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RESEARCH ARTICLE

Plant growth promoting rhizobacteria for improved water stress tolerance in wheat genotypes

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Abstract

A greenhouse experiment was carried out to assess the effect of the inoculation with rhizobacterial strains on the tolerance to water stress in wheat genotypes (*Triticum aestivum* L.) under two different water regimes. A drought resistant (Fontagro 8) and a susceptible (Fontagro 98) genotype were studied. Soil water content was kept at 100% and 45% field capacity. The treatments were inoculations with AG-70 (*Bacillus* sp.), AG-54 (*Pseudomonas* sp.), and a mixture of both (AG-70 + AG-54); a control treatment consisting of an autoclaved nutritive solution. When applied to Fontagro 8 genotype, the AG-70 + AG-54 treatment resulted in a higher increase in shoot (88%) and root dry weight (211%) compared to the control under drought conditions. The same treatment applied on the susceptible genotype (Fontagro 98) resulted in increases of 73% and 129% in shoot and root dry weight, respectively. In addition, the inoculated plants showed significant increases in root length, stomatal conductance and chlorophyll index. The AG-54 and AG-70 treatments increased NPK contents in the drought-resistant genotype, while the AG-54 and AG-70 + AG-54 treatments increased the P content in the susceptible genotype. The treatments that showed the most positive effects on the biological quality parameters of the soil (microbial activity, microbial respiration and urease enzyme activity) were AG-54 and AG-70 + AG-54. Therefore, the use of AG-70 and AG-54, applied separately or combined, increased tolerance to water stress in both wheat genotypes and constitute a biotechnological tool for the production of crops in water-deficit ecosystems.

Keywords: Inoculation, PGPR, water stress, wheat.

1. Introduction

Water represents between 80 and 90% of the biomass of non-woody plants, and it is the central molecule in all physiological processes of plants, as well as the principal means of transport for metabolites and nutrients. Water deficit is one of the main sources of plant stress (Lisar *et al.,* 2012). A drought situation reduces the water potential and turgor of plants, so that these face difficulties in carrying out their normal physiological functions (Lisar *et al.,* 2012). In fact, water is the main limiting factor for the growth and productivity of crops, playing a key role in the evolution and distribution of plant species (Ngumbi and Kloepper, 2016).

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops in terms of production and consumption. As drought is the main limitation for wheat yield (Budak *et al.,* 2013), the introduction of novel techniques to increase water stress tolerance is crucial for stabilizing and increasing wheat-based food production (Budak *et al.*, 2013).

The free-living bacteria found in the rhizosphere of plants, known as Plant Growth Promoting Rhizobacteria (PGPR), are highly efficient in the promotion of plant growth through direct and indirect mechanisms (Hassan *et al.,* 2015). Direct effects are related to the synthesis of phytohormones, such as auxins, gibberellins and cytokinins, which facilitate the absorption of nutrients in the plants and provide protection against various types of environmental stress (Glick, 2012; García-Fraile *et al.,* 2015).

Furthermore, the synthesis of enzymes, such as 1-aminocyclopropane-1-carboxylic acid (ACC deaminase), urease activity, modulate the development of plants by increasing plant force, growth and chlorophyll activity, as well as the total biomass of crops (Glick, 2012).

The indirect mechanisms include the inhibition of harmful microorganisms, such as root pathogens, and biological control of plant diseases through the production of antibiotics and siderophores (Widnyana and Javandira, 2016).

Therefore, PGPR increase crop yields, soil fertility and have the potential to contribute to sustainable agriculture because they increase the systemic tolerance to stress (García-Fraile *et al.*, 2015).

Strains with PGPR activity, especially those belonging to the genera *Bacillus* sp. and *Pseudomonas* sp., are particularly important in the soil-plant relationship (Widnyana and Javandira, 2016).

Both genera play key roles in the biosynthesis of phytohormones, such as the indole acetic acid (IAA), which is an auxin that promotes cell division and root elongation whose activity increases throughout the life cycle of plants (Widnyana and Javandira, 2016).

On the other hand, ethylene is a phytohormone that plays an important role in the senescence and abscission of leaves, germination and in the development of the seeds (Jha *et al.,* 2012). Ethylene produced endogenously by almost all plants is a growth regulator, but it can also cause negative effects on the development of plants, such as water stress, when synthesized by excess due to the inhibitory effect of the expansion and root elongation (Jha *et al.,* 2012). Previous studies have indicated that some of the PGPR strains belonging to the genera *Bacillus* sp. and *Pseudomonas* sp. are capable to produce the enzyme ACC deaminase, which is useful in reducing the levels of ethylene by the conversion of ACC to NH_4 and α-ketobutyrate in plants, promoting growth and increasing stress tolerance (Glick *et al.,* 2007).

Thus, microorganisms are essential to increase the exploration potential of roots, and improve nutrient and water uptake, and as physiological parameters

that enable plants to tolerate adverse environmental stresses, increase crop yields, and improve quality (Lim and Kim, 2013).

In this sense, the inoculation with PGPR to achieve increased stress tolerance can be a good tool to enhance production in water-deficit regions (Lim and Kim, 2013).

This study assessed the effects of the inoculation with plant growth promoting rhizobacteria on the water stress tolerance in two wheat genotypes (resistant and susceptible to water stress) grown in a volcanic soil (Andisol).

