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Consequences of the Water Deficit on Nitrogen Compounds in Pepper (cv. Vermelho Gigante) Plants

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Abstract: The aim of the study was to investigate the consequences provoked by the water deficit on nitrogen compounds in *Capsicum annuum* L. (cv. Vermelho Gigante) plants. The experimental design used was entirely randomized, with 2 water regimes (control and stress), as well as it was composed by 5 repetitions. The free ammonium levels suffered significant interference, as well as the control and stress treatments presented 25.9 and 20.1 μmol NH_4^+/g/DM, respectively. The proline level was significantly increased at 121.8%, when compared with control plants. The glycinebetaine level in stress plants presented significant increase at 39.8%. The total soluble proteins were in control and stress treatments were 5.72 and 3.89 mg/g/DM, respectively. The study revealed that the water deficit of 6 days was sufficient to provoke changes in all the nitrogen compounds evaluated, in which the reduction in amount of free ammonium is due to lower nitrogen influx and decrease of the total soluble proteins is promoted by the protease enzyme action. However, the proline and glycinebetaine levels in stress plants were showed accumulations, due to process of plant osmotic adjustment.

Key words: *Capsicum annuum* L., water deficit, nitrate, ammonium, proteins, osmotic adjustment

INTRODUCTION

The water deficit is one of the biotic stresses more studded, because this situation is common in several regions in world, as well as through of these studies can be evaluated the yield potential of cultivars and consequently, it to indicate the cultivars that support better conditions under limited water supplement.

The Nitrogen (N) is the element more abundant in earth surface, however, this inorganic form of nitrogen cannot be absorbed by the plants. Therefore, the nitrogen forms assimilated by higher plants are ammonium (NH_4^+) and nitrate (NO_3^-), as well as these compounds exert strong influences under the nitrogen metabolism (Lobato *et al*., 2008a).

Normality horticultural crops are more sensitives to high temperature, water deficit and salt stress, if compared with grain crops as *Vigna unguiculata* and *Sorghum bicolor* (Hamidou *et al*., 2007; Younis *et al*., 2000; Showemimo and Olarewaju, 2007), as well as the pepper crop is highly susceptible to water deficit effects during the growth, reproductive and fruiting stages (Olarewaju and Showemimo, 2002).

The aim of the study was to investigate, the consequences provoked by the water deficit on nitrogen compounds in *Capsicum annuum* L. (cv. Vermelho Gigante) plants.

MATERIALS AND METHODS

Growth conditions: The study was carried out in the Instituto de Ciencias Agraria (ICA) of the Universidade Federal Rural da Amazonia (UFRA), Belem City, Para State, Northern region, Brazil (01°27' S and 48°26' W) in the period of February and April of 2008. The plants remained in glasshouse environment under natural conditions day/night (air temperature minimum/maximum and relative humidity of 22.1-35.5°C and 65-93%, respectively. The photoperiod medium was of 12 h of light and photosynthesis radiation active maximum of 720 μmol/m^2/sec (at 12:00 h).

Plant materials: *Capsicum annuum* L., seeds of the Vermelho Gigante cultivar used in this study were harvested in the 2007 season and coming from Universidade Federal Rural da Amazonia (UFRA), estado

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Para, Brazil. The seed treatment was carried out through of immersion in solution of N-(triclorometil)-4 ciclohexan-1, 2 dicarbomixid ($C_9H_8Cl_3NO_2S$) at 3 ppm by 30 sec and drying in oven with forced air circulation at 30°C by 120 h (Machado, 2000). The seeds were kept in bags hermetically closed, in which it were remained in the dark and under the temperature of 10°C.

Substrate and pot: The substrate used to the plant growth was composed by sand and sialic in the proportion of 2:1, respectively, as well as this substrate was autoclaved at 120°C. atm.$^{-1}$ by 40 min. The container used to the plant growing was Leonard pot with 2 L capacity and it was adapted in the Laboratorio de Fisiologia Vegetal Avancada (LFVA).

Experimental design and treatments: The experimental design used was entirely randomized, with 2 water regimes (control and stress). The experiment was composed by 5 repetitions and 10 experimental units, as well as 1 plant in each unit.

Plant conduction: Five seeds pot^{-1} were sowed and after the germination were kept 1 plant pot^{-1}. The stress and control treatments received macro and micro nutrients in the form of nutritive solution of Schwarz (1995). The solutions were applied in plants by the period of 65 days, as well as the nutritive solution were changes with 5 days of interval, always at 09:00 h and the pH of the nutritive solution was adjusted to 6.0±0.1 with addition of HCl or NaOH. In the 65th day after the experiment implementation, the plants of the treatments under stress were submitted to period of 6 days without nutritive solution, in which the water deficit was simulated in the 65th until 71th day after of the experiment start. After this period, the plants were biochemically analyzed.

