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Responses of the Photosynthetic Pigments and Carbon Metabolism in *Vigna unguiculata* Cultivars Submitted to Water Deficit

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Abstract: The aim of this study was to evaluate the changes provoked by the water deficit on photosynthetic pigments and carbon metabolism in 2 *Vigna unguiculata* cultivars, besides indicating, which cultivar is more tolerant under water deficiency. The experimental design used was carried out at entirely randomized in factorial scheme, with 2 cultivars (Pitiuba and Pérola) and 2 water regimes (control and stress). The parameters evaluated were leaf relative water content, leaf number, chlorophylls a, b, total and carotenoids, as well as total soluble carbohydrates and sucrose. The decreases of total chlorophylls in Pitiuba and Pérola cultivars were at 20.7 and 34.1%, respectively. The total soluble carbohydrates levels in Pitiuba and Pérola cultivars presented significant increases at 67.6 and 42.8%, respectively. The study revealed that Pitiuba cultivar presents higher tolerance the water deficiency, because this cultivar had smaller variations in leaf relative water content, leaf number and pigments. In addition, it were showed that total soluble carbohydrates and sucrose suffer changes more intense in Pitiuba, when compared with Pérola, in which indicates that the mechanism of osmotic adjustment in Pitiuba is more efficient.

Key words: *Vigna unguiculata* (L.) Walp., water deficiency, chlorophylls, carbohydrates, pigments, tolerant

INTRODUCTION

The species *Vigna unguiculata* (L.) Walp. has as main characteristics to be highly rustic (Teofilo *et al*., 2001), greater protein content in grain, as well as it is a crop very used in Brazil and on the world in agricultural areas under influences of abiotic stresses (Islam *et al*., 2008; Lobato *et al*., 2006), in which these areas frequently present small rain index and high temperature. Besides of this, it is indicated to areas that the soil is susceptible at salinity or with fertility loss (Lobato *et al*., 2008a; Silveira *et al*., 2001).

The photosynthetic pigments are formed by chlorophylls and carotenoids, which it are responsibles to photo-reception process of light in the specify wavelengths (Gandul-Rojas *et al*., 2004). Therefore, the changes promoted in these structures can be limiting factors for growth, development and yield of the plant due the photosynthesis process to be depending on light capture.

Carbon metabolism is composed by several sugars as sucrose, glucose, fructose and starch, in which it are organic compounds found in higher amounts in the plants (Taiz and Zeiger, 1998). These sugars can carry out biochemical functions as transport, signalization and reserve plant (Pimentel, 2004). The accumulation of carbohydrates under conditions of abiotic stress is described by Lobato *et al*. (2008b).

The aim of this study was to evaluate the changes provoked by the water deficit on photosynthetic pigments and carbon metabolism in 2 *Vigna unguiculata* cultivars, as well as indicating, which cultivar is more tolerant under water deficiency.

MATERIALS AND METHODS

Growth conditions: The study was carried out in the Instituto de Ciências Agrária (ICA) of the Universidade Federal Rural da Amazônia (UFRA), Belém city, Pará state, Northern region, Brasil (01°27'S and 48°26'W) in the period of June and July of 2007. The plants remained in glasshouse environment under natural conditions day/night (air temperature minimum/maximum and relative humidity of 24.1/38.2°C and 72/89%, respectively. The photoperiod medium was of 12 h of light and photosynthesis radiation active maximum of 580 μ mol/m^2/sec (at 12:00 h).

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Plant materials: The *Vigna unguiculata* (L.) Walp. seeds of the Pitiuba and Pérola cultivars used in this study were harvested in the 2006 season and coming from Empresa Brasileira de Pesquisa Agropecuária/Meio Norte (Embrapa), estado Piauí, Brasil.

Substrate and pot: The substrate used to the plant growth was composed by sand and silic in the proportion of 2:1, respectively, as well as this substrate was autoclaved at 120°C atm^{-1} by 40 min. The container used to the plant growing was Leonard pot with 2 L capacity and it was adapted in the Laboratório de Fisiologia Vegetal Avançada (LFVA).

Experimental design and water regimes: The experimental design used was entirely randomized in factorial scheme, with 2 cultivars (Pitiuba and Pérola) and 2 water regimes (control and stress), with 10 repetitions and 40 experimental units, in which each experimental unit was constituted by 1 plant pot^{-1}.