2. Materials and Methods

2.1. Soil characteristics

Soil was collected from the Santa Rosa experimental station, INIA-Quilamapu (National Agricultural Research Institute) (36 ° 32 'S, 71 ° 55' W). The soil is classified as Typic Haploxerands, Andisols order, derived from volcanic ash, with a silk loamy texture on the surface and clay loam deep in the profile, and with high organic matter content and moisture retention capacity. Annual rainfall at sampling site ranges from 1,500 to 2,000 mm (Stolpe, 2006). The soil chemical parameters analyzed before the start of the experiment are presented in the following order: pH (water): 5.75; organic matter: 4.33%; N available: 32.40 mg kg $^{-1}$; P-Olsen: 38.60 mg kg $^{-1}$; available K: 240.60 mg kg $^{-1}$.

2.2. Wheat genotypes

Two genotypes were selected from a large set of genotypes, previously evaluated by the wheat breeding program of the National Agricultural Research Institute (INIA-Chile). The soil used to conduct the experiments was collected from two Mediterranean sites of Chile. Soil samples were taken in Cauquenes (35°58′ S, 72°17′ W; 177 m.a.s.l.), which was submitted to water stress (WS) as typically rainfed at this site; and in the Santa Rosa Experimental Station (36°32′ S, 71°55′ W; 220 m.a.s.l.), which was submitted to full irrigation. Fontagro 8 showed a high yield tolerance index (YTI= 0.6), and it was considered as resistant to water stress. On the other hand, Fontagro 98 had a low yield tolerance index (YTI= 0.18), and it was considered as susceptible.

2.3. Experimental procedure

The experiment was conducted under greenhouse conditions at the University of Concepcion, Chillan, Chile (36° 35' 43.2" S, 72° 04' 39" W, 144 m.a.s.l.), between October (2016) and January (2017). Harvest was carried out in stage 9 (physiological maturation), according to the Zadoks (1974) scale. Plastic pots (top diameter 20 cm; height 17 cm) were used as experimental units and filled with 2.5 kg of soil; six wheat seeds were sown per pot. After seed emergence (7 days), plants were thinned and only four plants were kept per pot. The treatments were the following: 1- autoclaved nutritive solution (Control); 2- Strain AG-70 (*Bacillus* sp.); 3- Strain AG-54 (*Pseudomonas* sp.) and 4-mixture of the two strains (AG-70 + AG-54). The four treatments were applied to two wheat genotypes and submitted to two water regimes (45% and 100% of field capacity). Soil moisture was measured using a sensor (MORPHO, GS-1) and readings were recorded in a data logger (DECAGON-EM50 Series). Water stress (45% field capacity) was applied from anthesis (flowering) according to the Zadoks scale (1974). The experimental design included four replicates per water regime, while four plants per treatment were evaluated in 64 experimental units.

2.4. Origin of the strains, preparation and inoculation

The bacterial strains used (AG-70 and AG-54) were isolated from the rhizosphere of lettuce (*Lactuca sativa* L.) and belong to the microbial collection of the School of Agronomy of the University of Concepción. IAA was determined through the Salkowski reaction (Sarwar and Kremer 1995) and ACC deaminase was determined according to the methodology described by Li *et al.*, 2011. The AG-70 strain produces 8.5 µg mL $^{-1}$ ACC deaminase and 14.03 µg mL $^{-1}$ IAA, while the AG-54 strain produces 35.18 µg mL $^{-1}$ of IAA (Sepúlveda, 2013). Strains were cultivated using the methodologies described by Miles *et al.* (1938), in Difco™ nutrient broth under constant agitation (Lab Companion, model IF-600) at 150 rpm and 25 °C for 2 days. The concentrations were measured by optical density in a spectrophotometer (UV/VIS Optizen POP, South Korea) at 600 nm and calculating their equivalence in CFU mL $^{-1}$. Once plants emerged (state of four leaves), they were inoculated with each of the strains at concentrations of 10^6 CFU mL^{-1}. Each plant was inoculated with 5 mL of bacterial suspension applied directly to the root zone, while the autoclaved nutritive solution was applied to the control treatment. Plants were inoculated every 30 days to guarantee the survival and activity of the microorganisms during the study.

2.5. Fertilization

Nitrogen (N) was applied at a rate of 200 kg ha^{-1}, using concentrations of 30% and 40% at the beginning and end of tillering, respectively, based on expected performance. No applications of P_2O_5 and K_2O were required because soil analyses revealed that nutrients levels in the soil were adequate for the proper development of the crop.

2.6. Plant parameters evaluated

The shoots and roots were separated and dried in an air force oven for 24 hours at 65 °C. Both shoot and root dry weights were determined using SHIMADZU analytical balances, model AUX220®. Root length were estimated using RootSnap software (version 1.3.2.25 Bio-Science, Inc.). The physiological variables evaluated were stomatal conductance (Sc) and chlorophyll index. Stomatal conductance was determined up to 28 days post-anthesis using a Porometer (DECAGON DEVICES model SC-1, USA), while chlorophyll index (soil plant analysis development (SPAD) values was determined using a portable meter 502 (Minolta Spectrum Technologics Inc., Plainfield, IL, USA). Both parameters were measured on flag leaves from each plant at noon. For chlorophyll index measurements, plants were previously irrigated in the morning.

