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Relationship Between Leaf Relative Water Content and Total Soluble Proteins in Soybean Exposed to Short Water Deficit

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Abstract: The aim of this study was to evaluate the impact of short water deficit on nitrate reductase activity and nitrogen contents in *Glycine max* plants (cv. Sambaiba), as well as to investigate the hypothesis that leaf relative water content exercises influence on total soluble proteins. Experimental design used was completely randomised, with 2 treatments (control and stress). Water deficiency promoted significant decrease on leaf relative water content in stress treatment. For nitrate reductase activity the period of 2 under water deficit provoked a reduction at 45% in stressed plants. In total soluble proteins the control and stress treatments presented 10.0 and 7.7 mg g^{-1} DM, respectively. The correlation analysis indicates that there is a positive and significant relationship between leaf relative water content and total soluble proteins ($r = 0.88$; $p<0.01$). This study revealed that *Glycine max* induced to short water deficiency presented significant decreases in leaf relative water content, nitrate reductase and total soluble proteins. Furthermore, total soluble amino acids also suffered significant disorders. In addition, the results obtained confirmed the hypothesis that leaf relative water content exercises influence on total soluble proteins.

Key words: *Glycine max*, nitrogen metabolism, nitrate reductase, proteins, water deficiency

INTRODUCTION

Environment inadequate conditions due to water (Oliveira *et al*., 2009), heat (Tan *et al*., 1999), salt (Martinez-Ballesta *et al*., 2004) and mineral stress (Monnet *et al*., 2001) can cause reductions in morphological and agronomical parameters, as well as disorders at physiological, biochemical and molecular levels (Lobato *et al*., 2009a).

Drought is a common component in agricultural regions due the irregular rainfalls and/or inadequate irrigation supply to the crop (Lobato *et al*., 2008a). Water deficiency can limit plant productive potential (Carlesso and Santos, 1998). In addition, water deficit during vegetative, reproductive and maturation periods result in lower growth and development rates (Lobato *et al*., 2008b), flower abortion (Pimentel, 2004) and decreased grain yield (Leport *et al*., 1998), respectively.

Nitrogen (N) is frequently considered the more important into essential elements, due participation in biosynthesis of molecules such as ATP, NADH and NADPH, as well as it contributes directly in formation of proteins, cytochrome, chlorophylls and several enzymes (Bredemeier and Mundstock, 2000) that will be used in fundamental processes to growth and development plant (Andrade *et al*., 2003).

Studies conducted by Ribas-Carbo *et al*. (2005) with *Glycine max* grown in differents water regimes reveals changes in gas exchanges such as stomatal conductance and photosynthetic rate. In addition, reductions in leaf and stomatal areas were reported by Inamullah and Isoda (2005) investigating the water deficit effects in this species. Lobato *et al*. (2008c) working with *Glycine max* plants exposed to progressive water deficiency found strong decreases in several nitrogen compounds such as free ammonium and total soluble proteins.

The aim of this study was to evaluate the impact of short water deficit on nitrate reductase activity and nitrogen contents in *Glycine max* plants (cv. Sambaiba), as well as to investigate the hypothesis that leaf relative water content exercises influence on total soluble proteins.

MATERIALS AND METHODS

Plant material: *Glycine max* (L.) Merril (cv. Sambaiba) seeds were collected in the 2005/2006 season, from Paragominas city, Para state, Northern region, Brazil

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(03°00'S and 47°21'W) and stored until the experiment implementation. Seed treatment was carried out through immersion in a solution of Methyl benzimidazol-2-ylcarbamate at 3 M for 30 sec and drying in an oven with forced air circulation at 30°C for 120 h (Machado, 2000). Harvested and treated seeds were stored in hermetically closed bags and remained in the dark and at 10°C, in the seed bank of Universidade Federal Rural da Amazania (UFRA) under lot identification number (UFRA/2006-245), until the execution of this experiment.