Conduction plant: Three seeds per pot were sowed and singled after germination. The control and stress treatments received macro and micro nutrients in the form of nutritive solution of Hoagland and Arnon (1950), by the period of 30 days, as well as the nutritive solution were changes with 2 days of interval, always at 09:00 h and the pH of the nutritive solution was adjusted to 6.0±0.1 with addition of HCl or NaOH. In the 30th day after the experiment implementation, the plants of the treatment under stress were submitted to period of 5 days without nutritive solution, in which the stress was simulated in the 30 until 35th day after of the experiment start. After this period, the plants were physiologically and biochemically analysed.

Leaf number and Leaf relative water content: The leaf number was evaluated through counting only of definite leafs, as well as the leaf relative water content was evaluated with leaf disks with 10 mm of diameter and it was carried out in each plant, in which were removed 40 disks and the calculation in agreement with the formula proposed by Slavick (1979):

$$\text{LRWC} = \frac{(\text{MF-MS})}{(\text{MT-MS})} \times 100$$

where:

MF = Matter Fresh

MT = Matter Turgid evaluated after 24 h and saturation in deionized water at 4°C in dark

MS = The dry matter determined after 48 h in oven with forced air circulation at 80°C

Chlorophyll contents: The determination of the photosynthetic pigments was carried out with 25 mg of leaf tissue, which the samples were homogenized in the dark and in the presence of 2 mL of acetone at 80% (Nuclear). Subsequently the homogenized was centrifuged at 5.000 g, by 10 min in the temperature of 5°C, in which the supernatant was removed and quantified the chlorophylls a, b, carotenoids and total using spectrophotometer Femto (700 S), in agreement with the methodology of Lichthenthaler (1987).

Biochemical analysis: The plants were harvested and placed in oven with forced air circulation at 70°C by 96 h. The leaf dry matter was triturated and the powder was kept in glass containers, in which it were remained in the dark and under the temperature of 15°C until the moment to carry out the biochemical analysis.

Total soluble carbohydrates: The total soluble carbohydrates were determined with 50 mg of powder coming from leaf dry matter, which it was incubated with 5 mL of ultra pure water at 100°C by 30 min, subsequently the homogenized was centrifuged at 2.000 g, by 5 min at 20°C and the supernatant was removed. The quantification of the total soluble carbohydrates were carried out at 490 nm, in agreement with the method of Dubois *et al.* (1956), as well as was utilized glucose (Sigma chemicals) as standard.

Sucrose: The determination of sucrose was carried out with 50 mg of powder coming from leaf dry matter, in which it was incubated with 1.5 mL of solution MCW (Methanol, Chloroform and Water), in the proportion 12:5:3 ($v\ v^{-1}$) at 20°C by 30 min and under agitation, subsequently the homogenized was centrifuged at 10.000 g by 10 min at 20°C and the supernatant was removed. The sucrose quantification was carried out at 620 nm, in agreement with Van Handel (1968), as well as was used sucrose (Sigma chemicals) as standard.

Data analysis: The data were submitted at variance analysis and when significant diferences occurred were applied to Tukey test at 5% level of error probability, as well as the standard errors were calculated in all evaluated points (Gomes, 2000). The statistical analysis were carried out with the software (SAS Institute, 1996).

RESULTS

Leaf relative water content and leaf number: The leaf relative water content in both cultivars presented similar behavior, which the Pitiuba cultivar presented 90 and 68% in control and stress plants, respectively (Fig. 1a). In addition, in the Pérola cultivar was showed in control and stress treatments the values at 82 and 53%, respectively. The results reveal that occurred reductions at 24.4 and 35.4% in Pitiuba and Pérola cultivars, respectively.

The leaf number in Pitiuba cultivar was significantly affected, which the control and stress treatments presented 27 and 17 leafs, respectively (Fig. 1b). The Pérola cultivar presented in control and stress plants the values 18 and 12 leafs, respectively. The study revealed that control plants of Pitiuba and Pérola cultivars presented increases at 58.8 and 50%, respectively, if compared with the plants under water stress.

Chlorophylls a and b: The chlorophyll a amounts present in control and stress plants of Pitiuba were 1.50 and 1.16 g/Kg/FM, respectively. Whereas, in Pérola were showed the amounts of 1.49 and 0.96 g/kg/FM in the control and stress treatments, respectively (Fig. 2a). These results reveal that occur significant decreases in 2 cultivars, in which Pitiuba and Pérola presented decreases at 22.7 and 35.6%, respectively.

