Mitigation of drought stress effects on soybean gas exchanges induced by *Azospirillum brasilense* and plant regulators

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**ABSTRACT**

Drought stress is a limitation for the agricultural production, having as a primary effect the reduction of plant gas exchanges, and the continuity of its incidence results in a lower yield. This study aimed to evaluate the photosynthetic responses and the soybean yield, concerning the seed inoculation and foliar spray with *Azospirillum brasilense* and plant regulator containing auxin, gibberellin and cytokinin. A randomized complete block design was used under greenhouse conditions, with five treatments: four under drought stress (control, seed inoculation and foliar spray with *A. brasilense* and plant regulator) and one irrigated treatment. The soil gravimetric moisture, relative water content, CO$_2$ net assimilation rate, apparent quantum efficiency, light compensation point and grain yield were evaluated. The water deficiency reduced the relative water content by 76.96 % and the soybean gas exchanges by 860.43 %, in the drought stress control. However, when using *A. brasilense* or plant regulator, the reduction of these values was mitigated, with maximum reductions of 52.40 % in the relative water content and 361.99 % in the gas exchanges. Thus, the mitigation of these effects was directly correlated with the grains yielded by plants, where the use of foliar spray with *A. brasilense* or plant regulator presented averages 19 % higher than the drought stress control. The applications of foliar spray with *A. brasilense* and plant regulator mitigate the effects of drought stress on the soybean photosynthesis and culminate in lower yield losses.

**KEYWORDS:** *Glycine max* L., photosynthesis, relative water content.

**INTRODUCTION**

Soybean occupies a prominent place among worldwide commodities. Drought stress periods are one of the factors that limit the crop yield, mainly when they affect the reproductive stage, due to the abortion of leaves and reproductive structures, lower grain filling and accelerated senescence (Vieira et al. 2013). The soybean crop has a water demand that varies from 450 mm to 800 mm during its cycle, with a greater need between flowering and grain filling (Souza et al. 2016). In order to tolerate drought stress, the plants developed adaptive strategies to reduce losses in low water availability resulted by

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physiological, morphological and structural levels (Chaves & Oliveira 2004).

Physiological adaptations are fast responses to reduce drought stress effects. In this line, different ways of handling crops are studied to increase the drought stress tolerance, especially the use of plant regulators and plant growth promoting bacteria, standing out the *Azospirillum* genus. Within this genus, the *A. brasilense* species is the most used for both grasses and legumes, due to its ability to excrete plant hormones such as auxins, gibberellins and cytokinins, as well as other effects related to growth promotion (Kuss et al. 2007, Perrig et al. 2007).

Plant growth promoting bacteria influence positively fundamental pathways to increase the drought stress tolerance (Ngumbi & Kloepper 2016, Vurukonda et al. 2016). Among them, the root development (Rampim et al. 2012), cell expansion (Radwan et al. 2004), gas exchange (Inagaki et al. 2015) and reduction of ethylene synthesis (Glick 2014) stand out. In addition, they minimize the loss of water by the stomatal closure promoted by ABA (Cohen et al. 2008 and 2009). Thus, the use of *Azospirillum* sp. mitigates the effects of drought stress in the wheat crop (Arzanesh et al. 2011) and corn plants (Rodriguez-Salazar et al. 2009, Bano et al. 2013).

Similarly, the plant growth promoting bacteria and plant growth regulators reduce the effects of drought stress on plants (Peres et al. 2009, Werner et al. 2010, Colebrook et al. 2014). Accordingly, the use of kinetin (cytokinin) promotes greater levels of relative water content in soybean plants under drought stress (Fioreze et al. 2013). Vieira et al. (2013) showed that the presence of auxins in plant tissues improves the maintenance of water status, classifying soybean cultivars with higher levels of auxin as drought stress tolerant.

Studies developed with soybean under drought stress conditions report that the maintenance of water content in leaves is essential for productivity (Fioreze et al. 2011). Chavarría et al. (2015) showed a reduction in the soybean photosynthetic capacity under drought stress, and this condition impacts negatively the crop yield (Naderi et al. 2013, Farooq et al. 2016).

Therefore, the use of plant growth promoting bacteria and plant regulators contribute to keep the soybean photosynthetic rate and water status during the occurrence of drought stress, thus minimizing losses in the crop production by morphological and physiological responses that modify the plant capacity of water use.

This study aimed to evaluate the photosynthetic and productive responses of soybean seeds inoculated with *A. brasilense*, as well as foliar spray with *A. brasilense* and plant regulator containing auxin, gibberellin and cytokinin, under induced severe drought stress.

