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ROOT CONTRIBUTION TO WATER RELATIONS AND SHOOT IN TWO CONTRASTING *VIGNA UNGUICULATA* CULTIVARS SUBJECTED TO WATER DEFICIT AND INOCULATION

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ABSTRACT

This study aimed to investigate if root exercises influence on water relations and shoot of *Vigna unguiculata* plants exposed to combined action of water deficit and inoculation. The experimental design used was completely randomised with 2 cultivars (tolerant and sensitive) combined with 2 water regimes (water deficit and control), and 2 inoculation forms (inoculated and non-inoculated), totalising 8 treatments. Parameters evaluated were leaf relative water content, stomatal conductance, transpiration rate, leaf dry matter, stem dry matter, and root dry matter. Stomatal conductance was reduced in plants exposed to water deficiency in tolerant and sensitive cultivars, but plants inoculated presented no significant difference, when compared with non-inoculated plants. Water deficit provoked decrease in root dry matter of both cultivars, comparing to control plants, while tolerant plants exposed to water deficiency presented better results, if compared with sensitive plants. Significant relationships between root dry matter and stomatal conductance, as well as root dry matter and shoot (leaf and stem) were found in this study, confirming the hypothesis on influence of roots on water relations and shoot.

Key words: *Vigna unguiculata*, water deficit, inoculation, stomatal conductance, root.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is a leguminous with high protein content, large capacity of fixation of the atmospheric nitrogen (N_2) and low requirements to soil fertility (Alves et al., 2009), being frequently cultivated by farmers in Northern and Northeastern regions of the Brazil. This species constitutes the main subsistence culture, the grain being used as protein source in feeding (Frota et al., 2008). Cowpea presents important agronomical character-ristics, such as rusticity and precocity, besides being considered a plant adapted to conditions of limited or insufficient water availability (Oliveira et al., 2002).

Water deficit is an abiotic factor that affects the agricultural production with high frequency and intensity, influencing aspects related to plant development, such as decrease in photosynthesis rate, reduction in leaf area (Fontana et al., 1992), and stomata closing (Santos and Carlesso, 1998).

As in other crops, performance in cowpea is affected by water deficiency, which can cause lower growth and development, with progressive reduction in leaf dry matter (Costa et al., 2011) and consequent repercussion on production parameters, such as number of grains and pods per plant.

Cowpea plants are considered tolerant to water deficit, and important mechanisms were developed by this species to tolerate inadequate water supply. For example, biochemical modifications in carbon metabolism, such as increase in sucrose (Lobato et al., 2009a), as well as significant interference in nitrogen metabolism, like reduction of soluble proteins (Costa et al., 2011) and increase in total amino acids (Lobato et al., 2008a) contribute to osmotic adjustment of *Vigna unguiculata* plants.

The biological fixation of nitrogen is the capacity of an organism to divide the

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molecule of nitrogen (N_2) and to combine hydrogen atoms (H^+), forming ammonium (NH_4^+) (Albino and Campo, 2001), being carried out by a distinct group of micro-organisms, singly or under symbiosis. The *Bradyrhizobium* gender is described as soil bacteria that have ability to infect root hair of leguminous plants, and it to induce nodule formation, with subsequent fixation of nitrogen (Mercante et al., 2002).

The interaction between leguminous plants and micro-organisms is an example of biological association, with beneficial effects on agricultural sustainability, linked to the process of biological fixation of nitrogen (Souza et al., 2008). Araujo et al. (2009) investigating *Glycine max* plants under inoculation of *Bacillus subtilis* and *Bradyrhizobium japonicum/elkanii* micro-organisms showed increase in nodulation and grain yield.

Root system presents complex strategy aiming to maintain water supply in conditions of water deficit, by increasing the root elongation rate and completely inhibiting the shoot (Sharp et al., 2004). On the other hand, plants growing in low water potentials normally present thinner roots (Sharp et al., 1988), and this morphological modification is an adaptation to increase water absorption efficiency. Therefore, a combination of changes in morphological, physiological and biochemical levels are necessary to plant survival in environments affected by drought.

Beneficial effects linked to inoculation on growth parameters as leaf, stem and root are largely explored and well known (Ramos et al., 1999; Silveira et al., 2001). However interference of this symbiotic process on water relation is little explored. In addition, several studies revealed that adequate shoot development is dependent on factors such as nutrient availability in substrate (Cruz et al., 2011), water supply (Lobato et al., 2008b), and root formation (Sharp et al., 1990), but there is insufficient information linked to contribution of root on leaf and stem in *Vigna unguiculata* plants.

