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Physiological and Biochemical Changes in Soybean (*Glycine max*) Plants under Progressive Water Deficit During the Vegetative Phase

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Abstract: The experiment had the aim to investigate and evaluate the responses provoked by the progressive water deficit on leaf relative water content, plant dry matter, free proline, total soluble carbohydrate, sucrose and reducing carbohydrates levels in *Glycine max* (L.) Merril plants cultivar sambaiba. The experimental design was at randomized entirely factorial, with 2 hydric conditions (stress and control) and 4 stress points (0, 2, 4 and 6 days). It was showed in plants under water deficit the decrease of the leaf relative water content and plant dry matter, however, occurred increase in the total soluble carbohydrates, sucrose and reducing carbohydrates at 33.7, 205.0 and 19.2%, respectively, besides accumulation of free proline at 67.2% as biochemical mechanism of plant osmotic adjustment. It were showed changes on leaf relative water content, total soluble carbohydrates, sucrose and reducing carbohydrates in only 2 days under water stress, besides plant dry matter and proline after the 4th day of water restriction, proving the sensitive high of this species in conditions of water deficiency.

Key words: Soybean, water stress, osmotic adjustment, carbohydrates, proline

INTRODUCTION

The water supplement inadequate in soil is considered one of the limitation factors to the productive potential in several specie (Boyer, 1982), as well as this factor can provoke growth small during the vegetative period, moreover to promote abortion of flowers during the bloom and to affect significantly the yield (Pimentel, 2004).

The deficit hydric is characterized by water losses that exceed the absorption rate and this way act directly in the plant water relations (Costa *et al.*, 2008), in which depending on intense and exposure period, besides to promote changes in the cell and molecular pathways (Zhu, 2002), occurr accumulation of organic solutes with the carbohydrates and proline (Lacerda *et al.*, 2001), differential gene expression of DNA (Casagrande *et al.*, 2001), quantity variation in the photosynthetic pigments, mainly chlorophylls and carotenoids (Chandrasekar *et al.*, 2000), in which the estomatal enclosed interfere in photosynthesis rates (Ribas *et al.*, 2005).

The osmotic adjustment is considered one important mechanisms developed by the plants for to tolerate water deficiency (Costa, 1999), in which promote protection of the plant cell structures with membranes and chloroplasts (Martínez *et al.*, 2004), as well as avoid the cell toxicity provoked by the free radicals, maximize the water retention in cell inside (Hare *et al.,* 1998), besides it have with advantage the possibility of to utilize carbohydrates with energy source under severe stress (Pimentel, 2004).

The soybean is considered a species sensitive the several abiotic stress (Van Heerden and Krüger, 2000), when compared with others legume with *Vigna unguiculata* and *Phaseolus vulgaris* (Roy *et al.*, 1992; Silveira *et al.*, 2003), as well as others specie as *Gossypium hirsutum* and *Sorghum bicolor* (Inamullad and Isoda, 2005; Younis *et al.*, 2000). In which the sensitivity at water deficit can be emphasized, mainly during the growth and development period, in which might occurr strong reduction in the yield (Van Heerden and Krüger, 2002).

The experiment had the aim to investigate and evaluate the responses provoke by the progressive water

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deficit on leaf relative water content, plant dry matter, total soluble carbohydrate levels, sucrose, reducing carbohydrates and free proline tenors in *Glycine max* (L.) Merril plants cultivar sambaiba.

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^{-2} s^{-1} (at 12:00 h), 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.

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 and the plants grown in pots with 6 L capacity.

Experiment design and treatments: The experimental design was at randomized entirely factorial, with 2 hydric conditions (stress and control) and 4 stress points (0, 2, 4 and 6 days), the experiment was composed by 8 repetitions and 64 experimental units, as well as each repetition had 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 greenhouse for 40 days, watered daily and 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 were submitted to the period of 6 days without irrigation, simulating the water stress until the 46th day. After this period the plants were taking away to the Laboratório de Fisiologia Vegetal Avançada belonging to Instituto de Ciências Agrárias (ICA) of Universidade Federal Rural da Amazônia (UFRA) for to measure the physiologic and biochemical parameters.