**MATERIAL AND METHODS**

The experiment was conducted under greenhouse conditions, in Marechal Cândido Rondon, Paraná state, Brazil (24°55’S and 54°04’W), from November 2016 to March 2017. During the experiment, the average indoor temperature was 28.8 °C, with relative humidity of 71.84 % and dew point of 20.8 °C.

Plastic pots (12 L) received soil substrate from the A horizon of an Oxysoil, which presented the following chemical and physical characteristics: base saturation = 62.68 %; pH = 5.67; organic matter = 5.47 g dm⁻³; P = 2.07 mg dm⁻³; K = 0.18 cmol dm⁻³; clay = 578 g kg⁻¹; silt = 348.58 g kg⁻¹; and sand = 3.42 g kg⁻¹. The natural population of diazotrophic bacteria, determined by the most probable number method, was 7.5 x 10⁴ CFU g⁻¹ of soil. The supply of 150 mg dm⁻³ of K₂O (potassium chloride) and 300 mg dm⁻³ of P₂O₅ (superphosphate) preceded the sowing of the crop.

A randomized complete block design, with 5 treatments and 5 replicates conducted in triplicate, was applied. The treatments were represented by irrigated and non-irrigated controls (with drought stress), seed inoculation with *A. brasilense* (with drought stress), foliar spray with *A. brasilense* (with drought stress) and foliar spraying with auxin, gibberellin and cytokinin (AX + GA + CK) (with drought stress).

Two soybean plants (NA 5909® cultivar) were conducted per pot. All treatments were inoculated with *Bradyrhizobium japonicum* spraying (spray volume of 200 L ha⁻¹) in the soil (600 mL ha⁻¹), containing SEMIA 5079 and 5080, with a concentration of 5 x 10⁹ CFU mL⁻¹. For this, the pots were arranged in rows and kept in the application total area, where a CO₂ pressurized sprayer was used (Magno 11002
ADGA, 3 m wide bar and six fan-type tips, working pressure of 2.2 bar, flow rate of 200 L ha⁻¹ and application height of 0.5 m above the apex of the plants.

The seed inoculation with *A. brasilense* (AbV5 + AbV6 strains) was carried out at a dose of 100 mL of inoculant (2 x 10⁸ CFU mL⁻¹) for each 50 kg of seeds, which were homogenized and kept in the shade for about 30 min.

When the plants reached the phenological stage V₆ (fifth fully developed trifoliolate leaf), the foliar application of the treatments was made between 7 p.m. and 8 p.m.

For the plant regulator treatment, a mixture (Stimulate®) of auxin (0.025 g ha⁻¹ of 4-indol-3-ylibutyric acid), gibberellin (0.025 g ha⁻¹ of gibberellic acid) and cytokinin (0.045 g ha⁻¹ of kinetin) was used, while, for the foliar spray with *A. brasilense*, a dose of 500 mL ha⁻¹ of *A. brasilense* (AbV5 and AbV6 strains), at a concentration of 2 x 10⁸ CFU mL⁻¹, was applied.

The pots were kept constantly irrigated, with daily replenishment of water. The plants were monitored to ensure their adequate development, and no application of nutrients was performed during the conduction of the test. Before the water deficit imposition, the accumulation of root and shoot dry mass was evaluated, in order to verify the homogeneity in the plant growth.

When the plants reached the R₃ stage (full flowering), the imposition of drought stress was performed. Initially, all treatments were irrigated and, after this, the plants that were destined to drought stress had their irrigation suspended up to the moment that the CO₂ net assimilation rate (*A*) approached zero, corresponding to three days after the drought stress imposition. After this period, the pots were rehydrated and constantly irrigated up to the full maturity time.

To characterize that the plants were under severe drought stress, the criterion of stomatal conductance (*gs*) was established for *C₃* plants, where hydrated plants have a *gs* ≥ 0.2 mol H₂O m⁻² s⁻¹ and plants under severe drought stress present a *gs* ≤ 0.1 mol H₂O m⁻² s⁻¹, being evaluated daily for this finding (Flexas et al. 2004).

The relative water content and gravimetric soil moisture were evaluated before dawn and at the end of the evaluations. The relative water content was based on the known leaf area segments, followed by the method of difference among wet mass, full water mass content after 6 h at 25 °C and dry mass after 48 h at 65 °C. The gravimetric soil moisture was determined by the sample collection of substrate and by the mass difference method at the collection time and dry mass after 24 h at 105 °C.