2.7. Soil chemical and biological analysis

The soil nutrient analysis (N, P, and K) was carried out using the methodology described by Sadzawka *et al.* (2006) at the Laboratory of Soil and Plant Testing of the Department of Soils and Natural Resources, School of Agronomy, University of Concepción. Soil microbial activity was determined by FDA (Fluorescein diacetate) hydrolysis. An amount of 1.0 g of wet soil was used; samples were prepared in triplicate together with a blank. A volume of 9.9 mL of sodium phosphate buffer (60 mM; pH 7.8) plus 0.1 mL of 2 mg fluorescein diacetate (FDA) mL $^{-1}$ acetone was added to the soil samples, while a volume of 10 mL of buffer was added to the blank. The tubes were shaken in a vortex and then incubated at 20 °C for 1 hour in a thermostatic bath. After incubation, samples were cooled in an ice water bath and a volume of 10 mL of acetone was added to all tubes (samples and

blanks); tubes were shaken and filtered through Whatman No. 40 filter paper. Then, the absorbance of the samples and blanks was read in the spectrophotometer (Rayleigh - Model UV1601 UV / VIS) at 490 nm. The results were expressed as µg Fluorescein g^{-1} dry soil (Alef and Nannipieri, 1995). Soil respiration was determined using an amount of 25 g of soil (in duplicate) per treatment placed in an incubation bottle. A volume of 7.5 mL of NaOH (0.5 M) was placed in a centrifuge tube and then placed in an incubation bottle. Jars without soil (blank) were used; these were hermetically closed and remained in an incubation chamber at 22 °C and constant humidity for 7 days. After the incubation time, a volume of 1 mL of NaOH (0.5 M) was taken from the centrifuge tube and mixed with a volume of 2 mL of $BaCl_2$ (1 M) phenolphthalein indicator was previously added (2 to 3 drops) to the solution. Subsequently, the solution was titrated with HCl (0.1 M) and the data were expressed as µg $C\text{-}CO_2$ g^{-1} soil oven dried (105 ºC), (Alef and Nannipieri, 1995). Soil urease enzyme activity was determined using an amount of 1.0 g of soil (duplicate samples plus blank) placed in screw cap test tubes. Volumes of 4 mL of phosphate buffer (pH 8) and 1 mL of 6.4% urea were added to the samples, while the blanks consisted of 4 mL of phosphate buffer and 1 mL of distilled water. They were vortexed and placed in a thermostatic bath at 37 °C for 2 hours. After the incubation time, the tubes were cooled in an ice water bath, and 5 mL of 2 M KCl were added and filtered. A volume of 5 mL aliquot was removed and transferred to 25 mL volumetric flasks. A solution consisting of 1 mL of 6% EDTA, 2 mL of phenol nitroprusside, 4 mL of hypochlorite buffer (added in the same order) was prepared and distilled water was added to fill up to 25 mL. The blanks, reactive white and sample flasks were incubated at 40 °C for 30 minutes and cooled in an ice bath. An aliquot was extracted and read in a spectrophotometer (Rayleigh - Model UV1601 UV / VIS) at 636 nm against the reactive target. The data were expressed as $N\text{-}NH_3$ µg g^1 h^{-1} as described by Alef and Nannipieri (1995).

2.8. Experimental design and statistical analysis

The study was conducted using a completely randomized design consisting of 3 factors (3x2x2). The first factor corresponded to the bacterial strains (*Bacillus* sp. and *Pseudomonas* sp.) and the mixture of both, while the second and third factors corresponded to water regimes (plants irrigated at 45% and 100% field capacity) and wheat genotypes (Fontagro 8 and Fontagro 98), respectively. The data were subjected to analysis of variance (ANOVA). Data normality were verified using the modified Shapiro-Wilks test and mean separation by the Duncan test (P <0.05). Statistical analyses were conducted using InfoStat software version 2016e described by Di Rienzo *et al.* (2016).

3. Results

3.1. Effect of inoculation on plant morphological parameters

No interactions between factors were found in terms of shoot dry weight. However, significant differences were observed when the experimental treatments were analyzed separately (Figure 1). In Fontagro 8, the inoculation with AG-70+AG-54 (*Bacillus* sp. with *Pseudomonas* sp.) resulted in the largest increase in shoot dry weight, followed by AG-54 (*Pseudomonas* sp.). The AG-70+AG-54 treatment recorded increases of 91 and 88%, while the AG-54 treatment increased shoot dry weight in 67% and 58% in plants submitted to full irrigation and water stress, respectively. Similarly, the AG-70+AG-54 treatment also recorded significant increases in Fontagro 98 of 73% under full irrigation and 72% under drought condition. The root dry

weight showed no interactions between the factors under study. However, there were significant differences between the strains inoculated (Figure 2). The highest increase in root dry weight resulted from the inoculation with AG-70+AG-54 in both genotypes, but with higher values in Fontagro 98 compared to the control. Fontagro 8 reached increases of 211% and 115%, while Fontagro 98 recorded increases of 235% and 129% in plants submitted to full irrigation and water stress, respectively. The second highest values resulted from the AG-54 treatment (147% and 89% in irrigated and drought conditions, respectively). Significant differences were observed in terms of root length (Figure 3). In the genotype Fontagro 8, the highest increases were obtained with the AG-70+AG-54 treatment compared to the control, with increases of 173% and 140% under irrigated and drought conditions, respectively. The second largest increase was achieved by the AG-54 treatment with increases of 107% and 91%. In the genotype Fontagro 98, the greatest increases were recorded in the treatments AG-70+AG-54 (131 and 68%), and AG-54 95 and 65%) compared to the control in plants under full irrigation and drought conditions respectively.