Based on this overview, this study aimed to investigate if root exercises influence on water relations and shoot of *Vigna unguiculata* plants exposed to combined action of water deficit and inoculation.

MATERIAL AND METHODS

Growth conditions

The study was carried out in Instituto de Ciências Agrárias (ICA) of the Universidade Federal Rural da Amazônia (UFRA), Belém city, Pará state, Brazil (01°27′S and 48°26′W). The plants were grown in greenhouse environment, with air temperature minimum and maximum of 24.1 and 38.2°C, respectively. Air relative humidity oscillated between 72 and 89%. The photoperiod medium was of 12 h of light.

Plant materials, substrate and pot

The *Vigna unguiculata* (L.) Walp. seeds used in this study were of Pitiuba and Pérola cultivars, which are tolerant and sensitive to water deficit, respectively (Lobato et al., 2009b). The substrate used for the plant growth was composed by a mix of sand and silica in the proportion of 2:1, respectively, and it was autoclaved at 120°C atm^{-1} for 40 min. The container used for the plant growing was Leonard pot with 2L capacity, being adapted for this purpose in Laboratório de Fisiologia Vegetal Avançada (LFVA).

Experimental design

The experimental design used was completely randomised with 2 cultivars (tolerant and sensitive), combined with 2 water regimes (water deficit and control), and 2 inoculation forms (inoculated and non-inoculated), totalising 8 treatments, with 6 repetitions and 48 experimental units, in which each experimental unit was constituted by one plant pot^{-1}.

Inoculation and conduction of plant

Three seeds per pot were sowed and thinned after germination. The seedlings were inoculated with 1 ml of *Bradyrhizobium* sp. bv. BR 3256 suspension with concentration of 1.0×10^9 CFU, 3 times, at regular intervals in the 5^{th}, 10^{th} and 15^{th} day after the experiment implementation.

The control and water deficit treatments received macro and micronutrients in the form of nutritive solution of Hoagland and Arnon (1950) without nitrogen, for the period of 30 days, the nutritive solution being changed at 2 days interval, always at 09:00 h. The pH of the nutritive solution was adjusted to 6.0 ± 0.1, with addition of HCl or NaOH. On the 30th day after the experiment implementation, the plants of the treatment under water deficit were submitted to a period of 5 days without nutritive solution, in which the water deficit was simulated from 30th until 35th day after the start of experiment. After this period, the plants were physiologically and biochemically analysed.

Leaf relative water content

Leaf relative water content was evaluated with leaf disks with diameter of 10 mm, from each plant; 40 disks were removed and the calculation was done 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 24 h saturation in deionized water at 4°C in dark, and DM is the dry matter determined after 48 h in oven with forced air circulation at 80°C.

Water relations

Transpiration rate and stomatal conductance were evaluated in leaves totally expanded, under light and from the 3rd main branch, using static state porometer (LICOR, model AM-300), with the gas exchanges being evaluated immediately during the period between 10:00 and 12:00 h in all the plants of the experiment. Photosynthetic active radiation oscillated between 530 and 820 $\mu mol\ m^{-2}\ s^{-1}$ (at 12:00 h) during evaluation of physiological parameters.

Dehydration and determination of dry matter

Leaf, shoot, and root were harvested and placed in an oven with forced air circulation at 70 ± 2ºC for 96 h. After this period, leaf dry matter, shoot dry matter and root dry matter were determined.

Data analysis

The data were submitted to variance analysis and when significant differences occurred, the Scott-Knott test at 5% level of error probability was applied. Standard errors were calculated in all evaluated points. Correlation analysis was performed by the Pearson parametric method (Steel et al., 2006), and statistical analyses were carried out with the SAS software (SAS Institute, 1996).

RESULTS

Changes produced by water deficit on water relations

Water deficit promoted significant decrease of leaf relative water content (Figure 1A) in tolerant and sensitive cultivars. Inoculated plants of control treatment presented higher values of leaf relative water content, when compared with same treatments of non-inoculated plants. The tolerant cultivar showed better performance in this parameter, if compared with same treatments in the cultivar sensitive to water stress.