The CO₂ net assimilation rate (*A*), as a function of the different levels of light, was determined using the infra-red gas analyzer (IRGA) model LI-6400XT (Licor Inc., Lincoln, NE). The readings were performed in the morning, using a concentration of 400 µmol mol⁻¹ of CO₂, flow rate in the chamber of 500 µmol s⁻¹ and block temperature of 25 °C. The evaluations were performed on leaves fully developed, photosynthetically active and with no injures, located in the middle canopy, which were chosen randomly before the imposition of drought stress and marked to carry out the evaluation always on the same leaves, at the following photosynthetic photon flux densities (PPFD - *Q*): 0 µmol m⁻² s⁻¹; 25 µmol m⁻² s⁻¹; 50 µmol m⁻² s⁻¹; 100 µmol m⁻² s⁻¹; 200 µmol m⁻² s⁻¹; 500 µmol m⁻² s⁻¹; 800 µmol m⁻² s⁻¹; 1,200 µmol m⁻² s⁻¹; 1,500 µmol m⁻² s⁻¹; 2,000 µmol m⁻² s⁻¹; and 2,500 µmol m⁻² s⁻¹, using the variable CO₂ net assimilation rate.

To calculate the apparent quantum efficiency [Φ (µmol CO₂/µmol fotons)], the concentrations of 0 µmol m⁻² s⁻¹; 25 µmol m⁻² s⁻¹; 50 µmol m⁻² s⁻¹ and 100 µmol m⁻² s⁻¹ of photons were used, adjusting the equation *A* = *a* + Φ*Q*, where *a* and Φ are coefficients and *Q* represents the photosynthetic photon flux density, with Φ being the inverse of the line angular coefficient. At the intersection of the line in the x-axis, the value for the light compensation point ([Φ] (µmol m⁻² s⁻¹)) was established. The response curve of *A*, as a function of the photosynthetic photon flux density, was adjusted by the rectangular hyperbola function (*A* = *A*max *Q*/“a” + Φ*Q*), where *A*max is the CO₂ maximum assimilation rate, “a” is an adjustment coefficient of the equation and *Q* the photosynthetic photon flux density (Machado et al. 2005).

At the harvest point, all the pods were collected, being then threshed and quantified in an analytical precision scale, to determine the yield per plant. The data of soil moisture, relative water content and yield were submitted to analysis of variance at 5 % of probability, and when significant differences were found, the means were compared by the Student-Newman-Keuls test at 5 % of probability. The Pearson’s correlation analysis was also determined among the variables evaluated.
RESULTS AND DISCUSSION

The soil moisture content was statistically influenced by the treatments, where the irrigated control was superior to the other treatments (Figure 1a), which did not present statistical differences among them. Thus, the available water in the treatments under drought stress was absorbed in a similar way, being the greater average of the irrigated control provided by the daily water replenishment.

The results obtained to the relative water content in the foliar tissue followed the soil gravimetric moisture response, being observed significant differences for the irrigated control, which presented a higher mean, if compared to the others treatments. Treatments under drought stress did not differ among themselves (Figure 1b). These decreased relative water contents, when compared to the irrigated control, were 76.96 %, 41.75 %, 44.22 % and 52.40 %, respectively for the non-irrigated control, seed inoculation with A. brasilense, foliar spray with A. brasilense and foliar spray with AX + GA + CK. It is worth mentioning that the value of the relative water content for the irrigated control is low due to the high temperatures inside the greenhouse, leading to high transpiration rates, and, thus, even with the availability of water in the soil, the water absorption was slower, resulting in a lower relative water content.

This result demonstrates a mitigation of leaf water loss during the evaluated period, considering the smaller reductions of the relative water content values. The relative water content observed for plants inoculated with plant growth promoting bacteria or plant regulator can be explained by the better water-use efficiency (Dimkpa et al. 2009), as well as by the better vegetal hormonal balance. Thus, the plants, when identified the lack of water, have a greater potential for synthesizing abscisic acid (Cohen et al. 2009), what leads to a faster stomatal closure in the plant, a way of signaling the drought stress. Another condition may be linked to the stimuli in the production of osmolyte compounds, such as proline, sugars and polyamines, which cause the plant to close its stomata more rapidly (Vurukonda et al. 2016), thus reducing the main water loss pathway: stomatal transpiration.

Studies on plant growth promoting bacteria report a better use of water in inoculated plants, evidencing that the plant growth promoting bacteria help in the plant survival, because they maintain the highest relative water content (Grover et al. 2014, Fan et al. 2015). Accordingly, higher relative water content values represent plants with an advanced capacity of physiological adaptation to the environment, maintenance of cell division and physiological activity (Ngumbi & Kloeppeper 2016).

