PLANT GROWTH-PROMOTING BACTERIA EFFECT IN WITHSTANDING DROUGHT IN WHEAT CULTIVARS

Efeito de bactérias promotoras de crescimento vegetal na resistência à seca em cultivares de trigo

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Resumo – Este trabalho avaliou genótipos de trigo em condições de escassez de água inoculados com Azospirillum brasilense e Herbaspirillum seropedicae, com e sem adubação nitrogenada. Para tanto, amostras para determinação do conteúdo relativo de água na folha (CRA) e do índice de estabilidade da membrana (IEM) foram coletadas no 1º e 8º dias de uma restrição total de água no estágio de emborrachamento. Além disso, os parâmetros de biomassa, nitrogênio total (NT) e produtividade foram determinados na colheita. Como resultado, os genótipos mostraram um desempenho distinto. Os dados de CRA e IEM revelaram que a inoculação de A. brasilense e H. seropedicae são capazes de conduzir o genótipo CD 120 à tolerância à seca. Além disso, o índice de grãos foi melhorado em todas as condições em que H. seropedicae estava presente em ambos os diferentes regimes de água. O H. seropedicae inoculado juntamente com adubo nitrogenado também aumentou o rendimento de grãos sob o regime de escassez de água. O cultivar Frontana exibiu a capacidade de manutenção do IEM e CRA apenas quando H. seropedicae ou ambas as bactérias mais adubo nitrogenado foram aplicados, no entanto, esta cultivar não apresentou diferenças em termos de massa fresca e seca da parte aérea e radicular, NT ou produtividade. Estes resultados apontaram H. seropedicae como promissora para inoculação em cereais e o CD120 como um bom modelo de planta para estudar a interação de plantas e bactérias.


Abstract – This work evaluated wheat genotypes under water deficit inoculated with Azospirillum brasilense and Herbaspirillum seropedicae, with and without nitrogen fertilization. Samples of the plants were collected to evaluate its relative water content (RWC) and membrane stability index (MSI) at the 1\textsuperscript{st} and 8\textsuperscript{th} day of total water restriction at the booting stage. The plant biomass, total nitrogen (TN) and grain yield were determined at harvesting. The genotypes showed different performances. According to the results of RWC and MSI, inoculation with A. brasilense and H. seropedicae can make the cultivar CD-120 more tolerant to drought. Grain index was improved with H. seropedicae in all conditions and water regimes. H. seropedicae with nitrogen fertilization increased grain yield under water deficit. A. brasilense with nitrogen fertilization improved the 1000-grain weight of plants under water deficit. The cultivar Frontana maintained its cellular integrity and RWC with nitrogen fertilization combined with H. seropedicae and with both bacteria, however the shoot and root fresh and dry weights, TN and yield of this cultivar showed no differences. These results show the inoculation with H. seropedicae as promising to cereals, and the cultivar CD-120 as a good plant model to study plant-bacteria interaction.

Keywords – water status, plant-microbe association, rhizobacteria
INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the main products in the human daily diet (JONES, 2005), whose production reached more than 730 million Mg in 2017/2018 (FAO, 2017). The most limiting factor for wheat productivity is water deficit, which affects yield depending on its intensity and wheat phenological stage (OKUYAMA et al., 2004; ARAUŞ et al., 2008). In the Southern hemisphere, the wheat season coincides with a low precipitation period. Thus, 5% of the wheat production was lost in South Brazil in 2013 (GODINHO, 2013), contributing to decreasing the internal production supply to about half of the expected (CONAB, 2015).

Plants have developed adaptive physiological responses to cope with drought stress, such as reduction of transpiration by fast stomatal closure, decreasing of photosynthetic activity and deepening of roots (ALI et al., 2013; BECK et al., 2007). Plants with high antioxidant levels usually have greater tolerance to oxidative damages due to water stress (APEL; HIRT, 2004). Moreover, the synthesis of osmolytes can increase the osmotic potential inside cells in response to drought (FAROOQ et al., 2009). Importantly, all of these responses may vary among plant cultivars.

