



# Gas exchange and water stress index in soybean cultivated under water deficit and soil compaction

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## ABSTRACT

Water stress is intrinsically related to soil water availability which is determinant in gas exchanges, as well as soil compaction in soybean growth and development. This study aimed to evaluate the leaf gas exchange, water stress index, and dry mass accumulation of soybean cultivated in different daily irrigation depths and bulk density. The treatments were distributed in pots subdivided in a 4×4 factorial scheme: four levels of bulk density of the soil (1.0, 1.15, 1.30 and 1.45 g cm<sup>-3</sup>) and four daily irrigation depths (4.0, 5.0, 6.0- and 7.0-mm day<sup>-1</sup>), with three replications. Physiological and morphological variables were analyzed. There were low transpiration rates due to water deficit in the 4 mm daily irrigation depth that resulted in stomatal closure and impaired the performance of the photosynthetic process in soybean. The increase in soil density provided gains in shoot and dry root mass in the layer above the compacted one. Low availability of water in the soil reduces the photosynthesis and growth of soybean plants. Increase in bulk density of the soil promotes greater root development, with the layer above the compacted being the one that concentrates most of the roots.

**Keywords:** *Glycine max* (L.) Merrill; bulk density; crop water stress; deficit irrigation; photosynthesis.

## INTRODUCTION

In recent times, the productive potential of soybean (*Glycine max* (L.) Merrill) has increased because of improved agricultural technology. However, low soil water availability is a major cause of reduced productivity, as well as soil compaction in the root system; since both factors are correlated with soil moisture (Santos *et al.*, 2005).

Conventional cultivation systems can change soil physical properties, cause compaction consequently affect crop productivity. As a result, it leads to an increase in soil density, which interferes with the temporal variability of soil moisture, interruption, continuity, frequency, and pore size (Hébrard *et al.*, 2006; Kuncoro *et al.*, 2014; Moraes *et al.*, 2015; Moraes *et al.*, 2016). Furthermore, it reduces hydraulic conductivity and soil water flow (Silva *et al.*, 2009; Moraes *et al.*, 2015). Thus, the depth and

volume of soil explored by the roots in search of water and nutrients can be limited (Valentine *et al.*, 2012). These effects can be seen when you have high levels of soil compaction in the 0.07– 0.15 m layer, which can directly interfere with root growth by reducing the rate of root elongation due to increased resistance to root penetration (Richart *et al.*, 2005; Lipiec *et al.*, 2012; Bengough *et al.*, 2011; Valentine *et al.*, 2012).

The low availability of water in the soil, as well as in the leaf tissues, alters the productive potential of soybean by reducing the number of branches, knots, and pods per plant, mainly under severe or moderate water stress (Brevedan & Egli, 2003; Catuchi *et al.*, 2012; Kirnak *et al.*, 2010). This is because of the progressive reduction of stomatal conductance that causes lower transpiration through the leaves and consequently a reduction in the photosynthetic rate (Lei *et al.*, 2006).

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However, when the density has values close to  $1.15 \text{ g cm}^{-3}$ , compaction can increase productivity since it can improve gas exchange. This increase is because the reduction in stomatal conductance provides a potential route for the improvement of water use efficiency and conservation of soil water levels (Hepworth *et al.*, 2015), which appears to be the most efficient way of reducing the water loss to the atmosphere by transpiration (Stiller *et al.*, 2008).

Majority of studies on water stress have failed to evaluate the effect of soil compaction on physiological processes as well as the water stress index in soybean. This culture can be favored by the greater availability of water in the layer above the compacted layer, which could be explained by the physiological parameters of the plant. For example, in their studies (Franchini *et al.*, 2012 and Machado Júnior *et al.*, 2017) did not observe any significant decrease in grain yield under water stress. Soil compaction can improve the regulation of gas exchange for soybean under stress on a temporal scale and in the direct relation of the transpiration demand to which the leaves are potentially (Kerbaudy, 2008; Blum, 2009; Taiz & Zeiger, 2017). It reduces the effects of severe water stress and prevents the dehydration of tissues (Ohashi *et al.*, 2006).

Therefore, considering that soil water is a determining factor in agricultural production, it is important to study the water requirements of soybean in order to properly manage cultivated areas. Thus, studies that evaluate the physiological and morphological changes in the formation of pods with variation in the availability of water as well as soil compaction, are important in elucidating the water stress index under these management conditions.

The objective of this study was to evaluate the leaf gas exchange, water stress index and dry mass accumulation of soybean cultivated in different daily irrigation depths and bulk density.