This way, when evaluating the soybean photosynthetic responses according to the different levels of photosynthetic photon flux density, it was verified that they did not suffer with water restriction and maintained high photosynthetic rates, reaching an $A_{max}$ of 27.62 μmol m$^{-2}$ s$^{-1}$, while the treatments kept under drought stress had reduced values.

The non-irrigated control presented $A_{max}$ of 3.21 μmol m$^{-2}$ s$^{-1}$, while the seed inoculation with A. brasilense and foliar spray with A. brasilense and AX + GA + CK showed values of 8.59 μmol m$^{-2}$ s$^{-1}$.
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7.63 μmol m⁻² s⁻¹ and 8.41 μmol m⁻² s⁻¹, respectively. In other units, these same treatments provided reductions of 860.43 %, 321.53 %, 361.99 % and 328.42 %, respectively, when compared to the irrigated control (Figure 2).

When considering the effects of drought stress mitigation and the *Amax* reduction of 860.43 % for the non-irrigated control, the treatments involving seed inoculation with *A. brasilense* and foliar spray with *A. brasilense* and AX + GA + CK showed increases of 267.60 %, 237.69 % and 261.99 %, respectively, if compared to the non-irrigated control, attenuating the effects on photosynthesis reduction.

The maintenance of a larger *Amax* in plants treated with *A. brasilense* and plant regulator reflects the mitigation observed in the relative water content, where these treatments reduced the leaf water loss (Figure 1b), what leads to a higher transpiration rate (data not shown), allowing a greater diffusion of CO₂ to the substomatal chambers (Chaves &

![Graph](image-url)

**Figure 2.** CO₂ net assimilation curve, due to different levels of luminosity for the treatments: irrigated control (a); non-irrigated control (b); seed inoculation with *Azospirillum brasilense* (c); foliar spraying inoculation with *A. brasilense* (d); foliar spraying with auxin + gibberellin + cytokinin (e), for soybean under severe drought stress, in the flowering stage. *A*: CO₂ net assimilation rate; *Q*: photosynthetic photon flux density; *Amax*: CO₂ maximum assimilation rate; Φ: apparent quantum efficiency; Γ: light compensation point.
Oliveira 2004). Associated with this, the water limitation results in a dynamic photoinhibition or protective photosynthesis by a limitation in the electron transport in the photosystem II, what leads to a decrease in the photosynthetic rate of short duration (Araújo & Deminicis 2009). Corroborating the present study, it is demonstrated that the drought stress reduces the photosynthetic rate of the plants in function of different levels of photosynthetic photon flux density (Chavarria et al. 2015).

Evaluating the apparent quantum efficiency (Φ) (Figure 2), the drought stress showed a Φ elevation, reducing the use efficiency of ATP and NADPH in the Calvin cycle, regardless of the treatments used. Ghanoum (2009) reports that the elevation of Φ also shows the deficient usability of light energy in the photochemical reactions of photosynthesis. Thus, the irrigated plants required 14.57 μmols of photons to fix 1 μmol of CO$_2$, while, in the dry control, 33.89 μmols of photons were required, whereas the seed inoculation with *A. brasilense* and foliar spray with *A. brasilense* and AX + GA + CK required 36.63 μmols of photons/μmol of CO$_2$, 45.45 μmols of photons/μmol of CO$_2$ and 31.64 μmols of photons/μmol of CO$_2$, respectively.

The increase of Φ is a consequence of the CO$_2$ limitation input by the stomatal closure (Machado et al. 2013), resulting in a lower efficiency to fix CO$_2$ and demanding a higher energy expenditure for fixing a CO$_2$ molecule, as a result of photorespiration improved by drought stress. This condition will result in plants with reduced yield capacity, evidenced by the negative correlation (-0.841) between Φ and plant production (Table 1).

Similarly to the apparent quantum efficiency, the light compensation point was increased in the plants maintained under drought stress, with emphasis on the non-irrigated control, which obtained a higher value, exceeding in 391.07 % the irrigated control. Corroborating the present results, the literature has demonstrated that soybean plants under drought stress present an increase in the light compensation point (Catuchi et al. 2011). Larcher (2003) reports that the increase in the light compensation point indicates that the plant respiration overcome the drought stress conditions, what will result in a decrease of the plant production (Figure 3) for the non-irrigated control.

**Table 1.** Person’s correlation among the photosynthetic variables, water content and production per plant (g plant$^{-1}$) of soybean submitted to seed inoculation with *Azospirillum brasilense* and foliar spraying with *A. brasilense* or plant regulators under drought stress, in the flowering stage.