In order to maintain productivity, it is necessary to find efficient low-cost technologies to reduce drought effects over crops. The maintenance of crop yields under water restriction is the major challenge facing agriculture where plant growth-promoting bacteria (PGPB) can play an important role. In the last decades, many authors have been studying soil microorganisms as crops helpers in withstanding abiotic stresses (CHAKRABORTY et al., 2013). The PGPB have a potential to increase agricultural productivity through biological nitrogen fixation, promotion of greater absorption of nutrients by plant roots and reduction of deleterious effects of pathogens (BECKERS; CONRATH, 2007, FARINA et al., 2012, NAIMAN et al., 2009, ROJAS-TAPIAS et al., 2012, SPAEPEN et al., 2008). Remarkably, PGPB can also promote tolerance to drought in plants by secreting compounds (including osmolytes) that increase osmotic potential of root cells (DIMKPA et al., 2009).

Herbaspirillum seropedicae (BALDANI et al., 1986) and Azospirillum brasilense (TARRAND et al., 1978) can colonize roots of cereals, and their efficiency to transfer fixed nitrogen to plants was already described in rice and sugarcane (BALDANI et al., 1997, BALDANI; BALDANI, 2005, BHATTACHARJEE et al., 2008, SENGUPTA; GUNRI, 2015). *H. seropedicae* in wheat crops can substitute nitrogen fertilization, according to a greenhouse experiment (NEIVERTH et al., 2014). Moreover, *A. brasilense* decreased grain yield loss in wheat under salt and drought stresses (CREUS et al., 1997; CREUS et al., 2004).

The beneficial effects of PGPB on plants depends on many factors, such as soil type, plant age, physiological stage and genotype, and the bacterial strain specificity (BALDANI; BALDANI, 2005, ROESCH et al., 2006, VARGAS et al., 2012). However, wheat genotypes have different capacities of association with bacteria. Root exudates act as chemical attractants for a vast number of heterogeneous, diverse and active metabolizing soil microbial communities (AHEMAD; KIBRET, 2014). These exudates differ according to the genotype, defining the microbiota around the roots (AIRA et al., 2010). These factors are essential to find solutions for specific potential application of these bacteria as bio-fertilizers.

Plants considered as models, showing a positive and negative plant-bacteria interaction, were described for cultivars of rice (IR42 and IAC 4440) (VARGAS et al., 2012), sugar cane (SP70 1143 and Chunee) (GOMES et al., 2005) and wheat (CD-120 and CD104) (NEIVERTH et al., 2014). However, few studies evaluated the performance of wheat model plants inoculated with PGPB and subjected to water deficit.

The cultivar CD-120 belongs to the Coodetec’s wheat germplasm and has Mexican origin (CIMMYT) (VENDRUSCOLO et al., 2008). This genotype is described as resistant to the major diseases and of high grain yield potential and soft wheat quality (MARCHIORO et al., 2011). Frontana is an old, tall, re-grained cultivar with high level of seed dormancy and resistance to leaf rust and fusarium head blight (FHB)
(ANDREOLI et al., 2006). This cultivar is an ancient genotype from which some Brazilian wheat varieties originated. It was included in the present study as control, since it probably underwent lower breeding pressure to respond to nitrogen fertilization and interactions with bacteria.

The objective of this study was to verify the performance of two wheat cultivars subjected to water deficit, under different inoculation with bacteria and fertilization conditions.

MATERIAL AND METHODS

The experiment was conducted at the Agricultural Research Central Cooperative COODETEC, Cascavel, State of Paraná, Brazil (24º53'10.7''S, 53º32'56.1''W), from May to September, 2012, using two wheat genotypes (CD-120 and Frontana).

The experiment was conducted in a complete randomized block design with five replications, consisting of two genotypes, eight different conditions (fertilizations and inoculations) and two water regimes (normal irrigation and water deficit). Results were subjected to analysis of variance (ANOVA) and compared by the Tukey's test at 5% significance level using the program GENES (CRUZ, 2013).

Six seeds were sown per pot, which contained approximately 4.5 kg of 5-mm sieved, not-autoclaved Red Latosol that were locally collected, arranged in a greenhouse to have the same solar radiation, temperature (25±2°C) and relative humidity (60%). No chemical fertilizers were applied due to the good soil characteristics (pH = 6.40, P = 60.00 mg dm⁻³, K = 1.14 cmol dm⁻³, Ca = 6.69 cmol dm⁻³, Mg = 3.03 cmol dm⁻³, H⁺Al = 3.18 cmol dm⁻³, Cu = 14.45 mg dm⁻³, Mn = 400.00 mg dm⁻³, Fe = 21.00 mg dm⁻³, Zn = 32.49 mg dm⁻³, BS = 10.86 cmol dm⁻³ and organic matter = 41.6 g dm⁻³).

