

ABA-mediated proline synthesis in cowpea leaves exposed to water deficiency and rehydration

Roberto Cezar Lobo da COSTA¹, Allan Klynger da Silva LOBATO^{2,*},

Joaquim Albenísio Gomes da SILVEIRA³, Haywood Dail LAUGHINGHOUSE IV⁴

¹Laboratory of Advanced Plant Physiology, Universidade Federal Rural da Amazônia, Belém - BRAZIL

²Nucleus of Basic and Applied Plant Research, Universidade Federal Rural da Amazônia, Paragominas - BRAZIL

³Laboratory of Metabolism and Stress Plant, Universidade Federal do Ceará, Fortaleza - BRAZIL

⁴Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC - USA

Received: 26.11.2009

Abstract: The aim of this study was (i) to evaluate the impact of water deficit and rehydration on nitrogen compounds and abscisic acid of *Vigna unguiculata* leaves, and (ii) to investigate the hypothesis that abscisic acid influences proline. The experimental design was entirely randomized in a factorial design, with 2 water conditions (control and stress) and 7 evaluation periods (0, 1, 2, 3, and 4 days under water deficit, followed by the 5th and 6th day under rehydration). The leaf relative water content presented significant reduction, presenting a lower value on the 4th day of treatment under water deficit. After rehydration, the plants reestablished the leaf relative water content. The correlation analysis suggested a significant relationship between the leaf relative water content and abscisic acid. The abscisic acid concentration of the plants under water deficiency were of 2.4, 45.9, 42.6, 47.9, 66.9, 9.2, and 5.5 mg kg⁻¹ DM on days 0, 1, 2, 3, 4, 5, and 6, respectively. Rehydration quickly decreased abscisic acid. In addition, the correlation analysis indicated a significant relationship between abscisic acid and proline. The proline levels of the plants under stress showed significant increases by 67%, 354%, 423%, and 532% on the 1st, 2nd, 3rd, and 4th day, respectively, when compared with control plants. This study with *Vigna unguiculata* plants reveals negative consequence of water deficiency over nitrogen metabolism, and fast recovery after rehydration. The nitrogen compounds and enzymes presented direct and indirect responses in the osmotic adjustment of the plant, as well as increasing abscisic acid concentration with the intent to decrease water loss in *Vigna unguiculata* induced by the water deficiency. Furthermore, a positive relationship between abscisic acid and proline synthesis was confirmed.

Key words: Abscisic acid, nitrogen metabolism, osmotic adjustment, rehydration, *Vigna unguiculata* L. (Walp.), water deficit

Introduction

Drought is one of the key sources of abiotic stress, since it induces smaller growth and development rates, flower aborting, and decreases crop yields during the vegetative, reproductive, and maturation stages

(Showemimo and Olarewaju 2007). Usually, drought consequences on plants are studied in controlled/artificial conditions through water restriction. The cowpea is a tropical legume with lower water and nutritional exigencies when compared to other

* E-mail: allanllobato@yahoo.com.br

legumes like soybean and common bean. In addition, the seeds can be used in human diet since they are sources of starch (500-650 g kg⁻¹) and proteins (80-350 g kg⁻¹), which are the major compounds in the grain (Olivera et al. 2003). These characteristics suggest that this crop should be used in regions with lower water supplementation (Lobato et al. 2009a).

Vascular plants present several strategies to minimize the negative effects induced by water deficiency, being morphological modifications like increase in root size (Lobato et al. 2008a) and reduction in leaf area (Anyia and Herzog 2004).

During water deficiency there are plant physiological mechanisms like stomatal closing and decrease in the transpiration and photosynthetic rates (Martinez-Ballesta et al. 2004; Krouma 2010), biochemical processes as osmotic adjustment characterized by accumulation of organic solutes used to maintain the cell turgor (Kuznetsov and Shevyakova 1997), and proteolysis process, which is the protein breakdown to form others nitrogenous compounds (Ezzine and Ghorbel 2006).

Proline is an amino acid that is usually synthesized in large amounts under conditions of biotic stress like pathogen infection (Lobato et al. 2009b; Lobato et al. 2010) and abiotic stress such as water deficiency (Costa et al. 2008), salt stress (Silveira et al. 2003a), and heavy metal stress (Chen et al. 2001). This accumulation is associated with osmoprotection functions (Cayley et al. 1992), osmotic adjustment (Oliveira Neto et al. 2009), and antioxidant activity (Sharma and Dietz 2006). Recent studies on the proline action in transgenic *Triticum aestivum* L. plants indicate that this nitrogenous compound decreases the damage induced by water deficiency, and consequently increases the plant tolerance (Vendruscolo et al. 2007). Husaini and Abdin (2008) studied the consequences of salt stress in transgenic *Fragaria x ananassa* Duch. plants and observed that the modified plants presented higher proline amounts and they were more tolerant to salt stress, when compared to wild plants.

