

Relationship between chlorophyll a and total soluble carbohydrates in pepper submitted to water deficiency

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Keywords: *Capsicum annuum* L., water deficit, photosynthetic pigments, soluble carbohydrates.

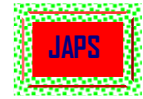
1 SUMMARY

The aim of this study was to evaluate the impact of water deficiency on *Capsicum annuum* plants, and it to investigate the hypothesis that chlorophyll influences total soluble carbohydrates. The experimental design was entirely randomized, with 2 water conditions (control and stress). Stomatal conductance was reduced at 87.6% after water deficit; transpiration rate presented 88.8 and 4.9 mmol/m²/s, in watered and induced to water deficiency, respectively. Chlorophyll total suffered also influence, and the values obtained were 50.24 and 26.98 mg/g FM in normal and stressed conditions, respectively. However, total soluble carbohydrates and sucrose were increased due to osmotic adjustment process. In addition, results suggest that there is significant and negative relationship between chlorophyll a and total soluble carbohydrates ($r = 0.78$; $P < 0.01$) and the hypothesis was confirmed.

2 INTRODUCTION

Environment inadequate conditions promoted by abiotic factors such as water, temperature, salt, and mineral elements can provoke reductions in growth and development in plants, disorders in physiological, biochemical, and molecular behaviors, besides yield losses in several crops (Tan *et al.*, 1999; Leport *et al.*, 1999; Oliveira Neto *et al.*, 2009a). Photosynthetic pigments are composed of chlorophylls *a*, *b* and *total*, and the main functions are reception and storage of light energy by inductive resonance through antenna complexes, and consequent electron transport carried out by the photosystem II (Candan & Tarhan, 2003). Oliveira Neto *et al.* (2009b) investigating *Sorghum bicolor* plants induced to water deficit obtained reductions in chlorophylls and carotenoids.

Carbon metabolism is responsible for regulation of compounds as sucrose, glucose, and starch, besides enzymes such as sucrose synthetase and invertase. In addition, sucrose is an important energy component exported from leaves to several plant organs (Kingston-Smith *et al.*, 1999). These carbohydrates are involved in processes such as energy transference, signal transduction, osmoprotection, and reserve. Study conducted by Lobato *et al.* (2008) reveals increase in carbohydrates, and this fact under inadequate situation is characteristic of compounds with capacity of protection osmotic (Verslues *et al.*, 2006). Therefore, the production and consequent compound function of carbon is determined by phenological stage, edaphoclimatic condition, and plant



nutritional status (Pimentel, 2004).

Pepper (*Capsicum annuum* L.) is a crop largely consumed worldwide, due to its agronomical and economical characteristics also due to its adaptability in several environments. Recent studies on physiological behavior of this species reveal that normally pepper is significantly affected by salt and water stress (Martínez-Ballesta *et al.*, 2004; Lobato *et al.*, 2009a). Therefore, quick detection of effects and an efficient parameter can help during selection process in breeding programs.

The aim of this study was to evaluate the impact of water deficiency on *Capsicum annuum* plants (cv. Vermelho gigante), and a possible influences of photosynthetic pigments over total soluble carbohydrates.

3 MATERIAL AND METHODS

3.1 Plant material: *Capsicum annuum* L. seeds of Vermelho gigante cultivar were used in this study which came from fruits harvested in 2007 season, during previous field experiment implemented under adequate nutritional and edafoclimatic conditions in Universidade Federal Rural da Amazônia (UFRA), state Pará, Brazil (01°27'S and 48°26'W). Seed treatment was carried out through immersion in a solution of N-(trichlorometil)-4 ciclohexan-1,2 dicarbomixid ($C_9H_8Cl_3NO_2S$) at 3 ppm for 30 s and drying in an oven with forced air circulation at 30°C for 120 h (Machado, 2000). The harvested and treated seeds were kept in hermetically closed bags, and remained in the dark and under temperature of 10°C, in the seed bank of Universidade Federal Rural da Amazônia (UFRA) under lot identification number (UFRA/2007-512), until the execution of this experiment.

3.2 Growth conditions: Study was done in the Instituto de Ciências Agrária (ICA) of Universidade Federal Rural da Amazônia (UFRA), Belém city, Pará state, Brazil (01°27'S and 48°26'W), from February to April 2008. The plants were grown in greenhouse and under natural conditions, air temperature minimum and maximum of 22.1 and 35.5 °C, respectively. The air relative humidity remained between 65 and 93% during experimental period. Medium photoperiod of 12 h of light, and photosynthetic active radiation (PAR) of 720 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, measured during maximum radiation and plant level, using steady-state porometer (LICOR AM-300, model 1600).

3.3 Substrate and pot: The Substrate used for plant growth and development was composed of sand and silica gel, (silica gel is a compound that is stable and chemically inert) in proportions of 2:1 (v/v), respectively. This mixture was autoclaved (120°C. atm.⁻¹ for 40 minutes) and distributed over Leonard-type pots. In each pot 2 L of mixture was

put and were adapted in Laboratório de Fisiologia Vegetal Avançada (LFVA).

