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Biochemical and Physiological Responses in Two *Vigna uguiculata* (L.) Walp. Cultivars Under Water Stress

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Abstract: The aim of this study was to evaluate and compare the effects of water stress on biochemical and physiological parameters of *Vigna unguiculata* (L.) Walp. (Sempre verde and Pitiuba), as well as reveal the cultivar that better adapts under water stress. The experimental design was randomized entirely in factorial, with 2 cultivars (Sempre verde and Pitiuba), combined with 2 water conditions (stress and control). Tenors of proline, free amino acids, total soluble carbohydrates and total soluble proteins were quantified and the nitrate reductase activity was measured. The plants submitted to stress suffered an increase in the amounts of proline, since this solute is an osmotic adjuster. There was an increase in the amounts of amino acids due to an increase in the protease enzyme activity and an increase in the total carbohydrate levels, caused by the increase of starch. A decrease in the amounts of total protein caused by the decrease in their synthesis and a fall in nitrate reduction activity caused by the low nitrate influx were reported. These responses were seen in both cultivars. The Pitiuba cultivar presented responses that show better osmotic adjustment in these conditions, revealing a greater adaptability of this cultivar in conditions of hydric stress.

Key words: *Vigna unguiculata* (L.) Walp., water stress, osmotic adjustment

INTRODUCTION

Water stress is commonly attributed to situations where the water loss exceeds sufficient absorption intensity causing a decrease in plant water content, turgor reduction and, consequently, a decrease in cellular expansion and alterations of various essential physiological and biochemical processes that can effect growth or productivity (Kramer and Boyer, 1995; Pimentel, 2004). Water deficiency causes various responses on plant metabolism, where osmotic adjustment is an extremely important physiological mechanism for preparing these plants to tolerate hydric stress (Morgan, 1991), where diverse organic compounds accumulate as osmoregulators (Zhang *et al.*, 1999).

Vigna unguiculata (L.) Walp. production has a strong influence on Brazilian commercial balance, many research institutions have been conducting studies with the species, aiming to produce cultivars that maximize productivity and adapt easily to cultured regions.

The Pitiuba and Sempre verde cultivars were developed for the edapho-climatic conditions of northern Brazil, where they possess high adaptation and productivity, as well as being used by local producers. However, there are only a few studies on how these cultivars respond to water deficiency. So, understanding the adaptation mechanisms, biochemical processes subjacent to damages provoked by environmental stress and physiological behavior of the cultivars under abnormal conditions, become important.

The aim of this study was to evaluate and compare water stress effects on proline, total soluble carbohydrates, total soluble proteins, free amino acids and nitrate reductase activity in plants of *Vigna unguiculata* from the Sempre verde and Pitiuba cultivars, as well as reveal which cultivar better adapts to water stress conditions using these parameters.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse under the mean environmental temperature of 25°C, with a minimum and maximum of 21 and 35°C, respectively and mean related humidity of 67.8%. The physiological analyses were carried out at the Laboratory of Advanced Plant Physiology at the Instituto de Ciências Agrárias (ICA) of the Universidade Federal Rural da Amazônia (UFRA), Belém, Pará State, Brazil, during the October and November 2006.

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Plants were grown in 6 L pots filled with black potting soil and aviary manure mixed at a 3:1 proportion. *Vigna unguiculata* seeds from the Sempre verde and Pitiuba cultivars collected and stored from the 2006 harvest were used in the experiment.

The experimental design was randomized entirely in factorial, with 2 cultivars (Sempre verde and Pitiuba), combined with 2 water conditions (stress and control), totalizing 4 treatments, with 8 repetitions, where each was made up of one plant. Three seeds were placed into each pot and after 7 days, the plants were thinned to one per pot only. The plants remained in the greenhouse for 40 days, watered daily and received macro and micronutrients every 5 days, using the nutritive solution by Hoagland and Arnon (1950) modified by the Laboratory of Advanced Plant Physiology of UFRA. Starting the 40th day post cultivation, the plants under stress treatment were submitted to a period of 5 days without irrigation, simulating water deficiency until the 45th day.

The plants were taken to the laboratory, where the leaves were collected, washed with distilled water and dried with filter paper. Determining nitrate reductase activity was carried out with disks of fresh leaves measuring 0.5 cm^2 in area and the spectrophotometer readings were 540 nm (Hageman and Hucklesby, 1971). To carry out the other biochemical analyses, the fresh leaves were oven dried at 65°C for 48 h. Proline was determined at 520 nm (Bates *et al.*, 1973), free amino acids at 570 nm (Peoples *et al.*, 1989), total soluble carbohydrates at 490 nm (Dbois *et al.*, 1956) and total soluble proteins at 595 nm (Bradford, 1976).