## MATERIALS AND METHODS

The experiment was conducted from November 2016 to January 2017 under greenhouse conditions at the Federal University of Lavras, state of Minas Gerais, Brazil (altitude 918,841 m, latitude  $21^{\circ}14'10''\text{S}$ ; longitude  $45^{\circ}00'10''\text{W}$ ). During experiment, the average temperature, relative humidity and solar radiation daily inside the greenhouse was  $26.9 \pm 2.1^{\circ}\text{C}$ ,  $58.22 \pm 5.1\%$  and  $663.2 \pm 104.3 \text{ J s}^{-1} \text{ m}^{-2} \text{ day}^{-1}$ , respectively.

Pots were filled with a very clayey Oxisol ("Latossolo Vermelho distroférico", according to the Brazilian classification system), collected from the 0-0.20 m layer in cattle pasture, dried in the air and ground to pass through a 2-mm sieve. Soil samples were analyzed for physical and chemical attributes which were 15 % sand, 18 % silt and 67 % clay;  $\text{pH}(\text{H}_2\text{O}) = 6.3$ ;  $\text{H} + \text{Al}^{3+} = 3.27 \text{ cmol}_c \text{ dm}^{-3}$ ;  $\text{Al}^{3+}$

$= 0.0 \text{ cmol}_c \text{ dm}^{-3}$ ;  $\text{P}(\text{Mehlich}^{-1}) = 1.13 \text{ mg dm}^{-3}$ ;  $\text{K} = 58.0 \text{ mg dm}^{-3}$ ;  $\text{Ca}^{2+} = 2.8 \text{ cmol}_c \text{ dm}^{-3}$ ;  $\text{Mg} = 0.6 \text{ cmol}_c \text{ dm}^{-3}$ ;  $\text{CEC} = 6.82 \text{ cmol}_c \text{ dm}^{-3}$ ;  $(\text{V}\%) = 52.0 \text{ g dm}^{-3}$  and organic matter (O.M.) =  $25.5 \text{ g dm}^{-3}$ .

Based on the chemical analysis, soil liming was carried out to increase the saturation by 60%, using calcium carbonate ( $\text{CaCO}_3$ ) and magnesium carbonate  $\text{MgCO}_3$  (PA – pure analytic) at a ratio of 4:1. The soil was held for 30 days with moisture close to 60% of the total pore volume. Fertilization was done according to the recommendations of Malavolta (1980): 80 mg of nitrogen (N), 300 mg of phosphorus (P), 200 mg of potassium (K) and 50 mg of sulfur (S) per  $\text{dm}^3$  of soil, supplied as monobasic ammonium phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ), monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), respectively. Micronutrient fertilization consisted of 0.5 mg of B, 1.5 mg of Cu, 0.1 mg of Mo and 5.0 mg of Zn per  $\text{dm}^3$  of soil, provided as boric acid ( $\text{H}_3\text{BO}_3$ ), copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), ammonium molybdate [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ] and zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), respectively. During the growing period, fertilization with 100 mg of K per  $\text{dm}^3$  of soil (divided in two applications) was carried out using potassium chloride (KCl) at 25 and 40 days after sowing.

Each experimental plot consisted of a pot with 29.6 cm in height and 26.2 cm of internal diameter in the compacted layer, divided into three layers: the upper with 12.0 cm and the lower with 10.0 cm, filled with sieved soil having bulk density of  $1.00 \text{ g cm}^{-3}$ , while the compacted layer was determined based on the volume of the metallic ring [ $(540 \text{ cm}^2 \times 6 \text{ cm}) = 3.234 \text{ cm}^3$ ] (Figure 1).

The soil was moistened and homogenized until reaching the optimum moisture for compaction, using a hydraulic press, according to the methodology of Santos



**Figure 1:** Schematic representation of the experimental pot, showing the top (layer A), compacted (layer B) and bottom layers (layer C) of the soil.

*et al.* (2005) for Oxisol (“Latossolo Vermelho distroférrico”, according to the Brazilian classification system). In order to avoid deformation or rupture of the pot by compressing the soil in the compacted layer, a metal ring (steel plate 6.0 cm high and 1.2 cm thick) was placed in each the pot. To prevent the preferential flow of water and root growth, cold asphalt was used between the compacted layer and the pot wall.

The experiment was conducted using a completely randomized design in a 4 x 4 plot scheme. The factors were: four levels of bulk density of the soil ( $1.0 \pm 0.013$ ;  $1.15 \pm 0.022$ ;  $1.30 \pm 0.035$  and  $1.45 \pm 0.040$  g cm<sup>-3</sup>) and four irrigation depths (4.0; 5.0; 6.0 and 7.0 mm day<sup>-1</sup>), with three repetitions. This compaction was defined according to Torres & Saraiva (1999), who considered 1.55 g cm<sup>-3</sup> a critical value for loamy to clayey soils.

In November 2016, five soybeans’ seeds (cultivar RK 8115 IPRO) were sown per pot at 0.02 m depth, and ten days after seedling emergence, the soybean seedlings were thinned to three plants per pot. Prior to sowing, soybean seeds of undetermined growth were treated with pyraclostrobin + methyl thiophanate + fipronil at a dose of 2 mL pc kg<sup>-1</sup> of seed, inoculated with *Bradyrhizobium japonicum*, strains SEMIA 5079 and 5080 ( $5.0 \times 10^9$  viable cells mL<sup>-1</sup>), at a dose of 4 mL pc kg<sup>-1</sup> of seed.

