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Physiological and biochemical behavior in soybean (*Glycine max* cv. Sambaiba) plants under water deficit

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Abstract

The experiment had the aimed at evaluating the effects of the progressive water deficit, as well as investigating the physiological and biochemical behavior in *Glycine max* cv. Sambaiba submitted to water restriction during the vegetative phase. The parameters that were measured are the leaf relative water content, plant dry matter, proline, total soluble carbohydrate, sucrose, reducing carbohydrates, free amino acids and total soluble proteins. The experimental design carried out was at entirely randomized factorial scheme, with 2 water regimes (stress and control) and 4 evaluation points (0, 2, 4 and 6 days). There was decrease in the leaf relative water content in plants under water deficit, however the total soluble carbohydrates, sucrose and reducing carbohydrates were increased at 40, 205.0, 19.2%, respectively, besides the accumulation of proline and free amino acids at 67 and 388.1%, respectively. Significant changes were shown on leaf relative water content, total soluble carbohydrates, sucrose and reducing carbohydrates with 2 days under water stress, indicating that the carbon metabolism is quickly modified and utilized as reserve source and membrane protector during the water deficit. Besides of this, the increase in free amino acids level occurred due to protein breakdown as consequence of the stress submitted to plants, however significant changes were not observed on the proline levels until the 4th day of water restriction. This fact reveals the inefficient osmotic adjustment and as consequence the high sensitivity of this species under conditions of water restriction.

Keywords: *Glycine max,* soybean, water deficit, carbon metabolism, nitrogen metabolism, carbohydrates, proteins

Introduction

The inadequate water supplement in soil is extremely damaging to the plant, in which can limit the productive potential in several specie (Santos and Carlesso, 1998), as well as provoking smaller growth during the vegetative period (Lobato et al, 2008a), moreover it promotes flower abortion during the reproductive period (Pimentel, 2004). The water deficit is characterized by water losses that exceed the absorption rate and of this way it acts directly in the plant water relations (Costa et al., 2008), in which the plant damages depend on the intensity and the exposure period, besides promoting changes in the cell and the molecular pathways (Zhu, 2002), as well as is reported

Table 1. Leaf relative water content in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days ofwater deficit. Averages followed by the same letter do not differ among themselves by the Tukey test at5% of probability.

Leaf relative water content (%)		
Days	**control**	**stress**
0	81.5 ± 1.59 a	81.5 ± 1.59 a
2	83.4 ± 0.78 a	72.8 ± 0.87 b
4	81.4 ± 0.81 a	66.8 ± 3.53 b
6	81.2 ± 1.15 a	60.9 ± 2.98 b

Table 2. Plant dry matter in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit.Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

Plant dry matter (g)		
Days	**control**	**stress**
0	0.94 ± 0.06 a	0.94 ± 0.06 a
2	1.03 ± 0.08 a	1.02 ± 0.05 a
4	1.37 ± 0.05 a	1.29 ± 0.02 b
6	1.91 ± 0.07 a	1.71 ± 0.03 b

accumulation of organic solutes as carbohydrates and proline (Lacerda et al., 2001), differential gene expression on nucleic acids as DNA and RNA (Casagrande et al., 2001), variation on the amount of photosynthetic pigments, mainly chlorophylls and carotenoids (Chandrasekar et al., 2000), in which the mechanism stomatal interferes on photosynthesis rates (Ribas-Carbo et al., 2005).

The osmotic adjustment is considered an important mechanism developed by the plants to tolerate water deficiency (Costa, 1999), which promotes protection of the plant cell structures as membranes and chloroplasts (Martínez et al., 2004). It also avoids cell toxicity provoked by free radicals and maximizes water retention inside the cell (Hare et al., 1998), moreover it presents the possibility of using the carbohydrates as energy source under severe stress (Pimentel, 2004). The soybean is considered a sensitive species to the several abiotic stress (Van Heerden and Krüger, 2000), when compared with other tropical legumes, such as Vigna unguiculata and Phaseolus vulgaris (Roy-Macauley 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). The soybean sensitivity under water deficit can be emphasized, mainly during the growth and development period, as a result, it might occur a strong reduction of leaf area, smaller nutrient assimilation and lower pod number and consequently the yield decrease (Van Heerden and Krüger, 2002). The experiment aimed at evaluating the effects of the progressive water deficit, as well as to investigating the physiological and biochemical behavior in *Glycine max* cv. Sambaiba submitted to water restriction during the vegetative phase.