The abscisic acid (ABA) hormone is synthesized in the plastids, and is linked to the stomatal mechanism (Kirda et al. 2007) and quickly responds to water deficiency (Liu et al. 2005). The ABA can be produced

in the roots and/or shoots, but this hormone is usually synthesized under water deficiency in the roots and translocated to leaves in order to improve stomatal control. The ABA signalization pathway depends on the Ca⁺ influx into the cytosol (Nayyar and Kaushal 2002) activating the K⁺, Cl⁻, and malate⁻² efflux channels to external medium, through plasmatic membranes and concomitantly blocking the K⁺ entrance to cytosol. Therefore, the cytosol solute flux in the direction of the cell wall results in a decrease of turgescence pressure in the guard cells and consequently the stomata are closed (Assmann 2003).

The aim of the present study was to evaluate the impact of the water deficiency and rehydration on nitrogen compounds and abscisic acid of *Vigna unguiculata* leaves. In addition, the hypothesis that abscisic acid concentration influences proline levels was also investigated.

Materials and methods

Plant material and experimental conditions

The *Vigna unguiculata* seeds of the Vita 7 cultivar were used in this study, which were treated with 2.5% sodium hypochlorite solution. This cultivar was developed and obtained from Empresa Brasileira de Pesquisa Agropecuária (Embrapa/Brazil). The plants were grown in a glasshouse situated in the Universidade Federal do Ceará (UFC), Fortaleza, Ceará State, Brazil, under natural photoperiod of 12 h and day/night temperature means of 29/25 °C, and 63%/85% of relative humidity, respectively. Maximum photosynthetic active radiation (PAR) at midday was approximately 1500 mmol m⁻² s⁻¹, measured at plant level (LI-COR, model 190SA).

Experimental design

The experimental design used was entirely randomized in a factorial design with 2 water conditions (control and stress) and 7 evaluation periods (0, 1, 2, 3, and 4 days under water deficit, followed by 5th and 6th day under rehydration). The experiment was composed of 6 replicates and 84 experimental units, where each plant was one experimental unit.

Plant growth and harvest

Five seeds were placed into pots with a capacity of 800 cm³ containing sand and vermiculite as substrate in proportion of 2:1, respectively. After 7 days, the seedlings were thinned to 1 seedling per pot. The plants (control and stress) were watered with 0.5 L pot⁻¹ of Hoagland and Arnon (1950) nutrient solution modified in the NO₃⁻ content at 5 M. The plants were maintained in a Leonard pot, and nutrient solution changed in constant intervals of 4 days, with pH adjusted at 6.0 ± 0.5 by adding HCl or NaOH. On the 8th and 12th days, the roots were inoculated with *Bradyrhizobium* spp. BR-3256 (CB-756) at 10⁹ cells plant⁻¹, supplied by Empresa Brasileira de Pesquisa Agropecuária (Embrapa/Brazil). All the plants were grown until the 28th day as described above. Subsequently, the plants under the stress treatment were submitted to 4 consecutive days without nutrient solution, and, after this period, rehydrated with distilled water for 2 days. The plants under the control treatment were watered with nutrient solution for 4 days, followed by 2 days with distilled water. In each period, the leaves present in middle region of the plant were removed, washed with distilled water, dried with filter paper, and the leaf relative water content was determined instantaneously. After that, the leaves were frozen in liquid nitrogen and kept at -20 °C for subsequent biochemical determinations.

Leaf relative water content

The leaf relative water content (LRWC) was evaluated in leaf disks of 10 mm diameter. For each plant 40 disks were removed and the RWC was calculated according to the formula proposed by Slavick (1979):

$$\text{LRWC} = [(\text{FM-DM})/(\text{TM-DM})] \times 100,$$

where FM is fresh matter, TM is turgid matter evaluated after 24 h and saturated in deionized water at 4 °C in the dark, and DM is the dry matter determined after 48 h in an oven with forced air circulation at 80 °C.

Glutamine synthetase activity

The extraction of the glutamine synthetase enzyme (E.C. 6.3.1.2) was carried out with 200 mg of leaf tissue ground in liquid nitrogen. The samples were then incubated in 5 mL of extraction mix (Tris-HCl buffer pH 7.6 containing 10 mM MgCl₂, 10

mM β-mercaptoethanol, 5% (w/v) PVP, and 5 mM EDTA), homogenized, centrifuged at 30,000 ×g for 10 min, and the supernatant was removed. All the procedures were carried out in the interval of 0-4 °C. The quantification of the enzyme activity was carried out using the method of Kamachi et al. (1991) with absorbance at 540 nm, and g-glutamylhydroxamate (Sigma Chemicals) was used as a standard.