Growth conditions: Study was implanted in Instituto de Ciencias Agraria (ICA) of Universidade Federal Rural da Amazonia (UFRA), Belem City, Para State, Brazil (012°27'S and 48°26'W), from September to October of 2006. Plants were grown under greenhouse environment, with natural conditions day/night (air temperature minimum/maximum and relative humidity of 22.4/37.6°C and 68/79%, respectively. The medium photoperiod was 12 h under light, with Photosynthetic Active Radiation (PAR) of 623 μmol/m^2/sec.

Substrate and pot: Substrate used for plant and development growth was composed of plantmax® and sand, which plantmax® is um substrate with pH adjusted and macro and micro nutrients in adequate amounts to this crop, in proportion of 2:1 (v:v), respectively. This mixture was autoclaved (120°C atm^{-1} for 40 min) and distributed over pots and each pot used 3 L of mixture.

Experimental design and treatments: Experimental design used was completely randomised, with 2 treatments (control and stress). This experiment used 6 replicates and 12 experimental units and each plant was considered one experimental unit.

Experimental conditions: Three seeds were placed per pot and the seedlings after 7 days were thinned out. Subsequently was kept only one plant per pot. All plants were watered daily with 400 mL of sterile distillated water. Treatments (control and stress) were watered as previously described, during the period of 40 days after experimental implementation. The stress treatment was exposed to water deficiency, for a period of 2 days without water supplementation. This water deficit was simulated from 40th until 42th day after sowing. All plants on the 42th day were harvested and evaluated.

Leaf relative water content: Leaf Relative Water Content (LRWC) was evaluated with leaf disks (10 mm diameter), on each plant. For each plant, 40 discs were removed in agreement with the formula proposed by Slavick (1979):

$$\text{LRWC (\%)} = \left[\frac{\text{(FM-DM)}}{\text{(TM-DM)}}\right] \times 100$$

Where:
FM = Fresh Matter
TM = Turgid Matter evaluated after 24 h and saturated in deionized water at 4°C in the dark
DM = Dry Matter determined after 48h in an oven with forced air circulation at 80°C

Nitrate reductase activity: Extraction of nitrate reductase enzyme (E.C. 1.6.6.1) was carried out with leaf disks until the weight of 200 mg was reached, the samples were incubated in 5 mL of extraction mix (0.1M KH_2PO_4, 50 mM KNO_3, isopropanol at 1% (v v^{-1}) and pH 7.5) for 30 min at 30°C and all the procedures were carried out in the dark. The quantification of the enzyme activity was made by the method of Hageman and Hucklesby (1971) with absorbance at 540 nm and using spectrophotometer (Quimis, model Q798DP), nitrite (Sigma Chemicals) was used as standard.

Plant dry matter and leaf powder: Plants were harvested, washed and placed in an oven with forced air circulation at 70°C for 96 h and subsequently was determined plant dry matter. Only leaf dry matter was triturated and leaf powder was kept in glass containers, which remained in the dark at 15°C, until biochemical analysis.

Amino acids and proline: In determination of amino acids was used 50 mg of leaf dry matter powder, which was incubated with 5 mL of sterile distilled water at 100°C for 30 min. After homogenization, it was centrifuged at 2.000 g for 5 min at 20°C and the supernatant was removed. Quantification of the total soluble amino acids was executed at 570 nm according to Peoples *et al.* (1989) and L-asparagine + L-glutamine (Sigma Chemicals) was used as a standard. Proline quantification was performed at 520 nm according to Bates *et al.* (1973), in which we used L-proline (Sigma Chemicals) as the standard.

Total soluble proteins: For determination of total soluble proteins was carried out with 100 mg of powder, in which it was incubated with 5 mL of extraction buffer (Tris-HCl at 25 mM and pH 7.6). The mixture was kept in agitation for 2 h, afterwards centrifuged at 2000 g for 10 min at 20°C and subsequently the supernatant was removed. The quantification of the total soluble proteins was carried out at 595 nm in agreement with Bradford (1976), albumin bovine (Sigma Chemicals) was used as standard.

