms of height, diameter and number of leaves.

The characteristic most representative of the productive potential of timber-producing species is biomass production and mainly the stem + branches. However, basing the classification on dry weight production did not identify hybrids tolerant and efficient to drought. This could be due to the stage at which the plants were evaluated. In the seedling phase, the plants invest proportionally more energy in the production of branches and leaves, at the expense of other compartments, so these characteristics are also the most sensitive to water stress. As the plant ages, more biomass is allocated to forming wood, so this behavior can change throughout the crop cycle, a fact corroborated by various authors (Chaves and Oliveira, 2004; Gindabaa *et al.*, 2005; Taiz and Zeiger, 2010; Pinheiro and Chaves, 2011; Paula *et al.*, 2012) who found that the effects of water deficit are variable according to the species, genotype, phenological stage and drought intensity and duration, as well as other factors.

These evaluations show that, of the hybrids classified as efficient and tolerant (H1, H4 e H5), H1 and H5 are among the hybrids that exhibited the highest increments in height and dry weight parameters, whereas H4 is among those with the lowest increments in dry weight at higher soil moisture levels (Fig. 2), which is unfavorable in terms of water use efficiency when water availability is not restricted.

It is worth pointing out that H5 has other interesting characteristics compared to H1. Despite its classification as sensitive, since it exhibits higher drops in the majority of characteristics, it is still the hybrid with the strongest growth in terms of height (Fig. 1) and since this characteristics is a good predictor of growth in the field (Coopman *et al.*, 2008), H5 is expected to perform satisfactorily, even under drought conditions.

Another advantage of H5 was the gradual increase in the root/shoot ratio (R/S) when subjected to water deficit, indicating that it is a genotype that invests more in shoot growth when water is available, and lowers this investment when water is scarce, with proportionally lower changes in root dried weight (Table 1), which could enhance water absorption. For hybrids 2, 3 and 4 that remained relatively unchanged in terms of R/S as the moisture level dropped could be linked to the equilibrium in growth between the root and shoot of these plants, since there was no need to invest specifically in root growth (Nascimento *et al.*, 2011). The root growth is considered a constitutive rather than a stress-induced characteristic (Chaves *et al.*, 2003; Blum, 2011) which is simply inherited and can have a decisive effect on the performance and productivity of plants under drought conditions, mainly because it prevents dehydration (Blum, 2011).

Evaluating resistance mechanisms in contrasting *Eucalyptus globulus* genotypes in regard to drought tolerance in greenhouse experiments, Costa e Silva *et al.* (2004) reported that the tolerant clone exhibited greater investment in root development under adequate water availability conditions and maintained root system growth for a longer period than the sensitive clone under drought conditions, optimizing the relation between transpiration and absorption areas, which explained its higher resistance to drought. The same authors also point out that in the field, where the volume of soil does not restrict root development, the benefits of higher investment in the root system during a drought are even greater, due to higher absorption of water.

The H2 was classified as not efficient but tolerant. It exhibited the lowest growth even with adequate water availability, which is a disadvantage in terms of productivity and resource use efficiency. On the other hand, it exhibited a rapid adaptive response to the first signs of drought (50% SML), reducing growth and transpiration surface area. Slower growth has been proposed as an adaptive characteristic for plant survival under water stress (Chaves and Oliveira, 2004), since with reduction in growth rate, there is less demand for water.

Cavatte *et al.* (2012) describe the paradoxical situation that exists between mechanisms that prevent excessive water loss and the accumulation of plant biomass. Leaf area and stomatal conductance are the main factors that determine transpiration rates, and lowering these variables allows the water potential to increase or be kept within certain limits so that plant development is possible. However, these factors also determine the amount of carbon accumulated by plants, since reduced leaf area means that less light is intercepted, lowering the photosynthesis rate and stomatal conductance, leading to a lower influx of CO_2 into the chloroplasts.

The situation described above shows that there is a trade-off when selecting tolerant and productive genotypes, but the results obtained in our experiment show that productive hybrids sensitive to drought could also perform better under water deficit conditions, maintaining satisfactory growth despite significant drops in these characteristics.

Conclusions

The hybrids evaluated exhibited varied performance in terms of biometric characteristics, irrespective of the water regime.

Water deficit resulted in a greater reduction in the production of dry weight compared to number of leaves, diameter and height.

Hybrids H2 and H5 have favorable characteristics for tolerating drought; The hybrid H2 shows a stronger slowdown in growth as soil moisture levels drop, although its growth rate is low, and H5 increases the root/shoot ratio but maintains growth in terms of height, even under drought conditions.

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