The pre-inoculum (of both *H. seropedicae* SMR1 and *A. brasilense* ABV5 strains) were prepared from a single colony in 5 mL of liquid DYGS medium maintained at 28 °C in a shaker (120 rpm overnight). One mL of the pre-inoculum was transferred to a 20-mL conic tube containing the liquid DYGS medium originating the inoculum. The inocula grew until the log phase (OD 660 nm) and the seed inoculation was performed before sowing, in order to provide 10⁶ cells seed⁻¹ for *H. seropedicae* and 10⁷ cells seed⁻¹ for *A. brasilense* (JUHNKE et al., 1989; SANTOS et al., 2010).

The treatments consisted of control (C1), inoculation with *H. seropedicae* (C2), inoculation with *A. brasilense* (C3), inoculation with *H. seropedicae* and *A. brasilense* (C4), nitrogen fertilization (C5), inoculation with *A. brasilense* and nitrogen fertilization (C6), inoculation with *H. seropedicae* and nitrogen fertilization (C7), inoculation with *H. seropedicae* and *A. brasilense* and nitrogen fertilization (C8).

Thinning was performed 30 days after planting, keeping four plants in each pot, followed by nitrogen fertilization (142 kg urea ha⁻¹). The plants were subjected to two water regimes, plants with normal irrigation (once a day) (1) and plants under total water restriction for 8 days in the early booting stage, from the 65th for the cultivar CD-120 and from the 75th day after germination for the cultivar Frontana (2) (Zadoks 4.5) (ZADOKS et al., 1974). After this period, plants from both water regimes were irrigated normally until harvesting. Leaf samples were collected from plants at the same time in both water regimes, in the 1st and 8th day after water restriction. Samples were used to quantify the relative water content (RWC), following the protocol proposed by Schonfeld et al. (1988), and membrane stability index (MSI), according to Chandra Babu et al. (2004). Harvests were performed at 139 (Frontana) and 120 (CD-120) days after germination, and the plants were evaluated according to production parameters, including plant fresh and dry weights, shoot total nitrogen (TN), grain weight per plant (grain yield) and 1000-grain weight (grain index) (BREMNER; MULVANEY, 1982).

RESULTS AND DISCUSSION

Relative water content and membrane stability index

RWC and MSI were used to assess the water status and membrane stability of plants subjected to water deficit and inoculation with *A. brasilense* and/or *H. seropedicae* and/or nitrogen fertilization. RWC and MSI data showed that the inoculation with *A. brasilense* and *H. seropedicae* promoted tolerance to drought for the cultivar CD-120. The same trend was observed for the cultivar Frontana, but at a lesser extent. The cultivar CD-120 maintained a high RWC until the 8th day of water restriction, under all inoculation conditions (Figure 1A).

The lowest RWC were found in the control (C1) (12%) and nitrogen fertilization (C5) (41%) of the cultivar CD-120. These results represent a decrease in RWC of 84 and 51%, respectively, compared with control plants, denoting the effect of water deficit in the plants. The RWC of plants of the cultivar Frontana under irrigation and water stress, with *A. brasilense* (C2) or *H. seropedicae* (C3) and with nitrogen plus the bacteria strains (C8) was similar (Figure 1B).
The MSI data of CD-120 clearly showed its maintenance of cellular membrane in most conditions, excepted control (C1) and nitrogen fertilization (C5), which had decreases in membrane integrity of 54% and 48%, respectively (Figure 2A).

The MSI of the cultivar Frontana showed the same trend of the RWC (Figure 2B). The data indicated a better membrane protection when inoculated with H. seropedicae (C2) and with both strains plus nitrogen fertilization (C8).

The RWC and MSI of plants under water deficit were similar to those reported by Lemos et al. (2011) for another COODETEC genotype under the same water regimes. These authors found significant decreases in RWC (31%) and MSI (15%) in plants under water deficit, compared with those under normal irrigation.

The low RWC and MSI found are indicators of severe membrane injuries, which compromised the cell recovery after the drought period. Reduction in electrolyte losses under water stress results in a better membrane integrity and tolerance to oxidative stress (LIU et al., 2011).
Inoculation with *A. brasilense* induced homeostatic mechanisms that generate tolerance to drought in prime wheat (KASIM et al., 2013). Moreover, drought increases reactive oxygen species production (BECK et al., 2007), which can damage cells and activate defense responses to water stress (DOORNBOS et al., 2012; FAROOQ et al., 2009). The MSI of inoculated plants of the cultivar CD-120 indicated a protection against oxidative damage using the bacteria as a bio-priming (BECKERS et al., 2007, COMPANT et al., 2005, ZAMIOUDIS; PIETERSE, 2012; MASWADA; EL-KADER, 2016). However, the treatment with nitrogen fertilization (C5) did not prevent damage to the membrane structure and loss of turgor in both genotypes subjected to water deficit.

