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Consequences of the Progressive Water Deficit and Rehydration on Nitrate Reductase Activity and Nitrogen Compounds in Soybean (*Glycine max* cv. Sambaiba)

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Abstract: The experiment had the aim of investigate the responses provoked by the progressive water deficit and evaluate as the rehydration can to contribute to the recovery of the leaf relative water content, plant water content, nitrate reductase enzyme activity, as well as changes in the nitrogen compounds as free ammonium, total soluble amino acids and total soluble proteins in *Glycine max* (L.) Merril cultivar Sambaiba. The experimental design was at randomized entirely factorial, with 2 water conditions (control and stress/ rehydration) and 5 evaluation points (0, 2, 4, 6th day under stress and 7th day under rehydration). The nitrate reductase activity had accumulated reduction of 70% after 6 days of water deficit and the rehydration promoted increase of 96% in this parameter. The total soluble proteins were reduced at 20.7% in the 6th day of water restriction and after the rehydration were showed increase of 43% in this variable. The results showed with this cultivar submitted the water restriction reveal that occurr significant reductions in the leaf relative water content, plant water content, nitrate reductase enzyme activity, free ammonium and total soluble proteins, however, was showed increase in the free amino acids levels. The leaf relative water content, nitrate reductase enzyme activity and total soluble proteins were recovery after the rehydration.

Key words: Soybean, water stress, rehydration, nitrogen

INTRODUCTION

The soybean (*Glycine max* (L.) Merril) is one of the more importants food crops in the world, because is protein source in the human food and has been utilized as animal ration (Lobato *et al*., 2008a), being this species more sensitive to abiotic stresses (Van Heerden and Krüger, 2000), that others with *Vigna unguiculata*, *Phaseolus vulgaris*, *Sorghum bicolor* and *Gossypium hirsutum* (Roy-Macauley *et al*., 1992; Silveira *et al*., 2003; Inamullad and Isoda, 2005; Younis *et al*., 2000).

The stress is defined as an external factor that practice negative influence on plant, this way the water deficit is normally attributed the situations that the water loss occur due the transpiration, in which exceed the absorption rate at intensity enough for to cause changes in several physiologic and biochemical process that can negatively to affect the growth, development, bloom, property grains and consequently the yield (Pimentel, 2004).

The nutritional supplement is fundamental for to reach maximum productivity (Costa, 1999), in which the nitrogen (N) is normally the mineral element more required by the plants (Maman *et al*., 1999), being absorbed than as ammonium (NH_4^+) that nitrate (NO_3-) (Marschner, 1995) in which the production of metabolics as nucleotides, amino acids, enzymes and proteins is dependent these nitrogen forms (Nelson and Cox, 2000).

The nitrate reductase is the first enzyme of the nitrogen metabolism, being extremely sensitive to water stress and is utilized as accurate physiologic indicator (Oliveira *et al*., 2005), as well as has the capacity of to catalyze the nitrate reduction (NO_3-) to nitrite (NO_2-) esides normally to be found in leaf and root (Brewitz *et al*., 1996).

The experiment had the aim of to investigate the responses provoked by the progressive water deficit and to evaluate as the rehydration can to contribute to the recovery of the leaf relative water content, plant water content, nitrate reductase enzyme activity, free ammonium, total soluble amino acids and total soluble proteins in *Glycine max* (L.) Merril plants cultivar sambaiba.

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MATERIALS AND METHODS

Growth conditions and plant material: The experiment was carried out in greenhouse 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 photosynthetical radiation of 623 μmol m^{-2} s^{-1} (at 12:00 h), located at the Instituto de Ciências Agrárias (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.

It was used seed of *Glycine max* (L.) Merril of cultivar sambaiba collected in the 2006 season, from city of Paragominas, state Pará, Brazil (03°00'S and 47°21'W) and stored until carrying out of the experiment. The substrate utilized to the plant growth and evaluation was composed by black potting soil and sand at 3:1 ratio, respectively. The plants grown in pots with 6 L capacity, being placed three seeds into each pot, in which the plants were thinned in the 7th day after the experiment implantation and was remained 1 plant pot^{-1}.

