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## INITIAL GROWTH AND COMPATIBLE SOLUTES CONCENTRATION IN SEEDLINGS OF TWO GENOTYPES OF *PSIDIUM ARACA* RADDI (MYRTACEAE) IN RESPONSE TO WATER SUPPRESSION

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**ABSTRACT** - The physiological characterization of plants is of great importance to the selection of suitable genotypes tolerant of low water availability. Therefore, the aim of this study was to evaluate the influence of water deficit on growth and compatible solutes concentration of two genotypes of *Psidium araca* Raddi seedlings. The IPA 10/2 and IPA 16/2 genotypes behaved similarly in terms of number of leaves, stem diameter and the increase of root formation over shoot formation under water deficit. IPA 10/2 produced higher total dry matter; however, IPA 16/2 accession produced higher root-shoot ratio, carbohydrates and free proline contents, conferring to IPA 16/2 a greater ability to grow and survive in environments subjected to low water availability.

**KEYWORDS:** *ARAÇA; BIOMASS PARTITIONING; CARBOHYDRATES; PROLINE.*

**CRESCIMENTO INICIAL E TEOR DE SOLUTOS COMPATÍVEIS EM MUDAS DE DOIS ACESSOS DE *PSIDIUM ARACA* RADDI (MYRTACEAE) EM RESPOSTA A SUPRESSÃO HÍDRICA**

**RESUMO** - A caracterização fisiológica das plantas é de suma importância para a escolha de genótipos mais indicados para área de baixa disponibilidade hídrica, com isso o objetivo deste estudo foi avaliar os efeitos da baixa disponibilidade de água no crescimento e concentração de solutos compatíveis em dois acessos de *Psidium araca* Raddi. Os acessos IPA 10/2 e IPA 16/2 se comportaram de maneira semelhante para número de folhas, diâmetro do caule, e promoveram uma maior formação de raízes em detrimento da parte aérea sob déficit hídrico. IPA 10/2 produziu maior matéria seca total, contudo o acesso IPA 16/2 produziu maior razão raiz/parte aérea e conteúdo de carboidratos e prolina livre, promovendo para o IPA 16/2 uma maior habilidade de crescer e sobreviver em ambientes com baixa disponibilidade hídrica.

**PALAVRAS-CHAVE:** *ARAÇA; CARBOIDRATOS; PARTIÇÃO DE BIOMASSA; PROLINA.*

**EL CRECIMIENTO INICIAL Y EL CONTENIDO DE SOLUTOS COMPATIBLES DE LAS PLÁNTULAS DE DOS ACCESOS DE *PSIDIUM ARACA* RADDI (MYRTACEAE) EN RESPUESTA A LA SUPRESIÓN DE AGUA**

**RESUMEN** - Las características fisiológicas de las plantas son muy importantes para la elección de la mayoría de los genotipos adecuados para el área de baja disponibilidad de agua. Por lo tanto, se objetivó en este estudio evaluar los efectos de la

baja disponibilidad de agua en el crecimiento y en la concentración de solutos compatibles en dos accesiones de *Psidium araca* Raddi. IPA IPA 10/2 y 16/2 accesos se comportaron de manera similar al número de hojas, diámetro del tallo y promovieron una mayor formación de raíces a expensas de rodaje en condiciones de sequía. IPA 10/2 produce la materia seca total más alta. Sin embargo, el acceso IPA 16/2 produce mayor proporción de área de la raíz/parte y el contenido de hidratos de carbono y prolina libre, la promoción de IPA 16/2 mayor capacidad de crecer y sobrevivir en ambientes con poca disponibilidad de agua.

**PALABRAS CLAVE:** GUAYABA; HIDRATOS DE CARBONO; PARTICIÓN DE BIOMASA; PROLINA.

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## INTRODUCTION

The Northeastern region of Brazil is particularly rich in diversity of native and exotic species, especially fruit trees due to its favorable ecological conditions. Despite their recognized qualities and economic potentials, *Psidium araca* Raddi (Myrtaceae) remain just semi-domesticated, without interest in its cultivation on a commercial scale. Within the several species of the genera *Psidium*, some have edible fruits of good quality as well as they may serve as primary trees for reforestation (Gomes 1975, Donadio 1991). *Psidium araca* (popularly known as “araçá”) is a native fruit tree from Brazil, where it can be found from the State of Rio Grande do Sul, passing through Minas Gerais up to the Amazon region, showing excellent production with high quality fruits. A typical “araçá” fruit has yellow color when ripe, yellow pulp, tastes sweet and slightly acidic and it is suitable to be consumed *in natura* or processed. Due to those qualities *P. araca* has been used as a source of income in poverty-stricken areas where their cultivation is possible.