Stomatal conductance had a significant reduction in plants exposed to water deficiency (Figure 1B) in both tolerant and sensitive cultivars. Plants inoculated presented not significant difference, when compared with same treatments in non-inoculated plants. Higher values of stomatal conductance were found in the tolerant cultivar. Correlation analysis revealed a positive and significant interaction between root dry matter and stomatal conductance (r=0.84; $P<0.01$) (Figure 2A).

Water restriction produced a significant decrease in transpiration rates in both cultivars (Figure 1C). The inoculation provoked not significant changes in tolerant and sensitive plants. In the tolerant cultivar submitted to water deficit, the values were higher than those found in the sensitive cultivar, this behaviour being similar in inoculated and non-inoculated plants.

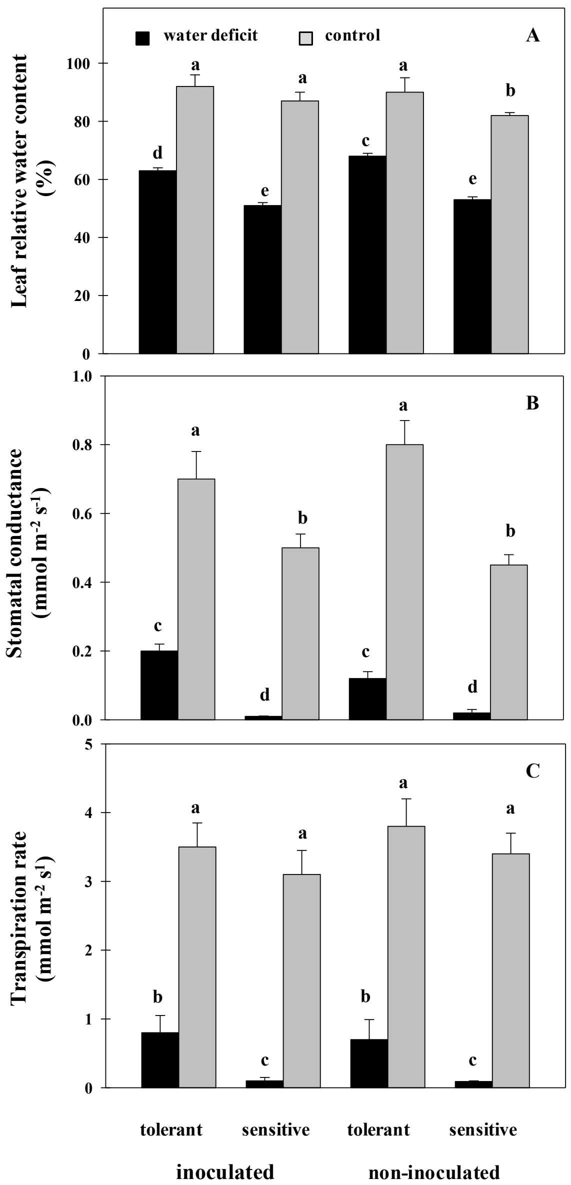

Figure 1. Leaf relative water content (A), stomatal conductance (B), and transpiration rate (C) in two contrasting *Vigna unguiculata* cultivars under water deficit and subjected to inoculation
(Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error)