Experiment design and treatments: The experimental design was at randomized entirely factorial, with 2 hydric conditions (control and stress/rehydration) and 5 evaluation points (0, 2, 4, 6th day under stress and 7th day under rehydration), with 8 repetitions, being utilized 64 experimental units, as well as each experimental unit was composed of 1 plant pot^{-1}.

The plants of both treatments (control and stress/rehydration) remained in greenhouse during 40 days, being watered daily at 09:00 h with water until to reach the field capacity, besides received macro and micronutrients every 5 days, using the nutritive solution of Hoagland and Arnon (1950). Starting 40th day after the implementation of the experiment, the plants from the treatment under stress/rehydration were submitted to the period of 6 days without irrigation and 1 day de rehydration, simulating the water stress of 40-46th day and rehydration of 46-47th day, after the beginning of experiment. The plants were take away to the Laboratório de Fisiologia Vegetal Avançada of Universidade Federal Rural da Amazônia (UFRA) for measure the physiologic and biochemical parameters.

Measurements: The Leaf Relative Water Content (LRWC) was measured utilizing disk leaf with 10 mm of diameter, being calculated as: LRWC = [(FW-DW)/(TW-DW)] ×100, where FW is the fresh weight, TW is the turgid weight measured after 24 h of saturation on distilled 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). The evaluation of the nitrate reductase enzyme activity in the leaf, were removed disks with 0.5 of area of fresh leaf and the quantification was carried out at 540 nm (Hageman and Hucklesby, 1971). The plant water content was calculated with the difference among the plant fresh matter and plant dry matter (Romero-Aranda *et al.*, 2006), being the plants submitted at 65°C by 72 h for to get the plant dry matter. The leaves were harvesteds and triturates, being utilized the powder for biochemical extraction and quantification of the parameters at spectrophotometer. It were determined the ammonium levels at 625 nm (Weatherburn, 1967), total soluble amino acids at 570 nm (Peoples *et al.*, 1989) and total soluble proteins at 595 nm (Bradford, 1976), being carried out the measurements with spectrophotometer (Quimis, model Q798DP).

Data analysis: The standard error were calculated for each point, it being applied the variance analysis in the results and the averages of the treatments were compared following Tukey test at the 5% significance level, using SAS Institute (1996) and based on statistical theories by Gomes (2000).

RESULTS

Leaf relative water content and plant water content: The Leaf Relative Water Conten (LRWC) of the plants under water deficit had significant reduction, with progressive drop of 81.5 until 60.9% of 0-6th day, respectively. Moreover, was possible to show (Fig. 1a) that occurred significant difference only in the 2, 4 and 6 days after the beginning of the water stress, comparing with the hydric conditions of the control plants. However, after the rehydration the LRWC increase of 60.9-83.2%, during the period among 6 and 7th day, revealing that occurred recovery of LRWC, compared with control plants (irrigateds) and in agreement with variance analysis.

Occurred significant difference in the Plant Water Content (PWC), according the variance analysis, in which the experiment reveal increases in the PWC of the plants kept under irrigation (control) of 6.6, 20.8, 56 and 15.4% in the 2, 4, 6 and 7th day, respectively. The plants under water deficit had increases of 5.6 and 0.7% in the 2nd and 4th day of water restriction, respectively, however in the 6th day occurred reduction of 0.2% in the PWC, being different statistically the treatments to 4, 6 and 7 days (Fig. 1b). The rehydration of the plants under stress make possible the increase of 26.9% in the PWC, but this increase not carried out the recovery of this parameter, according the variance analysis and showing the control plants.