**Fresh and dry weight and yield parameters**

Booting seems to be the most critical phenological stage for wheat subjected to water deficit (LEMOS et al., 2011; KHAN et al., 2015). High shoot fresh weight and TN indicate a plant growth with a normal metabolism. Drought affected the shoot weight and TN of both genotypes (Table 1), showing that water is essential to the physiological apparatus.

*H. seropedicae* (C2) increased the fresh and dry shoot weight of CD-120 under irrigation (Table 1). However, under water deficit, this cultivar showed increments in fresh shoot weight with nitrogen fertilization plus inoculation with *H. seropedicae* (C6) and *H. seropedicae* and *A. brasilense* (C8) with 1.8-fold and 2-fold respectively, compared with the control. The highest shoot dry weight (17%) and TN (35%) content was found in the treatment with both bacteria and N fertilization (C8) in plants under water stress.

Grain weight per plant and 1000-grain weight was improved in all conditions with *H. seropedicae* (C2) under normal water regime. Moreover, *H. seropedicae* with nitrogen fertilization (C6) improved grain weight per plant of the cultivar CD-120 under water deficit. The good productive performance and higher RWC and MSI of the cultivar CD-120 indicated a beneficial interaction with *H. seropedicae*, thus, this cultivar can be considered as a positive model (NEIVERTH et al., 2014) to evaluate plant-bacteria interaction in water stress conditions.

Furthermore, CD-120 showed increases in grain yield (8-fold) and 1000-grain weight (1.3-fold) with nitrogen fertilization plus inoculation with *H. seropedicae* (C6) and *A. brasilense* (C7) (Table 2). Its higher yields can be explained by a delayed water loss due to the presence of bacteria, involving several morphophysiological and biochemical changes, such as rises in abscisic acid (ABA), lipid peroxidation and proline (COHEN et al., 2015).

Although the results showed good responses of the cultivar CD-120, there are few works in the literature comparing its performances. However, Alamri and Mostafa (2009) evaluated the effect of N supply and inoculation with *A. brasilense* in wheat subjected to a saline condition (8%) and found an increase of 9% in 1000-grain weight without N fertilization and of 21% when N was applied. In the present work, the application of nitrogen fertilization (C5) did not increase grain yield and grain index of plants under water standard regime.

The cultivar Frontana has usually high shoot weight phenotype, which explains its highest fresh and dry shoot weight and TN (Table 1). The parameters of this cultivar did not differ significantly with the inoculations or water regimes (Tables 1 and 2). The Frontana shoot dry weight of plants inoculated with *A. brasilense* increased in approximately 2-fold with nitrogen fertilization (C7) under water deficit condition. Nevertheless, TN content decreased under the same condition. The grain weight per plant under water deficit of the cultivar Frontana, inoculated with *A. brasilense* and *H. seropedicae* (C2, C3 and C4) was also improved. *H. seropedicae* plus nitrogen fertilization (C7) promoted the highest grain weight per plant, but the 1000-grain weight did not increase (Table 2).

Rhizobacteria-induced drought endurance and resilience (RIDER) that includes changes in the levels of phytohormones, defense-related proteins and enzymes, antioxidants and epoxypolysaccharide have been observed for microbe-mediated plant responses (MEENA et al., 2017). According to the results of the present work, *A. brasilense* and *H. seropedicae* can be considered as RIDERs, since they improved the plants of both genotypes.

The cultivar CD-120 (commercial cultivar) seems to interact better with bacteria, minimizing the drought stress effects than the cultivar Frontana (ancestral cultivar). Probably, CD-120 presents a better anti-oxidative enzymatic profile and the presence of bacteria can optimize it, conferring a better tolerance to drought stress to this cultivar (HANDIA et al., 2004; HAYAT et al., 2010).
Table 1 – Effect of different conditions of fertilization and/or inoculation with *H. seropedicae* and *A. brasilense* on biomass parameters of wheat varieties submitted to different water regimes.