Leaf powder

The leaves were harvested and placed in an oven with forced air circulation at 70 °C for 96 h. The leaf dry matter was triturated and the powder was kept in glass containers, which remained in the dark at 15 °C until biochemical analysis.

Amino acids and proline

The amino acids were determined in 50 mg of leaf dry matter powder incubated in 5 mL of sterile distilled water at 100 °C for 30 min. After being homogenized, it was centrifuged at 2000 ×g for 5 min at 20 °C and the supernatant was removed. The 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. The quantification of proline was performed after measuring the absorbance at 520 nm according to Bates et al. (1973) based on L-proline (Sigma Chemicals) as standard.

Total soluble proteins

The determination of the total soluble proteins was performed in 100 mg of leaf powder incubated in 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 according to Bradford (1976) with albumin bovine (Sigma Chemicals) used as standard.

Abscisic acid determination

The samples of leaf dry matter were extracted in 80% (v/v) acetone containing BHT (butylated hydroxy-toluene) at 0.45 mM and BHA (butylated hydroxy-anisole) (Sigma chemicals) at 0.55 mM, during 7 days under 4 °C, according to Norman et al. (1990) with minor modifications. Subsequently,

the extract was incubated in saline buffer (Tris-NaCl at 50 mM pH 7.5) (Merck Reagents), centrifuged at 7000 ×g for 10 min at 10 °C, and the supernatant removed. The quantification was carried out using Phytodetek ABA kits (Phytodetek reagents).

Data analysis

The data were subjected to variance analysis and significant differences between means were determined by Tukey test at 5% level of error probability. The correlation analysis was performed by the Person parametric method, and the statistical procedures were carried out with the SAS software.

Results

Leaf relative water content and leaf dry matter

The leaf relative water content results revealed that water deficiency induced significant decrease in this parameter, and the values showed in the stress treatment were at 88%, 86%, 80%, 72%, 68%, 90%, and 91% during the 0, 1st, 2nd, 3rd, 4th, 5th, and 6th day, respectively. The lower value was observed on the 4th day of the treatment under water deficit. After rehydration (5th and 6th day), the plants under water deficiency reestablished the leaf relative water content to the same levels as the control plants (Figure 1a).

Leaf dry matter was severely affected by the simulated water deficit, while the control treatment presented an increase in all the evaluated points (Figure 1b). The plants under water stress presented a decrease in leaf dry matter only on the 3rd and 4th days after water restriction. The rehydration of the plants under water deficit promoted an increase in leaf dry matter but the increase was not significant.

The correlation analysis suggests that there is a significant relationship between leaf relative water content and abscisic acid concentration ($r = 0.88$; $P < 0.01$) (Figure 2).

Glutamine synthetase activity, total soluble amino acids, and total soluble proteins

Glutamine synthetase activity was affected by water deficiency, and 2 days of stress were sufficient to induce a significant increase (Figure 3a). The maximum value observed was 115 μmol GGH g⁻¹ FM on the 4th day under water stress. Rehydration promoted a decrease in the glutamine synthetase

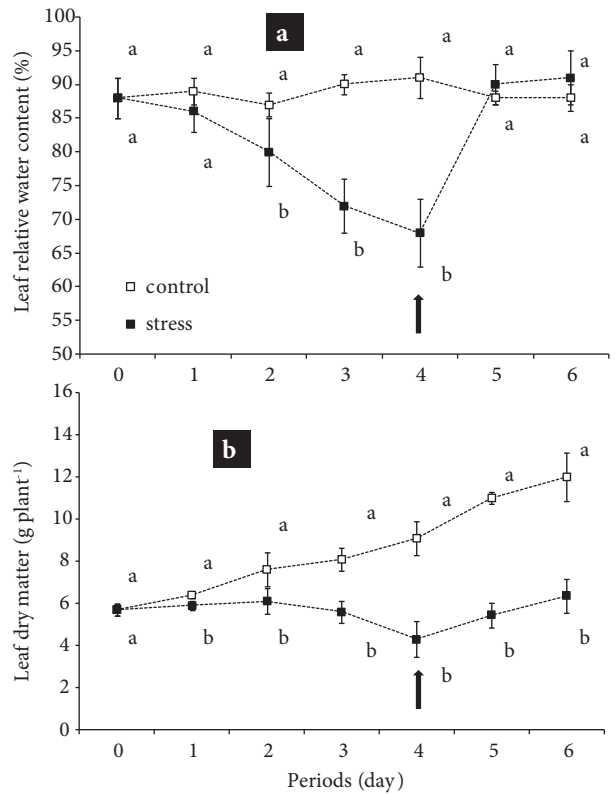


Figure 1. Leaf relative water content (A) and leaf dry matter (B) in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. Means followed by the same letter are not significantly different by the Tukey test at 5% of probability. The bars represent the mean standard error and the arrow indicates the rehydration point.