Materials and methods

Growth conditions and plant material

The experiment was carried out in greenhouse ambient and Laboratório de Fisiologia Vegetal Avançada (LFVA) of the Universidade Federal Rural da Amazônia (UFRA), city of Belém, state of Pará, region Northern, Brazil (01º27'S and 48º26'W) during the months of September and October of 2006. The plants grow in greenhouse ambient under natural conditions day/night (minimum/maximum air temperature and relative humidity were: 22.4/37.6 ºC and 68/79%, respectively, as well as the average photoperiod was of 12 h of light and maximum active photosynthetical radiation of 623 µmol $^{-2}$ s^{-1} (at 12:00 h). The *Glycine max* (L.) Merril (cultivar Sambaiba) seeds were collected in the 2006 season, from Paragominas city, Pará state, Northern region, Brazil (03º00'S and 47º21'W) and stored until the experiment implementation. The plants grown in

Table 3. Total soluble carbohydrates in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

Soluble carbohydrates (mg. g^{-1} DM)		
Days	**control**	**stress**
0	58.7 ± 1.8 a	58.7 ± 1.8 a
2	59.3 ± 1.2 a	62.6 ± 1.6 b
4	60.2 ± 1.0 a	71.1 ± 2.8 b
6	63.4 ± 0.6 a	78.6 ± 1.4 b

Table 4. Sucrose in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averagesfollowed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

Sucrose (mg. g^{-1} DM)		
Days	**control**	**stress**
0	6.1 ± 0.3 a	6.1 ± 0.3 a
2	6.3 ± 0.6 a	7.8 ± 0.4 b
4	6.5 ± 0.9 a	8.4 ± 0.5 b
6	6.4 ± 0.7 a	18.6 ± 1.1 b

pots with 6 L capacity and the substrates used were composed by black potting soil and sand at 3:1 ratio, respectively.

Experiment design and treatments

The experimental design used was carried out at randomized entirely factorial scheme, with 2 water regimes (stress and control) and 4 evaluation points (0, 2, 4 and 6 days). The experiment was composed by 8 repetitions and 64 experimental units, which each repetition had one plant. Three seeds were placed into each pot and after 7 days, which the seedling were thinned and was kept only one seedling per pot. The plants remained in greenhouse ambient for 40 days, watered daily and received macro and micronutrients every 5 days, using the nutritive solution of Hoagland and Arnon (1950). The stress treatment started at the 40^{th} day after the implementation of the experiment and the plants under stress were submitted to the period of 6 days without irrigation. The plants after this period were conduced to Laboratório de Fisiologia Vegetal Avançada (LFVA) to measure the physiologic and biochemical parameters.

Measurements

The leaf relative water content (LRWC) was carried out with 10 mm disks of diameter, it was calculated as: LRWC = [(FW-DW)/(TW-DW)] x 100, in which FW is fresh weight, TW is the turgid weight measured after 24h of saturation on deionised water at 4ºC in the dark, and DW is the dry weight determined after 48 h in oven at 80ºC (Slavick, 1979). The plants were placed in dried oven with air forced circulation (Quimis, model Q314M) under 65 ºC for 72 h and after of the dehydration, it was measured the plant dry matter. The biochemical analysis were carried out with only the dry leaves, which the leaves were triturated and the powder was submitted the extraction and quantification of the biochemical parameters with spectrophotometer (Quimis, model Q798DP). The free proline level was determined at 520 nm (Bates et al. 1973), the total soluble carbohydrate level at 490 nm (Dubois et al. 1956), the sucrose at 620 nm (Van Handel, 1968), the reducing carbohydrates was calculated as the difference among the total soluble carbohydrate total and sucrose (Chaves and Stacciarini-Seraphin, 2001), the free amino acids at 570 nm (Peoples et al., 1989) and the total soluble proteins at 595 nm (Bradford, 1976).

Data analysis

The standard error were calculated for each point, as well as in the results were applied the variance analysis and the averages of the treatments were compared by the Tukey test at the 5% significance level (Gomes, 2000), using the software SAS (SAS Institute, 1996).

