

# RESPONSES OF PARICÁ SEEDLINGS TO WATER STRESS

Daiane Marques Duarte<sup>1</sup>, Gabriela Teodoro Rocha<sup>2</sup>, Fernanda Borges de Lima<sup>2</sup>,  
Fabio Santos Matos<sup>1</sup>, Fabricio Rodrigues<sup>1\*</sup>

<sup>1</sup>Universidade Estadual de Goiás, Programa de Pós-Graduação em Produção Vegetal, Ipameri, Goiás, Brasil –  
daiane.marques.floresta@gmail.com; fabio.matos@ueg.br; fabricio.rodrigues@ueg.br\*

<sup>2</sup>Universidade Estadual de Goiás, Curso de Engenharia Florestal, Ipameri, Goiás, Brasil – gabriela\_teodoro.rocha@yahoo.com.br;  
fernandalima08@hotmail.com

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## Abstract

The present study objective was identifying mechanisms of response to water deficit of paricá seedlings. The experiment was conducted in a greenhouse, in 8-liter pots, with substrate consisting of soil (dystrophic Red-Yellow Latosol), in randomized block design, with five different irrigations based on the evapotranspiration of the plants (20, 40, 60, 80 and 100%), with six replications. The plants were irrigated daily, with a water volume corresponding to 100% of the evapotranspired water, and, at 90 days after germination, subjected to ten days of water deficit, after that irrigated during ten days with the different percentages of evapotranspiration, then evaluated. The following variables were measured: number of leaves (NL), plant height (PH), stem diameter (SD), total biomass (TB), total daily transpiration (TDT), total chlorophyll (TC), carotenoids (CAR), root mass ratio (RMR), leaf mass ratio (LMR) stem mass ratio (SMR). Concluded that paricá seedlings response to water deficit with slowing down of dehydration, with reduction of content of carotenoids and increase in root volume. As a consequence of water stress, they reduce the number of leaves, stem diameter, plant height, and, of course, total biomass, aiming at maintaining the internal water, indicating low ability to tolerate stress.

**Keywords:** Evapotranspiration; *Schizolobium parahyba* var. *amazonicum*; stress and biomass.

## Resumo

*Efeito responsivo de mudas de paricá ao estresse hídrico.* O presente estudo teve como objetivo identificar mecanismos de resposta ao déficit hídrico em mudas de paricá. O experimento foi conduzido em casa de vegetação, em vasos de oito litros, com substrato constituído por solo (Latosolo Vermelho-Amarelo distrófico), em delineamento experimental de blocos casualizados, utilizando cinco irrigações diferentes, com base na evapotranspiração das plantas (20, 40, 60, 80 e 100%), com seis repetições. As plantas foram irrigadas diariamente, com volume de água correspondente a 100% da evapotranspiração e, aos 90 dias após a germinação, submetidas a dez dias de déficit hídrico. Em seguida, irrigadas por mais dez dias com as diferentes porcentagens de evapotranspiração e, então, avaliadas. Foram realizadas as mensurações número de folhas (NF), altura da planta (PH), diâmetro do caule (SD), biomassa total (TB), transpiração diária total (TDT), clorofila total (TC), carotenoides (CAR), razão de massa radicular (RMR), razão de massa foliar (LMR) e razão de massa caulinar (SMR). Conclui-se que mudas de paricá respondem ao déficit hídrico com o retardo da desidratação, com redução de carotenoides, além do aumento do volume de raiz. Como consequência do estresse, a planta reduz o número de folhas, o diâmetro do caule, a altura da planta e, claro, a biomassa total, objetivando a manutenção da água interna.

**Palavras-chave:** Evapotranspiração; *Schizolobium parahyba* var. *amazonicum*; estresses e biomassa.

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## INTRODUCTION

The export and domestic consumption of timber grows each year in Brazil and, with it, the necessity to increase the production area and product quality (ASSOCIAÇÃO BRASILEIRA DE PRODUTORES DE FLORESTAS PLANTADAS (ABRAF), 2013). The competitiveness of the Brazilian forest sector, result of the climatic conditions and of the technology developed by the companies and research institutions, makes the country occupy a unique position on the international scene, even compared with countries like Canada (ALMEIDA *et al.*, 2013).

