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Biochemical and Physiological Behavior of *Vigna unguiculata* (L.) Walp. Under Water Stress During the Vegetative Phase

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Abstract: The aim of this experiment was to investigate the responses caused by progressive water stress and the necessary time for have biochemical and physiological changes of *Vigna unguiculata* (L.) Walp. cv: manteguinha during the vegetative phase. Leaf Relative Water Content (LRWC), Nitrate Reductase Activity (NRA), free proline, total soluble carbohydrates, free amino acids and total soluble proteins were quantified. The plants under the control treatment maintained stable variables; however, those under water stress suffered an increase of 97.3% in proline due to osmotic adjustment, increase of 78.4% in free amino acids, caused by the break down of proteins by protease enzymes, increase in total carbohydrates by 94.2% due to a decrease in the photosynthetic capacity and low synthesis of sucrose used for exporting this solute. There was a decrease in 25.7% of the leaf relative water content in virtue of low water availability in the soil, reduction of 43.8% in Nitrate Reductase Activity (NRA) due to the regulation of this enzyme being induced by its substratum and 47.3% of total proteins due to the paralyzation of protein and proteolytic protein degradation, being maximized the variations in the parameters evaluated according to the increase in the period the plants were exposed to water stress. Physiological changes can be carried out in only a few days. However, biochemical responses should be evaluated starting at 4 days under water restriction.

Key words: Physiology, biochemical, water stress, vegetative phase

INTRODUCTION

Water availability is considered the climatic factor with greatest effect on agricultural productivity, being responsible to govern species distribution in the different climate zones around the globe (Rockström and Falkenmark, 2000). The effects of drought vary and depend on the intensity, development stage and duration of the hydric stress, as well as, the adaptive strategies that the species possess to tolerate this abnormal condition (Kramer and Boyer, 1995).

During the vegetative phase, water deficit causes leaf and plant growth reduction, besides altering the process of nutrient absorption due to the low water availability in the environment and low photosynthetic activity, since with water stress an increase in stomatal resistance occurs, decreasing the capacity of gaseous exchange between the environment and the plant (Kerbauy, 2004).

The water deficit influences various biochemical and physiological processes (Sircelj *et al*., 2005), where proline and carbohydrates constitute some of the organic solutes, which accumulate in the plant cells for adjusting plant osmosis (Taiz and Zeiger, 1998; Zhang *et al*., 1999). Many researchers have reported alterations in the functioning and speed of enzymatic activity, like nitrate reductase, amino acid synthesis (Andrews *et al*., 2004) and decrease in protein levels (Zhu and Xiong, 2002), as metabolical responses to water restrictions (Pimentel, 2004).

Cowpea (*Vigna unguiculata* (L.) Walp.) is an edible dicotiledoneous legume with the capacity to store large quantities of protein in its grain, having an excellent capacity at fixing nitrogen, as well as not needing very fertilized soil (Lobato *et al*., 2006; Peksen and Artik, 2004).

Studies indicate that cowpea tolerates low water availability and high temperatures, besides revealing that the species supports up tp 10 days under water defficiency, simulated in a greenhouse (Costa, 1999; Silveira *et al*., 2001, 2003); however, the consequences on the physiological and biochemical metabolisms involved in conditions of light, moderate and severe stress are not well-known for manteguinha cultivars.

The aim of the experiment was to investigate the responses caused by progressive water stress and the necessary time for have changes on the LRWC, NRA, proline, total soluble carbohydrates, free amino acids and total soluble proteins of plants of *Vigna unguiculata* (L.) Walp. cv: manteguinha during the vegetative phase.

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MATERIALS AND METHODS

The experiment was conducted in a greenhouse, located at the Instituto de Ciências Agrária (ICA) of the Universidade Federal Rural da Amazônia (UFRA), city of Belém, state of Pará, Brazil (01°27'S and 48°26'W), during the months of September and October of 2006, under natural conditions: day/night (minimum/maximum air temperature and relative humidity were: 22.4/37.6°C and 68/79%, respectively, verified during the experiment), where the average photoperiod was 12 h and the maximum active photosynthetic radiation, 525 μmol^{-2} sec^{-1} (at 12:00 h).