At 35 days after emergence (DAE), replacement of the pre-established daily irrigation of 4, 5, 6 and 7 mm day<sup>-1</sup> was maintained manually from 9:00 am to 10:00 am. The smallest daily irrigation depth of 4.0 mm day<sup>-1</sup> represents half of the critical value (7-8 mm day<sup>-1</sup>) required by soybean in the phases of greatest transpiration demand between flowering and grain filling under normal growing conditions, according of Farias *et al.* (2007).

At 55 DAE, gas exchange was determined using a portable infrared gas analyzer (IRGA - LICOR 6400, LiCOR, Nebraska, USA), carried out between 9:00 am and 10:30 am, in two plants per pot using the third fully expanded leaf from top to bottom, photosynthetically active and without lesions, located in the third leaf from top to bottom. Photosynthetically active radiation was standardized in artificial saturating light of 1,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (intensity similar to that natural light) and ambient CO<sub>2</sub> concentration. Stomatal conductance ( $g_s$ , mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), was measured, transpiration rate ( $E$ , mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), internal CO<sub>2</sub> concentration ( $C_i$ ,  $\mu\text{mol mol}^{-1}$ ), as well as the net photosynthetic rate ( $A$ ,  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ), leaf temperature ( $T_l$ ) and vapor pressure deficit (VPD).

The instant efficiency of carboxylation ( $CE - A/C_i$ ) [ $(\mu\text{mol m}^{-2} \text{s}^{-1}) (\mu\text{mol mol}^{-1})^{-1}$ ] was calculated from the relationship between CO<sub>2</sub> assimilation rate and internal CO<sub>2</sub> concentration. The soybean water stress index (WSI) was determined using the formula  $WSI = [(t_c - t_{air}) - D_2 / (D_1 - D_2)]$ , as described by Idso *et al.* (1981), in which  $D_2 =$

$7.6046 - 2.725 * VPD$ , is the equation obtained by the correlation between  $D_2 = (t_c - t_{air})$  (°C) and vapor pressure deficit (VPD) (kPa),  $t_c$  is the culture temperature,  $t_{air}$  is the temperature of the greenhouse,  $D_1$  is the maximum difference between  $t_c$  and  $t_{air}$ , which was 2.6 °C for soybean.

After taking gas exchange readings, the SPAD index (Soil Plant Analysis Development - SPAD-502, Minolta, Japan) was determined with the chlorophyll meter, in two leaf per pot (two different plants). Thereafter, the plants were sectioned at the base of the neck, followed by the measurement of height of plants. Subsequently, they were taken to the laboratory and their leaf area determined using the LI-3100 Area Meter (LI-COR Inc. Lincoln, NE, USA) and number of pods determined. Then stems, leaves and pods were oven dried with forced air circulation at 65 °C for 72 h to determine dry mass on a precision balance scale ( $\pm 0.001$ ).

After removing the aerial part of the plants at 55 DAE, the root dry mass was determined in the upper layer (A), compacted layer (B) and below the compacted layer (C). Dry mass and percentage of roots were per plant determined on a precision scale ( $\pm 0.001$ ) after being washed and dried in an air circulation oven at 65 °C for 72 h.

Thereafter, the data obtained for each variable were subjected to the analysis of variance ( $p \leq 0.05$ ), in which means of qualitative factor levels were compared by the Scott-Knott test at 5% significance level. The quantitative factors were submitted to regression analysis compared by the Student’s “t” test at 5% significance level using the R software version 3.5.1. (R Development Core Team, 2020).