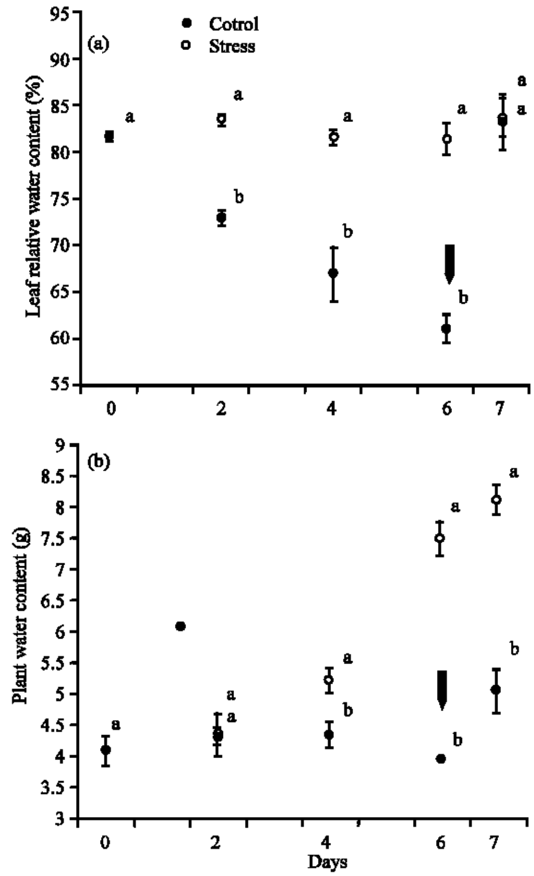

Fig. 1: Leaf relative water content (a) and plant water content (b) in plants of *Glycine max* cultivar sambaiba in the 0, 2, 4, 6th day under water deficit and in the 7th day under rehydration. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability. The arrow represents the point of rehydration and the bars represent the mean standard error

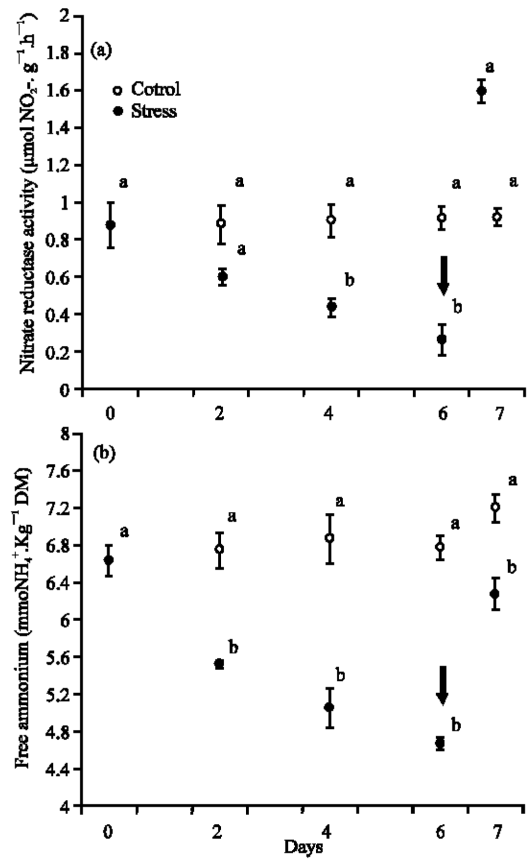

Fig. 2: Nitrate reductase activity (a) and free ammonium (b) in plants of *Glycine max* cultivar sambaiba in the 0, 2, 4, 6th day under water deficit and in the 7th day under rehydration. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability. The arrow represents the point of rehydration and the bars represent the mean standard error

Nitrate reductase and free ammonium: The Nitrate Reductase Enzyme Activity (NREA) was affected by the progressive water deficit in the plants under water stress, as well as the control and stress treatments are different statistically, in which the plants of the control treatment keep normal and stable levels of the NREA, however, the plants of the treatment under stress had activity reduced, with can to be showed in the Fig. 2a, being the reductions of 32.1, 18.3 and 19.6% of the NREA in the points relating to 2, 4 and 6 days, respectively, besides accumulated reduction of 70% after 6 days of water deficit. The rehydration effect make with the this parameter had increase of 96%, as well as after of 24 h of the rehydration to be carried out, the NREA level of the rehydration plants was above of control plant level, reveal that this species has the capacity of at period short to promote the recovery of the activity of this enzyme.

It was showed significant difference in the free ammonium, as well as the hydric deficit of 2 days was capable of to provoke decrease significant of free ammonium (Fig. 2b), in which the control plants fluctuates between 6.6 and 7.2 mmol NH_4^+ Kg^{-1} DM, however the plants submitteds to progressive stress and rehydration had total reduction of 30% in the free ammonium levels during the 6 days of water stress and after the rehydration occurred increase of 5.4%, revealing that the period of 24 h after the rehydration promote the increase of ammonium level, but was not sufficient for to keep the normal supplement of ammonium, remaining down and different statistically of control plant levels.