Measurements: The leaf relative water content (LRWC) performed with 10 mm disks in diameter, it being calculated as: LRWC = ((FW-DW)/(TW-DW)) × 100, where FW is the fresh weight, TW is the turgid weight measured after 24h of saturation on deionised water at 4°C in the dark and DW is the dry weight determined after 48 h in an oven at 80°C. The plants were placed in oven dried (Quimis, model Q314M) under at 65°C for 72 h and after the dehydration, it was measured the plant dry matter. In which the biochemical analysis were utilized only the dry leaves, it being ground until to get the powder, in which was submitted the extraction and subsequent quantification of the biochemical parameters by spectrophotometer (Quimis, model Q798DP). It were determined free proline at 520 nm (Bates *et al*., 1973), the total soluble carbohydrates levels at 490 nm (Dubois *et al*., 1956), sucrose (Van Handel, 1968) at 620 nm, reducing carbohydrates were calculated with the difference among total soluble carbohydrates totais and sucrose (Chaves and Stacciarini-Seraphin, 2001).

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 (SAS Institute, 1996) and based on statistical theories by Gomes (2000).

RESULTS

Leaf relative water content: Occurred significant decrease in the LRWC (Fig. 1), as well as the treatment under water deficiency had progressive fall of 81.5 to 60.9% in the LRWC of the 0 to 6 days, respectively. Moreover, was possible show significant difference after of 2, 4 and 6 days under water deficit, when compared with the control plants.

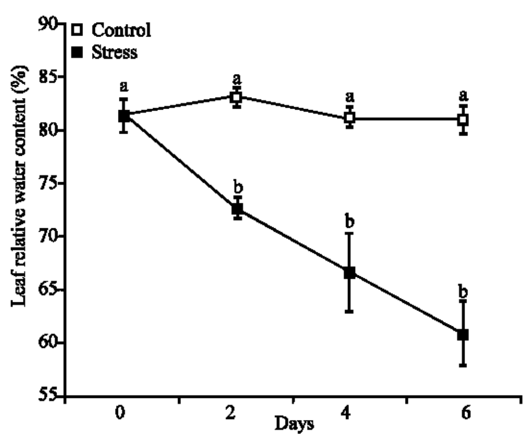

Fig. 1: Leaf relative water content in plants of *Glycine max* cultivar sambaiba under 0, 2, 4 and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

Plant dry matter: Occurred significant difference in the plant dry matter, it being statically different the points under 4 and 6 days of water deficit, moreover, the control treatment than stress treatment had increase in this variable (Fig. 2). The plants kept under irrigation (control) had increases in 9.6, 36.2 and 57.4% in the plant dry matter during the periods between 0-2, 2-4 and 4-6 days, respectively, besides accumulated increase in 103.2%. However, it was showed in the treatment under water stress the total increase in dry matter of only 81.9%, during the period between 4-6 days of water restriction, it being got the higher accumulation of dry matter in the period between 4-6th days, with 44.7%. Despite behavior of both the treatments (control and stress) to be similars, increase of the dry matter, the control treatment had greater plant dry matter in all the measured points, when compared with the stress treatment.

Total soluble carbohydrates: The levels were progressively increase in the water deficit and control, as well as the variance analysis reveal that occurred significant difference among the treatments, it being showed the increase of 6.6, 14.4 and 12.7 in total soluble carbohydrates in the points under water restriction of 2, 4 and 6 days, respectively, it being kept increases de 6.6, 14.4 and 12.7% total soluble carbohydrates in the points under water restriction points of 2, 4 and 6 days, respectively, as well as the higher accumulation was showed in the period of 2-4 days under water deficiency. It was showed in the stress treatment increase strong in total soluble carbohydrates during the measured period, in which under same points, remaining above control treatment levels (Fig. 3), however the control treatment had small increase in carbohydrate concentration.