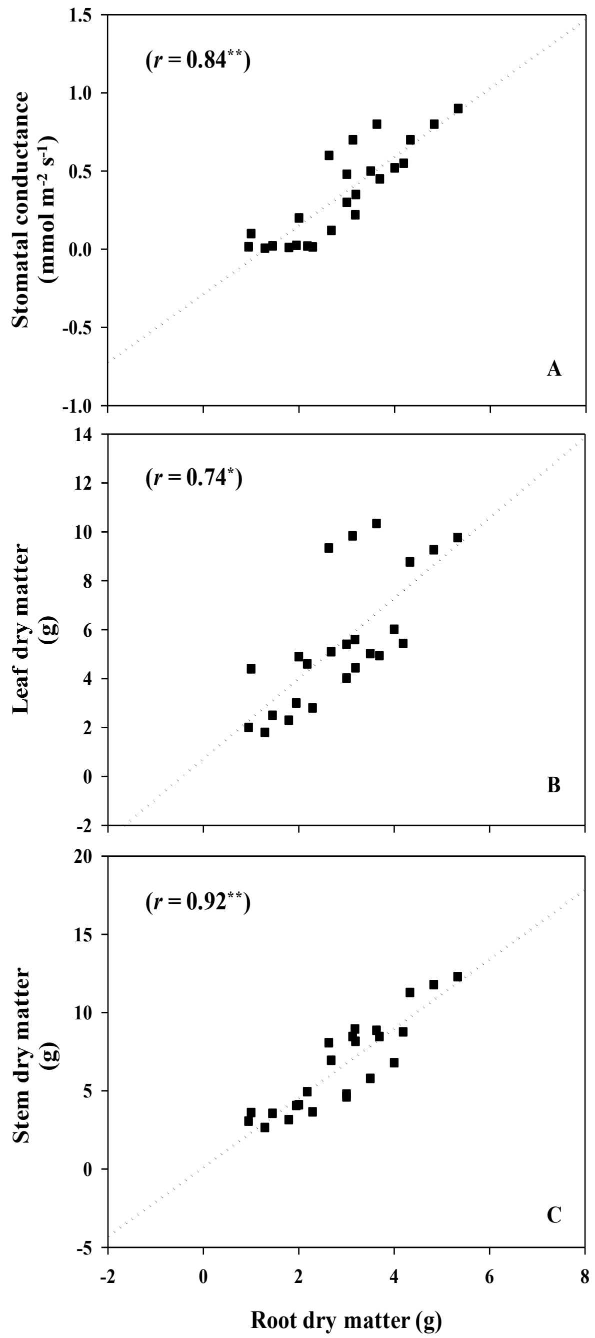

Figure 2. Linear relationships between root dry matter and stomatal conductance (A), root dry matter and leaf dry matter (B), and root dry matter and stem dry matter (C) in two contrasting *Vigna unguiculata* cultivars under water deficit and subjected to inoculation
[Asterisks (*) and (**) indicate significance at 0.05 and 0.01 probability levels, respectively.]

Morphological responses linked to water restriction

Leaf dry matter was decreased due to the water restriction evaluated in this study in both cultivars (Figure 3A). The tolerant and sensitive cultivars under inoculation presented higher values of leaf dry matter in control treatment, if compared with non-inoculated plants of same treatments. In the tolerant cultivar higher values of this parameter were recorded, when compared with same treatments in the sensitive plants. Correlation analysis demonstrated a positive.

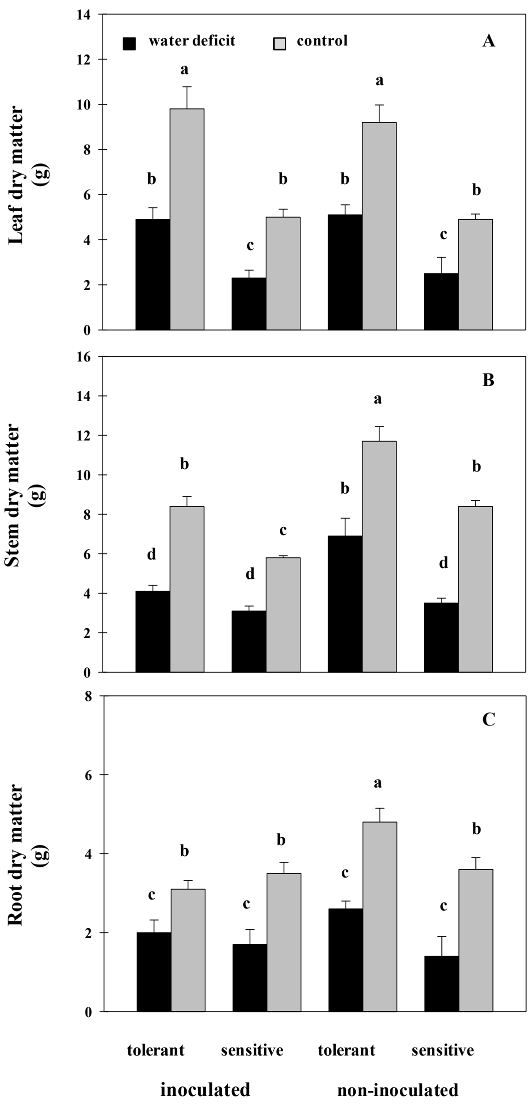

Figure 3. Leaf dry matter (A), stem dry matter (B), root dry matter (C) in two contrasting *Vigna unguiculata* cultivars under water deficit and subjected to inoculation (Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error.)