## RESULTS AND DISCUSSION

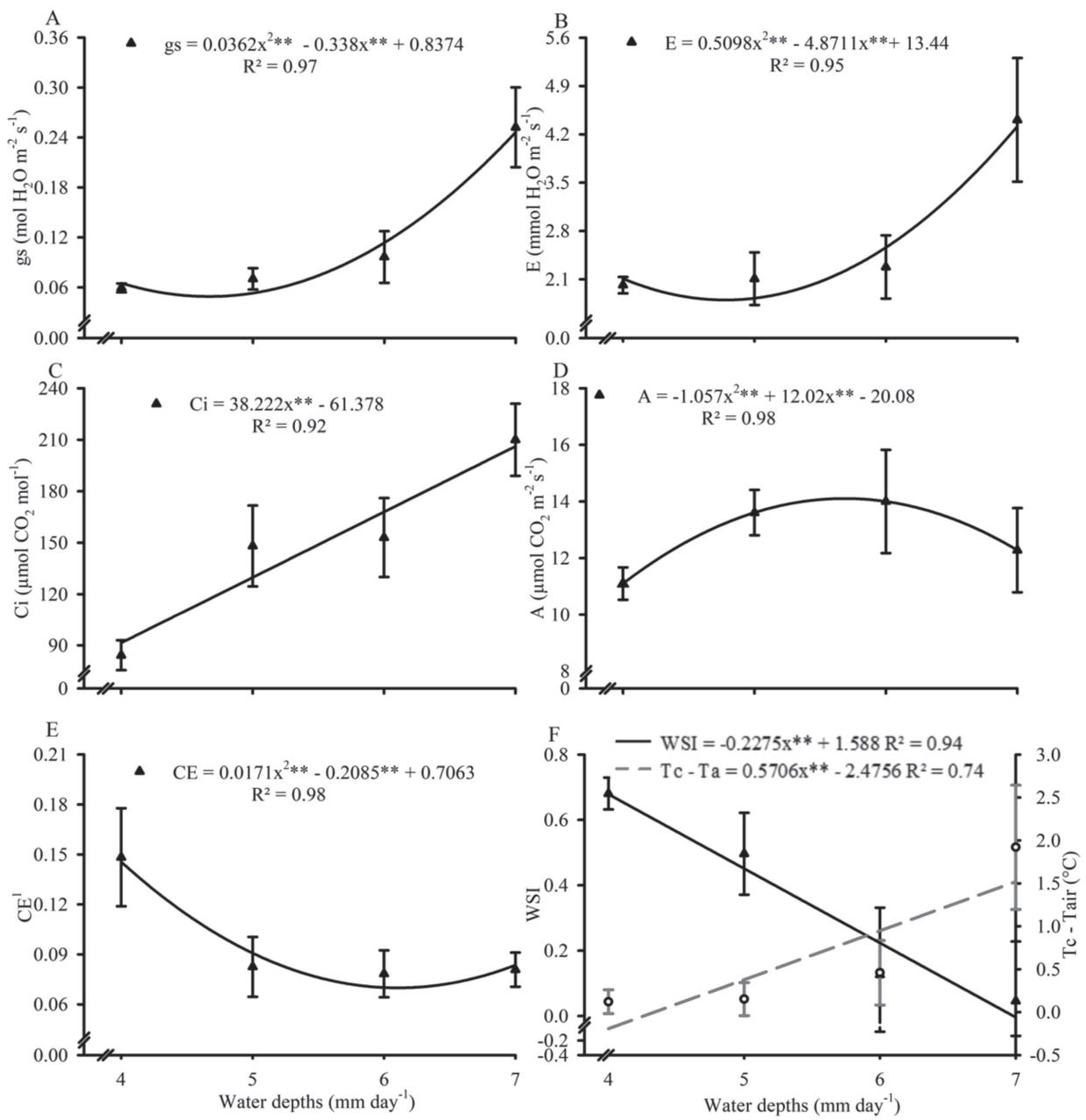
The irrigation depths significantly ( $p < 0.01$ ) altered stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ) and net photosynthetic rate ( $A$ ) (Figures 2 A-D). Stomatal closure was less than 2.5 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in irrigation depths 4 to 6 mm day<sup>-1</sup> caused by water restriction in the leaves mesophyll, (Figure 2 B), partially corroborating the results of Chavarria *et al.* (2015), who also observed transpiration in soybean cultivars subjected to severe water deficit. This might be related to the fact that plants are in a situation of low water availability in leaf tissues under severe water deficit conditions (Ribas-Carbo *et al.*, 2005).

The low photosynthetic activity observed in the daily irrigation depth of 4 mm day<sup>-1</sup> is explained by the inability of the plants to raise the carbon absorption rate (Figure 2 C) in this irrigation regime. The increase in leaf temperature due to water restriction in the soil in this irrigation depth induces a significant reduction in the normal flow of CO<sub>2</sub> towards the carboxylation site and, consequently, the rate of liquid photosynthesis, as observed in Figure 2 D. Soybean is a C3 cycle plant and therefore has low

efficiency in the use of water (Kerbaui, 2008). Thus, under conditions of hydric restriction, there is the closure of stomata and consequent decrease in the photosynthetic rate, as observed in this study. The higher transpiration rate observed in the 7 mm day<sup>-1</sup> water depth, did not result in a higher photosynthetic rate, which is explained by Han *et al.* (2018) when stating that the rate photosynthetic does not increase under conditions of total irrigation, even with an increase in the transpiration rate of the plants.

The water stress (water depth 4 mm day<sup>-1</sup>) caused the instant efficiency of carboxylation (CE) (Figure 2 E) to

reach higher values. However, it was observed that the value of the photosynthetic rate had the opposite behavior. According to Marenco *et al* (2014), there is a relationship between photosynthesis and stomatal conductance that must be positive for most species, however, in the present study, soybeans showed the opposite behavior. The stress condition to which the plants were subjected may have caused dehydration of the mesophilic cells, inhibited photosynthesis and causing losses to the metabolism, consequently affecting the efficiency of carboxylation (Taiz & Zeiger, 2017).