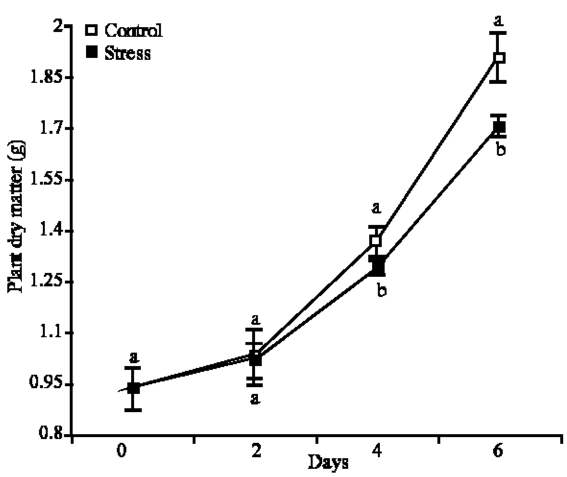

Fig. 2: Plant dry matter of *Glycine max* cultivar sambaiba under 0, 2, 4 and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

Sucrose: It was showed significant changes (Fig. 4), in which occurred progressive increase of the sucrose in the plants submitted to water stress and statistical differences among the treatments (control and stress) related with the days 2, 4 and 6. The treatment under water deficit had

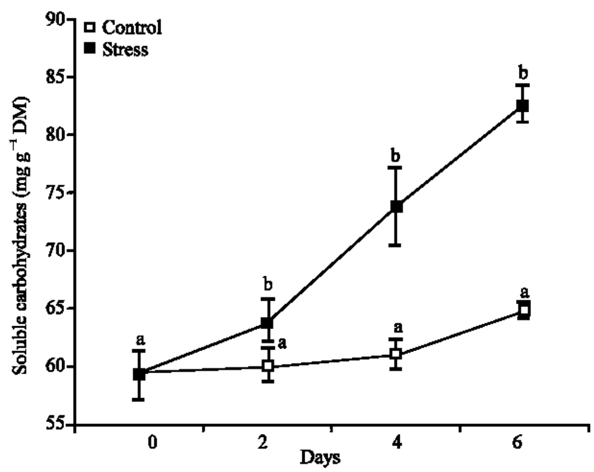

Fig. 3: Total soluble carbohydrates in plants of *Glycine max* cultivar sambaiba under 0, 2, 4 and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

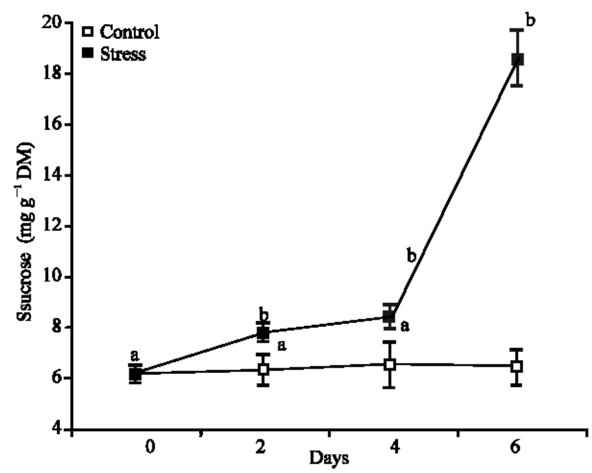

Fig. 4: Sucrose in plants of *Glycine max* cultivar sambaiba under 0, 2, 4 and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

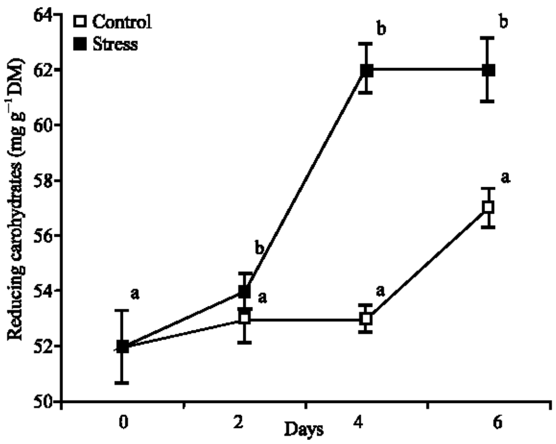

Fig. 5: Reducing carbohydrates in plants of *Glycine max* cultivar sambaiba under 0, 2, 4 and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