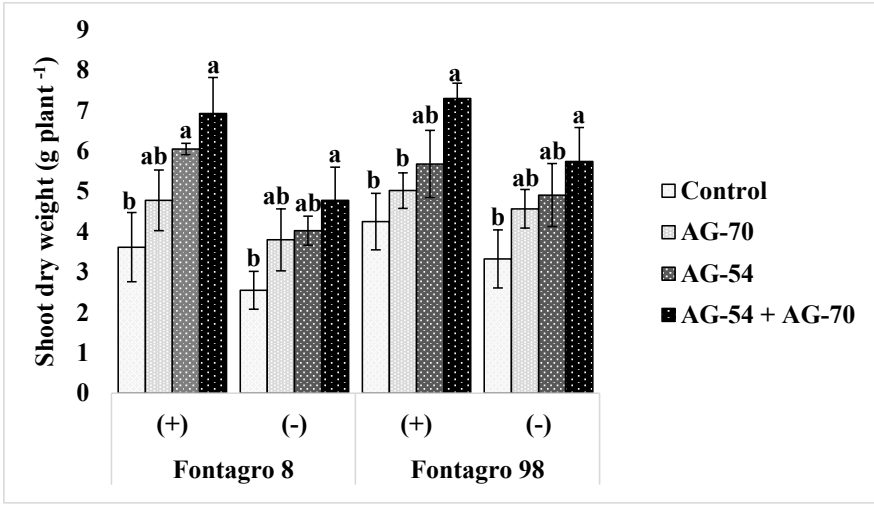

Figure 1. Shoot dry weight (g plant^{-1}) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. The error bars represent the standard error of mean. (+): Plants submitted to irrigation; (-): Plants submitted to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Bars of similar color within the same genotype and water regime are not significantly different by the Duncan test ($P > 0.05$).

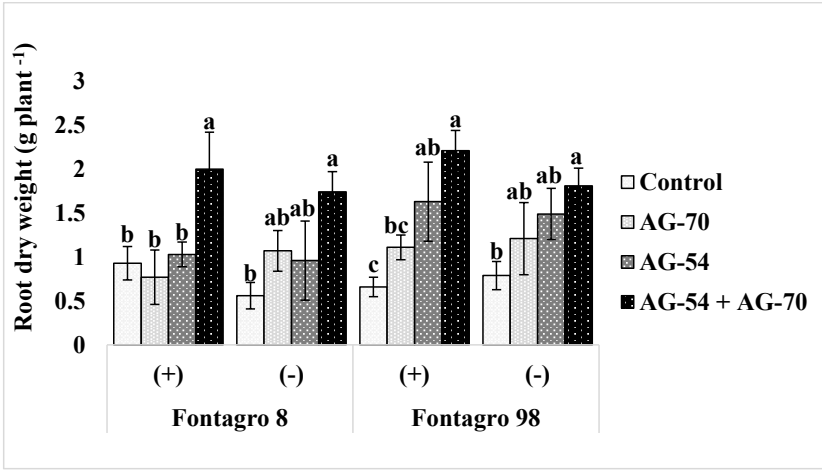

Figure 2. Root dry weight (g plant^{-1}) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. The error bars represent the standard error of mean. (+): Plants submitted to irrigation; (-): Plants submitted to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Bars of similar color within the same genotype and water regime are not significantly different by the Duncan test (P> 0.05).

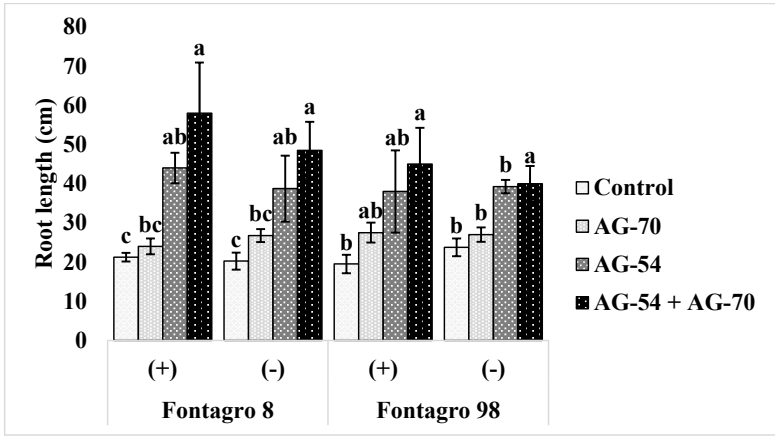

Figure 3. Root length (cm) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. The error bars represent the standard error of mean. (+): Plants submitted to irrigation; (-): Plants submitted to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Bars of similar color within the same genotype and water regime are not significantly different by the Duncan test (P> 0.05).