Total soluble amino acids and total soluble proteins: Occurred increase in the total soluble amino acids with the progress of water restriction. The accumulated increase

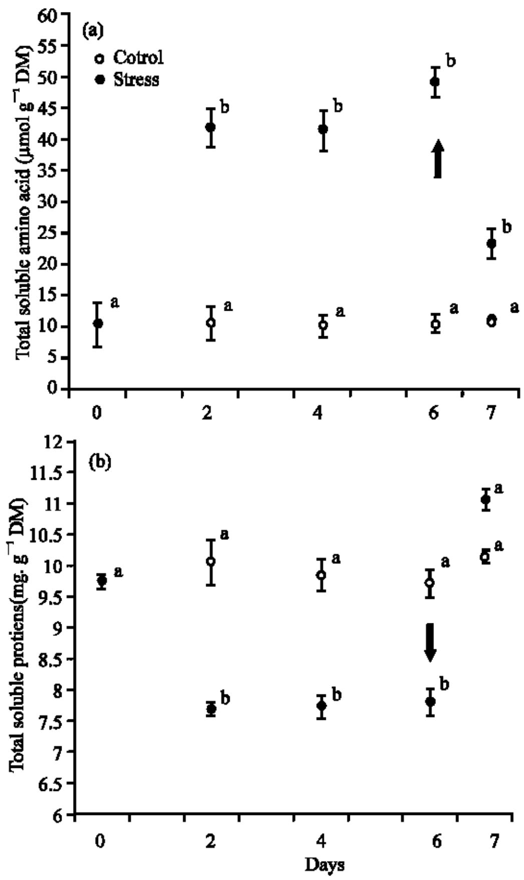

Fig. 3: Total soluble amino acids (a) and total soluble proteins (b) in plants of *Glycine max* cultivar sambaiba in the 0, 2, 4, 6th day under water deficit and in the 7th day under rehydration. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability. The arrow represents the point of rehydration and the bars represent the mean standard error

showed in the plants under water deficit was of 385%, when compared with the control treatment, being the control and stress treatments different statistically, in agreement with variance analysis, in the points corresponding to 2, 4 e 6 days of water restriction (Fig. 3a). The control plants had its total soluble amino acids levels fluctuating between 9.9 and 10.4 μmol g^{-1} DM, in the points of minor and higher levels, respectively. The rehydration make possible the drop of the amino acids levels of 49 until 23.2 μmol g^{-1} DM, relating to period of 6 and 7 days, in which this reduction not was sufficient for to equilibrate the total soluble amino acids concentration, showing the variance analysis and comparing with control treatment.

The total soluble proteins of the plants under water stress had significant reduction of 20.7% during the 6 days of water stress, occurring higher drop in this variable in the period between 0 and 2 days of water restriction (Fig. 3b). Moreover, the treatments were statistically differents of 2 until 6 days of water shortage. The plants keep under irrigation (control) had stables the total soluble protein levels, with fluctuations of 9.7 until 10.13 mg g^{-1} DM. After the rehydration was showed the increase of 3%, in which the rehydrated plants had the total soluble protein level more than of control treatment level, as well as the control and rehydration treatments were statistically similars.

DISCUSSION

The water deficit brought with direct consequence changes in the LRWC, dropping progressively this parameter, because with the transpiration and photosynthesis process occurr water loss through of the stomata and the reposition/assimilation rate not is sufficient for to keep the turgid leaves (Verslues *et al.*, 2006). The rehydration promote the recovery of the LRWC levels, reveal the high capacity of this species at to assimilate water and this way to return quickly the leaf turgescence, in which only 1 day demonstrate to be sufficient.

The reduction in the PWC of treatment under water deficit occurred due to water supplement small available in the substrate (Lobato *et al.*, 2008a). Moreover, was showed that the rehydration not recovery this parameter to control plant level, demonstrating that this period is not sufficient and consequently the possibility of to have significant increase in PWC. The PWC is dependent of the fresh and dry matter of plant, in which under water restriction the cell turgor pressure that promotes to plant tissue extension is small (Reddy *et al.*, 2003), promoting directly minor growth and amount of the dry matter, besides indirectly the small water amount in the tissues provoked smaller amount of fresh matter (Taiz and Zeiger, 1998). Similar results on the drop in the PWC was showed by Romero-Aranda *et al.* (2006) investigating the silicon effect in *Lycopersicon esculentum* plants under water stress.