The chlorophyll b levels in both cultivars suffered significant decreases, which Pitiuba presented 1.35 and 1.10 g/kg/FM in control and stress treatments, respectively, as well as it was showed total reduction at 18.5% after the application of the water deficit (Fig. 2b). The Pérola cultivar presented decrease at 37% in this parameter and revealed that the drop was more intense, when compared with Pitiuba. In addition, the control and stress treatments of Pérola presented 1.27 and 0.80 kg FM^{-1}, respectively.

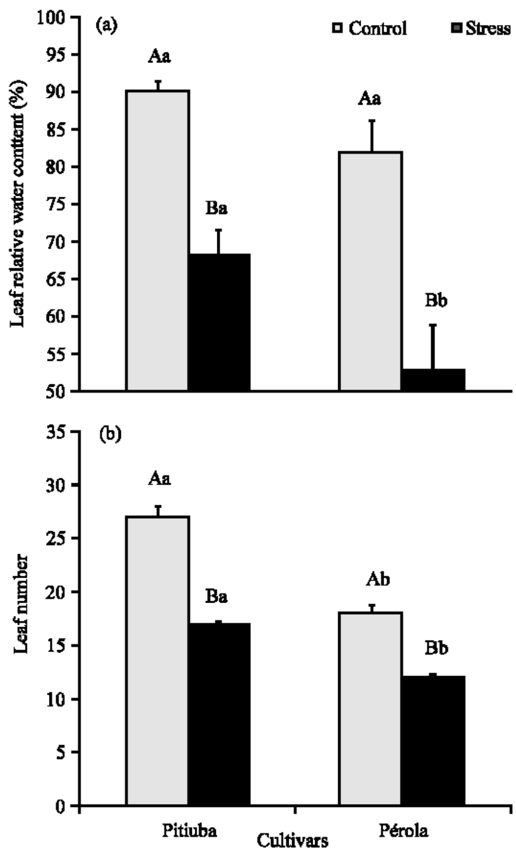

Fig. 1: Leaf relative water content (a) and leaf number (b) in *Vigna unguiculata* plants cultivars Pitiuba and Pérola submitted to 5 days of water restriction. Averages followed by the same uppercase letter within of the cultivars and lowercase letter among the treatments, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error

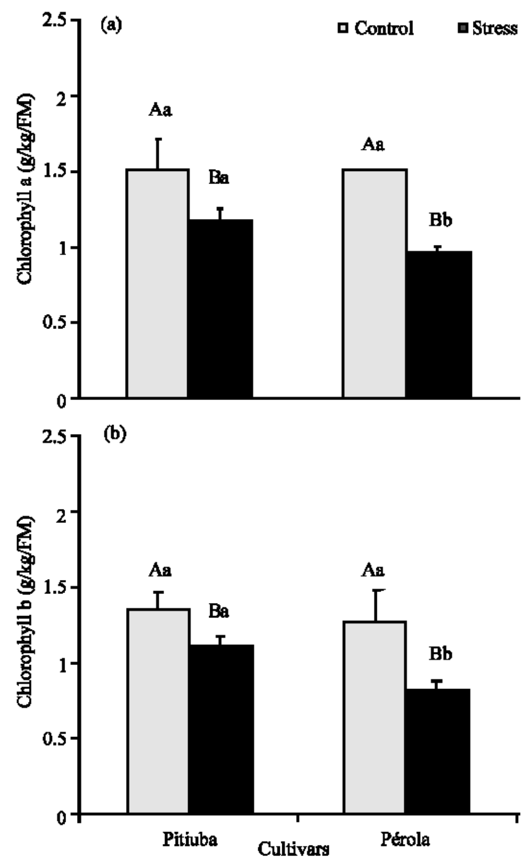

Fig. 2: Chlorophyll a (a) chlorophyll b (b) in *Vigna unguiculata* plants cultivars Pitiuba and Pérola submitted to 5 days of water restriction. Averages followed by the same uppercase letter within of the cultivars and lowercase letter among the treatments, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error

Carotenoids and total chrolophyll: The carotenoids level was influenced by the water restriction, in which Pitiuba presented 0.47 and 0.26 g/kg/FM in control and stress plants, respectively, besides to present reduction at 44.7% (Fig. 3a). In Pérola cultivar were showed in control and deficit treatments the carotenoids amounts of 1.48 and 0.48 g/kg/FM, respectively, as well as the decrease at 67.6%. Despite decrease in both cultivars, the Pérola had reduction more intense, if compared with Pitiuba.