The water deficiency promoted a decrease in stem dry matter, as compared to the control plants of same treatments (Figure 3B). In addition, in both treatments, the non-inoculated plants presented larger stem dry matter, in relation to inoculated plants. In the sensitive cultivar lower values of stem dry matter were recorded, when confronted with same treatments in the tolerant cultivar. The correlation analysis revealed positive and significant relationship between root dry matter and stem dry matter (r=0.92; P<0.01) (Figure 2C).

Results obtained in this investigation showed significant reduction of root dry matter in both sensitive and tolerant cultivars submitted to water deficit (Figure 3C), as compared with the same treatments in control plants. The tolerant cultivar, when inoculated and exposed to water deficit, had higher values in relation to same treatments in the sensitive cultivar. When exposed to water deficiency tolerant plants showed better results than sensitive plants.

DISCUSSION

The reduction in leaf relative water content is due to lower absorption rate of water by plant via roots, and water loss occasioned by gas exchanges through stomata (Lobato et al., 2009a). Similar results were reported by Maia et al. (2007) working with *Zea mays* plants.

Water deficit promoted a significant fall in stomatal conductance of the two cultivars, but tolerant plants presented higher values of this variable, probably by maintaining better plant water condition. This study revealed that root dry matter exercises influence on stomatal conductance in *Vigna unguiculata* plants submitted to 5 days of water deficiency, and this fact is based on the indirect effect produced by root on stomata mechanisms. In other words, an insufficient root system developed during water deficiency will supply lower amount of water to shoot, and consequently will promote reduction in stomatal conductance.

Reduction in stomatal conductance can also be explained by reduction in water availability in substrate, which produces a decrease in leaf water potential, resulting in stomata closing. Results revealed that in conditions of limited water supply in substrate there is increase in abscísic acid (ABA) concentration in xylem sap, and this promotes stomata closing (Santos and Carlesso, 1998). In addition, Gholz et al. (1990) reported that stomataclosing reduces the CO_2 influx to leaf, affecting production, transport and utilization of photo-assimilates, and therefore yield.

Results similar with those found in this study were found by Santos et al. (2009) in study with five *Phaseolus vulgaris* genotypes subjected to water deficiency.

Decrease in transpiration rate of *Vigna unguiculata* plants can be attributed to stomata behaviour, as under water deficit stomata are kept partially closed, contributing to change of transpiration behaviour of plant (Oliveira et al., 2005). Leite and Filho (2004) reported that reduction of transpiration works as an important mechanism of tolerance to drought. Values of transpiration demonstrated direct relation with stomatal conductance and also with leaf relative water content. Similar results were showed by Nogueira et al. (1998) studying two *Arachis hypogeae* cultivars exposed to water deficiency.

The reduction in leaf dry matter can be attributed to the decrease in root dry matter, as confirmed by the relationship found in this study. Other factor considered as secondary, is the decrease is leaf area in plants submitted to water deficit (Leite and Filho, 2004), and this is linked to a strategy of survival, with the objective to reduce area available to transpiration (Correia and Nogueira, 2004). Costa et al. (2011) obtained similar results in a study on impact of water deficit in *Vigna unguiculata* plants, showing that leaf dry matter was strongly affected and consequently reduction of this parameter occurred.

Stem dry matter was negatively affected by water deficit, and this decrease is associated directly to root dry matter. Modifications in root will probably promote lower absorption and transport of water in xylem, interfering in formation and functions of stem. Nascimento et al. (2004) working with *Vigna unguiculata* reported that water stress induced several decreases, of which the reduction in stem dry matter was more intense. Similar results were found by Diallo et al. (2001) studying *Vigna unguiculata* under water deficiency.

This study revealed significant reduction in root dry matter, and this result is explained by Leite and Filho (2004) as being a morphological response to water deficit, induced in higher plants, which will also interfere in water and nutrient absorption. Ramos et al. (2005), studying the application of *Bradyrhizobium japonicum* in *Glycine max* under water stress conditions, also reported a decrease of this variable.

CONCLUSION

This investigation revealed negative impacts induced by water deficit on water relations and morphological parameters in tolerant and sensitive plants of *Vigna unguiculata* exposed to inoculation. Significant relationships between root dry matter and stomatal conductance, as well as root dry matter and shoot (leaf and stem) were found in this study, confirming the hypothesis on interference of root on water relations and shoot.

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