In the statistical data analyses, the residues were tested using the Anderson-Darling test and the variance equality using the Barlett test. Variance analysis was applied on the results, comparing the means with the Tukey test at 5% significance level, using SAS Institute (1996) and ESTAT 2.0, following the theory by Gomes (2000).

RESULTS AND DISCUSSION

Proline: A significant interaction within the treatments was found in this variable, where proline in the Sempre verde cultivar increased 45.32%, while in the Pitiuba cultivar the increase was 57.57% (Table 1). These results revealed that Pitiuba cultivar presented a greater variation within the control and stress treatments, having a larger capacity of adjusting to the unfavorable conditions in which it was found and consequently reducing the effects on plant metabolism (Nogueira *et al.*, 1998). Similar results were found by Turan *et al.* (2007) studying *Triticum aestivum* L., Ismail *et al.* (2004) with *Musa* sp. and Costa (1999) with *Vigna unguiculata* (L.) Walp., demonstrating that this metabolic works as an osmotic adjuster in plants under abnormal abiotic conditions. The observed behavior in their cultivars is due to a decrease in leaf hydraulic potential, where, on the counterpart, there is an increase in proline levels (Nanjo *et al.*, 1999), caused by an increase in P-5CR enzyme activity or concentration and a decrease on the degradation of these amino acids in the mitochondria (Taiz and Zeiger, 1998; Kerbauy, 2004).

Table 1: Tenors of proline in the Sempre verde and Pitiuba cultivars of *Vigna unguiculata* (L.) Walp.

	Proline (µmol g^{-1} DM^{-1})	
	Cultivar	
Hydric condition	Sempre verde	Pitiuba
Control	0.214Ab[1]	0.132Aa
Stress	0.311Bb	0.208Ba
Variation (%)*	45.320	57.570
CV (%)	6.400	
DMS (Tukey)	0.058	
Standard error	0.012	

[1]: Averages followed by the same letter in the line and upper case in the column do not differ within them by the Tukey test at 5% probability; *: Comparing the control and stress treatment in the same cultivar

Table 2: Tenors of free amino acids in the Sempre verde and Pitiuba cultivars of *Vigna unguiculata* (L.) Walp.

	Free amino acids (µmol g^{-1} DM^{-1})	
	Cultivar	
Hydric condition	Sempre verde	Pitiuba
Control	0.287Ab[1]	0.121Aa
Stress	0.391Bb	0.208Ba
Variation (%)*	36.23	71.90
CV (%)	5.55	
DMS (Tukey)	0.060	
Standard error	0.009	

[1]: Averages followed by the same letter in the line and upper case in the column do not differ within them by the Tukey test at 5% probability; *: Comparing the control and stress treatment in the same cultivar

Amino acids: According to the variance analysis, there was a significant difference among the treatments, as well as a significant interaction within evaluated factors (Table 2), in which the hydric stress increased the free amino acids in both cultivars, where a 36.23% increase in amino acid levels was observed in cultivar Sempre verde and in Pitiuba cultivar the increase was 71.90%. Smaller alterations were observed in the Sempre verde cultivar revealing a higher sensibility to water stress. From the results, it is possible to verify that water restriction increased the activities of the protease enzymes. These enzymes are responsible for breaking the protein reserved that increase the activity according to the plant exposure time and stress intensity (Costa, 1999; Verslues *et al.*, 2006).

Carbohydrates: The variance analysis revealed a significant difference among the treatments, where the stress of five days elevated the total soluble carbohydrates for both cultivars (Table 3), where an increase of 57.81% in the Sempre verde cultivar and 72.15% in the Pitiuba cultivar were reported. A greater variation in total soluble carbohydrates was observed in the Pitiuba cultivar, revealing greater biochemical ability of this cultivar for this type of studied stress. This increase in carbohydrates was observed by Pimentel (1999), studying *Zea mays* L., when a progressive rise of this variable increased the water stress duration. These observed increases in plants under stress are associated to the fact that with dehydration, there is a decrease in cell starch content, as well as a fall in the photosynthetic capacity that decrease the cellular growth and reduces the synthesis of sucrose for export, raising the total soluble carbohydrates (Rossiello *et al*., 1981; Vassey and Sharkey, 1989).