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Fresh Shoot Mass (g)</td>
<td>10.1</td>
<td>Bb*</td>
<td>22.2</td>
<td>Aa*</td>
<td>7.7</td>
<td>Bb*</td>
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<tr>
<td>Shoot Dry Mass (g)</td>
<td>15.2</td>
<td>Aa*</td>
<td>13.2</td>
<td>Aa*</td>
<td>13.1</td>
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<tr>
<td>Shoot Total Nitrogen Content (g.kg⁻¹)</td>
<td>1.4</td>
<td>Aab</td>
<td>1.9</td>
<td>Aa</td>
<td></td>
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<tr>
<td>CD 120</td>
<td>Frontana</td>
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<td>CD 120</td>
<td>Frontana</td>
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<tr>
<td>C1</td>
<td>5.1</td>
<td>Bab</td>
<td>11.3</td>
<td>Aa</td>
<td>4.4</td>
<td>Aab</td>
</tr>
<tr>
<td>C2</td>
<td>5.3</td>
<td>Bab</td>
<td>12.1</td>
<td>Aa</td>
<td>3.9</td>
<td>Bb</td>
</tr>
<tr>
<td>C3</td>
<td>5.7</td>
<td>Bab</td>
<td>14.2</td>
<td>Aa</td>
<td>4.4</td>
<td>Bab</td>
</tr>
<tr>
<td>C4</td>
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<td>Bab</td>
<td>15.3</td>
<td>Aa</td>
<td>4.3</td>
<td>Bab</td>
</tr>
<tr>
<td>C5</td>
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<td>Aa</td>
<td>4.0</td>
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</tr>
<tr>
<td>C6</td>
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<td>Aab*</td>
<td>10.2</td>
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<tr>
<td>C7</td>
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<td>5.1</td>
<td>Bab</td>
</tr>
<tr>
<td>C8</td>
<td>10.3</td>
<td>Aa</td>
<td>10.2</td>
<td>Aa</td>
<td>6.0</td>
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</tr>
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</table>

Table 2 – Effect of different conditions of fertilization and/or inoculation with *H. seropedicae* and *A. brasilense* on yield parameters of wheat varieties submitted to different regimes of water support.

<table>
<thead>
<tr>
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<tr>
<td>Grain Mass/plant (g)</td>
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<td>Abc*</td>
<td>2.4</td>
<td>Aab*</td>
<td>3.4</td>
<td>Ad*</td>
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<tr>
<td>1000-Grain Mass (g)</td>
<td>3.1</td>
<td>Ba</td>
<td>3.1</td>
<td>Ba</td>
<td></td>
<td></td>
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<tr>
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<td>Frontana</td>
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<tr>
<td>C1</td>
<td>6.9</td>
<td>Aa*</td>
<td>3.1</td>
<td>Ba*</td>
<td>3.8</td>
<td>Aabc*</td>
</tr>
<tr>
<td>C2</td>
<td>2.8</td>
<td>Abc*</td>
<td>2.4</td>
<td>Aab*</td>
<td>3.6</td>
<td>Abcd*</td>
</tr>
<tr>
<td>C3</td>
<td>3.7</td>
<td>Ab*</td>
<td>2.4</td>
<td>Aab</td>
<td>4.0</td>
<td>Aa*</td>
</tr>
<tr>
<td>C4</td>
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<td>Ac*</td>
<td>0.6</td>
<td>Ac</td>
<td>3.5</td>
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<tr>
<td>C5</td>
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<td>Abc*</td>
<td>2.0</td>
<td>Aab*</td>
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<td>Aab</td>
</tr>
<tr>
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<tr>
<td>C8</td>
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<td>Ab</td>
<td>0.2</td>
<td>Ad</td>
<td>2.9</td>
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</table>

For both tables means followed by the same capital letter in the line (correspond to genotypes – CD120 and Frontana) and small letter on the column (correspond to different conditions of fertilization and/or inoculation) did not differ statistically by the Tukey test (*p<0.05*). Means of water regimes followed by * are statistically different by Tukey test (*p<0.05*).
Comparing CD120 (commercial cultivar) and Frontana (ancestral cultivar), the former seems to interact better to the presence of bacteria minimizing the drought stress effects. Probably, CD 120 presents a better anti-oxidative enzymatic profile and the presence of bacteria could optimize it conferring better tolerance to drought stress (HANDIA et al., 2004; HAYAT et al., 2010). 

CONCLUSION

The cultivar CD-120 can be used as a plant model in studies evaluating the effect of plant growth-promoting bacteria on tolerance to drought and yield performance in wheat.

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REFERENCES