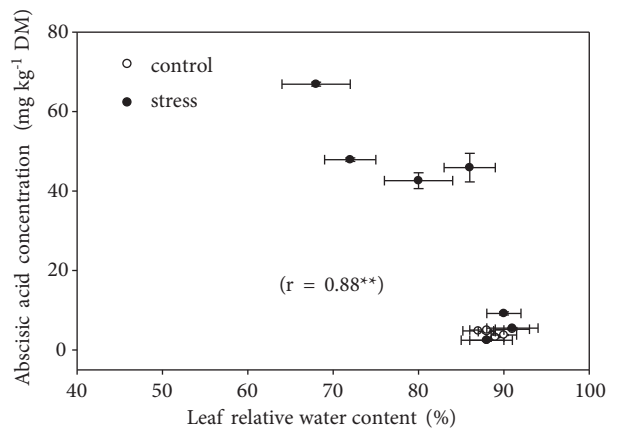


Figure 2. Relationship between leaf relative water content and abscisic acid concentration in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. The bars represent the mean standard error and the asterisks (**) indicate significance at 0.01 probability level.

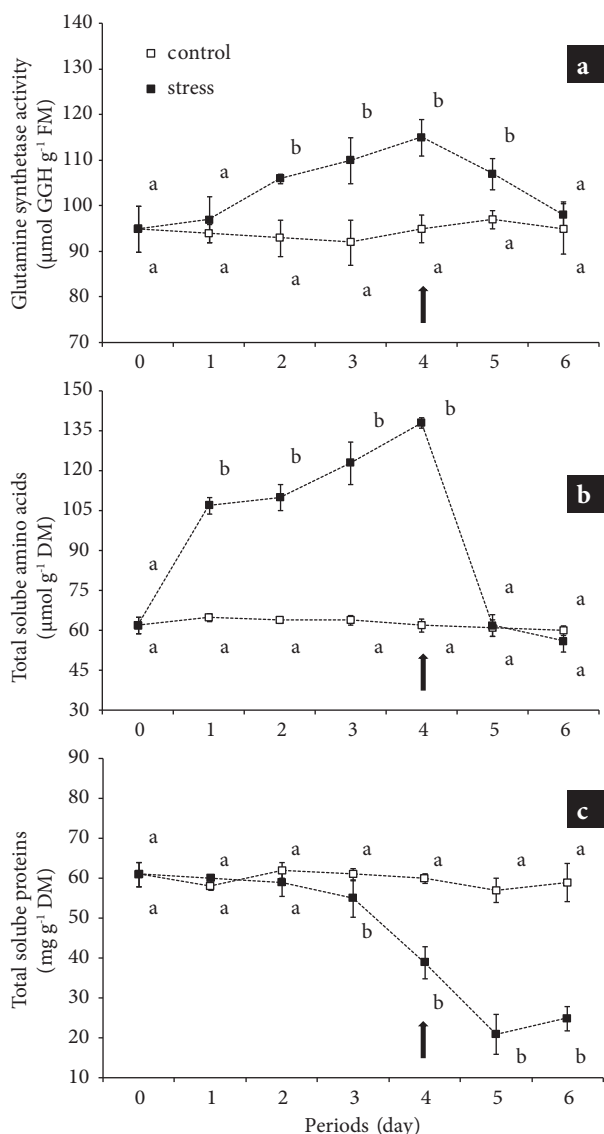


Figure 3. Glutamine synthetase activity (a), total soluble amino acids (b) and total soluble proteins (c) in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. Means followed by the same letter are not significantly different by the Tukey test at 5% of probability. The bars represent the mean standard error and the arrow indicates the rehydration point.

activity with the 6th day presenting statistically equal activities between the control and stress treatments.

The levels of amino acids were significantly increased on the 1st day. The plants under water deficit presented the amounts of 62, 107, 110, 123, 138, 62, and 56 $\mu\text{mol g DM}^{-1}$ on the 0, 1st, 2nd, 3rd,

4th, 5th, and 6th day, respectively, while the plants of the control treatment presented amino acids amount between 65 and 60 $\mu\text{mol g}^{-1}$ DM (Figure 3b). Rehydration of the plants under stress reduced the amino acid levels to the same level as the control.

Water deficiency induced progressive changes in the protein levels, where on the 3rd, 4th, 5th and 6th day significant decreases of 10%, 35%, 63%, and 56%, respectively, were observed when comparing the stress treatment with the control (Figure 3c). These results revealed that despite of the rehydration of the water restricted plants on the 4th day, the plants were not able to recover the content of protein to the same level as the control after 2 days of rehydration.