The plants under water restriction suffers reduction in the NREA, moreover, this experiment prove the high capacity of this enzyme in to reestablish its activity, within of short period after the rehydration. The progressive drop in the NREA showed during the period under stress is due the degradation end inactivation of the enzyme nitrate reductase induced of the water deficit

(Foyer *et al.*, 1998.), whereas the quick recovery after the rehydration, occurred due to intense synthesis of protein responsible by the production of the mRNA of enzyme RN (Ferrario-Mery *et al.*, 1998). Similars results were showed by Marur *et al.* (2000) studying the effects of water defiict in *Grossypium hirsutum* plants, as well as occurred reduction in the enzyme activity in cultivars of *Vigna unguiculata* under water deficiency (Costa *et al.*, 2008).

The free ammonium of the plants under water stress were progressively reduced, reaching smaller level after 6 days under water restriction, in which this decrease occurred due the drop in the NREA and consequently assimilation of NO_3^-, as well as this study demonstrate the insufficiency of ammonium pathway in this species under water deficit, in which probably not assimilate amounts sufficient of NH_4^+ for to keep under normal levels this parameter. Several studies prove that plants submitted the abnormal conditions utilize the ammonium route for N assimilation (Frechilla *et al.*, 2002; Kant *et al.*, 2007), because the energy request for NO_3^- assimilation are of 20 ATP molecules, whereas the NH_4^+ assimilation are necessities only 5 ATP molecules, this way the plants get around the of nitrate route and reduce the energy expense. The rehydration promoted increase of amino acids levels, however in this period measured, only 1 day, it was insufficient for to equilibrate this variable, when compared with the control treatment.

The increase showed in the total soluble amino acids in the plants under water deficit occurred due the breakdown of the proteins, carried out by the proteases enzymes (Lobato *et al.*, 2008b) under adverses conditions of environment (Costa *et al.*, 2008). Moreover, occurred probably the increase of proline production, a nitrogen compound that has the capacity of to carried out the plant osmotic adjustment under conditions of water supplement low, reducing the effects of water deficit (Yadav *et al.*, 2005). After the rehydration the total soluble amino acids suffer reduction; however the period measured in this study not was sufficient for to keep this parameter under normal levels, when compared with the level of control plants (irrigateds). The results got in this parameter are similars with the found by Sankar *et al.* (2007) investigating *Abelmoschus esculentus*, as well as Kasturi Bai and Rajagobal (2000) working with *Cocos nucifera*, both researches on effects of the water restriction.

The progressive water restriction promoted drop in the total soluble proteins, because under this conditions occurr the paralyzation of the protein biosynthesis (Kramer and Boyer, 1995) and proteolysis degradation (Roy-Macauley *et al.*, 1992). However, under rehydration occurred recovery and the level of the rehydrated plant was higher the control treatment, due probably to increase of NREA, in which this increase maximized the assimilation and reduction nitrogen, promoting higher production of the amino acids and consequently of proteins. Studies conducted by Nejidat *et al.* (1997) with plants genetically modified of *Arabidopsis thaliana* over-expressing the enzyme nitrate reductase, had increase in the total soluble protein levels, besides to be showed increase in the sub-units that form the rubisco protein. Moreover, the investigation carried out by Lobato *et al.* (2008b) com *Vigna unguiculata* under water deficit corroborate the drop showed in the total soluble proteins of the plants submitted to water deficiency, however the increase in the total soluble protein levels after to be carried out the rehydration not agree with the results showed by Silveira *et al.* (2003) working with *Vigna unguiculata*, reveal that this parameter can to be studded again in others researches.

The results got with *Glycine max* cultivar sambaiba submitted to water deficit reveal that occurred significant reductions in the leaf relative water content, plant water content, nitrate reductase enzyme activity, free ammonium and total soluble proteins, however was showed increase in the total soluble amino acids levels. On the other hand, the leaf relative water content, nitrate reductase enzyme activity and total soluble proteins had recovery after the rehydration.

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