Since 1987, the Instituto Agrônômico de Pernambuco-IPA has developed a genetic prospecti work by collecting germplasm of fruit species, which the *P. araca* has been included, initially with 27 genotypes, in order to evaluate and characterize the materials with superior features, as well as preserve those species on the verge of permanent extinction (Bezerra et al. 1990). On behalf of the genetic improvement of crops for the semi-arid region, assessment of productive performance and physiological behavior of the plants is of fundamental importance to the processes of selecting resistant varieties, since water deficit is a widespread problem in our region.

Drought or water deficit stress is the major environmental factor adversely limiting the development of cultures throughout the world. On a global scale, the water available for crops is a limited resource, being available for irrigation only in 20% of the total area used for agriculture (Rosegrant et al. 2009). Plants experience water stress either when taking water from the soil becomes more difficult for the plant, due to an increase in the osmotic pressure of the soil solution as compared to that of roots, or when the transpiration rate becomes so intense that it might present a substantial negative impact on growth and development of the plants (Ghane et al. 2012).

The negative effects of water shortage over biochemical, physiological, and morphological processes can be verified in all parts of the plant at several organizational levels. The irreversible effects and modifications in plant behavior when they are subjected to water stress will depend on the intensity, duration, and speed enforcement of stress as well as the stage of development and genetic capacity of plants to cope with the environmental changes (Santos and Carlesso 1998, Pimentel 2004). Drought stress responses are multigenic so that the mechanism of tolerance is altered by changes in growth and concentration levels of several compatible

solutes/osmolytes. Osmotic adjustment involves the accumulation of compatible solutes (low-molecular-weight organic osmolytes), such as proline, mannitol, sorbitol, fructans, sucrose and oligosaccharides (Wang 2014). These larger amounts of compounds play a key role in maintaining the osmotic equilibrium and protecting membranes as well as macromolecules (Couee et al. 2006).

Due to little information in the literature on the physiological responses of *P. araca* to drought, the aim of the present study was to evaluate and quantify growth, dry biomass production and concentration of compatible solutes in two genotypes of *P. araca* seedlings from the gene bank of the IPA submitted to water suppression.

## MATERIAL AND METHODS

The experiment was conducted from August to November 2010, in a greenhouse of the Laboratory of Plant Physiology, Department of Biology in the Universidade Federal Rural de Pernambuco (UFRPE). For this purpose, three-month-old seedlings of two genotypes of *P. araca* from the Experimental Station of the Agronomic Institute of Pernambuco (IPA), Itapirema (PE), were transplanted into polyethylene pots containing a three-kilogram mixture of organic compost, clay and cattle manure in the ratio of 2:1:1 (v/v) and with one seedling of *P. araca* per pot.

Field capacity (FC) in pot was determined, as previously described by Souza et al. (2000), considering the difference between wet soil weight after saturation and free drainage, and weight of air-dried soil. Maintenance of water treatments was made daily by weighing the pots and replacing the volume of water lost by transpiration, using a precision scale with capacity for 15 kg.

The experimental design was completely randomized, using two genotypes (IPA 10/2 and IPA 16/2) and three water treatments (control-80%FC, moderate stress-40%FC and severe stress-20%FC) with four replicates per treatment, totaling 24 plants. During the trial period of 60 days, biometric evaluations were performed at 10-day intervals, measuring plant height, with the aid of a measuring tape; leaf number was counted and stem diameter was taken with a digital caliper with accuracy of  $\pm 0.01$  mm.

To determine dry mass, plants were collected and separated into leaves, stem and roots and placed into a forced-air-circulation oven at 65° C until constant weight, then, leaves, stem, root and total dry mass were measured. Complementarily leaves, stem and root biomass partitioning were calculated according to methodology described by Benincasa (2003).