**Figure 2:** Stomatal conductance (*gs*) (A), transpiration rate (*E*) (B), intercellular CO<sub>2</sub> concentration (*Ci*) (C), net photosynthetic rate (D), instant carboxylation efficiency (*CE*) (E), crop and leaf temperature (*Tc - Tair*) and water stress index (*WSI*) (F) of soybean subjected daily irrigation depths. Vertical bars on each observed mean are the respective standard errors.

As observed, the highest photosynthetic rate was obtained in the daily irrigation depths of 6 mm day<sup>-1</sup> with a value of 13.98  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Figure 2D). In that same water depth, the value of the difference between the culture temperature and the air temperature ( $t_c - t_{\text{air}}$ ) was 0.94 °C, and the WSI 0.22 (Figure 2F). In the lower water depth (4 e 5 mm day<sup>-1</sup>), the WSI values were less than 0.4 and the gas values less than 0.10 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. However, the lowest WSI value was observed in the 7.0 mm day<sup>-1</sup> irrigation depth, around 0.22 at the upper limit, corroborating with Candogan *et al.* (2013), who observed similar results before soybean irrigation when two years were evaluated. The result also corroborates with Waller & Yitayew (2016), who found it above 0.24, classifying it as having good water availability, without water stress. Ribas-Carbo *et al.* (2005) considered  $g_s$  values higher than 0.20 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>.

The water deficit applied to the soybean crop with the 4.0 mm daily irrigation depth resulted in a lower leaf area and the leaf dry mass (Figures 3 A, B). This negative effect of the water deficit also was verified by Sincik *et al.* (2008), Jaleel *et al.* (2009) and Rhine *et al.* (2009) in soybean. This reduction in leaf area is a strategy for coping with low soil water availability because it reduces transpiration rate, leading to water savings until the formation of pods (Stolf-Moreira *et al.*, 2010; Tardieu, 2013). The maximum accumulation of leaf area and dry mass of the soybean leaf were observed with the application of the 6.0 mm daily irrigation depth. This result is a reflection of the maximum photosynthetic rate, that is, maximum conversion of the assimilated CO<sub>2</sub> per unit of water, with a subsequent decrease in the daily irrigation depth of 7 mm day<sup>-1</sup>.

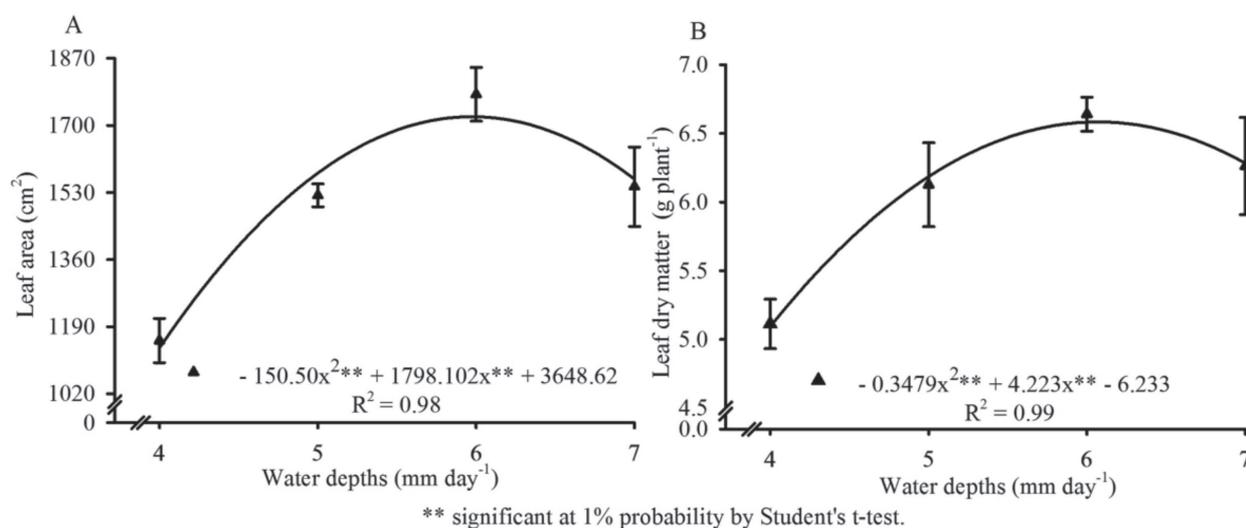
The highest SPAD index values were observed in the highest soil densities and larger daily irrigation depths (Figure 4 A). However, in loose soil, the SPAD index varied

up to 42 in irrigation depths of 4.0 and 5.0 mm day<sup>-1</sup>. It was evident the correlation between the highest soil densities with the largest daily irrigation depths, with a progressive increase in the capacity of soybeans to produce chlorophyll, since the soil compaction influences the increase in the stem neck diameter and, consequently, in the flow of water in the xylem, the area involved in transporting water to the leaves. This improved the performance of the photosynthetic process, as according to Taiz & Zeiger (2017) chlorophylls are responsible for capturing light energy for photochemical reactions.