Data analysis: Data were subjected to analysis of variance and means compared to F-test at 0.05 level of error probability. In addition, standard errors were calculated in all evaluated treatments (Steel *et al.*, 2006). Correlation analysis was performed by the Pearson parametric method and the statistical procedures were carried out using SAS.

RESULTS AND DISCUSSION

Consequences on leaf relative water content, plant dry matter and nitrate reductase activity: Water deficiency promoted significant decrease in leaf relative water content in stress treatment (Fig. 1a), which control and stress plants presented 83.4 and 72.8%, respectively. In addition, these results indicate that the experimental period of 2 days was sufficient to promote changes in leaf relative water content and consequently in other parameters.

In plant dry matter were not showed significant changes and the values showed in control and stress treatment were of 1.03 and 1.02 g, respectively (Fig. 1b).

For nitrate reductase activity the period of 2 under water deficit provoked statistical differences among treatments (Fig. 1c). In addition, in this parameter was showed lower activity in stress than control plants, as well as stress treatment had a reduction at 45%. In addition, the results showed in correlation analysis indicates that there is positive and linear interaction (Fig. 2a) between leaf relative water content and nitrate reductase activity ($r = 0.78$; $p<0.01$).

Changes in total soluble amino acids, proline and total soluble proteins: Total soluble amino acids suffered significant modifications after the water stress simulated in this study (Fig. 3a) and control and stress treatments presented the values of 10.2 and 41.9 μmol g^{-1} DM, respectively. Furthermore, correlation analysis presented a linear and negative interaction (Fig. 2b) between nitrate reductase activity and total soluble amino acids ($r = 0.76$; $p<0.01$).

Proline levels in control and stress treatments were statistically equals, but this nitrogen compound was lower in control than stress plants (Fig. 3b). The values of 2.6 and 2.7 μmol g^{-1} DM were obtained in watered and stressed plants, respectively.

In total soluble proteins the water restriction promoted significant disorders (Fig. 3c) and the control and stress plants presented 10.0 and 7.7 mg g^{-1} DM, respectively. In addition, this reduction in stressed plants is at 23%, in comparison with control plants. The correlation analysis (Fig. 4a) suggests that there is a positive and significant relationship between leaf relative water content and total soluble proteins ($r = 0.88$; $p<0.01$).