One gram of fresh leaves was also collected at the end of the experiment to evaluate the effects of water stress; the samples were stored at -20°C prior to the extraction. Extracts were prepared by grinding up plant tissue with 4 mL of 0.1 M monobasic phosphate buffer (pH 7.0). The homogenate was filtered, centrifuged to 14,676 x g for 15 min at 4°C and the supernatant was utilized for further analyses.

Total soluble carbohydrates were determined using the phenol-sulphuric acid method with D(+)-glucose as standard (Dubois et al. 1956). To 20  $\mu$ L of the crude extract, 500  $\mu$ L of 5% phenol plus 480  $\mu$ L of distilled water and 2.5 mL of concentrated sulphuric acid were added and the readings of samples were carried out using a spectrophotometer at wavelength 490 nm. To quantify free proline, 1 mL of extract added with 1 mL of acetic acid plus 1 mL of Ninhydrin acid was left in water bath during one hour at 100°C. After the development of colour, the samples were cooled down in ice bath, adding 2 mL of toluene to the sample for the separation of phases. Disregarding the colorless fraction, the fraction samples reading red and the blanks were read using a

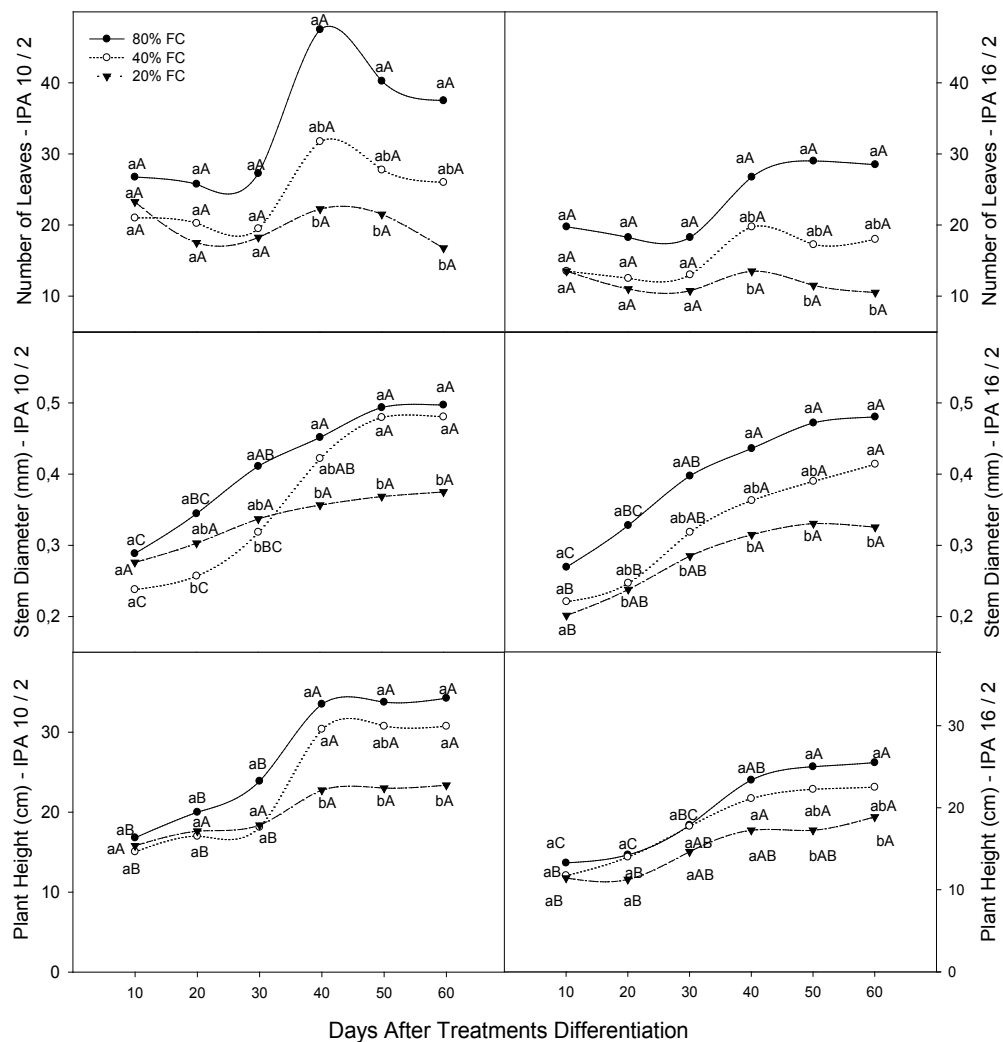
spectrophotometer at wavelength 520 nm according to methodology described by Bates et al. (1973).