3.2. Effect of inoculation on the physiological parameters of the plant

Values obtained in terms of stomatal conductance in Fontagro 8 showed no interactions between the factors (PGPR strains and water regimes) or differences between the treatments (Table 1). However, there was interaction between the PGPR strains and the water regime in Fontagro 98, which implies that the strains did not interact alone. Plants submitted to full irrigation also showed significant differences (Table 1). In this sense, the treatment AG-70 + AG-54 (*Bacillus* sp. with *Pseudomonas* sp.) recorded the highest increase of 244% in plants submitted to full irrigation, followed by AG-54 (141%) under the same water regime. Plants submitted to drought conditions showed no increase in stomatal conductance. The results for chlorophyll index (SPAD values) indicate that there were significant interactions between the factors in bothgenotypes (Table 2).The AG-70 + AG-54 treatment recorded the highest increases in both genotypes, with increases of 25% and 48% in Fontagro 8, and 22 and 39% in Fontagro 98 under irrigated and drought conditions, respectively.

Table 1. Effect of the inoculation with PGPR strains and its interaction with the water regimes on stomatal conductance (mmol $m^{-2}s^{-1)}$ in water-stress resistant and susceptible wheat genotypes (Fontagro 8 and Fontagro 98)

Genotype		**Water regime**		**Interaction Tr x Wr (p-value)**
	Treatments	**Irrigation**	**Drought**	
Fontagro 8	Control	316.00±64.00 a	90.71±11.80 a	0.5615
	AG-70	367.11±55.50 a	77.04±21.33 a	
	AG-54	327.46±33.82 a	96.46±20.00 a	
	AG-70+AG-54	403.88±30.24 a	92.36±20.57 a	
Fontagro 98	Control	99.99±12.33 c	126.65±20.73 a	0.0158
	AG-70	191.52±18.12 b	148.43±23.08 a	
	AG-54	240.63±24.14 b	100.44±28.59 a	
	AG-70+AG-54	344.03±38.63 a	197.59±61.20 a	

Average values of stomatal conductance (mmol $m^{-2}s^{-1}$) of four replicates. Standard error: ±;Tr: treatments; Wr: water regime. Control: non-inoculated; AG-70: *Bacillus* sp. strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Values followed by the same letter in the columns do not differ significantly ($P \leq 0.05$) by Duncan test. p-value: most relevant interactions of the study.

Table 2. Effect of inoculation with PGPR strains and its interaction with the water regimes on the SPAD chlorophyll index in water-stress resistant and susceptible wheat genotypes (Fontagro 8 and Fontagro 98).

Genotype		Water regime		Interaction Tr x Wr (p-value)
	Treatments	**Irrigation**	**Drought**	
Fontagro 8	Control	42.42±1.83 b	41.88±1.96 c	0.0267
	AG-70	51.79±0.86 a	52.35±2.17 b	
	AG-54	50.71±0.67 a	50.49±0.68 b	
	AG-70+AG-54	52.99±0.57 a	62.16±3.50 a	
Fontagro 98	Control	45.78±1.22 c	37.58±2.83 d	0.0272
	AG-70	49.06±0.91 bc	51.77±2.31 abc	
	AG-54	50.27±0.78 abc	52.01±2.55 abc	
	AG-70+AG-54	55.90±2.50 a	52.29±1.50 a	

Average values of SPAD (index) of four replicates. Standard error: ±; Tr: treatments; Wr: water regime. Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: Mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Values followed by the same letter in the columns do not differ significantly ($P \leq 0.05$) by Duncan test. p-value: most relevant interactions of the study.

3.3. Effect of inoculation on the chemical and biological properties of soil

No significant differences were observed in terms of the macronutrients (NPK) in the Fontagro 8 genotype submitted to full irrigation. However, plants of the same genotype but submitted to drought conditions showed significant differences in the three elements evaluated (Table 3).When compared to the control treatment, the largest increase in soil total N was observed with AG-54 (*Pseudomonas* sp.), followed by AG-70 (*Bacillus* sp.), reaching increases of 18% and 11%, respectively. Regarding the content of phosphorus Olsen (P), the highest increase was also obtained with the treatment AG-54 (4%), followed by AG-70 (3 %). Similarly, the largest increase of potassium (K) resulted from the inoculation with AG-54 (9%). In case of Fontagro 98, treatments submitted to full irrigation did not show significant differences in terms of NPK (Table 3). However, significant differences were found in those submitted to a water deficit regime; treatments AG-54 and AG-70 + AG-54 resulted in P increases of 5 and 2%, respectively. On the other hand, the values of soil microbial activity indicate no interaction between the Fontagro 8 genotype and water regimes (Table 4). However, the Fontagro 98 plants presented significant interaction between those two factors (Table 4). The AG-70 treatment recorded the highest increase of 76% in soil microbial activity, followed by the AG-54 treatment with an increase of 53% on the plants submitted to drought conditions. The plants submitted to full irrigation did not show significant differences compared to the control. In addition, the soil microbial respiration