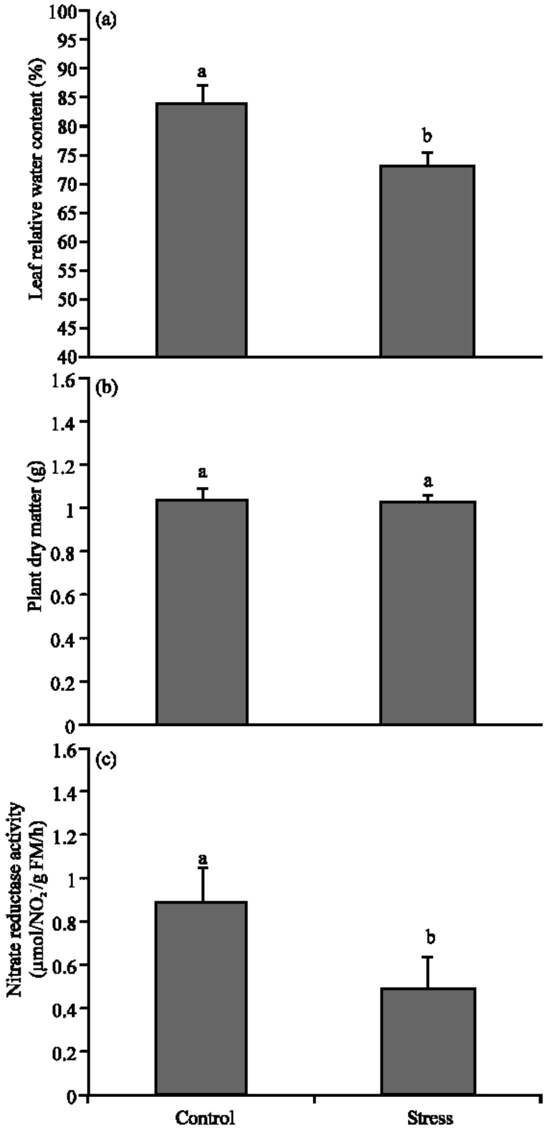

Fig. 1: (a) Leaf relative water content, (b) plant dry matter and (c) nitrate reductase activity in *Glycine max* cv. Sambaiba subjected to 2 days of water deficit. Averages followed by the same letter do not differ among themselves by the F-test at 0.05 level of error probability. The bars represent the standard error of the mean

Other result linked to correlation analysis, which indicate a linear and negative relationship (Fig. 4b) between protein soluble total and total soluble amino acids ($r = 0.95$; $p<0.01$).

Reduction in leaf relative water content of stressed plants is consequence of two factors, which the main factor was artificially water restriction, because it was removed water supplementation. In addition, the

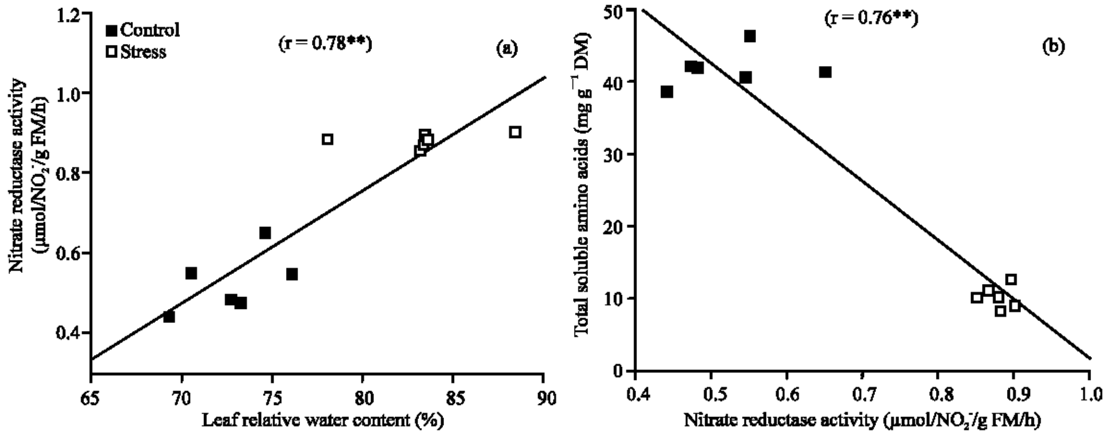

Fig. 2: (a) Relationships between leaf relative water content and nitrate reductase activity, (b) as well as nitrate reductase activity and total soluble amino acids in *Glycine max* cv. Sambaiba subjected to 2 days of water deficit. The bars represent the standard error of the mean and the asterisks (**) indicate the significance at 0.01 probability level