The Brazilian forest sector is responsible for 3.5% of the gross domestic product (GDP) and creates 4.7 million direct and indirect jobs (ABRAF, 2013). Among the main requirements of planted forests to satisfy market demand, we find rapid growth, large species diversity, high density, productivity and specific mass of the wood (BIANCHE *et al.*, 2012). In this context, *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby (henceforth parica) is an excellent alternative from a forestry point of view, besides producing wood with technological traits favorable to the production of wood plates and panels (MELO *et al.*, 2013).

Wood in the lamination process presents physical and texture properties that render cooking unnecessary, with yellowish white coloration, smooth, light, and soft surface, with density around  $0.40 \text{ g.cm}^{-3}$ , offering an excellent finish product at the end of the lamination process (IWAKIRI *et al.*, 2010). Moreover, *Schizolobium amazonicum* wood can be used to generate energy, since the calorific value and charcoal traits show values similar to those of wood commonly used as fuel. According to Vidaurre (2012), the calorific value of *Schizolobium amazonicum* wood resembles that of eucalyptus, traditionally used for energy purposes.

The area occupied by the parica in 2014 was approximately 89,000 hectares, representing 1.15% of the total planted forests, with an average increase of 902 hectares year<sup>-1</sup>, considering the period 2010-2015 (INDÚSTRIA BRASILEIRA DE ÁRVORES (IBÁ), 2015).

Establishing commercial plantations causes some alterations in the abiotic factors, like high irradiance and low water or nutritional availability, deeply affecting the physiological activity of the plants in the different phases of their growth, which can make their establishment in the field difficult (GONÇALVES *et al.*, 2009).

According to Chaves *et al.* (2009), the environmental factor that most limits plant growth and yield in the whole world is low water availability, so that all growth and development variables of the plants are affected by water deficit. In tissues, it causes an excessive evapotranspirative demand or can limit water supply, so, it compromises various parts of the plant and can even drive some species to extinction (MARTINS *et al.*, 2010).

Responsive effect of paricá seedlings to drought is an important tool for the genetic improvement of plants, important to accelerate breeding programs and also improve crop management in areas with frequent water deficit. In this way, the present study objective was identifying mechanisms of response to water deficit of *Schizolobium amazonicum* var. *amazonicum* (Huber ex Ducke) Barneby seedlings and their behavior under controlled water deficit.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse, in 8-liter-pots, using a randomized block design with five different treatments (irrigated with 20, 40, 60, 80 and 100% of the evapotranspiration) and six replications.

Initially, the soil, classified as dystrophic Red-Yellow latosol (Oxisol), was sieved, then, 3g of dolomitic limestone that were applied, in accordance with the chemical analysis of the soil (Table 1). Soon after, it was incubated and irrigated for about 30 days, later, plantation fertilization was carried out in all the soil with 3g urea, 2.5 g triple superphosphate and 2.2 g potassium chloride per kg soil<sup>-1</sup>. The pots were filled, weighed, and standardized at 5.5 kg of soil; later, were placed in a greenhouse.

The seeds came from the Aimex Laboratory of Seeds and Seedlings of the Amazon Forest Species, before, subjected to dormancy break, using sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and water in the ratio of 70 and 30%, respectively. Three seeds were sown in each pot, thinning being made at 30 days after emergence (DAE). Later, at approximately 60 DAE, top dressing was carried out with 2 g of the 10-25-15 formula.

Table 1. Main chemical attributes of the soil (0-20 cm depth) without fertilizer or limestone application.

Tabela 1. Principais atributos químicos do solo (0-20 cm), sem a aplicação de fertilizante ou calcário.

Traits	pH	O.M.	P <sub>resin</sub>	H+Al	K	Ca	Mg	BS	CEC	V%
	CaCl <sub>2</sub>	g dm <sup>-3</sup>	g dm <sup>-3</sup>	mmoldm <sup>-3</sup>						
Soil	4.8	24	9	30	2.1	18	7	27	57	47

pH = active acidity; O.M. = organic matter; P = available phosphorus; H+Al = potential acidity; K = available potassium; Ca = exchangeable calcium; Mg = exchangeable magnesium; BS = base saturation; CEC = effective cation exchange capacity; V% = base saturation.