Abscisic acid concentration and proline

Water deficit simulation induced a significant increase in the abscisic acid concentration, and values verified in the stress treatment were 2.4, 45.9, 42.6, 47.9, 66.9, 9.2, and 5.5 mg kg^{-1} DM on days 0, 1, 2, 3, 4, 5 and 6, respectively (Figure 4a). The control plants presented values between 2.4 and 5.2 mg kg^{-1} DM. The rehydration induced a rapid decrease in the abscisic acid concentration, but the concentration of the treatment stress was equal to the control only on the 6th day.

Proline levels of the plants under stress increased due to water restriction, in which the stress treatment presented significant increases of 67%, 354%, 423%, and 532% on the 1st, 2nd, 3rd, and 4th day, respectively, when comparing with the control (Figure 4b). After rehydration, the proline levels of the plants subjected to water deficit were reduced; however, only on the 6th day was it statistically similar to the control. In addition, the correlation analysis shows a significant relationship between abscisic acid concentration and proline level ($r = 0.97$; $P < 0.01$) (Figure 5).

Discussion

Reduction in leaf relative water content of the water restricted plants reveals that the simulated stress was sufficient to promote significant changes in this parameter with biochemical and physiological consequences for the plant. Subsequently, the rehydration reestablished the amount of water in the leaf and demonstrates the fast absorption and translocation of water coming from substrate to

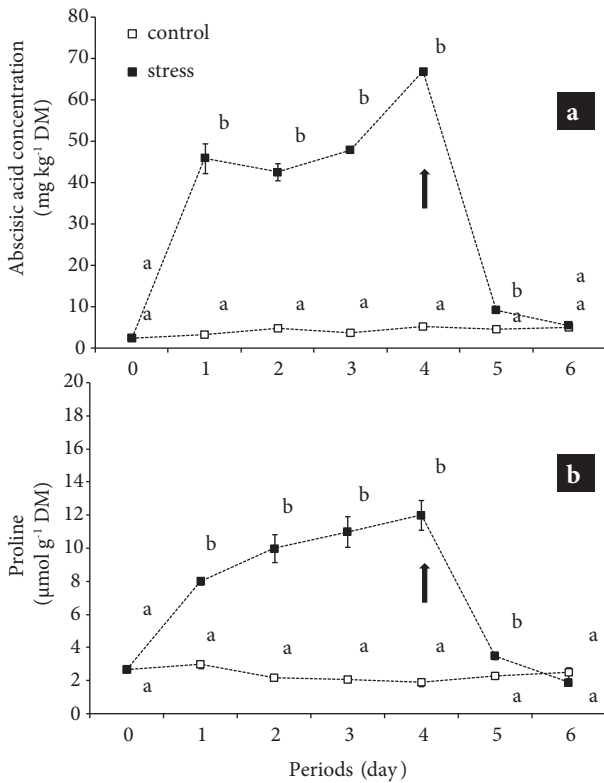


Figure 4. Abscisic acid concentration (a) and proline (b) in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. Means followed by the same letter are not significantly different by the Tukey test at 5% of probability. The bars represent the mean standard error and the arrow indicates the rehydration point.

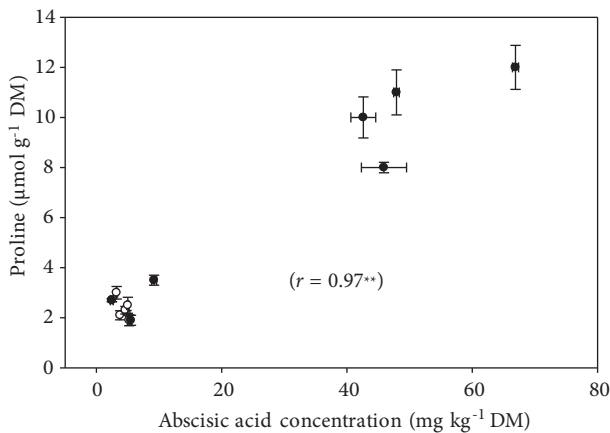


Figure 5. Relationship between abscisic acid concentration and proline in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. The bars represent the mean standard error and the asterisks (**) indicate the significance at 0.01 probability level.

leaf. Similar results on decrease in leaf relative water content were reported by Lobato et al. (2008b) in *Vigna unguiculata* plants under water deficiency.