The Analysis of variance (ANOVA) and comparison of averages by a Tukey's Multiple Range test ( $p < 0.05$ ) were performed using ASSISTAT 7.7 software.

## RESULTS

In a general way, severe water stress represented by the treatment 20% FC negatively affected the most growth parameters. The water stress reduced the number of leaves for both *P. araca* genotypes. From 40DAD (days after differentiation) until the end of the experimental period, plants under severe stress began to present a smaller amount of leaves. This reduction was 33.49% for IPA10/2 and 37.6% for IPA16/2 when compared to control treatment at 60 DAD, as shown in Figure 1.

**Figure 1 - Number of leaves, stem diameter and plant height on seedlings of two *Psidium araca* Raddi (Myrtaceae) genotypes subjected to water deficit. Identical letters, lower case among treatments and upper case among evaluation times, do not significantly differ by Tukey's Multiple Range test ( $p < 0.05$ ).**



The same behavior was observed to stem diameter, which presented differences within the treatments from 20DAD. However, accession IPA 10/2 initially demonstrated a smaller diameter in the moderate stress

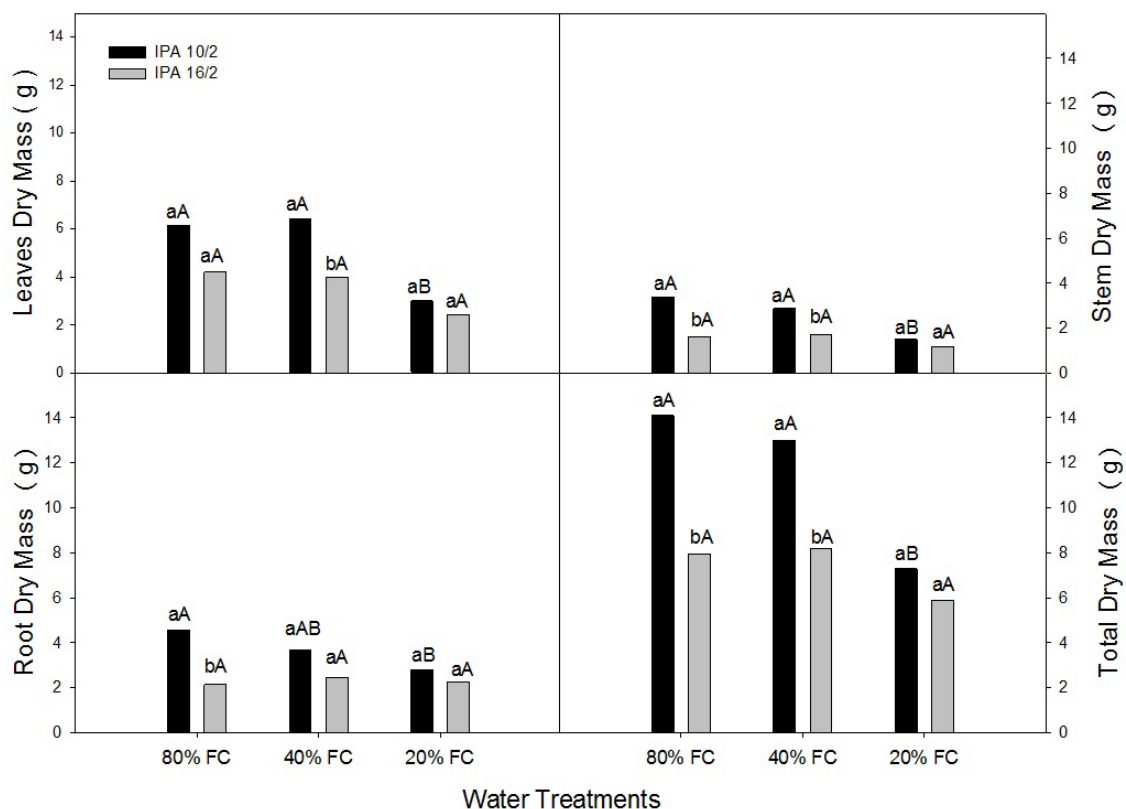
treatment, having a resumption of growth and matching the control at the end of the period, leaving to the severe stress the largest reductions. However, accession IPA 16/2 presented the greatest reductions in severe stress. As shown in Figure 1, the diameters of plants under 20%FC were 24.55 and 32.23% smaller compared to control (80%FC) respectively to genotypes IPA 10/2 and IPA 16/2 at 60DAD.