Table 5. Reducing carbohydrates in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

Reducing carbohydrates (mg. g^{-1} DM)		
Days	**control**	**stress**
0	52 ± 1.3 a	52 ± 1.3 a
2	53 ± 0,8 a	54 ± 0.6 b
4	53 ± 0.5 a	62 ± 0.9 b
6	57 ± 0.7 a	62 ± 1.1 b

Table 6. Proline in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

Proline (µmol. g^{-1} DM)		
Days	**control**	**stress**
0	2.61 ± 0.09 a	2.61 ± 0.09 a
2	2.66 ± 0.14 a	2.72 ± 0.16 a
4	2.63 ± 0.16 a	3.85 ± 0.15 b
6	2.78 ± 0.17 a	4.36 ± 0.22 b

Results

Leaf relative water content

The leaf relative water content (LRWC) was reduced significantly (Table 1), as well as in 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, significant changes were showed with 2, 4 and 6 days under water deficit, when compared with the control plants.

Plant dry matter

Significant difference was showed in the plant dry matter, in which the treatment kept under irrigation (control) had increase at 103.2% in the plant dry matter during the period measured. However, the treatment under water stress was showed the total increase in dry matter of only 81.9%, (Table 2). The stress and control treatments had similar behavior, but the increase of the dry matter of the control treatment was greater in all the measured points, when compared with the treatment under stress.

Total soluble carbohydrates

The total soluble carbohydrate levels were progressively increased on the treatments control and stress, as well as the variance analysis reveals that occur significant difference among the treatments. In the plants submitted to stress was showed the increase at 40% (Table 3) after 6 days of water restriction, as well as great accumulation was showed in the period of 2 to 4 days under water deficiency.

Sucrose

The sucrose level was significantly changing (Table 4) in the plants submitted to water stress, which the treatment under water deficit had total increase at 205% in this parameter during the 6 days under water restriction. The control treatment fluctuating the sucrose levels between 6.1 and 6.5 mg. g^{-1} DM.

Reducing carbohydrates

The reducing carbohydrate level was affects by the water restriction, in which the plants submitted on water stress had total increase at 19.2% in reducing carbohydrates, as well as the stress treatment suffers strong increase in the period between 2 to 4 days.

Proline

The proline level had significant changes, as well as in the plants under stress was showed total increase at 67% in free proline during the period of 6 days

Table 7. Free amino acids in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

	Free amino acids (µmol. g^{-1} DM)	
Days	**control**	**stress**
0	10.1 ± 3.5 a	10.1 ± 3.5 a
2	10.2 ± 2.8 a	41.6 ± 3.0 b
4	9.9 ± 1.9 a	41.2 ± 3.1 b
6	10.1 ± 1.5 a	49.3 ± 2.4 b

Table 6). Moreover, the treatment stress suffers significant increase only after 4 of water stress.

Free amino acids

The amino acids level suffers significant increase, in which the water deficit promotes total increase at 388.1% in amino acids level after of 6th days of water restriction (Table 7), moreover in the stress treatment were showed major increase in period between 0 and 2 days.

Total soluble proteins

The total soluble protein level was significantly reducing, which the stress treatment had accumulated fall at 20%, as well as strong decrease in period between 0 and 2 days (Table 8). The plants under stress present behavior similar, stable and smaller after the 2nd day, when compared with control plants.

Discussion

The reduction in leaf relative water content was provoked by the water deficiency in soil, because during the photosynthesis occur water loss through of the stomatal mechanism and the water assimilation rate is negatively affecting during water stress (Verslues et al., 2006). The smaller plant dry matter was shows in plants submitted to water restriction, when compared with control plants, in which despite the increase of this parameter in plants under stress, this increase is not of shoot, but is consequence of the root growth. The root growth is a strategy used by the plants to water capture in substrate under water deficit conditions (Lobato et al., 2008b), in which the growth and development plant is dependents of the cell turgor, as well as the water fills the cell space and practice a positive pressure that promotes through this mechanism the tissue extension (Kerbauy, 2004).