Stress plants maintained the dry matter in the earliest periods; however, in the late period this parameter was reduced. Maintenance is probably due to lower water volume in the tissue, because the water acts as substrate to expansins activities (Kerbaux 2004), and as a response paralysation of shoot growth will occur under these conditions (Lobato et al. 2008a). Additionally, the decrease verified in this parameter is linked to leaf loss obtained in the experiment. The results reveal that rehydration does not result in the total recuperation of the leaf dry matter, because stressed plants have a rate of growth smaller than the control, demonstrating the irreversible damage of the water deficit. Lobato et al. (2008c) studying the effects of the water deficiency in *Glycine max* L. Merr plants found similar results.

The increase in the glutamine synthetase activity of the plants under water deficit is probably linked to its osmotic adjustment process because this enzyme acts by consuming ammonium and producing amino acids that can contribute directly to the plant's osmotic adjustment (Oliveira Neto et al. 2009). The rehydration induced a significant reduction in glutamine synthetase level only on the 6th day, and this fact contributes to the previously cited hypothesis, due to the slow recuperation of this enzyme to normal level. The increase in glutamine synthetase activity obtained in this study is similar to that observed by Silveira et al. (2003a) investigating the metabolic consequences of salt stress in leaves and roots of *Anacardium occidentale* L.

The increase in the amount of total soluble amino acids in the water deficiency-induced plants is due to the biosynthesis of amino acids, such as glycinebetaine and proline, that can be used as organic compounds with high affinity by the water, and, consequently, decrease the negative effects of water deficiency (Lobato et al. 2009c). The water availability provoked fast reestablishing of this parameter. Sircelj et al. (2005) working with 2 *Malus domestica* Borkh. cultivars described similar results as shown in this investigation on the increase in amino acids levels.

The progressive reduction of total soluble proteins during water deficiency in the plants was induced by proteolysis (Debouba et al. 2006) with the liberated amino acids used during the plant osmotic adjustment. The rehydration did not promote the return of the total soluble proteins to the same level as the control plants. This fact indicates a slow recuperation of this parameter probably because the proteins depend on other nitrogen compounds for synthesis. The behavior shown in proteins during water deficit was described by Hamidou et al. (2007) evaluating 5 genotypes; furthermore the results presented during rehydration are similar to those reported by Silveira et al. (2003b), both being studies performed with *Vigna unguiculata* plants.

The progressive increase in abscisic acid concentration in the plants of the stress treatment is related to the stomatal mechanism, because this hormone, under these conditions, provokes stomatal closing (Stikic and Davies 2000), consequently reducing the water losses during the gas exchanges in essential physiological processes like transpiration and photosynthesis (Liu et al. 2005). Based on this study, the leaf relative water content influences abscisic acid concentration present in the leaf. Therefore, the relative water content acts as a signal and the abscisic acid works during the signal transduction due to the easy and fast movement of this compound into plant tissue, and as a response the stomatal closing occurs in *Vigna unguiculata* plants subjected to water deficit. The fast decrease in the abscisic acid concentration after rehydration indicates the efficiency of the signalization pathway, transduction, and consequent response of this compound. The results reported in this study on abscisic acid are corroborated by Hsu et al. (2003) evaluating the consequences of water stress in *Oryza sativa* L., and the effects of heavy metal stress in *Cicer arietinum* L. (Atici et al. 2005).

Water deficit promoted a progressive increase in proline levels, and this fact was due to the rise in the enzymatic activity of Δ -pyrroline-5-carboxylate synthase (P5CS) that is induced under water deficiency (Lei et al. 2007). Proline is accumulated

during inadequate environment conditions, occurring without toxicity or damage in cells, membranes, and/or tissues (Pimentel 2004). In addition, the results obtained suggest that abscisic acid mediates actively and significantly the proline accumulation and consequent osmotic adjustment in *Vigna unguiculata* leaves induced to water deficit and rehydration. A recent study indicated that *Vigna unguiculata* plants considered resistant to water deficit presented proline accumulation (Costa et al. 2008) and consequently are more adapted to environments with low water supplement, when compared to sensitive plants. The rehydration reduced the proline levels, suggesting that this nitrogen compound participates actively in the osmotic adjustment in this species. The proline accumulation during water deficit presented in this study is similar with results reported by Sarker et al. (1999) investigating *Triticum aestivum*, and by Smita and Nayyar (2005) evaluating *Cicer arietinum*.

Our study indicates a negative interference promoted by water deficit in nitrogen metabolism of *Vigna unguiculata* plants, and also a fast recovery after rehydration. The water restriction promoted decreases in leaf relative water content and leaf dry matter. The nitrogen compounds as total soluble amino acids and proline and the enzymatic activity of glutamine synthetase presented significant increases, which are direct and indirect responses involved in the osmotic adjustment of the plant, in addition to an increase in abscisic acid concentration with the objective to relieve water loss in *Vigna unguiculata* induced by water deficiency. However, the total soluble proteins were reduced probably due the proteolysis process. Furthermore, a positive relationship between abscisic acid and proline synthesis was confirmed.