The plants were grown in 6 L pots filled with black potting soil and aviary manure, mixed at 3:1 proportion. *Vigna unguiculata* seeds collected in the 2006 season were stored until the experiment and used.

The experimental design was randomized, with 2 water conditions (stress and control) and 4 evaluation points (0, 3, 6 and 9 days), with 8 repetitions and 64 experimental units, where each repetition was composed by 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 a greenhouse for 35 days, watered daily and received macro and micronutrients every 5 days, using the nutritive solution by Hoagland and Arnon (1950). Starting the 35th day after the implementation of the experiment, the plants from the treatment under stress were submitted to a period of 9 days without irrigation, until the 44th day, simulating water deficit (stress).

Physiological analyses: The plants were taken to the Laboratory of Advances Plant Physiology (UFRA) to perform the analyses. The nitrate reductase activity was performed with disks of fresh leaves measuring 0.5 cm^2 in area and the spectrophotometer readings were 540 nm (Hageman and Hucklesby, 1971). The determination of the Leaf Relative Water Content (LRWC) was performed with 10 mm disks in diameter, as well as RWC was calculated as:

$$LRWC = [(FW-DW)/(TW-DW)]\times 100$$

Where:

FW = The fresh weight

TW = Turgid weight measured after 24 h of saturation in de-ionized water at 4°C in the dark and DW is the dry weight determined after 48 h in an oven at 80°C (Slavick, 1979)

Biochemical analyses: The fresh leaves were oven dried at 65°C for 48 h, until obtaining dry matter for quantification in the spectrophotometer. The free 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 (Dubois *et al*., 1956) and total soluble proteins at 595 nm (Bradford, 1976).

Statistical analyses: The data was submitted to ANOVA and when there was a significant difference, the Tukey test at 5% significance was applied, as well as was calculated standard error for each point, where the statistical analyses were performed with SAS (1996).

RESULTS

Leaf relative water content and nitrate reductase activity: The LRWC suffered a significant reduction of 25.7%, according to the variance analysis, in which the treatment under stress decreased from 89.7 to 66.7% at 0 and 9 days of stress, respectively (Fig. 1A). This reveals that an

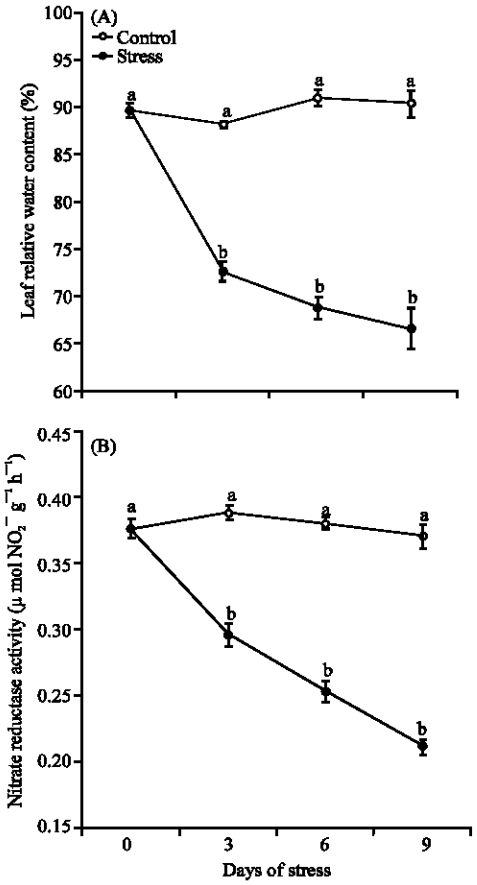

Fig. 1: Relative water content (A) and nitrate reductase activity (B) in plants of *V. unguiculata* under 0, 3, 6 and 9 days of hydric stress. Averages followed by the same letter(s) do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