The total soluble carbohydrates amount was progressively increasing in plants under water deficit due the soluble carbohydrates as sorbitol, sucrose and starch that are extremely soluble and permeable, in which can are accumulated in cell and this way improving 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 may*s plants and Lobato et al. (2008a) studying the behavior the *Vigna unguiculata* plants under deficit water during the vegetative phase. The sucrose level was progressively increasing in the plants under water deficit due the sucrose biosyntheses, in which this increase was probably promoted by the increasing in sucrose phosphate synthase enzyme activity that acts in photosynthetic cell citosol (Huber and Huber, 1996) with the intention protects membranes and integrity proteins (Hoekstra et al., 2001) in water deficiency conditions. The sucrose has important role, as well as is the main photo-assimilated exported of the synthesis sites as leaves from consumes sites as flowers, buds and stem, besides it is kept during light and moderate water restriction and consumed under severe water deficit (Pimentel, 2004).

The increase in the reducing carbohydrates levels in plants under water restriction occur due the two biochemical events that simultaneously happen in plants under these conditions. The starch pathway is the main, because the starch is degraded and this degradation is promoted by the amylase enzyme action (Chaves Filho and Stacciarini-Seraphin, 2001). Besides of this, the sucrose pathway there be and is considered secondary, because the sucrose suffers from invertase enzyme actions and this way go to liberates hexoses that might are utilized in anabolic or catabolic processes and if went not used might contributes to the reducing carbohydrates accumulation (Kingston-Smith et al., 1999). The proline level was significantly increasing only after of the 4th day and this accumulation is a response characteristic of plants under abiotic stresses, which it works as osmotic adjustor with the objective reduces the negative effects provokes in the plants

Table 8. Total soluble proteins in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability.

	Soluble proteins(mg. g^{-1} DM)	
Days	**control**	**stress**
0	9.74 ± 0.11 a	9.74 ± 0.11 a
2	10.05 ± 0.37 a	7.69 ± 0.09 b
4	9.87 ± 0.26 a	7.73 ± 0.19 b
6	9.71 ± 0.22 a	7.79 ± 0.21 b

under adverse conditions (Kishor et al., 1995), besides of this, it promotes higher resistance in cells under these circumstances (Zhu and Xiong, 2002). This results prove the inefficient plant osmotic adjustment and suggest the smaller plant capacity to keep the water in the tissue, in which the water (H_2O) is the fundamental compound to carried out adequately the metabolic and physiologic activity as assimilation and transport of nutrients, as well as production of essential metabolics (Lobato et al, 2008b). Similar results on the proline accumulation in plants under water deficiency were showed by Sarker et al. (1999) working with cultivars of *Triticum aestivum* and Costa (1999) studying *Vigna unguiculata*.

The increase showed in the free amino acids is due to high synthesis of amino acids from protein hydrolyses, in which the free amino acids are utilized by the plant to reduced the effects of the water deficit through organic solute accumulation and this way increased the water retention capacity (Sircelj et al., 2005). Under water stress the free amino acids as proline and glycinebetaine are strongly influenced and consequently quickly accumulated (Carceller et al., 1999; Nakamura et al., 2001), as well as of secondary form occur the increase of aspartate, glutamate and alanine (Ramos et al., 2005). Result on increase in the free amino acids was found by Asha and Rao (2002) working with *Arachis hypogaea* under water deficit corroborate the results of this study.

The reduction in the total soluble proteins showed in the plants under water stress is due to probable increase of the proteases enzyme activity, in which this proteases enzyme promote the breakdown of the proteins and consequently decrease the protein amount presents in the plant under abiotic stress conditions (Debouba et al., 2006). In inadequate conditions to the plant is active the pathway of proteins breakdown, because the plant use the proteins to the synthesis of nitrogen compounds as amino acids that might auxiliary the plant osmotic adjustment (Sankar et al., 2007). Similar results on reduction in the proteins were found by Ramos et al. (1999) investigating the effects of the water stress in *Phaseolus vulgaris*.

Significant changes were shown on leaf relative water content, total soluble carbohydrates, sucrose and reducing carbohydrates with 2 days under water stress, indicating that the carbon metabolism is quickly modified and utilized as reserve source and membrane protector during the water deficit. Besides of this, the increase in free amino acids level occurred due to protein breakdown as consequence of the stress submitted to plants, however significant changes were not observed on the proline levels until the 4th day of water restriction. This fact reveals the inefficient osmotic adjustment and as consequence the high sensitivity of this species under conditions of water restriction.

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