Proteins: Decreases of 42.46 and 54.22% in the Sempre verde and Pitiuba cultivar, respectively, were observed (Table 4). In relation to the total protein tenors, Pitiuba cultivar presented a greater variation, however this cultivar supports and adapts better to water stress. Similar results were found by Costa (1999) working with this species. Such results are due to an increase in the proteolytic enzymes, which break down the stored proteins in the plant and from the decrease in protein synthesis, it is shown that such deficiency affects all the biochemical process while trying to maintain the water level in the leaves, as well as the cellular osmotic balance. Moreover some proteins involved in the hydrophilic interaction with cellular macromolecules are synthesized to establish the metabolism and act on recuperating damages caused by water stress (Yordanov *et al*., 2000; Xiong *et al*., 2002).

Table 3: Tenors of total soluble carbohydrates in the Sempre verde and Pitiuba cultivars of *Vigna unguiculata* (L.) Walp.

	Total soluble carbohydrates (μmol g^{-1} DM^{-1})	
	Cultivar	
Hydric condition	Sempre verde	Pitiuba
Control	0.320Ab[1]	0.255Aa
Stress	0.505Bb	0.439Ba
Variation (%)*	57.810	72.150
CV (%)	9.680	
DMS (Tukey)	0.094	
Standard error	0.015	

[1]: Averages followed by the same letter in the line and upper case in the column do not differ within them by the Tukey test at 5% probability; *: Comparing the control and stress treatment in the same cultivar

Table 4: Tenors of total soluble proteins in the Sempre verde and Pitiuba cultivars of *Vigna unguiculata* (L.) Walp.

	Total soluble proteins (mg. g^{-1} DM^{-1})	
	Cultivar	
Hydric condition	Sempre verde	Pitiuba
Control	0.478Ab[1]	0.284Aa
Stress	0.275Bb	0.130Ba
Variation (%)*	42.460	54.220
CV (%)	3.990	
DMS (Tukey)	0.082	
Standard error	0.006	

[1]: Averages followed by the same letter in the line and upper case in the column do not differ within them by the Tukey test at 5% probability; *: Comparing the control and stress treatment in the same cultivar

Nitrate reductase: The variance analysis pointed out that there was a significant difference within the treatments and the Tukey test showed interaction within the evaluated factors. The variations in activities of the enzyme nitrate reductase in the Sempre verde and Pitiuba cultivars were 44.73 and 40.94%, respectively (Table 5). This variable attained a higher variation in the Sempre verde cultivar, where it can be inferred that the physiological mechanism of the Sempre verde is more sensitive, compared to the Pitiuba, under hidric deficiency. This parameter is widely used as an excellent physiological indicator, since it has a simple and fast methodology that is easy to measure (Oliveira *et al*., 2005). The obtained results suggest that in virtue of the water stress, there are decreases in the leaf hidric potential and consequently lower transpiration levels, reducing the influx of nitrate and the enzyme activity in both cultivars, since this enzyme is induced by the substrate (Sharner and Boyer, 1976). Studies carried out by Marur *et al*. (2000) with *Grossypium hirsutum* corroborated with the results attained under the influence of environmental stresses in reducing the activity of the enzyme. Nitrate, being the most abundant form of inorganic nitrogen available for plants and since nitrate reductase is the first enzyme of the N-inorganic assimilation pathway, makes this step fundamental in plant metabolism, besides its activity being regulated by the availability of water in the soil (Meguro and Magalhães, 1982).

Table 5: Nitrate reductase enzyme activity in the Sempre verde and Pitiuba cultivars of *Vigna unguiculata* (L.) Walp.

	Nitrate reductase activity (μmol NO_2 g^{-1} FM^{-1})	
	Cultivar	
Hydric condition	Sempre verde	Pitiuba
control	0.532Ab[1]	0.232Aa
stress	0.294Bb	0.137Ba
variation (%)*	44.730	40.940
CV (%)	11.230	
DMS (Tukey)	0.088	
Standard error	0.019	

[1]: Averages followed by the same letter in the line and upper case in the column do not differ within them by the Tukey test at 5% probability; *: Comparing the control and stress treatment in the same cultivar

CONCLUSIONS

Even though the cultivars had similar behaviors, Pitiuba cultivar presents responses that proves the best osmotic adjustment in this condition, revealing a better adaptation of this cultivar while enduring water stress.

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