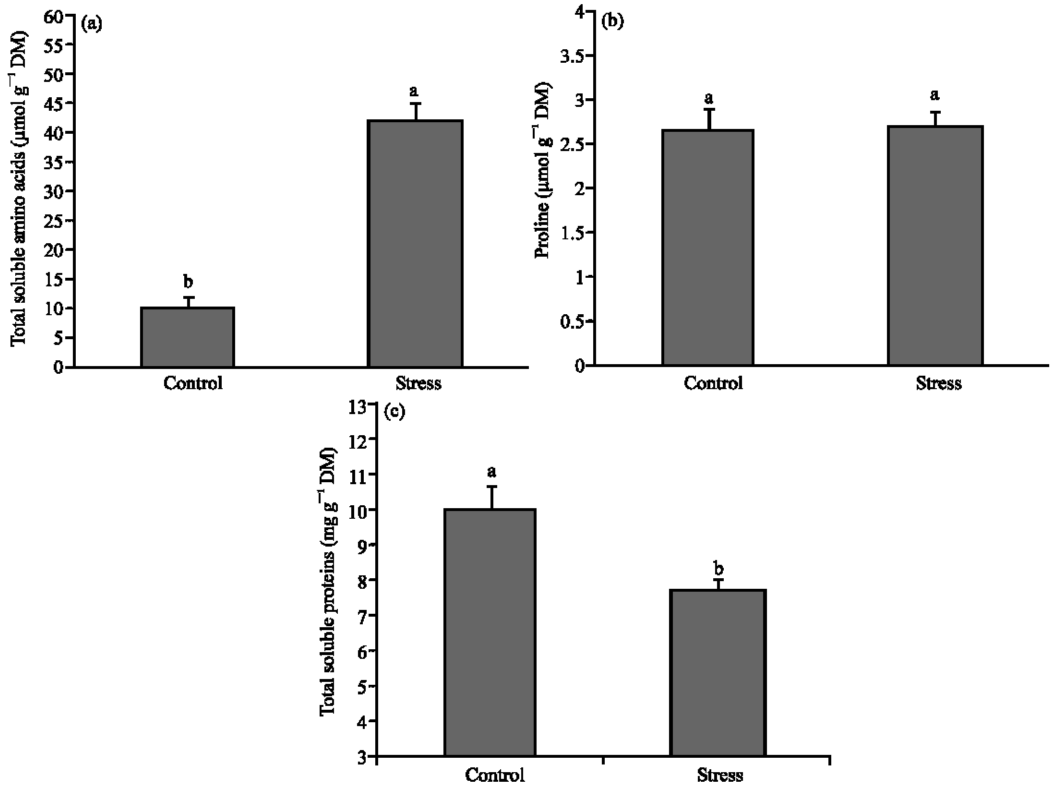

Fig. 3: (a) Total soluble amino acids, (b) proline and (c) total soluble proteins in *Glycine max* cv. Sambaiba subjected to 2 days of water deficit. Averages followed by the same letter do not differ among themselves by the F-test at 0.05 level of error probability. The bars represent the standard error of the mean

secondary factor is linked to gas exchanges during photosynthesis and transpiration processes, those it promotes progressive water losses coming from leaf tissue to air/environment. Lobato *et al.* (2008a) studying *Glycine max* plants induced to water deficit described that this species less tolerant than other legumes.

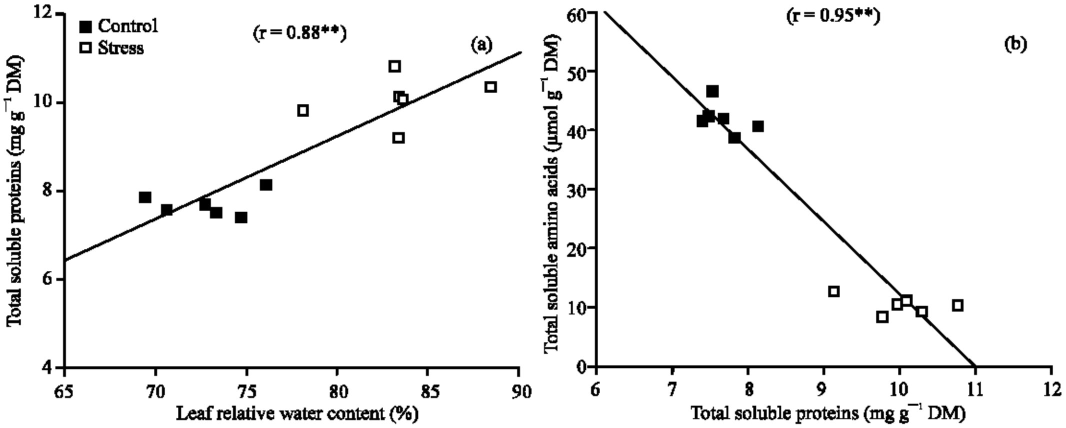

Fig. 4: (a) Relationships between leaf relative water content and total soluble proteins, (b) as well as total soluble proteins and total soluble amino acids in *Glycine max* cv. Sambaiba subjected to 2 days of water deficit. The bars represent the standard error of the mean and the asterisks (**) indicate the significance at 0.01 probability level