The water suppression treatment influenced plant height of *P.araca* as from 40DAD for IPA 10/2, whereas for IPA 16/2 the influence started from 50DAD. This may have occurred because IPA 10/2 presented larger phenotypic size in relation to IPA 16/2 as shown in Figure 1. The control treatment showed maximum plant height value of 33.75 cm for IPA 10/2 and 25.50 cm for IPA 16/2.

There were no differences among the water treatments studied in relation to leaves, stem, root and total dry mass production in IPA 16/2 access, as shown in Figure 2. However, in IPA 10/2, the severe stress treatment (20%FC) reduced their leaves dry mass (42.13%), stem dry mass (55.85%), root dry mass (38.93%) and the total dry mass (48.33%) when compared to the control (80%FC) (Figure 2).

Comparing between the genotypes, IPA 10/2 had more root, stem and total dry mass than IPA 16/2 to control plants (80% FC). The same was observed to root, stem and total dry mass to plants subjected to 40% FC (Figure 2). However, under 20%FC both genotypes were similar.

**Figure 2 - Leaves, stem, root and total dry mass in seedlings of two *Psidium araca* Raddi (Myrtaceae) genotypes subjected to water deficit. Identical letters, lower case between genotypes and upper case among treatments, do not significantly differ by Tukey's Multiple Range test ( $p < 0.05$ ).**



There was a genotypic variation for leaves biomass partitioning only at 80%FC, in which the IPA 16/2 accession increased 18.77% compared to IPA 10/2. No statistical difference was found between the genotypes under the other water treatments (Table 1).

**Table 1 - Leaves, stem, root biomass partitioning and root/shoot ratio in seedlings of two *Psidium araca* Raddi (Myrtaceae) genotypes subjected to water deficit. Identical letters, lower case between genotypes and upper case among treatments, do not significantly differ by Tukey's Multiple Range test ( $p < 0.05$ ).**

Water Treatments	Leaves Biomass Partitioning (%)		Stem Biomass Partitioning (%)		Root Biomass Partitioning (%)		Root/Shoot Ratio	
	IPA10/2	IPA16/2	IPA10/2	IPA16/2	IPA10/2	IPA16/2	IPA10/2	IPA16/2
	80%FC	43.91 bA	52.15 aA	24.06 aA	20.44 aA	31.97 Aab	27.41 aB	0.474 aAB
40%FC	49.16 aA	47.73 aAB	22.03 aA	21.02 aA	28.81 Ab	31.25 aB	0.407 aB	0.465 aB
20%FC	41.07 aA	40.22 aB	20.71 aA	20.53 aA	38.22 Aa	39.25 aA	0.619 aA	0.651 aA

The IPA 16/2 accession showed a greater leaves biomass partitioning to control plants, nearly 30% larger than the severe stress. Based on isolated treatments there were no differences for stem biomass partitioning in any of the genotypes. However, for root biomass partitioning both genotypes at 20%FC (most severe treatment) showed a greater allocation to roots when compared to other treatments in the order of 19.55% for IPA 10/2 and 43% for IPA 16/2. This greater biomass allocation to roots transduces directly in larger values of root-shoot ratio under the most severe treatment.

The contents of soluble carbohydrates in leaves showed no significant difference among water treatments and between the genotypes used. The carbohydrate content ranged from 3.47 to 3.53  $\mu\text{mol g}^{-1}$  FM to 80 and 20%FC, respectively, for the IPA 10/2 accession. With regard to values obtained to genotype IPA 16/2, these values ranged from 4.24  $\mu\text{mol g}^{-1}$  FM to 4.78  $\mu\text{mol g}^{-1}$  FM to 80%FC and 20%FC, respectively (Table 2).

Although increases in free proline contents have been observed (in order of 60% under the most severe treatment for IPA 16/2, while in IPA 10/2 this buildup was only 27%), it was not significantly different.