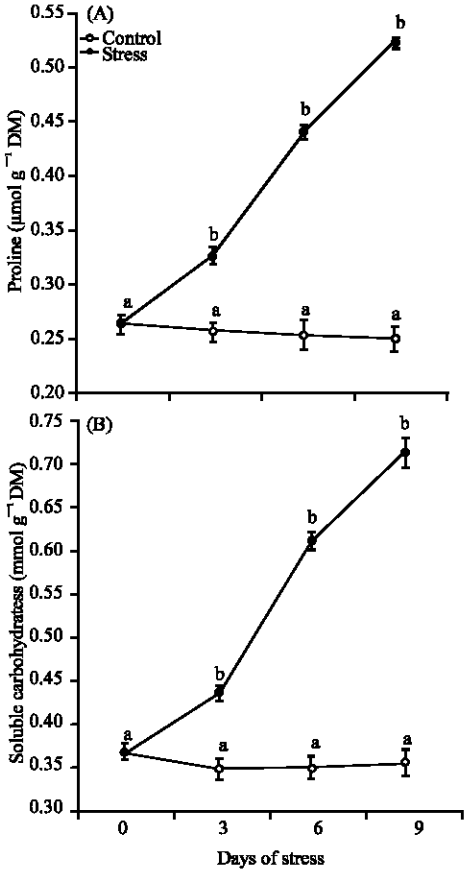

Fig. 2: Tenors of free proline (A) and total soluble carbohydrates (B) in plants of *V. unguiculata* under 0, 3, 6 and 9 days of hydric stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

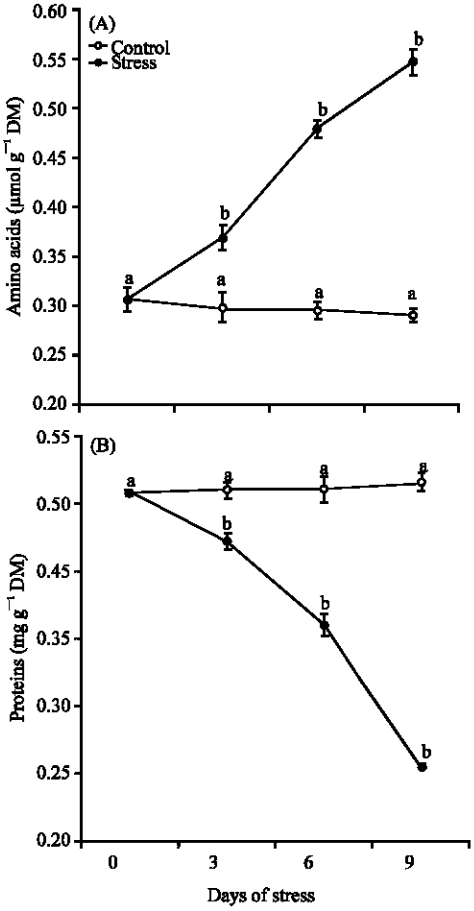

Fig. 3: Tenors of free amino acids (A) and total soluble proteins (B) in plants of *V. unguiculata* under 0, 3, 6 and 9 days of hydric stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

accentuated drop occurred in this variable during the period between 0 and 3 days, contributing with 19% of the reduction total. However, the control treatment remained stable and above the stress, varying between 88 and 91%.

The NRA decreased significantly, 43.8% (Fig. 1B), when the period under water restriction increased (9 days). It has been observed that there is a relation between the relative water content and nitrate reductase, because the larger variation occurred during the period of 0 to 3 days, with 21.4% of the total reduction. As well as similar to what was observed with the LRWC. The control kept the levels of nitrate reductase stable. Our results are in agreement with Marur *et al.* (2000), who worked with *Grossypium hirsutum* and Chandrasekar *et al.* (2000), studying *Triticum* spp. on the influence of abiotic stresses in the reduction of enzyme activity.

Proline and carbohydrates: In the plants submitted to 9 days of water stress, a significant increase of 97.3% in free proline was observed (Fig. 2A). It was seen that in the period between 3 and 6 days a larger variation, 43% increase, occurred in the proline while under water stress. Similar results were observed by Costa (1999) investigating *Vigna unguiculata*.

Carbohydrate levels suffered a significant increase of 94.2% in the plants submitted to 9 days of water stress (Fig. 2B), in which 48% of the increase was observed in the period of 3 to 6 days under water restriction; however the plant carbohydrate levels under the control treatment remained stable.