The experiment was placed in a greenhouse with an average temperature of 27.8° C, with mesh with 50% shade of light. The plants were irrigated daily with a volume of water corresponding to the maximum capacity of the daily evapotranspiration. At 90 DAE, the plants were subjected to 10 days of water deficit, then irrigated with different percentages of evapotranspiration (irrigated with 20, 40, 60, 80 and 100%, calculated based on the weight of the plant, which had its container protected by transparent plastic bags and closed to the base of the plant), for 10 days more, in order to analyze their recovery capacity and water efficiency, which were analyzed regarding the traits below.

### Growth variables

The number of leaves (NL) was determined by counting all compound leaves of the plant. Plant height (PH) was measured from the neck of the plant to its apex, by means of a graduated ruler. Stem diameter (SD) was measured by means of a caliper, at the base of the plant stem. Total biomass (TB) was measured by determining the dry matter mass of the whole plant, weighing up leaves, stems and roots in precision scale. Leaf mass ratio (LMR), following the equation: LMR = dry matter of leaf/TB; stem mass ratio (SMR), by the equation: SMR = dry matter of stem/TB; root mass ratio (RMR), according to the equation: RMR = dry matter of root system/TB.

## Physiological variables

Carotenoids (CAR) and total chlorophylls (TC) were measured by means of leaflet disks of known area removed, then placed in closed glass tubes containing dimethyl sulfoxide (DMSO); later, extraction was carried out in a bain-marie at 65° C for four hours and finally, the aliquots were removed for spectrophotometric reading at 480, 649, and 665 nm. Leaf concentrations of carotenoids and chlorophylls *a* and *b* were determined following the equation proposed by Wellburn (1994).

The total daily transpiration (TDT) was estimated by gravimetry, by means of the weight difference of the pots with a one-hour interval between 07:00 and 18:00 as described by Cavatte *et al.* (2012).

The relative water content (RWC) was measured in five leaf disks of 12 mm diameter each were removed, then weighed and placed for four hours to saturate with distilled water on Petri dishes. After that, the disks were weighed again and put to dry at a temperature of 70 °C for 72 hours, the dry matter mass being obtained afterwards. To calculate the relative water content, we used the following formula:  $RWC = (fresh\ mass - dry\ mass / turgid\ mass - dry\ mass) \times 100$ .

Data were subjected to analysis of variance and, in the cases where were significant, conducted the regression analysis ( $p < 0,05$ ), using the SISVAR statistical software (FERREIRA, 2011).

## RESULTS AND DISCUSSION

In the analysis of variance, tables 2 and 3, one can observe that among variables, RWC did not present any significant difference, which shows that it was not influenced by stress and that after water replacement, with the different percentages of evapotranspiration, the plant had a behavior similar to the control, even under low water availability. On the other hand, the other variables showed significant differences ( $p \leq 0,05$ ), influenced by the different percentages of irrigation.

The RWC, for being a quantitative reference of the water content in the cell, is naturally associated with its volume and, consequently, with the capacity of lengthening and cell division (LARCHER, 2006). On the other hand, the absence of significant difference shows that the cells maintained the internal water content or that the 10-day stress, even with low water replacement, was not capable of reducing the water content of the cell, according to table 2.

Table 2. Summary of the analysis of variance of number of leaves (NL), plant height (PH), stem diameter (SD), total biomass (TB), total daily transpiration (TDT) and total chlorophyll (TC) evaluated in plants of *Schizolobium amazonicum* subjected to different water supplies.

Tabela 2. Resumo da análise de variância de número de folhas (NL), altura de planta (PH), diâmetro do caule (SD), biomassa total (TB), transpiração diária total (TDT) e clorofila total (TC) avaliadas em plantas de paricá [*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby] submetidas a diferentes suprimentos hídricos.

V.S.	Mean squares						
	DOF	NL	PH	SD	TB	TDT	TC
Treatment	4	1.87*	44.1*	2.6*	68.68*	0.01*	7.04*
Block	5	0.07	8.19	0.08	0.80	0.01	0.48
Error	20	0.08	3.75	0.14	0.91	0.01	0.52
C.V. (%)		11.58	7.13	5.95	13.04	23.13	7.20

DOF = degree of freedom; C.V. = coefficient of variation; \* = significant at 5% of probability.