Acknowledgments

This research had financial support from Conselho Nacional de Pesquisa (CNPq/Brazil) for R.C.L. Costa and J.A.G. Silveira and from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brazil) for A.K.S. Lobato. The manuscript was reviewed by H.D. Laughinghouse IV.

References

- Anyia AO, Herzog H (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Europ J Agron* 20: 327-339.
- Assmann SM (2003) *OPEN STOMATA1* opens the door to ABA signaling in *Arabidopsis* guard cells. *Trends Plant Sci* 8: 151-153.
- Atici O, Agar G, Battal P (2005) Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. *Biol Plant* 49: 215-222.
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-207.
- Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
- Cayley S, Lewis BA, Record Jr MT (1992) Origins of the osmoprotective properties of betaine and proline in *Escherichia coli* K-12. *J Bacteriol* 175: 1586-1595.
- Chen C, Chen L, Lin C, Kao C (2001) Regulation of proline accumulation in detached rice leaves exposed to excess copper. *Plant Sci* 160: 283-290.
- Costa RCL, Lobato AKS, Oliveira Neto CF, Maia PSP, Alves GAR, Laughinghouse IV HD (2008) Biochemical and physiological responses in two *Vigna unguiculata* (L.) Walp. cultivars under water stress. *J Agron* 7: 98-101.
- Debouba M, Gouia H, Suzuki A, Ghorbel MH (2006) NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato "*Lycopersicon esculentum*" seedlings. *J Plant Physiol* 163: 1247-1258.
- Ezzine M, Ghorbel MH (2006) Physiological and biochemical responses resulting from nitrite accumulation in tomato (*Lycopersicon esculentum* Mill. cv. Ibiza F1). *J Plant Physiol* 163: 1032-1039.
- Hamidou F, Zombre G, Braconnier S (2007) Physiological and biochemical responses of cowpea genotypes to water stress under glasshouse and field conditions. *J Agron Crop Sci* 193: 229-237.
- Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. California Agricultural Experiment Station, San Francisco.
- Hsu SY, Hsu YT, Kao CH (2003) Ammonium ion, ethylene, and abscisic acid in polyethylene glycol-treated rice leaves. *Biol Plant* 46: 239-242.
- Husaini AM, Abdin MZ (2008) Development of transgenic strawberry (*Fragaria x ananassa* Duch.) plants tolerant to salt stress. *Plant Sci* 174: 446-455.
- Kamachi K, Yamaya T, Mae T, Ojima K (1991) A role for glutamine synthetase in remobilization of leaf nitrogen during natural senescence in rice leaves. *Plant Physiol* 96: 411-417.
- Kerbauy GB (2004) *Plant Physiology*. Guanabara Koogan S.A., Rio de Janeiro.
- Kırda C, Topaloglu F, Topçu S, Kaman H (2007) Mandarin yield response to partial root drying and conventional deficit irrigation. *Turk J Agric For* 31: 1-10.
- Krouma A (2010) Plant water relations and photosynthetic activity in three Tunisian chickpea (*Cicer arietinum* L.) genotypes subjected to drought. *Turk J Agric For* 34: 257-264.
- Kuznetsov VV, Shevyakova NI (1997) Stress responses of tobacco cells to high temperature and salinity. Proline accumulation and phosphorylation of polypeptides. *Physiol Plant* 100: 320-326.
- Lei Y, Yin C, Ren J, Li C (2007) Effect of osmotic stress and sodium nitroprusside pretreatment on proline metabolism of wheat seedlings. *Biol Plant* 51: 386-390.
- Liu F, Jensen CR, Shahanzari A, Andersen MN, Sven-Erik J (2005) ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. *Plant Sci* 168: 831-836.
- Lobato AKS, Costa RCL, Oliveira Neto CF, Santos Filho, BG, Cruz FJR, Freitas JMN, Cordeiro FC (2008a) Morphological changes in soybean under progressive water stress. *Int J Bot* 4: 231-235.
- Lobato AKS, Oliveira Neto CF, Costa RCL, Santos Filho BG, Cruz FJR, Laughinghouse IV HD (2008b) Biochemical and physiological behavior of *Vigna unguiculata* (L.) Walp. under water stress during the vegetative phase. *Asian J Plant Sci* 7: 44-49.
- Lobato AKS, Oliveira Neto CF, Santos Filho BG, Costa RCL, Cruz FJR, Neves HKB, Lopes MJS (2008c) Physiological and biochemical behavior in soybean (*Glycine max* cv. Sambaiba) plants under water deficit. *Australian J Crop Sci* 2: 25-32.
- Lobato AKS, Costa RCL, Oliveira Neto CF, Santos Filho BG, Gonçalves-Vidigal MC, Vidigal Filho PS, Silva CR, Cruz FJR, Carvalho PMP, Santos PCM, Gonela A (2009a) Consequences of the water deficit and symbiosis in *Vigna unguiculata* cultivars. *Plant Soil Environ* 55: 139-145.
- Lobato AKS, Gonçalves-Vidigal MC, Vidigal Filho PS, Costa RCL, Lopes MJS, Cruz AS, Meirelles ACS, Gonçalves AMO (2009b) Nitrogen compounds responses in two cultivars of common bean inoculated with *Colletotrichum lindemuthianum*. *Res J Biol Sci* 4: 293-297.
- Lobato AKS, Costa RCL, Neto MAM, Oliveira Neto CF, Santos Filho BG, Alves GAR, Costa KC, Silva LI, Conrado TV, Maleia MP (2009c) Consequences of the water deficit on nitrogen compounds in pepper (cv. Vermelho gigante) plants. *Res J Biol Sci* 4: 760-764.
- Lobato AKS, Gonçalves-Vidigal MC, Vidigal Filho PS, Andrade CAB, Kvitschal MV, Bonato CM (2010) Relationships between leaf pigments and photosynthesis in common bean plants infected by anthracnose. *New Zealand J Crop Hort Sci* 38: 29-37.