**Table 2 - Soluble Carbohydrates and Free Proline concentrations ( $\text{mmol.g}^{-1}$  FM) in leaves of seedlings of two *Psidium araca* Raddi (Myrtaceae) genotypes subjected to water deficit. Identical letters, lower case between genotypes and upper case among treatments, do not significantly differ by Tukey's Multiple Range test ( $p < 0.05$ ).**

Water Treatments	Soluble Carbohydrates		Free Proline	
	IPA10/2	IPA16/2	IPA10/2	IPA16/2
80%FC	3,47 aA	4,24 aA	0,15 aA	0,23 aB
40%FC	3,48 aA	4,78 aA	0,16 aA	0,19 aB
20%FC	3,53 aA	4,34 aA	0,19 bA	0,37 aA

## DISCUSSION

Drought is a multifaceted stress condition that causes serious crops yield limitations depending on plant growth stage, stress duration, and severity (Muscolo et al. 2014). In the present study the severe level of water suppression (20% FC) induced reductions in almost all growth parameters. However, it was more conspicuous to accession IPA 10/2 when compared with IPA 16/2.

Some different aspects of initial growth can be observed in the present study between IPA 10/2 and IPA 16/2, as differences in plant height and number of leaves. There were largest fall in number of leaves in IPA 10/2 compared to IPA 16/2. It does indicate an increased ability, in the latter, to maintain leaves in stressful

conditions. However, this behavior requires high energy costs, which will probably not result in a higher dry matter accumulation. IPA 10/2 naturally showed a higher plant height and number of leaves than IPA 16/2, demonstrating the conservative characteristic during initial growth of this latter genotype.

The number of leaves depends on the emergence of new leaves as well as the rate of senescence of older leaves. In high evaporative demand environments, these two factors influencing the leaves number, while in environments with low evaporative demand senescence contributes more than the appearance of new leaves. So the number of leaves may be a good indicator of water stress (Holanda et al. 2014). Falling of older leaves and the consequent reduction in their number is considered, according to Taiz and Zeiger (2013), a common reaction of plants to water deficit situations, when the leaf abscission is stimulated by increased synthesis and greater sensitivity to ethylene.

The first effect of a water shortage is drastically reduce the growth of expanding tissues. Expansive growth is one of the processes most sensitive to water deficit in leaves. This occurs because turgor - the driving force for cell expansion- is reduced in the case of water deficit, but also because of other indirect processes such as reduction in the rate of cell division and/or the extensibility of cell walls (Tardieu 2013).

The stem diameter and plant height had the same behavior, significantly reducing in plants under severe water deficit to both genotypes. Nevertheless, plant height in genotype IPA 10/2 was more expressive when cultivated with 80%FC and even when submitted to water deficit than IPA 16/2. This faster initial growth to IPA 10/2 reflected in a greater reduction when water availability was drastically reduced in treatment 20%FC (almost 32% of reduction). Martins et al. (2010), studying *Azadirachta indica* under water stress found that plants subjected to 20%FC had similar reduction when compared to the control, which was around 38% of its stem diameter, corroborating to our findings. The same driving force necessary to plant cell elongation to expanding leaves and shoot in extension is required to lateral growth due the secondary expansion in the stem.

Water stress affects the strength of source and sink and their effects on photosynthesis, growth, translocation and overall metabolism, usually causing reductions in growth as a whole. A number of studies report the negative effect of drought on plant growth and development (Martins et al. 2010, Scalon et al. 2011, Fini et al. 2013, Rocha et al. 2016, Silva et al. 2016). However, for *P. araca* studies are still scarce. The only one was performed by Freire et al. (2009), in which they observed the greatest reductions in *P. araca* for leaf number, stem diameter and plant height occurred under severe water restriction (25%FC), supporting the results obtained in this research.

The increased water suppression caused reductions in the dry mass of different organs in the IPA 10/2 accession. Those marked reductions were probably due to decreases in processes such as plant growth, which in turn results from diminished plant turgor, since water stress caused by drought develops gradually in plants and affects the expansion of cells. At least in part, the loss of turgor induces stomatal closure (data unshowed) and it does reflect in carbon assimilation, compromising the growth rate. When growing with good supply of available water (80%FC) IPA 10/2 genotype favored its production of total dry mass making it 77.5 and 58.85% higher under 80%FC and 40%FC treatments respectively, when compared to the IPA 16/2 genotype. But, on the other hand, when cultivated with low water availability, IPA 10/2 was more sensitive than IPA 16/2.