Table 3. Summary of the analysis of carotenoids (CAR), root mass ratio (RMR), stem mass ratio (SMR), leaf mass ratio (LMR), and relative water content (RWC), evaluated in seedlings of *Schizolobiumamazonicum* subjected to different water supplies.

Tabela 3. Resumo da análise de carotenoides (CAR), razão massa radicular (RMR), razão massa caulinar (SMR), razão massa foliar (LMR) e teor relativo de água (RWC) avaliadas em mudas de paricá [*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby] submetidas a diferentes suprimentos hídricos.

V.S.	Mean squares					
	DOF	CAR	RMR	SMR	LMR	RWC
Treatment	4	0.32*	0.01*	0.02*	0.01*	123.5 <sup>n.s.</sup>
Block	5	0.17	0.01	0.01	0.01	31.21
Error	20	0.03	0.01	0.01	0.01	54.03
C.V. (%)		8.30	9.81	9.76	9.07	13.86

DOF = degrees of freedom; C.V. = coefficient of variation; \* = significant at 5% probability; n.s. =not significant.

The figure 1 features the graphs of the variables observed in the different replacements of evapotranspiration, with the respective coefficients of determination, as well as the observed curves and average values. Figure 1A shows that NL was more affected in the treatments with lower evapotranspiration value, presenting leaf abscission, which is a defense mechanism of plants under stress conditions, limiting water losses through transpiration.

It is important to emphasize that the control, when compared to the plants with lower water percentage, shows a reduction of about 40% of NL, reducing the leaf area, which confirms the results of Lenhard *et al.* (2010), who studied the leaf area of *Caesalpinia ferrea* and also observed a positive and significant correlation between lower water availability and decrease in leaf growth. Similarly, Vieira and Gomes (2011), working with initial development of *Qualea grandiflora* under different water regimes, observed the same results. Leaf fall is considered a common reaction of plants in situation of water deficit.

The PH variable was also affected by water availability, as showed in figure 1B, verified by the growth of the plant and the increase in evapotranspiration. The defense mechanisms of the plant invest most photoassimilates in the maintenance, thus guaranteeing survival, which is confirmed by the fact that the hedge was 9 cm higher in treatments with higher water availability than in those with lower water availability. Similar results were found by Nascimento *et al.* (2011), who studied different irrigation levels in seedlings of *Hymenaea courbaril*, and noticed that plants subjected to higher water availability were higher. Similarly, studies conducted by Martins *et al.*, (2010), who studied the growth of young plants of nim-indiano (*Azadirachta indica* A. Juss).

Figure 1C shows that there was a reduction of about 2 cm in the SD of the plants with total replacement of evapotranspiration in relation to those with only 20%; on the other hand, for treatments with 40 to 80% of replacement, variation is lower than 1%. Such results confirm those of Tatagiba *et al.* (2009), who studied six commercial clones of *Eucalyptus* spp. in response to water availability and observed that water deficit also restricted the growth in height and diameter of the studied clones.