The total chlorophyll amounts in Pitiuba cultivar were 2.85 and 2.26 g/kg/FM in control and stress plants, respectively (Fig. 3b), besides to reveal significant decrease at 20.7%. The Pérola cultivar had significant reduction at 34.1%, in which it were showed 2.73 and 1.80 g/kg/FM in control and affected plants, respectively. These results demonstrate that in normal conditions and under water deficit the Pitiuba cultivar has higher amount of total chlorophyll, when compared with Pérola, besides to reveal that Pitiuba is more adapted for environments under water deficiency.

Total carbohydrates and sucrose: The total soluble carbohydrates level presents in Pitiuba cultivar in the control and stress treatments were 1.48 and 2.48 g/kg/DM, respectively (Fig. 4a). In addition, this result reveals that plants under stress suffer significant increase at 67.6%. The Pérola cultivar had significant increase at 42.8%, in which it were showed 1.12 and 1.60 g/kg/DM in control and stress plants, respectively. Both cultivars presented significant increase. However, the Pérola presented smaller variation, if compared with Pitiuba.

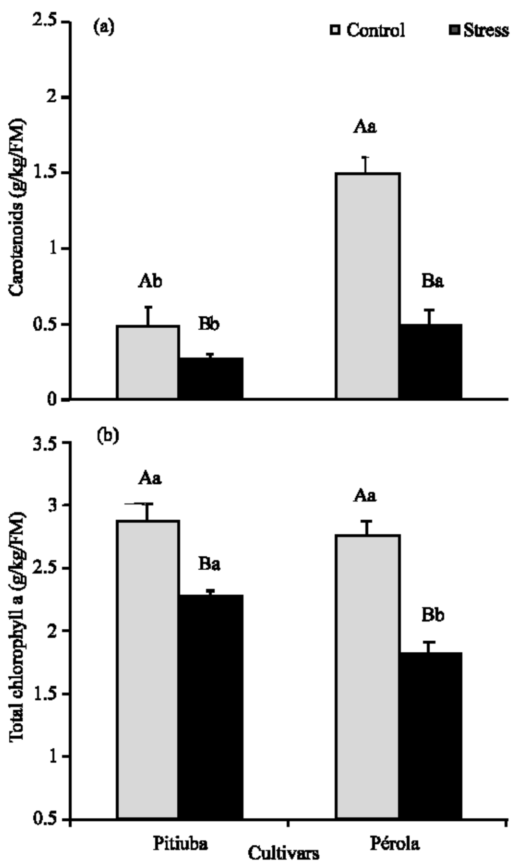

Fig. 3: Carotenoids (a) and total chlorophyll (b) in *Vigna unguiculata* plants cultivars Pitiuba and Pérola submitted to 5 days of water restriction. Averages followed by the same uppercase letter within of the cultivars and lowercase letter among the treatments, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error

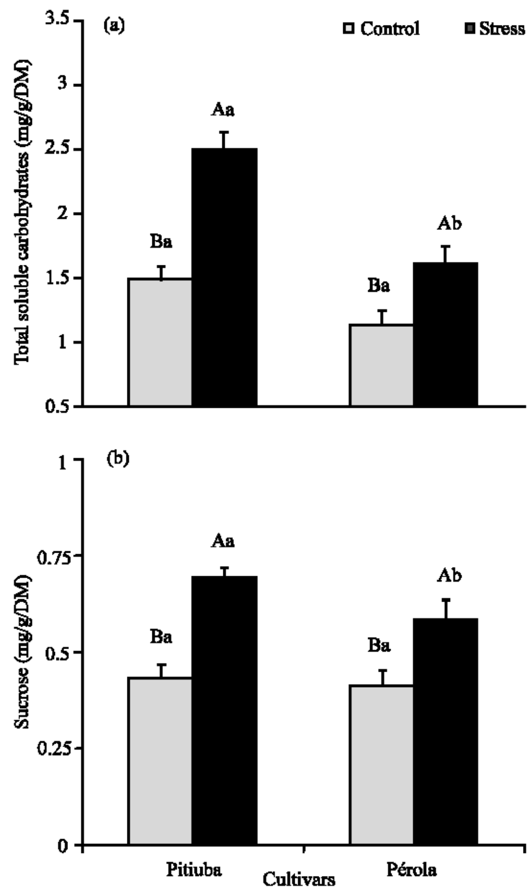

Fig. 4: Total soluble carbohydrates (a-b) in *Vigna unguiculata* plants cultivars Pitiuba and Pérola submitted to 5 days of water restriction. Averages followed by the same uppercase letter within of the cultivars and lowercase letter among the treatments, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error