showed significant interactions between the treatments under irrigated conditions, indicating a joint action of the two factors (Table 5). In Fontagro 8, the AG-70 treatment resulted in the highest increase in soil respiration (73%) under drought conditions, but the same treatment resulted in no increase under full irrigation compared to the control. In the same genotype, the treatment AG-70 + AG-54 recorded the second largest increases of 45 and 43% under drought and irrigated conditions, respectively. When comparing the results obtained in Fontagro 98 (susceptible to water stress) to those in the control treatment, the largest increase was recorded with the treatment AG-70 + AG-54 (202%) submitted to full irrigation. However, the greatest increase under drought conditions was observed with the AG-70 (55%) treatment. On the other hand, the results in terms of soil urease activity indicate no interactions between the factors under study (Table 6). Nevertheless, the treatments showed highly significant differences. In Fontagro 8, the largest increases in soil urease activity were recorded in the AG-54 treatment, followed by AG-70. Increases of 195 and 70% were obtained under full irrigation, and 53 and 19% under drought conditions for both treatments, respectively. In the Fontagro 98, the same treatments also maintained the best records with increases of 100 and 178% (full irrigation) and 74 and 177% (drought), respectively.

Table 3. Macronutrients (NPK) of the soil inoculated with two strains of PGPR and a mixture of both in two wheat genotypes submitted to two water regimes

Genotype	Water regime	Variables	Treatments			
		(mg kg $^{-1}$)	**Control**	**AG-70**	**AG-54**	**AG-54+AG-70**
Fontagro 8	(+)	N	532.53±41.33 a	489.98±24.25 a	554.10±65.01 a	502.63±32.25 a
		P	44.65±0.73 a	43.93±0.64 a	43.48±0.42 a	45.10±0.45 a
		K	190.95±6.06 a	207.18±6.39 a	205.93±7.14 a	192.70±2.92 a
	(-)	N	479.98±15.87 b	534.28±17.08 ab	567.30±20.34 a	481.55±33.63 b
		P	45.43±0.25 bc	46.65±0.20 ab	47.18±0.65 a	44.80±0.74 c
		K	212.15±4.02 ab	205.20±2.60 b	222.88±2.23 a	202.45±6.69 b
Fontagro 98	(+)	N	294.78±19.75 a	306.03±13.73 a	277.43±24.16 a	278.95±51.22 a
		P	41.18±0.34 ab	40.78±0.61 a	40.20±0.12 a	40.63±0.60 a
		K	125.55±7.44 a	124.80±5.79 a	105.33±6.59 a	110.58±11.44 a
	(-)	N	319.05±11.90 a	343.13±11.01 a	361.38±40.07 a	317.93±29.58 a
		P	41.13±0.57 ab	40.48±1.06 b	43.03±0.70 a	41.95±0.57 ab
		K	113.58±15.34 a	123.55±5.47 a	114.83±13.30 a	143.30±7.29 a

Average values of NPK (mg kg $^{-1}$) of four replicates. Standard error: ±; (+): plants submitted to irrigation; (-): Plants submitted to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Values followed by the same letter in the row do not differ significantly ($P \leq 0.05$) by Duncan test.

Table 4. Values for microbial activity (FDA) in the soil inoculated with two strains of PGPR and the mixture of both in two wheat genotypes submitted to two water regimes.

Genotype		Water regime		Interaction Tr x Wr (p-value)
	Treatments	**Irrigation**	**Drought**	
	Control	22.46±1.16 ab	25.70±1.08 a	
Fontagro 8	AG-70	27.71±1.16 a	32.11±2.21 a	0.2967
	AG-54	20.11±1.68 ab	44.62±6.04 a	
	AG-70+AG-54	16.79±0.23 b	24.50±4.52 a	
	Control	33.41±3.92 a	26.00±6.38 b	
Fontagro 98	AG-70	33.07±0.70 a	45.66±0.74 a	<0.0001
	AG-54	32.61±2.82 a	39.83±1.06 a	
	AG-70+AG-54	43.52±6.08 a	13.93±0.37 c	

Average values of soil microbial activity (µg F dry soil^{-1}) of four replicates. Standard error: ±; Tr: treatments; Wr: water regime. Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Values followed by the same letter in the columns do not differ significantly ($P \leq 0.05$) by Duncan test. p-value: most relevant interactions of the study.

Table 5. Values for microbial respiration (µg C-CO_2 g^{-1}) in the soil inoculated with two strains of PGPR and the mixture of both in two wheat genotypes submitted to two water regimes.

Genotype		Water regime		Interaction Tr x Wr (p-value)
	Treatments	**Irrigation**	**Drought**	
	Control	3.39±0.42 b	1.98±0.21 c	
Fontagro 8	AG-70	3.06±0.46 b	3.42±0.31 a	0.0149
	AG-54	3.01±0.35 b	2.20±0.17 bc	
	AG-70+AG-54	4.91±0.56 a	2.83±0.13 ab	
	Control	1.10±0.05 b	1.51±0.05 b	
Fontagro 98	AG-70	2.72±0.34 a	2.34±0.25 a	0.0872
	AG-54	2.83±0.69 a	2.11±0.22 ab	
	AG-70+AG-54	3.32±0.17 a	2.04±0.27 ab	

Average values of soil microbial respiration (µg C-CO_2 g^{-1}) of four replicates. Standard error: ±; Tr: treatments; Wr: water regime. Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Values followed by the same letter in the columns do not differ significantly ($P \leq 0.05$) by Duncan test. p-value: most relevant interactions of the study.