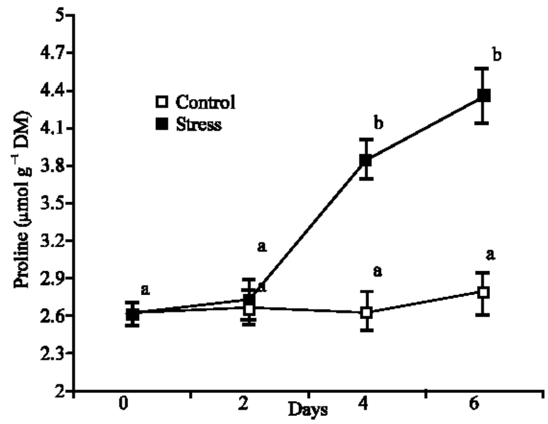

Fig. 6: Free proline in plants of *Glycine max* cultivar sambaiba under 0, 2, 4 and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability and the bars represent the mean standard error

total increase of 205% in the sucrose levels, of the 0-6th day under water restriction, it being showed increases of 28 and of 10%, in the periodsbetween 0-2 and 2-4 days, respectively. However, in the period between 4 and 6 days occurred increase of 167% in the sucrose levels. The plants belonging to control treatment kept stable the sucrose levels, fluctuating between 6.1 and 6.5 mg g^{-1} DM.

Reducing carbohydrates: The reducing carbohydrates was affect by the water restriction, according the variance analysis, occurred significant difference among control and stress at 2, 4 and 6 days (Fig. 5). The plants submitted on water stress had total increase of 19.2% in reducing carbohydrates, fluctuating of 52 until 62 mg g^{-1} DM, moreover this increase was strongly affected in the period between 2-4 days under water deficit, with 15.4% of the increase of this variable. However, the plants normally irrigates had small fluctuation in the reducing carbohydrates levels, it being showed variation of 52 until 57 mg g^{-1} DM during the experiment.

Free proline: It was showed significant increase in the plants under deficit water, it being the increase of 4.3, 43.4 and 19.5% in free proline in the assessments to carry out at 2, 4 and 6 days, respectively (Fig. 6). Moreover, occurred accumulate great in proline in the period among 2 and 4 days under water deficiency and significant difference among the water conditions at 4 and 6th day under water restriction. However, the control treatment kept the proline levels stables.

DISCUSSION

The reduction in LRWC was provoked by the water deficiency in soil, in which the water stress simulated artificially in this experiment cause as direct consequence changes in LRWC, because during the transpiration process and photosynthesis occur water loss through of the stomata and the assimilation/reposition rate is strongly affected, occurred probably decrease of the conductance estomatal for reduce the water loss to the atmosphere (Verslues *et al*., 2006).

The smaller dry matter showed in plants submitted on water restriction, when compared with control plants, occurred by consequence of the smaller growth of the plants under stress in this circumstance, because the growth and development of several specie is dependent of the cell turgor, in which the water fill in cell space and practice a positive pressure that promote through this mechanism, the extension tissue (Taiz and Zeiger, 1998; Kerbauy, 2004). These results to coincide with showed by Sankar *et al.* (2007) studding the effects of the water restriction in *Abelmoschus esculentus*, in which occurr reduction of the plant dry matter.

The amount of total soluble carbohydrates was progressively increased in plants under water deficit with the objective to protect the leaf cell structures against the damage provoked by the stress, because the soluble carbohydrates with sorbitol and sucrose, besides starch that are extremely soluble and permeable, in which it can are accumulate in cell and this way to improve the resistance of the plants to water deficit (Li and Li, 2005).