3.4 Experimental design and treatments:

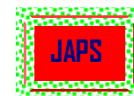
The experimental design used was completely randomised, with 2 treatments (control and stress). This experiment was composed by 5 repetitions, and 10 experimental units. Each plant was considered as one experimental unit.

3.5 Plant conduction and evaluation period:

Five seeds per pot were placed and after the emergence, within 10 days, but only one plant per pot were kept. All plants were watered, and received macro and micro nutrients from nutritive solution described by Schwarz (1995), 400 mL per pot. The changes of the nutritive solution were performed in equal periods, with 5 interval days, always at 09:00 h and pH was adjusted to 6.0 ± 0.1 with addition of HCl or NaOH.

Treatments (control and stress) were watered with the nutritive solution, and the control was kept with nutritive solution through all the experimental period, with the objective of simulating normal conditions. In the 65th day after experiment implementation, this period is characterized by end of the vegetative stage for the cultivar used in this study. The stress treatment was exposed to water deficiency, which was exposed to a period of 6 days under total absence of nutritive solution. This water deficiency was simulated in the 65th until 71st day after the experiment started. All plants on the 71st day were harvested for subsequently will be physiologically analyzed.

3.6 Leaf relative water content: The leaf relative water content was evaluated with leaf disks (10 mm diameter), carried out on each plant, and for each plant 40 disks were removed calculated in agreement with the formula proposed by Slavick (1979):



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

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

3.7 Water relations: Water relations were measured by stomata conductance and transpiration rate in mature and full expanded leaves, under light and localized in the branch medium third, and were measured through steady-state porometer (LICOR AM-300, model 1600), with gas exchanges evaluated immediately during period between 10:00 and 12:00 h in all experiment plants.

3.8 Photosynthetic pigments: Chlorophyll determinations were carried out with 25 mg of leaf tissue used to measure water relations, the samples were homogenised in the dark, and extracted with 2 mL of acetone at 80% (Nuclear). Subsequently, the homogenized liquid was centrifuged at 5000 RPM, for 10 minutes at 5°C. The supernatant was removed and quantified chlorophylls a, b, and total chlorophyll using a spectrophotometer at 646.8 and 663.2 nm, respectively (Lichthenthaler, 1987).

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

analysis.

3.10 Total soluble carbohydrates:

Carbohydrate contents were determined with 50 mg of powder coming from leaf dry matter, and incubated with 5 mL of pure ultra water at 100°C for 30 minutes. Subsequently the homogenized solution was centrifuged at 2000 RPM, for 5 minutes at 20°C and supernatant removed. Quantifications of total soluble carbohydrates were carried out at 490 nm, according to Dubois *et al.* (1956), using glucose (Sigma chemicals) as standard.

3.11 Sucrose: For sucrose determination 50 mg of powder from leaf dry matter was incubated with 1.5 mL of solution MCW (methanol, chloroform and water), in the proportion of 12:5:3 (v/v) at 20°C by 30 minutes and under agitation. Subsequently the sample was homogenised by centrifuging at 10000 RPM for 10 minutes at 20°C and the supernatant removed. Sucrose quantification was done under 620 nm, in accordance with Van Handel (1968), using sucrose (Sigma chemicals) as standard.

3.12 Data analysis: Data were subjected to analysis of variance, and means compared to Tukey test at 5% level of error probability. In addition, standard errors were calculated in all evaluated treatments (Steel *et al.*, 2006). Correlation analysis was performed by the Person parametric method and the statistical procedures were carried out using SAS.

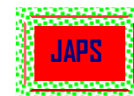
4 RESULTS AND DISCUSSION

4.1 Consequence on leaf relative water content: Water deficiency promoted significant decrease over leaf relative water content, and control and stress treatments presented 92.6 and 51.8%, respectively (Figure 1 A). This result obtained in stress plants is a consequence of water losses during transpiration and photosynthesis processes (Jones, 1998). This result on leaf relative water content indicates that period of 6 days used in this study was efficient to simulate water deficiency in *Capsicum annuum* plants, and this parameter was responsive to measure the water volume in plant tissue. Lobato *et al.* (2009b) investigating *Capsicum annuum* plants under water deficiency reported similar results.

4.2 Influence on stomatal conductance: This parameter in plants induced to water deficiency was strongly affected, as well as was obtained a reduction at 87.6% after 6 days under water

restriction, when compared to control plants (Figure 1 B). Stomatal conductance was reduced probably due to stomatal mechanism, which under water deficiency situations the acid abscisic is over-expressed (Liu *et al.*, 2005) through of K^+ , Cl^- and malate²⁻, promoting stomatal closing to avoid/decrease water loss from leave to environment (Assmann, 2003). Study conducted by Lobato *et al.* (2009c) with *Vigna unguiculata* plants exposed to water deficit describes similar results on decrease in stomatal conductance.