Table 6. Values for soil urease activity (µg N-NH_3 $g^{-1}h^{-1}$) in the soil inoculated with two strains of PGPR and by the mixture of both in two wheat genotypes submitted to two water regimes

Genotype		Water regime		Interaction Tr x Wr (p-value)
	Treatments	**Irrigation**	**Drought**	
Fontagro 8	Control	44.64±13.54 b	56.91±8.49 a	0.2097
	AG-70	75.93±22.83 ab	67.44±7.39 a	
	AG-54	131.87±26.6 a	86.98±34.18 a	
	AG-70+AG-54	38.09±3.58 b	74.71±14.53 a	
Fontagro 98	Control	37.73±3.80 b	18.06±1.42 b	0.6527
	AG-70	65.73±3.05 a	50.03±4.44 a	
	AG-54	75.30±5.52 a	49.82±1.90 a	
	AG-70+AG-54	42.78±6.85 b	18.84±3.91 b	

Average values of soil urease activity (µg N-$NH_3$$g^{-1}h^{-1}$) of four replicates. Standard error: ±; Tr: treatments; Wr: water regime. Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *Pseudomonas* sp. strain; AG-70 + AG-54: mixture of both strains. Fontagro 8: resistant to water stress; Fontagro 98: susceptible to water stress. Values followed by the same letter in the columns do not differ significantly ($P \leq 0.05$) by Duncan test. p-value: most relevant interactions of the study.

4. Discussion

The present study evaluated the effect of PGPR (*Bacillus* sp. and *Pseudomonas* sp.) on water stress tolerance in two wheat genotypes under two irrigation regimes.

The highest dry matter content was obtained with the inoculation of a mixture of both strains (AG-70 + AG-54) (Figure 1), which agrees with the results obtained in previous studies that have described that soil beneficial bacteria would promote plant growth under abiotic stress. In this sense, Durán *et al.* (2016) found similar results in lettuce inoculated with PGPR strains (*Bacillus* sp. and *Klebsiella* sp.). They obtained higher shoot dry weight compared to non-inoculated plants.

According to Glick *et al.* (2007), the increase in shoot dry weight may be influenced by the ability of these bacteria to lower ethylene levels by the activity of the enzyme ACC deaminase, leaving NH_4^+ and α-ketobutyrate available for the plant. Furthermore, Sánchez López (2011) described that auxins, such as IAA, generate positive effects on the development of secondary roots, which may not necessarily be related to increased root length, but to the total biomass of the plant.

In terms of root dry weight, the highest increases were also obtained by the mixture of the two strains (AG-70 + AG-54) in both genotypes and under the two water regimes (Figure 2).

Similar results were also found by Naiman *et al.* (2009) when inoculating strains of *Azospirillum* and *Pseudomonas* in wheat; and by Banerjee *et al.* (2017),

who conducted a study in rice and indicated that the increase in shoot and root biomass resulting from the inoculation with these microorganisms has beneficial effects on crop and soil quality.

Therefore, Glick (2014), affirms that ACC deaminase degrades ethylene, while IAA has the ability to promote cell division and, consequently, increase root expansion and elongation, thus facilitating nutrient and water uptake. This is directly related to the tolerance to water stress, and therefore it has a direct impact on yield.

These results are in agreement with those reported by Mishra *et al.* (2012). These authors described that an increase in the concentration of auxins promotes the formation of adventitious roots, which favors an increase in the root surface area and root mass.

This agrees with our results as the bacterial strains under study promoted positive effects on the root system by increasing the production capacity of auxin, and thus promoting an increase in root length (Figure 3); this indicate that the inoculation with these strains can be a morphological strategy to increase tolerance to water stress. Glick (2014) explained that this occurred because low levels of IAA stimulate root elongation, whereas elevated levels of this hormone stimulate lateral and adventitious root, have longer shoots and root, in addition, are more resistant to growth-inhibition by a variety of ethylene-inducing stresses. Furthermore, Chen *et al.* (2014) have also described that the inoculation with plant growth promoting bacteria in crops generally results in larger root length compared to non-inoculated plants.

This is closely related to a study conducted by Galland *et al.* (2012), who inoculated strains of PGPR (*Phyllobacterium brassicacearum*) in *Arabidopsis thaliana* seedlings, and indicated that PGPR promote positive effects on the root system by reducing ethylene levels and, consequently, increasing root hairs. In addition, a recent study conducted by Chen *et al.* (2017) reported significant increases in root length by the inoculation with PGPR strains (*Pantoea alhagi* sp. nov.) in wheat plants under two irrigation regimes.

Regarding stomatal conductance (Table 1), our study found no effects of the microorganisms on Fontagro 8. The combined action of the native microbial activity of the soil and the PGPRs inoculated could have synthesized phytohormones by excess, resulting in the inhibition of the synthesis of metabolites, which exert a key function in the physiological parameters that contribute with mechanisms that influence the tolerance to abiotic stress. On the other hand, Fontagro 98 plants submitted to irrigation presented an increase in the stomatal conductance index. This increase can produce an improved rate of photosynthesis and, consequently, increase leaf water potential, which plays an important role in the tolerance of plants to water stress (Durán *et al.*, 2016).