Similar results on increase in the total soluble carbohydrates were showed by Pimentel (1999) measuring the water stress effects in *Zea mays* plants and Lobato *et al.* (2008) studying the behavior the *Vigna unguiculata* plants under deficit water during the vegetative phase. Recent researches to relate to the carbon metabolism, specify the carbohydrates distribution and utilization, with the production of ABA endogen hormone, in which it is produced in the root with a response to water stress (Li and Li, 2007), as well as the ABA functioned with fundament element in the molecular signalization and consequent regulation of gene expression to get involved in carbon metabolism (Verslues and Zhu, 2005).

The progressive increase in the sucrose levels of the plants under water deficit occurred due to increase of the sucrose biosyntheses, probably promoted by the increase in sucrose phosphate synthase enzyme activity that act in photosynthetic cell citosol (Huber and Huber, 1996) with the intention to protect membranes and integrity proteins (Hoekstra *et al.*, 2001) in water deficiency conditions. The significant increase in the sucrose levels also was showed in *C. annuum* L. plants under saline stress (Martinez-Ballesta *et al.*, 2004), however these results are to disagree with showed by Sánchez-Rodríguez *et al.* (1999) investigating the effect of the water stress in *C. equisetifolia*. The carbon in higher plants is fixed through photosynthesis and distributed mainly in the sucrose form, hexoses as fructose and glucose, besides starch (Escobar-Gutiérrez *et al.*, 1998) and others alcohol sugars as mannitol, in which these compounds can be accumulated during the water deficit without to interfere in the plant metabolism (Williamson *et al.*, 2002). The sucrose has important role, it being the main photo-assimilated exported of the synthesis sites as leaves, at direction consume sites as flowers, buds and stem, besides it are utilized to the storage during light and moderate water restriction and consumed under severe water deficit (Pimentel, 2004).

The increase in the reducing carbohydrates levels in plant under water restriction occurr due the 2 biochemical events that simultaneously happen in plants under these conditions. The starch pathway is the main, because the starch is degraded in the sites that accumulate, it being this degradation promoted by the amylase enzyme action (Chaves Filho and Stacciarini-Seraphin, 2001), besides it to have the sucrose pathway that is considered secondary, because the sucrose suffer from invertase enzyme actions and this way go to liberate hexoses that might are utilized in anabolic or catabolic processes and if went not used, it contribute to the accumulation of reducing carbohydrates (Kingston-Smith *et al.*, 1999). Chaves Filho and Stacciarini-Seraphin (2001) studying the effects of the water deficit in *Solanum lycocarpum* found similar results.

The proline accumulation is a metabolic response characteristic of plants under abiotic stresses, it being showed the increase in this experiment because the free proline work as osmotic adjustor that objective reduce the negative effects provoked in the plants under adverse conditions (Kishor *et al.*, 1995), besides promote higher resistance in cells under these circumstances (Zhu and Xiong, 2002). The results of this experiment corroborate the behavior relative to proline accumulation in plants under water deficiency, it showed by Sarker *et al.* (1999) working with cultivars of *Triticum aestivum* L. and Costa (1999) studying *Vigna unguiculata*. The proline is synthesized from glutamate and ornitine, in which the production of this organic solute, under conditions of the water shortage, occurr at major part from glutamate (Delauney and Verma, 1993), it being this pathway possible by the Δ^1-pyrroline-5-carboxylase (P5C) enzyme, besides P5C Syntetase (P5CS) and P5C redutase (P5CR) enzyme actions, responsibles by to catalyze the reaction through of two successive reductions (Hare *et al.*, 1999; Zhu *et al.*, 1998).

It were showed changes on leaf relative water content, total soluble carbohydrates, sucrose and reducing carbohydrates in only 2 days under water stress, besides plant dry matter and proline after the 4th day of water restriction, proving the sensitive high of this species in conditions of water deficiency.

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