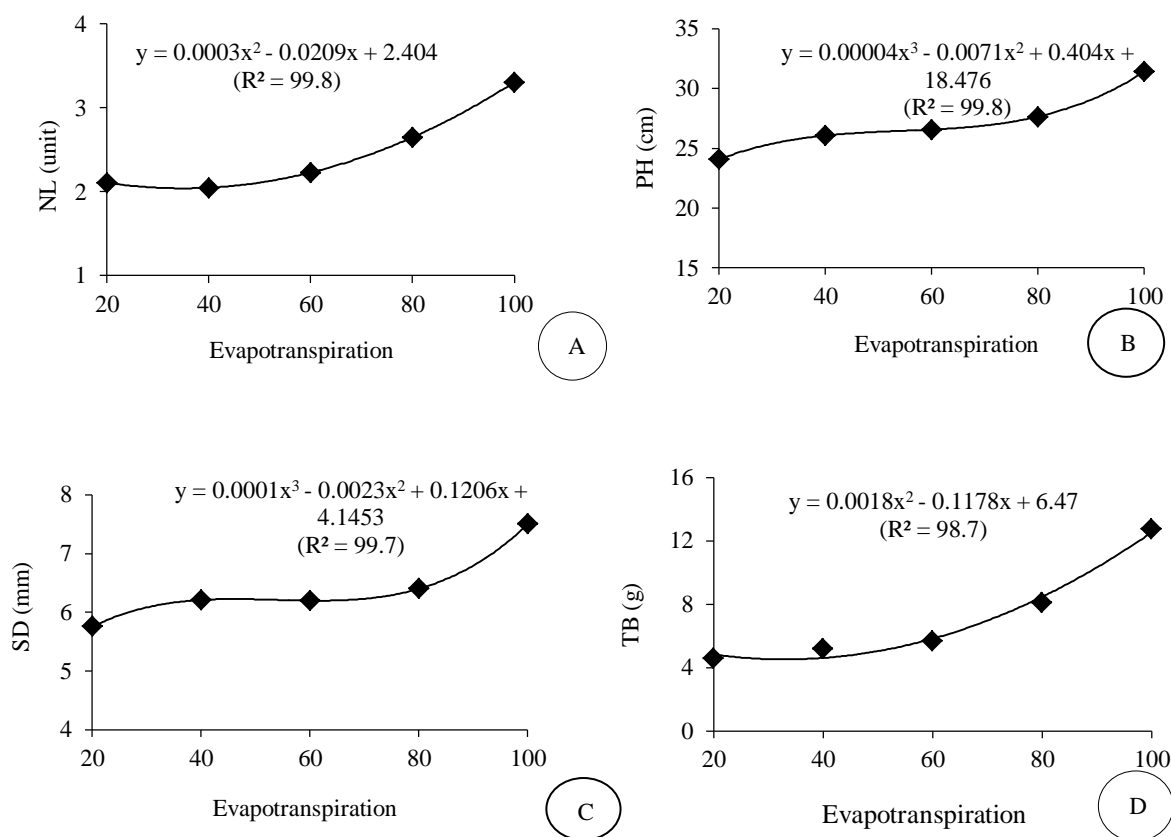


Figure 1. Number of leaves (A), plant height (B), stem diameter (C) and total biomass (D) of seedlings of *Schizolobium amazonicum*, subjected to ten days of water deficit, then irrigated with five different percentages of evapotranspired water.

Figura 1. Número de folhas (A), altura de planta (B), diâmetro do caule (C) e biomassa total (D) de mudas de paricá [*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby] submetidas a dez dias de déficit hídrico e posteriormente irrigadas com cinco diferentes porcentagens da água evapotranspirada.

Shows that the TB variable (Figure 1D) had a reduction of nearly 68% of mass between the control and the plants with 80% replacement, which shows a quadratic trend as water availability increases, but an almost linear trend between replacements of 20 to 80%. According to SOUZA *et al.* (1997), this is due to the stress, even low, which can cause a decrease of the net photosynthesis rate and, consequently, the production of carbohydrates, which can result in a decrease of biomass accumulation of the plants.

It may be seen that the plant transpiration rate (Figure 2A) was almost twice as high under maximum water availability as under replacement of only 20%, which allows us to claim that these plants manage to control efficiently water loss by transpiration, regulated possibly through stomatal aperture. SCALON *et al.* (2011) observed in seedlings of *Guazuma ulmifolia*, with transpiration higher when the seedlings were subjected to a higher water supply, and by GONÇALVES *et al.* (2009), observed a higher transpiration of seedlings of *Carapa guianensis* with higher water availability, where, both works, this control is related to control of the stomata.

Stomatal closure is considered a defense against drought, being important for stomatal control, as emphasized by Santos *et al.* (2012), with the aim of fighting leaf dehydration, as it is one of the first adjustments developed by plants to prevent continue water loss. Loss of turgidity due to water stress causes stomatal closure, reducing photosynthesis and breathing, interfering with many basic metabolic processes; it is thus a mechanism created to interrupt continue water loss to the external environment.