A number of studies showed similar results decreasing the dry matter production in response to water deficit as found by Silva et al. (2002) studying *Melaleuca alternifolia* Cheel under four irrigation blades. The authors found out that the dry mass content of shoot and root decreased when subjected to water suppression. The same results was found by Gonçalves and Passos (2000) studying five Eucalypt species subjected to two levels of

water suppression proved that a lower water availability led to the decline of leaf, twig, root and total production, in the ratio of 64.0, 56.9, 40.89 and 58.92% respectively. Lower dry matter accumulation in plants under water stress is a consequence of the own plant mechanisms to prevent excessive water loss, such as a decrease in the leaf number, resulting in the reduction of the radiation intercepted and stomatal conductance, which causes a decrease in CO<sub>2</sub> assimilation and thus the accumulation of biomass as well (Cavatte 2011).

The root-shoot ratio is a correlation of development expressing the fact that roots growth can affect the shoot and the other way around. Plants subjected to severe water deficit tends to invest more in root elongation than in shoot, to facilitate absorption of water and nutrients from deeper soil zones and maintain osmotic pressure (Chavarria and Santos 2012, Robins II and Dinneny 2015). The ability to develop extensive root systems contributes to differences among genotypes on behalf of drought tolerance, being considered an important trait in selection of plants resistant to drought (Abd Allah et al. 2010), and this behavior was achieved in both *P. araca* genotypes. This point has been proven in studies conducted by Figueirôa et al. (2004), Villagra and Cavagnaro (2006) and Medeiros et al. (2012), studying respectively *Myracrodruon urundeuva*, *Prosopis* sp. and *Malpighia emarginata* DC with all species subjected to water suppression treatments.

Accumulation of carbohydrates in plants is enhanced in response to a variety of environment stresses. Besides direct involvement in the synthesis of other compounds and production of energy, sugars are also involved in the stabilization of membranes. The carbohydrates formed are important to protection against dehydration and may function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor as well (Devi and Sujatha 2014). These data differ from the results of this research, which found no significant difference, probably by the short trial period in which the plants were exposed. Despite the values found to IPA 16/2, apparently higher than IPA10/2, carbohydrate content was not different between genotypes and water treatment, suggesting that this important organic solute do not act as a drought stress regulator to *P. araca*.

On the other hand, proline showed different results compared with carbohydrate. Proline increased its content in plants under severe water deficit. In the present study, the intensity of drought stress increased the level of proline significantly in both genotypes that could be linked to their ability to perform tissue osmotic adjustment to lower the osmotic potential and protect plants from dehydration damages (Lehmann et al. 2010, Hossain et al. 2014). Several studies suggest that the accumulation of proline represents a compensatory mechanism during the stress period; plants have a greater survival rate, according to the osmotic adjustment induced by proline accumulation, which also works as a protection to enzyme denaturation, protecting protein turnover machinery and yet contributes to the removal of free radical (Szabados and Savouré 2010, Liang et al. 2013, Silva et al. 2013).

Osmotic adjustment and turgor regulation are well-illustrated functions of these compounds in plants since their high solubility in water acts as a substitute for water molecules released from leaves. The primary function of compatible solutes is to prevent water loss to maintain cell turgor and maintain the water potential gradient between plant and soil for water uptake into the cell. The accumulation of these metabolites in cells led to a decrease in osmotic potential and finally resulted in a higher capacity of water absorption by roots and water retention in the cells (Lisar et al. 2012). Although leaf water potential was not evaluated and the role of proline as osmoregulator cannot be confirmed, at least in part proline certainly performs an important role against desiccation. The accumulation of proline under stress supplies energy for the plant to survive and grow and



thereby helps the plants to tolerate the stress condition (Kumar et al. 2011). Thus, the proline content is a good indicator for screening drought tolerant genotypes in water stress condition (Rahdari et al. 2012) to *P. araca*.

## CONCLUSION

Severe water suppression affect the growth of the two *P. araca* genotypes, but IPA 10/2 is more drought sensitive than IPA 16/2, yet in no time the cell elongation ceased. Smaller percentage of reduction in the number of leaves under stressed treatments, as well as, higher root-shoot ratio and compatible solutes content in the IPA 16/2 accession confers to it a greater ability to be grown and survive in environments with low water availability.

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