The sucrose level in Pitiuba suffered significant increase at 60.5%, which the control and stress plants presented 0.43 and 0.69 g/kg/DM, respectively. The Pérola cultivar presented significant increase at 41.5%, as well as the values obtained were 0.41 and 0.58 g/kg/DM in control and stress treatments, respectively (Fig. 4b). These results reveal that Pérola had minor variation, when compared with Pitiuba.

DISCUSSION

The decrease showed in leaf relative water content of both cultivars is due the 2 simultaneous factors, which the primary factor is the lower rate of water absorption into substrate to the plant using as via the root, during the period under water stress. In addition, the gas exchanges through of the stomatal promote the water loss of the plant to environment during the photosynthesis and respiration processes. These results prove that the period evaluated was sufficient to promote significantly reduction in both cultivars, as well as it reveals that the Pitiuba cultivar suffered minors changes in this parameter. Results similars were reported by Silveira *et al.* (2001) investigating water deficit in *Vigna unguiculata* plants.

The smaller leaf number showed in the 2 cultivars under water deficit was promoted by the minor water amount presents in the leaf and probably into plant, as it can be proved by the reduction of the leaf relative water content. The water volume into cell is responsible of indirect form by the growth and development plant, in which the water (H_2O) actives expansin enzymes that promote the extension of the cell wall and consequent increase of the cell volume/size (Kerbauy, 2004). Similar results on smaller leaf number were found by Lobato *et al.* (2008c) working with *Glycine max* plants submitted to water deficit.

The lower amount of chlorophylls a, b and total in plants under water stress of the 2 cultivars demonstrate that these pigments are affected by stress applied, as well as the decrease of the chlorophylls is due the peroxidative enzymatic activity, in which promotes damages in the thylakoid membranes (Gandul-Rojas *et al.*, 2004). Besides of this, it there be as consequence the reduction of the light absorption and indirectly lower plant photosynthesis (Santos and Carlesso, 1998). Similar results on decrease of the chlorophylls a and b were obtained by Mohammadkhani and Heidari (2007) investigating the effects of the water deficit in 2 maize cultivars.

The changes showed in carotenoids levels of both cultivars indicate that these pigments also were affected by the water deficiency. The carotenoids are photosynthetic pigments present within chloroplasts, in which a function is the photo-system protection due the occurrence of reactive oxygen species during the photosynthesis (Ledford and Niyogi, 2005), as well as the stabilization of lipid membranes (Havaux, 1998). Younis *et al.* (2000) working with 2 cultivars of *Sorghum bicolor* submitted to water deficit shown not significant changes in carotenoids levels.

The increase showed in the amount of total soluble carbohydrates is a classical response of the plants when submitted to water deficit, because these organic compounds can be utilized in situations of soft and moderate stresses as osmotic adjustments (Rejskova *et al.*, 2007). In addition, under severe stress are used as storage/reserve (Pimentel, 2004). Costa *et al.* (2008) studying 2 cultivars of *Vigna unguiculata* obtained increase in total soluble carbohydrates levels. Study conducted by Li and Li (2007) revealed similar results on carbohydrates accumulation in *Malus domestica* plants under water deficiency.

The sucrose levels in both cultivars presented significant increases, as well as this study revealed that sucrose was an of the sugars responsibles by the changes in total soluble carbohydrates. The sucrose is an important molecule into carbon metabolism, in which can act in the signalization and consequently regulation under situations of abiotic and biotic stresses (Roitsch, 1999). Studies carried out by Lobato *et al.* (2008d) on effect of the *Colletotrichum lindemuthianum* pathogen in *Phaseolus vulgaris* plants revealed modifications in sucrose.

CONCLUSION

The study revealed that the Pitiuba cultivar presents higher tolerance the water deficiency, because the leaf relative water content, leaf number, chlorophylls a, b and total, as well as carotenoids suffer smaller changes, if compared with Pérola cultivar. In addition, it were showed that the total soluble carbohydrates and sucrose suffer changes more intense in Pitiuba, when compared with Pérola, in which indicates that the mechanism of osmotic adjustment in Pitiuba is more efficient.

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