Leaf relative water content: The leaf relative water content was evaluated with leaf disks with 10 mm of diameter and it was carried out in each plant, in which were removed 40 disks and the calculation in agreement with the equation proposed by Slavick (1979):

$$\text{LRWC} = \frac{(\text{MF-MS})}{(\text{MT-MS})} \times 100$$

where:

MF = Matter Fresh

MT = Matter Turgid evaluated after 24 h and saturation in deionized water at 4°C in dark

MS = The dry matter determined after 48 h in oven with forced air circulation at 80°C

Leaf dehydration: The leaves were harvested and placed in an oven with forced air circulation at 70°C by 96 h. After this period, the leaf dry matter was triturated and the powder was kept in glass containers. The containers remained in the dark at temperature of 15°C until the moment to carry out biochemical analysis.

Free ammonium and proline: The free ammonium and proline were determined with 50 mg of leaf dry matter powder, which was incubated with 5 mL of sterile distilled water at 100°C by 30 min, after the homogenized was centrifuged to 2.000 g by 5 min at 20°C and the supernatant was removed. The quantification of the free ammonium was carried out at 625 nm in agreement with Weatherburn (1967), as well as was used $(NH_4)_2SO_4$ (Sigma Chemical) as standard. The quantification of proline was carried out at 520 nm according to Bates *et al.* (1973), in which was utilized L-proline (Sigma Chemicals) as standard.

Glycinebetaine: The glycinebetaine was determined with 25 mg of leaf dry matter powder, which it was incubated with 2 mL of sterile distilled water at 25°C by 4 h and under agitation, after the homogenized was centrifuged to 10.000 g by 10 min at 25°C and the supernatant was removed. The glycinebetaine quantification was carried out at 365 nm according to Grieve and Grattan (1983), in which was utilized glycinebetaine (Sigma Chemicals) as standard.

Total soluble proteins: The determination of the total soluble proteins was carried out with 100 mg of powder, in which was incubated with 5 mL of extraction buffer (Tris-HCl at 25 mM and pH 7.6). The homogenized was kept in agitation by 2 h, after this period centrifuged to 2000 g by 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), as well as was used albumin bovine (Sigma Chemicals) as standard.

Data analysis: The data were submitted at variance analysis and when significant differences occurred were applied to Scott-Knott test at 5% level of error probability, as well as the standard errors were calculated in all evaluated treatments (Gomes, 2000). The statistical analysis were carried out with the software SAS Institute (1996).

RESULTS AND DISCUSSION

Consequences on leaf relative water content: The leaf relative water content was significantly modified after the water restriction, in which the control and stress plants presented 92.6 and 51.9%, respectively (Fig. 1).

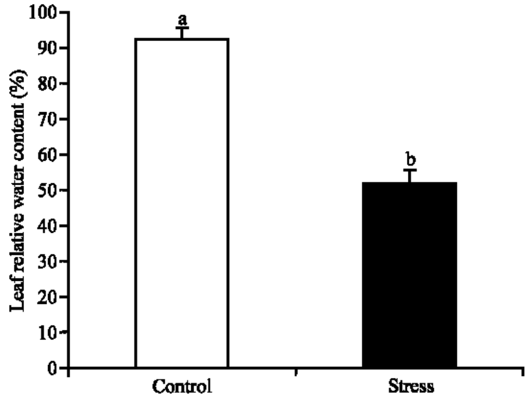

Fig. 1: Leaf relative water content in *Capsicum annuum* cv. Vermelho Gigante under water deficiency. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

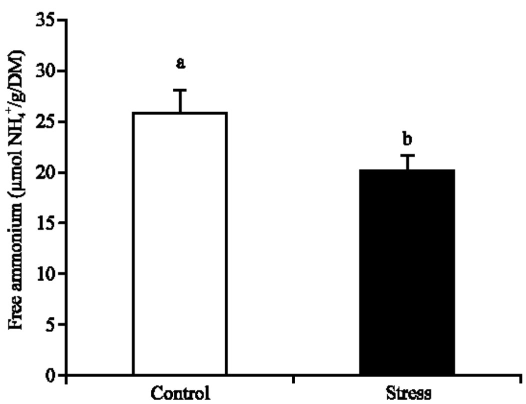

Fig. 2: Free ammonium in *Capsicum annuum* cv. Vermelho Gigante under water deficiency. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

Consequences on free ammonium: The free ammonium levels suffered significant interference promoted by the water deficit (Fig. 2), as well as the control and stress treatments presented 25.9 and 20.1 μmol NH_4^+/g/DM, respectively.

Responses on proline: The proline levels significantly changed, in which it showed 6.57 and 14.57 μmol/g/DM, respectively (Fig. 3). In addition, this increase obtained corresponding to 121.8%, when compared with control plants.