Amino acids and proteins: A significant elevation of 78.4% in the levels of free amino acids occurred under

9 days of water restriction, when the major period increased from 3 to 6 days with 36.3%. However, the control treatment showed stable levels of amino acids (Fig. 3A). These results corroborate with the studies by Silveira *et al.* (2001) with *Vigna unguiculata* under saline stress and Pimentel (1999) on the behavior of *Zea mays* under water stress.

Significant reduction, 47.3%, in the levels of total soluble proteins under 9 days of water stress, as well as the period between 6 and 9 days showed a major drop and contributed with 24.3% of the total reduction. The control maintained little variation in the protein levels (Fig. 3B).

DISCUSSION

The drop in the leaf relative water content is a result of low water availability in the soil, where the osmosis in the environment was progressively becoming negative, causing many biochemical and physiological alterations which aimed at decreasing the plant water loss to the environment during transpiration, to maintain the metabolic function and to adjust the species osmotically (Kerbauy, 2004).

The nitrate reductase enzyme activity decreases due to a drop in nitrate absorption carried out by plants under water stress; hence the inorganic nitrogen is assimilated by the root system using water as a vehicle (Larsson, 1992). Nitrate reductase is considered an excellent physiological indicator, since it is the first enzyme in the nitrogen metabolism, as well as its activity being regulated by NO_3^- and glutamine, which is the final product of the metabolic nitrogen pathway (Foyer *et al.*, 1998; Ferrario-Méry *et al.*, 1998). These results reveal that the plants under water stress assimilate small quantities of nitrogen (Crawford, 1995). Under normal conditions the nitrate is used to form proteins, nucleic acids and other cellular components, this being the biggest source of nitrogen for vascular plants (Ezzine and Ghorbel, 2006).

The increase of free proline in this experiment is attributed to the osmotic adjustment (Verslues *et al.*, 2006), in which *V. unguiculata*, under water stress, has low hydric potential and in reply elevated the levels of proline aiming at tolerating the abnormal situation to which it is submitted (Vendruscolo *et al.*, 2007). Since proline is an organic solute, it accumulates in the cytoplasm, protecting cellular structures and macromolecules from denaturation (Vanrensburg *et al.*, 1993), aside from being a source of energy (Serrano and Gaxiola, 1994). On the other hand, the plants in the control treatment maintained the levels of proline low and stable, because free proline is easily converted into glutamic acid and other components, under the normal irrigation conditions (Sarker *et al.*, 1999).

The total soluble carbohydrates were progressively accumulated in the plant leaves during water stress, due, initially, to the hydric restriction. This provoked a reduction in the quantity of starch by the amylase enzyme activity and increase in the stress level (Chaves Filho and Stacciarini-Seraphin, 2001), probably occurring due to the accumulation of other soluble carbohydrates, principally fructose, triose and glucose, besides sacarose in lower quantity compared to the other sugars, being responsible of elevating this variable even more (Lawlor and Cornic, 2002).

The decrease observed in soluble proteins occurred primarily due to the paralyzation of protein biosynthesis (Kramer and Boyer, 1995) and as the period under water restrictions increases, a proteolytic degradation of proteins occurs (Roy-Macauley *et al.*, 1992) decreasing, even further, the protein levels. It has to be taken into consideration that between the proteins present in plants, rubisco (ribulose 1,5 bisphosphate carboxilase-oxigenase) is the most important, because it composes more than 50% of the leaf cellular protein and constitutes the biggest nitrogen reserve in plant tissues, aside from being fundamental in the photosynthesis process (Lawlor, 2002). Studies conducted by Flexas *et al.* (2006) indicate that the activity regulation of this protein is correlated with the stomatal conductance, being observed an accentuated drop plant activity under water stress caused by higher stomatal resistance.

An increase in free amino acids was observed, where the increase was attributed to the damages caused by the water deficit, which initially promoted the increase of this metabolic provoked by protein degradation by proteases. With the increase in water stress severity, a degradation of the enzymatic systems occurred, where it was necessary to synthesize the amino acids again, which made the amino acid concentration in the leaves even more elevated with the objective of osmotically adjusting the plant under hydric deficit conditions (Yordanov *et al.*, 2000; Hoekstra *et al.*, 2001).

This study revealed that experiments aiming to obtain physiological changes in *V. unguiculata* can be carried out in only a few days. However, the biochemical responses should be evaluated starting at 4 days under water restriction.

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