- Martinez-Ballesta MC, Martinez V, Carvajal M (2004) Osmotic adjustment, water relations and gas exchange in pepper plants grown under NaCl or KCl. *Environ Exp Bot* 52: 161-174.
- Nayyar H, Kaushal SK (2002) Alleviation of negative effects of water stress in two contrasting wheat genotypes by calcium and abscisic acid. *Biol Plant* 45: 65-70.
- Norman SM, Poling SM, Maier VP (1990) An indirect enzyme-linked immunosorbent assay for (+)-abscisic acid in *Citrus*, *Ricinus* and *Xanthium* leaves. *J Agric Food Chem* 36: 225-231.
- Olivera L, Canul RR, Pereira-Pacheco F, Cockburn J, Soldani F, McKenzie NH, Duncan M, Olvera-Novoa MA, Grant G (2003) Nutritional and physiological responses of young growing rats to diets containing raw cowpea seed meal, protein isolate (Globulins), or starch. *J Agric Food Chem* 51: 319-325.
- Oliveira Neto CF, Lobato AKS, Costa RCL, Maia WJMS, Santos Filho BG, Alves GAR, Brinez B, Neves HKB, Lopes MJS, Cruz, FJR (2009) Nitrogen compounds and enzyme activities in sorghum induced to water deficit during three stages. *Plant Soil Environ* 55: 238-244.
- Peoples MB, Faizah AW, Reakasem BE, Herridge DF (1989) Methods for evaluating nitrogen fixation by nodulated legumes in the field. Australian Centre for International Agricultural Research, Canberra.
- Pimentel C (2004) The relationship of the plant with the water. EDUR Seropédica.
- Sarker AM, Rahman MS, Paul NK (1999) Effect of soil moisture on relative leaf water content, chlorophyll, proline and sugar accumulation in wheat. *J Agron Crop Sci* 183: 225-229.
- Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J Exp Bot* 57: 711-726.
- Showemimo FA, Olarewaju JD (2007) Drought tolerance indices in sweet pepper (*Capsicum annuum* L.). *Int J Plant Breed Genet* 1: 29-33.
- Silveira JAG, Viégas RA, Rocha IMA, Moreira ACOM, Moreira RA, Oliveira JTA (2003a) Proline accumulation and glutamine synthetase activity are increased by salt-induced proteolysis in cashew leaves. *J Plant Physiol* 160: 115-123.
- Silveira JAG, Costa RCL, Viégas RA, Oliveira JTA, Figueiredo MVB (2003b) N-compound accumulation and carbohydrate shortage on N₂ fixation in drought-stressed and rewatered cowpea plants. *Spanish J Agric Res* 1: 65-75.
- Sircelj H, Tausz M, Grill D, Batic F (2005) Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. *J Plant Physiol* 162: 1308-1318.
- Slavick B (1979) Methods of studying plant water relations. Springer-Verlang, New York.
- Smita, Nayyar H (2005) Carbendazim alleviates effects of water stress on chickpea seedlings. *Biol Plant* 49: 289-291.
- Stikic R, Davies WJ (2000) Stomatal reactions of two different maize lines to osmotically induced drought stress. *Biol Plant* 43: 399-405.
- Vendruscolo ECG, Schuster I, Pileggi M, Scapim CA, Molinari HBC, Marur CJ, Vieira LGE (2007) Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J Plant Physiol* 164: 1367-1376.