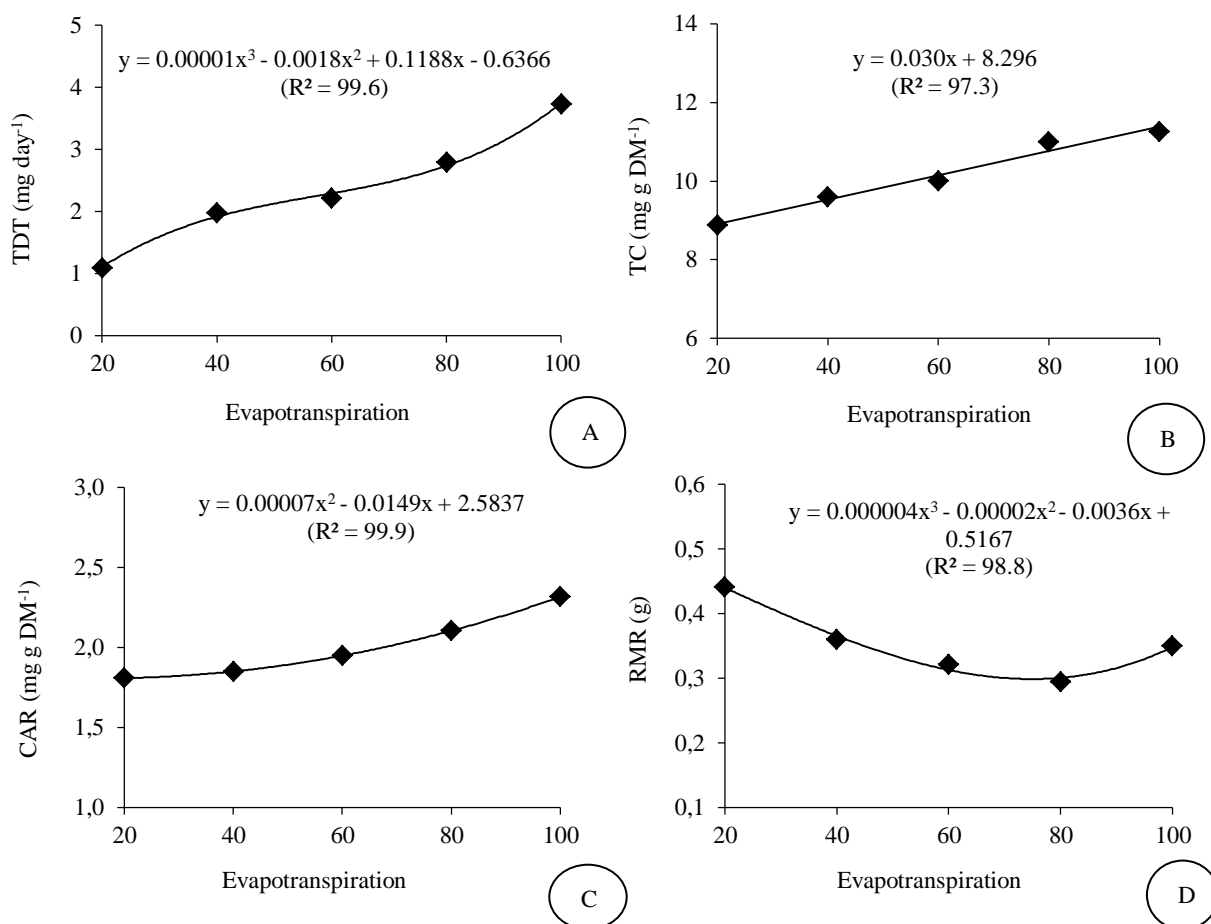


Figure 2. Transpiration (A), total chlorophyll (B), carotenoids (C), and root mass ratio (D) of seedlings of *Schizolobium amazonicum*, subjected to ten days of water deficit, then irrigated with five different percentages of evapotranspired water.

Figura 2. Transpiração (A), clorofila totais (B), carotenoides (C) e razão massa radicular (D) de mudas de paricá [*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby] submetidas a dez dias de déficit hídrico e posteriormente irrigadas com cinco diferentes porcentagens da água evapotranspirada.

As for the TC variable (Figure 2B), we observed a gradual fall, 0.6 mg DM<sup>-1</sup> for each 20% of reduction of evapotranspiration replaced; such reduction may have occurred due to chlorophyll degradation under stress conditions. Lenhard (2010) observed that seedlings of *Caesalpinia ferrea*, subjected to four distinct water

treatments, showed an increase in chlorophyll as the water regime was increased. Low chlorophyll concentrations are often associated with stressed plants, such variation in total chlorophyll and carotenoid content being used as a sign or indicator of stress (NETTO *et al.*, 2005).