4.3 Influence on transpiration rate: Water restriction imposed to plants provoked significant decrease in transpiration rate, and the values shown were 88.8 and 4.9 mmol/m²/s, respectively (Figure 1 C). Correlation analysis indicated a significant relationship (Figure 2 A) between leaf relative water content and transpiration rate ($r = 0.99$; $P < 0.01$). In other correlation analysis (Figure 2 B) was



obtained significant interaction between stomatal conductance and transpiration rate ($r = 0.95$; $P < 0.01$). In addition, the relationship between leaf relative water content and transpiration rate is explicated based in Figure 2 B, which water restriction induces stomatal closing and consequently lower stomatal conductance, and this fact provokes direct interference in transpiration rate.

4.4 Change on chlorophyll a: The chlorophyll level was significantly modified after the period under water absence (Figure 3 A), which control and stress plants presented 8.12 and 4.08 mg/g FM, respectively. Stressed plants suffered reduction in chlorophyll level probably due action of reactive oxygen species (ROS), mainly hydrogen peroxide (H_2O_2) that is accumulated under water deficiency, because it provokes the inactivation/oxidation of the pigments pre-existing in chloroplasts (Almási *et al.*, 2000).

4.5 Change on chlorophyll b: chlorophyll b showed also significant reduction, following analyze of variance, and the stress treatment presented a decrease in 44.5% (Figure 3 B), if compared to control treatment. In addition, normally chlorophyll a and b are found in large amount into chloroplasts, but absorption wavelengths are different. Sircelj *et al.* (2005) studying two *Malus domestica* cultivars exposed to water restriction described similar results on chlorophyll b.

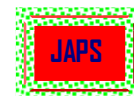
4.6 Consequence on chlorophyll total: Chlorophyll total suffered influence of water deficit simulated in this study, and the values obtained in pepper plants were 50.24 and 26.98 mg/g FM in control and treatments, respectively (Figure 3 C). The correlation analysis (Figure 4 A) suggests that there is a significant relationship between leaf relative water content and total chlorophyll ($r = 0.95$; $P < 0.01$). In addition, the figure 4 B presents a significant and positive relationship between chlorophyll a and total chlorophyll ($r = 0.98$; $P < 0.01$). Water deficiency provoked reduction leaf relative water content and also in chlorophyll total, but these results coming from chlorophyll total are linked to fall in chlorophyll a (Figure 4 B). Therefore, this study with *Capsicum annuum* plants suggests that leaf relative water content induced decrease in chlorophyll total, due low amount of chlorophyll a into chloroplast. Similar results linked

to reduction in leaf pigments were described by Cha-um *et al.* (2007) working with tolerant and susceptible lines of *Oryza sativa* exposed to abiotic stress.

4.7 Effect on total soluble carbohydrates:

Stressed plants presented were significantly changed (Figure 5 A). In control and stress treatments was obtained the values of 69.8 and 80.4 mg/g DM, respectively. Correlation analysis suggests a negative and significant relationship (Figure 6 A) between leaf relative water content and total soluble carbohydrates ($r = 0.73$; $P < 0.05$). In addition, results demonstrate through other analysis of correlation, that also there is significant interaction (Figure 6 B) between chlorophyll a and total soluble carbohydrates ($r = 0.78$; $P < 0.01$). This study reveals the interference of leaf relative water content on total soluble carbohydrates, beside to indicate that significant increase in total soluble carbohydrates must be associated with reduction in chlorophyll a. Therefore, increase of carbon compounds in leaf after water deficiency can have connection with reduction in chlorophyll a, and possibly over reduction in photosynthetic rate. The sugar accumulations, mainly glucose, into cell are responsible by the regulation of photosynthetic process (Moore *et al.*, 1999). In other words, lower flux of sugars from source (leaf) to sink (stem, root, and grain) during inadequate situations, combined with reduction in photosynthesis and oxidation/loss leaf pigments, it explain the relationship reported between chlorophyll a and carbohydrates. Increases in total soluble carbohydrates of plants exposed to water deficiency are frequently reported (Sircelj *et al.*, 2005; Martinelli, 2008).

4.8 Effect on sucrose: The sucrose level was influenced by water deficiency (Figure 5 B), as well as plants induced to stress presented a significant increase at 171.7%, if compared with control plants. The increase in stress treatment is linked to osmotic adjustment process (Rejskova *et al.*, 2007), because sucrose is inert organic compounds that can be accumulated and consequently to increase water retention during water deficiency (Williamson *et al.*, 2002), and it concomitantly can work as energy source after inadequate situation (Pimentel, 1999). In recent studies inducing *Glycine max* plants to water deficiency were reported similar results on sucrose (Lobato *et al.*, 2008).



5 CONCLUSION:

This study revealed several damages promoted by the water deficiency and obtained significant changes in all parameters measured. So, in leaf relative water content, stomatal conductance, transpiration rate, chlorophyll a, b and total suffered decrease after 6 days under water restriction.

6 ACKNOWLEDGMENTS

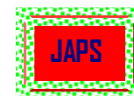
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However, total soluble carbohydrates and sucrose were increased due to osmotic adjustment process. In addition, results suggest that there is significant and negative relationship between chlorophyll a and total soluble carbohydrates and the hypothesis was confirmed.

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