Similarly, to the SPAD index, the values of stem dry mass and plant height also increased because of the increase in soil density and the applied irrigation depth (Figures 4 B, C), due to the greater development of the stem. This is due to increased availability and increased flow of water in the xylem to cells undergoing cell division and expansion of young meristems in stem cell extension (Gunes *et al.*, 2008; Munawarti *et al.*, 2014), with increasing diameter in the stem neck, the area involved with water transport and, consequently, in apical growth. Farias *et al.* (2007) explained it by the close relationship with the specific mass of the soil and the soil water content, which are good indicators of soil physical quality.

Dry mass and percentage of roots in the layer (A) were higher in the highest soil densities (Figure 5A, B<sub>1</sub>), being 1.5 times higher at the highest value of bulk density (1.45 g cm<sup>-3</sup>) compared to the lowest value (1.00 g cm<sup>-3</sup>). However, the root percentage decreased with an increase in the irrigation depth (Figure 5 B<sub>2</sub>).

Such responses are related to soil compaction, reducing porosity and increasing soil resistance to root penetration (Franchini *et al.*, 2009; Moraes *et al.*, 2014; Moraes *et al.*, 2016), which limits the depth and volume of

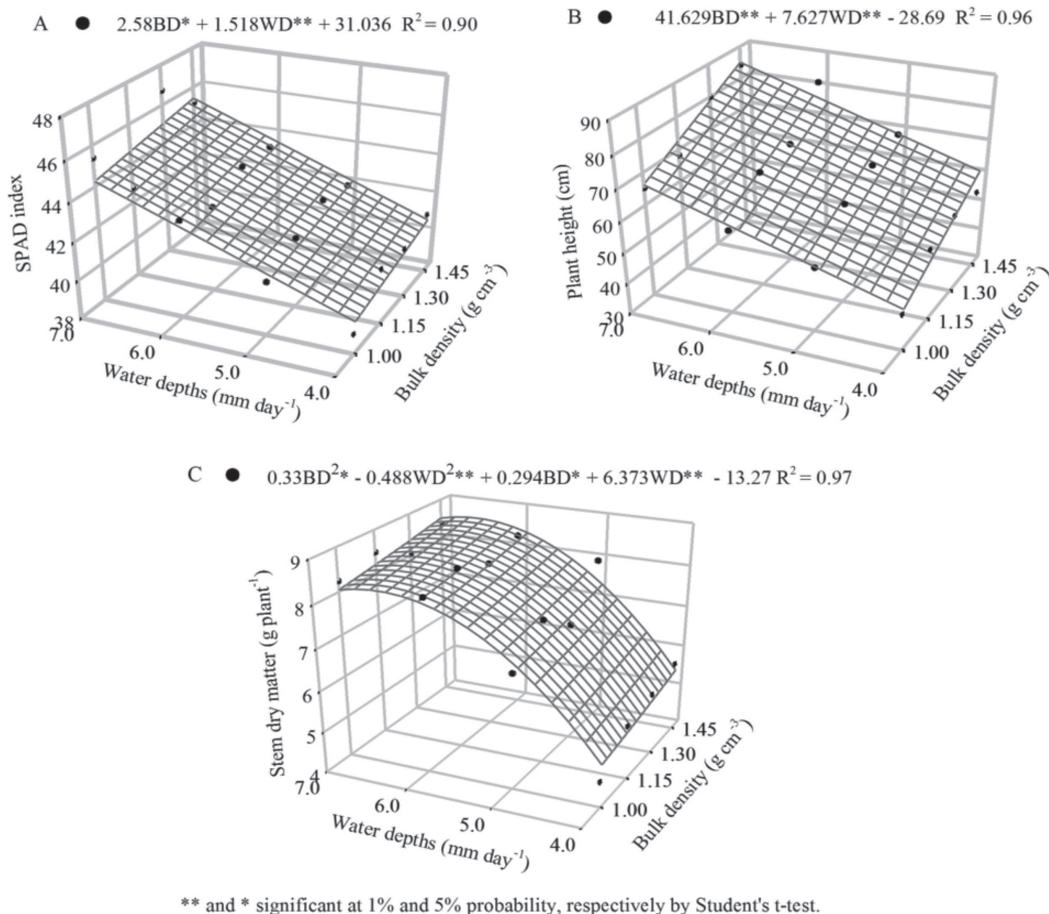


**Figure 3:** Leaf area (A) and leaf dry mass (B) of soybean submitted to different daily irrigation depths. Vertical bars on each observed mean are the respective standard errors.