Carotenoid content (CAR) increased as more water was made available (Figure 2C), which allows to claim that *Schizolobium amazonicum* has a low tolerance or sensibility to water stress, since with the increase in carotenoid production, the plant starts protecting itself against free radicals, in order to protect biochemically the seedlings of most severe stresses. Such results differ from those of CHA-UM *et al.* (2010), studied the effect of induced stress on Eucalyptus plants (*Eucalyptus camaldulensis* Dehnh.) and observed an increase in carotenoid production as water stress increased. It is well known that carotenoids play a crucial role in the protection against photo-oxidation, dissipating the excess of excitation energy, and that it also regulates the energy flow into or out of the photosynthetic system (NETTO *et al.*, 2005).

As for RMR (Figure 2D), one notices that the results are specific of plants tolerant to water stress, because, with water shortage, the plants developed the root system in search of more water availability in the soil; the greater the stress, the higher the root volume. Such results confirm those of Figueiroa *et al.* (2004) who studied *Myracrodruon urundeuva* in three different stress levels, using 25, 50 and 75% of the field capacity, and also those of Scalon (2011) in seedlings of *Guazuma ulmifolia*, who tested four different water regimes.

Higher root growth causes a reduction of leaf growth, observed in LMR, because the plant tends to invest less in the production of new leaves, in order to reduce water losses (Figure 3A). Our study confirms the results, Nascimento *et al.* (2011), who studied the development of seedlings of *Hymenaea courbaril*, in different water levels in the soil, and got the same results.

As for the SMR variable (Figure 3B), one sees that such behavior is common in the different species, even after the seedling phase. According to Sette Júnior *et al.* (2010), for right establishment of forest plantations, water regime and rainfall level are of extreme importance for maximum productivity of the plants, since water is necessary during all the development and plays a crucial role in the cellular physiological processes.

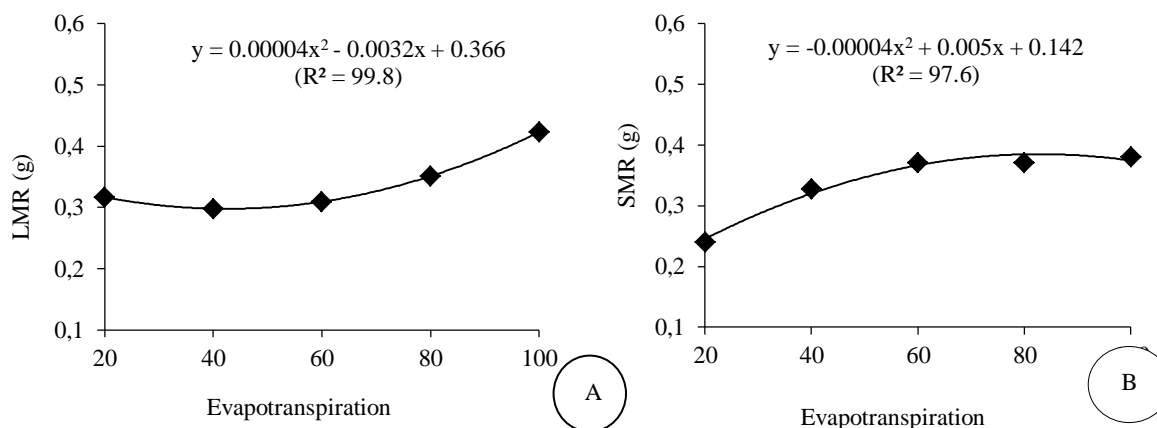


Figure 3. Leaf mass ratio (A) and stem mass ratio (B) of seedlings of *Schizolobium amazonicum*, subjected to ten days of water deficit, then irrigated with five different percentages of evapotranspired water.

Figura 3. Razão massa foliar (A) e razão massa caulinar (B) de mudas de paricá [*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby] submetidas a dez dias de déficit hídrico e posteriormente irrigadas com cinco diferentes porcentagens da água evapotranspirada.

## CONCLUSION

- Seedlings of paricá response to water deficit with slowing down of dehydration, with reduction of content of carotenoids and increase in root volume. As consequence of water stress, they reduce the number of leaves, stem diameter, plant height, and, of course, total biomass, aiming at maintaining the internal water, indicating low ability to tolerate stress.

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