In plant dry matter was obtained statistically equal results and it is indicative that period of 2 days without water available provokes not interference on this parameter. These results can be explained due to increase in root and maintenance in shoot growth. In addition, recent studies evaluating *Glycine max* under water deficiency describes an increase in root dry matter with objective of re-establish plant water necessity and consequently increase the absorption range into substrate (Lobato *et al.*, 2008b).

Nitrate reductase activity presented reduction after water deficit applied and this result was provoked by the inadequate water supplement. So, nitrate acts as substrate to this enzyme during the biochemical reaction and this nitrogen form is assimilated by the higher plants using the water as transporter from soil to plant. Therefore, under conditions of water deficit the nitrate amount translocated is limited and enzyme activity also and this explanation is confirmed by the relationship between leaf relative water content and nitrate reductase activity. Similar results on fall in nitrate reductase activity were reported by Lobato *et al.* (2009a) investigating two *Vigna unguiculata* cultivars exposed to water deficit.

Total soluble amino acids suffered increase after water deficiency, as well as this metabolic response can be linked to production of amino acids specifics such as proline, glycinebetaine and other that are used during cell osmotic adjustment or due to protease enzyme actions. Therefore, the negative relationship between amino acids and proteins presented in this study corroborates the results and functions previously described by other researches (Oliveira *et al.*, 2009; Vendruscolo *et al.*, 2007). Proteases are enzymes that act breakdown proteins with objective to form essential amino acids that will used in several functions as such adjust osmotic, osmoprotection and antioxidant activity (Lobato *et al.*, 2009b; Cayley *et al.*, 1992; Sharma and Dietz, 2006), those it are characteristics commonly presents in tolerant plants to abiotic stresses. Silveira *et al.* (2003a) studying *Vigna unguiculata* plants under water deficiency and rehydration also described increase in total soluble amino acids.

The proline level in stressed treatment was significantly similar with control and it indicates that this species present not efficient adjustment osmotic in period of 2 days under water deficiency. Proline is an amino acid normally accumulated in adverse conditions such as abiotic or biotic stresses, which this strategy promotes osmotic adjustment and consequently higher plant tolerance. Therefore, under the stress period exposed to *Glycine max* plants in this study, proline contributes not to increase the total soluble amino acids and this fact is an indicative on lower tolerance of *Glycine max* plants, if compared with other legume specie such as *Vigna unguiculata* and *Cicer arietinum*.

In tress treatment was showed decrease in total soluble proteins and this fact is linked two simultaneous events, which the primary is due to disorders in nitrogen metabolism and consequent lower protein formation confirmed through relationship between leaf relative water content and total soluble proteins. Concomitantly, protease activity is maximized under water deficit, because during inadequate conditions to plant there is a specific signalization that over-expression genes responsibles by the regulation and subsequently enzyme production (Rodrigues *et al.*, 2009). Furthermore, results obtained by Silveira *et al.* (2003b) reveal increase in protease activity and consequent reduction in proteins in *Anacardium occidentale* plants exposed to short and long salt stress.

CONCLUSION

This study revealed that *Glycine max* induced to short water deficiency presented significant decreases in leaf relative water content, nitrate reductase and total soluble proteins. Furthermore, total soluble amino acids also suffered significant disorders. In addition, the results obtained confirmed the hypothesis that leaf relative water content exercises influence on total soluble proteins.

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