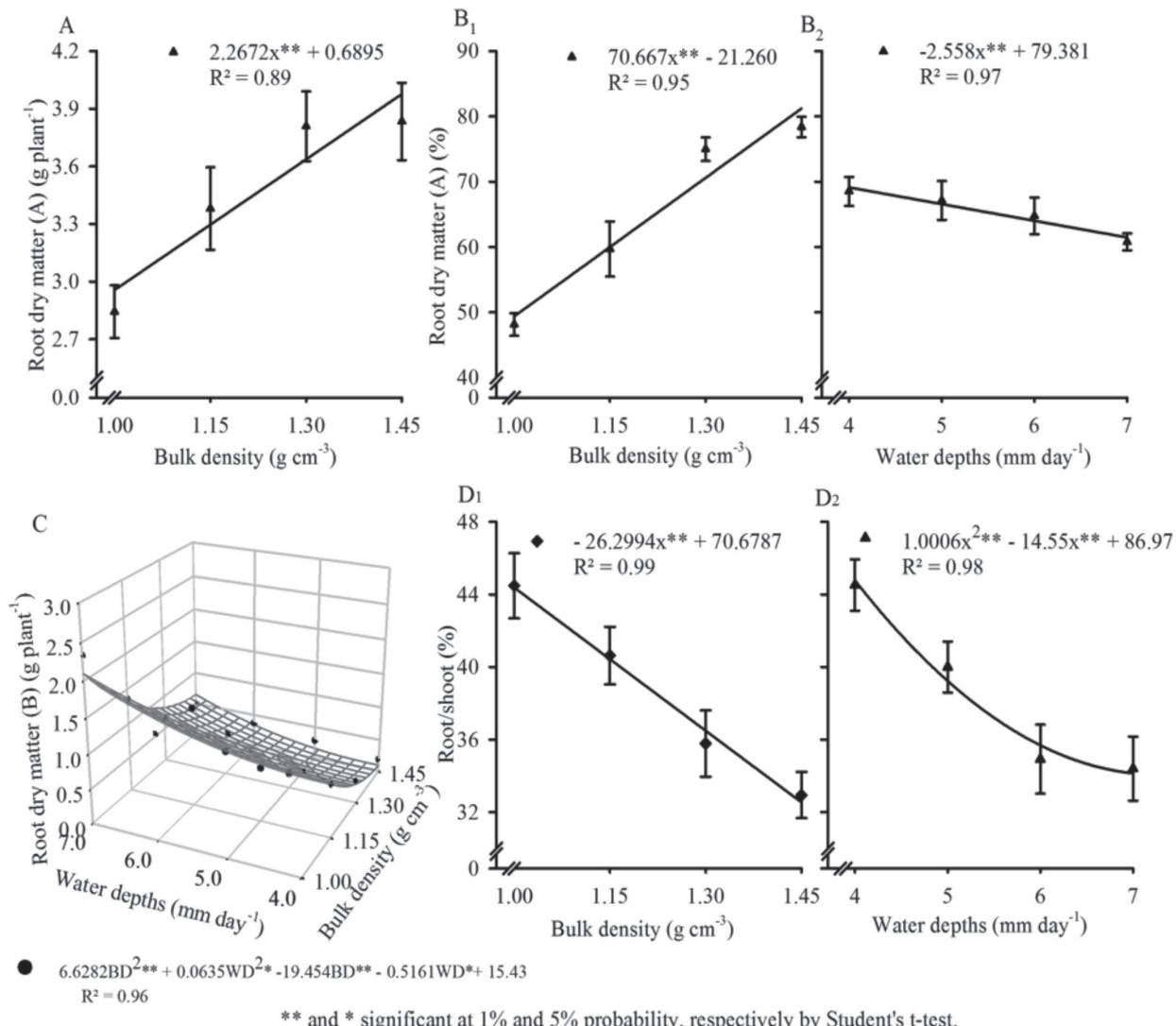
the explored soil (Hamza & Anderson, 2005; Gonçalves *et al.*, 2006; Modolo *et al.*, 2008; Franchini *et al.*, 2009; Bengough *et al.*, 2011; Jin *et al.*, 2013). Thus, there is greater proliferation and concentration of secondary roots superficially disposed when reaching the compacted layer. It was also observed in the compacted layer (B) when considering the lowest soil densities and the highest irrigation depths (Figure 5 C). However, there was an increase in soil resistance to root penetration in the highest densities of the soil at the smallest irrigation depths. Buttery *et al.* (1998) also observed similar results in soybean cultivation at two levels of soil density. This increase in root dry mass at the highest irrigation depths in the compacted layer is due to the formation of biopores in the soil by the roots, thereby reducing the effect of soil resistance on root penetration.