<table>
<thead>
<tr>
<th></th>
<th>$A_{max}$</th>
<th>Φ</th>
<th>Γ</th>
<th>Yield</th>
<th>RWC</th>
<th>Ug</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{max}$</td>
<td>1</td>
<td>-0.841•</td>
<td>-0.806•</td>
<td>0.919*</td>
<td>0.988**</td>
<td>0.982**</td>
</tr>
<tr>
<td>Φ</td>
<td>-0.841•</td>
<td>1</td>
<td>0.368</td>
<td>-0.621</td>
<td>-0.756</td>
<td>-0.893*</td>
</tr>
<tr>
<td>Γ</td>
<td>-0.806•</td>
<td>0.368</td>
<td>1</td>
<td>-0.935*</td>
<td>-0.876*</td>
<td>-0.698</td>
</tr>
<tr>
<td>Yield</td>
<td>0.919*</td>
<td>-0.621</td>
<td>-0.935*</td>
<td>1</td>
<td>0.936</td>
<td>0.838*</td>
</tr>
<tr>
<td>RWC</td>
<td>0.988***</td>
<td>-0.756</td>
<td>-0.876*</td>
<td>0.936</td>
<td>1</td>
<td>0.952*</td>
</tr>
<tr>
<td>Ug</td>
<td>0.982**</td>
<td>-0.893*</td>
<td>-0.698</td>
<td>0.838*</td>
<td>0.952*</td>
<td>1</td>
</tr>
</tbody>
</table>

**, *, and •: significant by the t test at 1 %, 5 % and 10 % of probability, respectively. $A_{max}$: CO$_2$ maximum assimilation rate; Φ: apparent quantum efficiency; Γ: light compensation point; RWC: relative water content; Ug: gravimetric moisture.

Figure 3. Yield after the imposition of severe drought stress on soybean, in the flowering stage. Bars indicate the standard deviation of the means. Different letters between them indicate a significant difference by the Student-Newman-Keuls test at 5 % of probability.
levels, in plants under drought stress conditions, assist in the plant development, mitigating the stress effects.

Following the behavior demonstrated in the previous variables, the drought stress during the soybean flowering stage influenced the final crop production. The highest average was obtained for the irrigated plants, while the lowest one was observed in the non-irrigated control, with reduction of 28.31%, if compared to the irrigated control. However, the non-irrigated control did not differ from the seed inoculation with *A. brasilense*. In turn, the foliar spray with *A. brasilense* and AX + GA + CK presented higher values of 20.34% and 19.07%, respectively, if compared to the non-irrigated control (Figure 3). The same treatments did not differ from the seed inoculation with *A. brasilense*.

The occurrence of drought stress in the flowering stage reduced the plant production, due to the abortion of reproductive structures and less availability of photoassimilates by leaf abortion, driving to a lower pod formation. According to the literature, the incidence of drought stress in this stage may result in abortion rates of 15% (Gava et al. 2016), 49% (Fioreze et al. 2013) or 26.8% (Fioreze et al. 2011).

The highest yields obtained using foliar spray with *A. brasilense* or plant regulator are related to the photosynthetic activity maintenance. This fact explains the lower yield per plant for seed inoculation with *A. brasilense* by reduced water-use efficiency, which culminated in a greater flower abortion, resulting in a lower number of pods (data not measured).

The maintenance of the grain production in soybean plants is directly linked to the physiological activity maintenance and relative water content in the plant tissue, confirmed by the positive correlations obtained between the variables *A. brasilense* x production and *A. brasilense* x relative water content, as well the negative correlations between production x apparent quantum efficiency and production x light compensation point (Table 1).

The highest averages using foliar spray with *A. brasilense* and plant regulators resulted from the association of the productive factors and the hormonal stimuli, represented in this study by *A. brasilense* and the closing of the soybean cycle with a higher production, thus confirming that soybean plants that continue to perform photosynthesis under drought stress conditions present higher yields (Liu et al. 2005).

In short, seed inoculation or foliar spray with *A. brasilense*, as well as plant regulator foliar spray with auxin, gibberellin and cytokinin, alleviate the effects of drought stress during the soybean flowering on the $CO_2$ assimilation rate, but only *A. brasilense* and plant regulator foliar spray reduce the decrease in the soybean production caused by drought stress in the flowering stage.

CONCLUSION

The foliar spray with *Azospirillum brasilense* or plant regulator are options to alleviate the drought stress effects in the soybean flowering stage, thus minimizing the reduction in the $CO_2$ net assimilation and resulting in lower losses in yield.

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