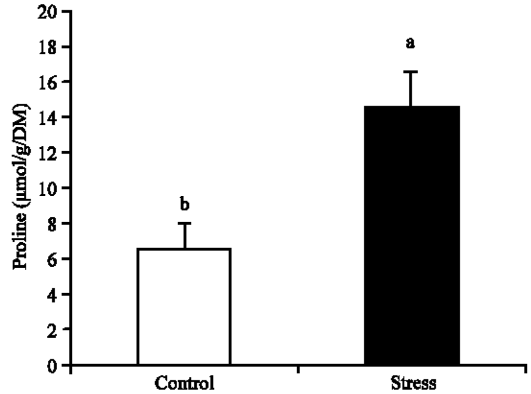

Fig. 3: Proline in *Capsicum annuum* cv. Vermelho Gigante under water deficiency. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

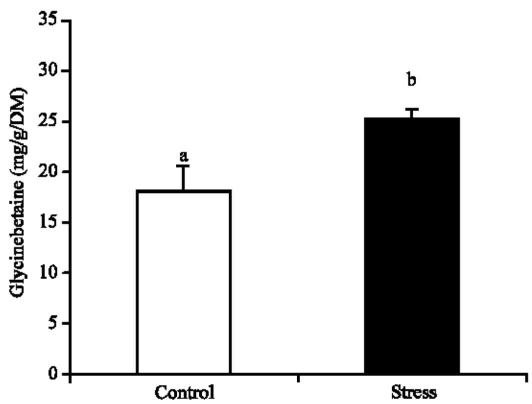

Fig. 4: Glycinebetaine in *Capsicum annuum* cv. Vermelho Gigante under water deficiency. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

Responses on glycinebetaine: The glycinebetaine levels in stress plants presented significant increase at 39.8% (Fig. 4), as well as the values showed in control and stress plants were of 18.1 and 25.3 mg/g/DM, respectively.

Responses on proteins: The total soluble proteins were significantly modified, as well as the values obtained in control and stress treatments were 5.72 and 3.89 mg/g/DM, respectively (Fig. 5).

The reduction in leaf relative water content after the application of the water deficit reveals that the period of 6 days is sufficient to promote changes in this parameter and consequently in others, as well as the decrease is due

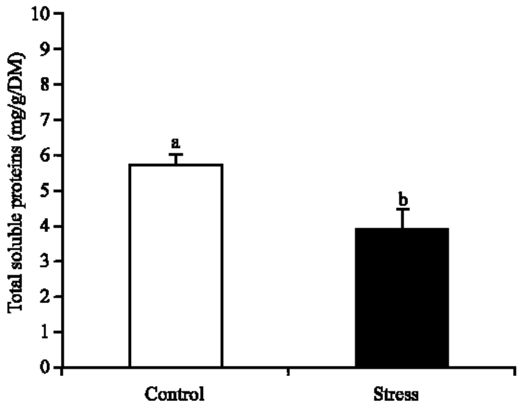

Fig. 5: Total soluble proteins in *Capsicum annuum* cv. Vermelho Gigante under water deficiency. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

the water loss to environment through photosynthesis and respiration processes. The leaf relative water content is used to measure the water volume presents in leaf tissue in situations that there not are specific equipments. Similar results on decrease in leaf relative water content were obtained by Lobato *et al.* (2008b) working with *Vigna unguiculata* plants under water deficiency.

The decrease in free ammonium levels after the simulation of the water deficit was promoted probably by lower nitrogen influx and consequent reduction in enzyme activities nitrate reductase and nitrite reductase, because these two enzymes are responsibles to reduce of catalytic form the nitrate to ammonium and these can be present into leaf and root (Taiz and Zeiger, 1998). Lobato *et al.* (2009) showed similar results on decrease of the free ammonium levels in two cultivars of *Phaseolus vulgaris* infected by the pathogen *Colletotrichum lindemuthianum.*

The accumulation in proline level is linked to process of osmotic adjustment of the plant under situations of water deficiency. This amino acid presents high affinity by the water molecule (H_2O) and this way increase the water retention in tissue and consequently the plant tolerance to water deficit. Studies conducted by Lobato *et al.* (2008c) investigating the consequences of the water stress in *Glycine max* plants corroborate the results obtained in this experiment.

The glycinebetaine level suffered increase in plants submitted to water stress, in which this amino acid acts as organic solute that can be accumulated without energy consumption and without interference in the plant metabolic activity, as well as studies carried out by Allakhverdiev *et al.* (2003) reveal that this compound is a protector of the photosynthetic apparatus. The increase in glycinebetaine also was reported by Cha-um *et al.* (2007) working with two cultivars of *Oryza sativa* induced to salt stress.

The significant reduction in total soluble proteins was promoted by the action of the protease enzymes, because theses enzymes are responsibles by the protein breakdown to form amino acids as proline and glycinebetaine, in which it is used in mechanism of plant osmotic adjustment (Costa *et al.*, 2008). In addition, the protein synthesis is paralyzed under circumstances of water deficiency and this fact also, contributes for the reduction in the amount of total soluble proteins. Results obtained by Debouba *et al.* (2006) on the decrease in total soluble proteins corroborate the results showed in this study.

CONCLUSION

The study revealed that the water deficit of 6 days was sufficient to provoke changes in all the nitrogen compounds evaluated, in which it was obtained reduction in amount of free ammonium due by the lower nitrogen influx and decrease of the total soluble proteins promoted by the protease enzyme action. However, the proline and glycinebetaine levels in stress plants were showed accumulations, due to process of plant osmotic adjustment, as well as it was proved that in this species the proline presented higher variation, when compared with glycinebetaine and this fact indicates that this amino acid contributes of form more intense in the osmotic adjust.

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