The restriction of root growth as a result of the increased resistance to penetration in compacted soils reflected in the shoot/root ratio (Figure 5 D), with greater accumulation of dry mass in the aerial part. However, for the smallest irrigated layers, the strategy of the plant was to prioritize the root system with allocation of most photo-assimilates, as observed in the results of the leaf area.

The number of pods per plant and the dry mass of pods (Figure 6 A, B) were two and five times lower at the irrigation depth of 4.0 mm day<sup>-1</sup>, respectively, compared with the irrigation depth of 7.0 mm day<sup>-1</sup> at 55 DAE. The greater number and mass of pods in the largest irrigation depth is related to how plants during the reproductive phase require a large amount of water to fill the grains, therefore the linear increase in these parameters with an increase in irrigation depth. These results partly corroborate those of Brededan & Egli (2003) in the formation of soybean pods, under severe water deficit for 13 days. However, it differs from the results obtained by Machado Júnior *et al.* (2017) in soybean grain yield under water stress for 15 days. These results are controversial, can be, due to the fact that soybean, when subjected to a daily water deficit, has a great facility in recovering stomatal conductance and transpiration after irrigation, without inhibiting the photosynthetic apparatus and, consequently, gain in biomass. Therefore, considering proportional gains in soybean biomass under water deficit can lead to erroneous conclusions, when using exclusively water deficit assessments.

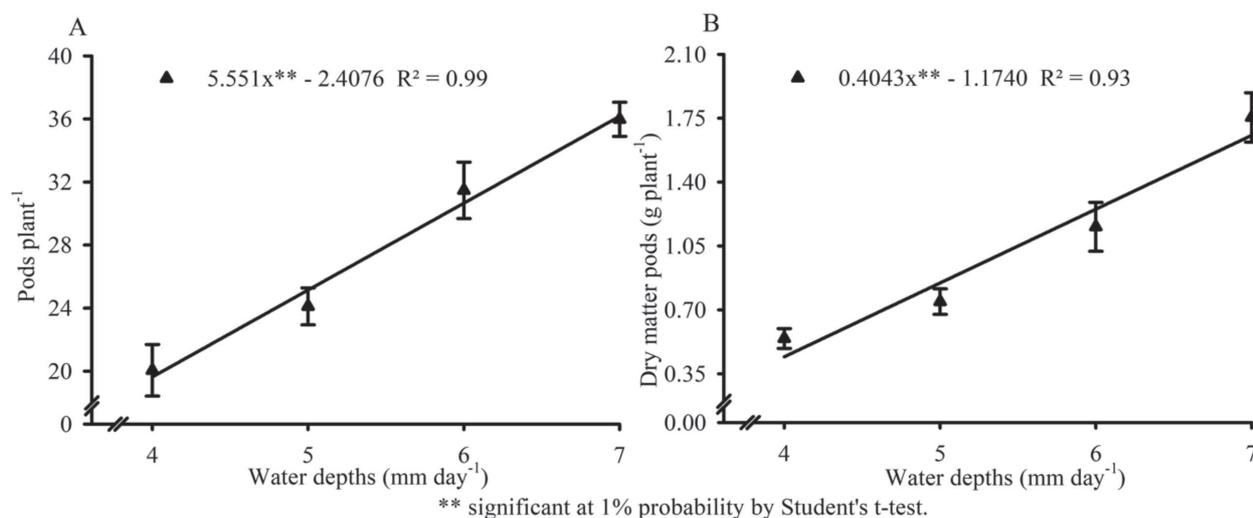


**Figure 4:** SPAD index (A), plant height (B) and stem dry mass (C) of soybean submitted to different bulk density (BD) of soil and daily water depths (WD).



\*\* and \* significant at 1% and 5% probability, respectively by Student's t-test.

**Figure 5:** Root dry mass in layer A (A), percentage of root dry mass in layer A (B), root dry mass in layer B (C) and ratio root/shoot dry mass (D) in soybean submitted to different bulk density (BD) and daily water depths (WD). Vertical bars on each observed mean are the respective standard errors.



\*\* significant at 1% probability by Student's t-test.

**Figure 6:** Number of pods (A) and dry matter pods (B) of soybean submitted to daily irrigation depths. Vertical bars on each observed mean are the respective standard errors.

## CONCLUSIONS

There were low transpiration rates and lower thermal energy dissipation due to water deficit in the 4-mm daily irrigation depth that resulted in stomatal closure and impaired the performance of the photosynthetic process in soybean. These results were confirmed by the values of leaf area and leaf dry mass verified in the irrigation depth of 6.0 mm day<sup>-1</sup>.

The increase in soil density provided gains in shoot and dry root mass in the layer above the compacted one. Low availability of water in the soil reduces the photosynthesis and growth of soybean plants. Increase in bulk density of the soil promotes greater root development, with the layer above the compacted being the one that concentrates most of the roots.

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Authors declare there is no conflict of interests in carrying the research and publishing this manuscript.

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