Chlorophyll content has also been considered as a suitable parameter for the physiological evaluation of stress intensity. However, the genotypes under study showed significant increases in SPAD chlorophyll index. The highest values were recorded with the inoculation with the mixture of the two strains (AG-70 + AG-54), followed by AG-70 and AG-54 treatments in both genotypes (Table 2), under drought conditions.

This is directly related to the nitrogen content in the leaf since this macronutrient is essential for plant development. The reduction of chlorophyll content under abiotic stress conditions is mainly caused by chloroplast damages that are caused by active oxygen species, which contribute to important physiological changes during the plant growth cycle and, consequently, lead to reduced yield (Manivannan *et al.*, 2007). Similar results were found by Ahmadi *et al.* (2013), who obtained significant increases in two wheat genotypes inoculated with different strains of *Azospirillum* and *Pseudomonas* sp. These authors

indicated that the inoculation with different strains produces beneficial effects on physiological parameters, directly contributing to increases in SPAD chlorophyll index.

In our study, the increase in NPK content due to the use of the inoculants was one of the various strategies that enhanced water stress tolerance in plants. The inoculation with strains of *Bacillus* sp. and *Pseudomonas* sp applied separately increased levels of NPK in Fontagro 8, but only in plants submitted to drought (Table 3).

Therefore, the inoculation with a mixture of the two strains was not effective in the solubilization of the nutrients evaluated. This indicates that the inoculation with any of the two strains allowed for a more efficient use of water or the enzymatic reduction of the ethylene concentrations because of the production of ACC deaminase. Conversely, it seems that the combined action of native soil microorganisms and the inoculation with a mixture of both strains (AG-70 + AG-54) may have resulted in nutrient competition and, consequently, in less efficiency in nutrient solubilization.

Our results in terms of macronutrient increase are in agreement with those found by Durán *et al.* (2016). They reported that the inoculation with different strains of *Bacillus* sp. and *Klebsiella*, increased P and K in lettuce plants. In this sense, Romheld and Kirkby (2010) indicated that K is an important element in the relief of water stress, protecting chloroplasts from oxidative damage and affecting water absorption by roots.

There was also an increase in soil biological quality parameters. Soil microbial activity increased with the AG-70 and AG-54 treatments (Table 4). The synchronicity between the microorganisms inoculated and the plant species resulted in a higher synthesis of exudates by the plant and, consequently, in an increase in the active microbial activity of the soil. Zhang *et al.* (2011) indicated that the type of plant species not only plays an important role in the physicochemical properties of the soil, but also in the abundance and composition of the soil microbial community. In addition, Hou *et al.* (2015) found significant increases in soil microbial activity by the inoculation with strains other than PGPR (*Lysobacter, Pseudoxanthomonas, Planctomyces, Nocardioides*) in the cultivation of *Festuca arundinacea*, which agrees with the results found in the present study.

In terms of soil microbial respiration, we found a significant interaction between the activities of the inoculated strains on plants submitted to drought in the water-stress resistant genotype (Table 5). However, the results show that there were no interactions between the evaluated factors in case of Fontagro 98, indicating that moisture did not influence the increase in soil respiration, but increased through the activity of inoculated strains and root exudates that were probably synthesized in the soil rhizosphere.

Similar results on soil microbial respiration were described by Mengual *et al.* (2014) by the inoculation with strains of *Enterobacter* sp. genus under field conditions. On the other hand, Aparna *et al.* (2014) indicated that plant growth affects soil properties due to the effect of the rhizosphere on root growth and activity, which selectively encourages the proliferation and activity of specific microorganisms through root exudates.

Apart from soil biomass and microbial respiration, urease enzyme activity was also evaluated as one of the indicators of soil biological quality. The results indicate that the AG-70 and AG-54 treatments resulted in the highest increases in urease activity in both strains (Table 6). These results could explain the increased availability of N by the action of the inoculated strains used in this study.

According to Aparna *et al.* (2014), the soil urease enzyme is an indicator of the microbial activity of the

soil caused by the effect of the rhizosphere, as well as the inoculation of the microorganisms. Gianfreda (2015) determined that inoculation with PGPR in soil induces the development of enzymatic activity of the existing beneficial microbial population, which affects even more the basic productive capacity of the soil.

Therefore, the results found concerning morphological and physiological parameters, and those related to the chemical and biological properties of the soil are in agreement with previous studies on the use of PGPR inoculation as an alternative to enhance tolerance to different types of biotic and abiotic stress. However, further research is required to understand how PGPRs can positively affect parameters related to the biological quality of the soil, such as microbial activity, respiration and soil enzymatic activity, through mechanisms that increase tolerance to water stress under field conditions.

5. Conclusions

The inoculation with a mixture of *Bacillus* sp. and *Pseudomonas* sp. proved to be the most effective treatment to enhance tolerance to water in both genotypes, by increasing plant biomass and other morphological and physiological parameters. The separate inoculation with AG-70 (*Bacillus* sp.) and AG-54 (*Pseudomonas* sp.) strains had beneficial effects on the two wheat genotypes, improving the chemical and biological properties of the soil. Based on these data, the separate and combined application of these PGPRs can be a biotechnological tool to enhance crop growth in ecosystems that present water deficit problems by increasing tolerance to water stress for sustainable agriculture.

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