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RIZOBACTERIAS PROMOTORAS DE CRECIMIENTO VEGETAL COMO INDUCTORES DE TOLERANCIA AL ESTRÉS HÍDRICO EN TRIGO

(PLANT GROWTH PROMOTING RHIZOBACTERIA FOR IMPROVED WATER STRESS TOLERANCE IN WHEAT GENOTYPES)

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RESUMEN

El agua es el principal medio de transporte de metabolitos y nutrientes. Su carencia constituye una de las principales fuentes de estrés. En este estudio, se evaluó el efecto de la inoculación con cepas de rizobacterias, AG-70 (*Bacillus* sp.), G-54 (*Pseudomonas* sp.), y la combinación de ambas (AG-70+AG-54), sobre la inducción a la tolerancia del estrés hídrico en dos genotipos de trigo (*Triticum aestivum* L.), uno resistente (Fontagro 8) y otra susceptible al estrés hídrico (Fontagro 98). Las plantas fueron regadas a 45% de capacidad de campo y otras a 100%. El tratamiento AG-70+AG-54 proporcionó a Fontagro 8 mayor incremento de peso seco aéreo (88%) y peso seco radicular (211%) en relación al control bajo condiciones de secano, mientras que la Fontagro 98 obtuvo 73% y 129% de peso seco aéreo y radicular respectivamente. Además, las plantas inoculadas presentaron aumentos significativos de longitud y volumen radicular, conductancia estomática e índice de clorofila. Los tratamientos AG-54 y AG-70 incrementaron contenidos de NPK en el genotipo resistente, mientras que en el susceptible el P fue incrementado por los tratamientos AG-54 y AG-70+AG-54. Los tratamientos AG-54 y AG-70+AG-54 fueron los más efectivos en los parámetros de calidad biológica del suelo (actividad microbiana del suelo y respiración microbiana, actividad de la enzima ureasa). Por lo tanto, la inoculación separada y combinada de estas rizobacterias promovieron aumento de tolerancia al estrés hídrico en los dos genotipos de trigo, y pueden ser herramientas biotecnológicas para la producción de cultivos en ecosistemas con problemas de déficit hídrico.

Palabras clave: Inoculación, PGPR, tolerancia, estrés hídrico.

ABSTRACT

Water is a principal means of transport for metabolites and nutrients. Water deficit is one of the main sources of plant stress. 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% of field capacity. The treatments were inoculated 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 was also included. 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 and volume, 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.

Key words: Inoculation, PGPR, tolerance, water stress.

CAPÍTULO 1

INTRODUCCION GENERAL

El agua comprende el 80 a 90% de la biomasa de las plantas no leñosas y es la molécula central en todos los procesos fisiológicos, por ser el principal medio para el transporte de metabolitos y nutrientes. Su carencia constituye uno de los principales factores de estrés (Lisar *et al.,* 2012). La ONU pronostica que la demanda de agua aumentará un 40 % para el 2030, y con un clima cada vez más impredecible, las reservas de agua son cada vez más bajas, fruto del calentamiento global (Harrison, 2002). La sequía es una situación que reduce el potencial hídrico de la planta y la turgencia a medida que las plantas se enfrentan a dificultades de ejecución de sus funciones fisiológicas normales (Lisar *et al.,* 2012). De esta manera, el agua constituye el principal factor limitante del crecimiento y de la productividad de los cultivos, actuando como una fuerza selectiva de primer grado para la evolución y distribución de las especies vegetales (Ngumbi y Kloepper, 2016). Se plantea que cerca del 10 % de la superficie del planeta está afectada por estrés hídrico y muchas superficies arables son abandonadas constantemente por esta causa. La tolerancia al estrés hídrico es la capacidad que poseen las plantas en mantener un contenido relativamente más alto de agua en los tejidos, a pesar de su reducción en el suelo (Basu *et al.*, 2016). Esto se consigue mediante una serie de rasgos adaptativos, que implican minimizar la pérdida de agua y la optimizar su absorción, por el mantenimiento de la turgencia celular mediante al ajuste osmótico, elasticidad celular, aumento de la resistencia protoplasmática, través del aumento del sistema radicular (Basu *et al.,* 2016).

Por otro lado, el trigo (*Triticum aestivum* L.*)* es uno de los cereales de mayor producción y consumo global, junto al maíz y el arroz, sin embargo, su carencia constituye una grande demanda en la fuente de alimentación humana siendo la sequía es principal amenaza que limita el rendimiento del cultivo (Budak *et al.*, 2013). Por esta razón, la introducción de técnicas tendientes a proporcionar tolerancia al estrés hídrico, resulta crucial para estabilizar y aumentar la producción de alimentos derivados del trigo (Budak *et al.,* 2013). No obstante, las bacterias de vida libre encontradas en la rizósfera de las plantas, denominadas por Rizo-bacterias Promotoras del Crecimiento Vegetal (PGPR, por sus siglas en inglés, *Plant Growth Promoting Rhizobacteria*), son altamente eficientes en la promoción de crecimiento vegetal mediante los mecanismos directos e indirectos (Hassan *et al*., 2015). Los mecanismos directos están relacionados a la síntesis de fitohormonas tales como auxinas, giberelinas y citoquinina que facilitan la absorción de los nutrientes en las plantas y por la protección frente a diversas condiciones de estrés ambientales, contrarrestando así los impactos negativos causados por el estrés (Glick, 2012; García-Fraile *et al.*, 2015). Además, sintetizan enzimas como la ACC deaminasa (ácido 1-aminociclopropano-1-carboxílico), actividad ureasa, que modulan el desarrollo de las plantas, aumentando su vigor, crecimiento y mejoran la actividad de la clorofila, así como la biomasa total de los cultivos (Hayat *et al.*, 2010; Glick, 2012). Mientras que los mecanismos indirectos se destacan por la inhibición de microrganismos nocivos, patógenos de las raíces y por el control biológico de enfermedades de los cultivos por la producción de antibióticos y sideróforos (Ahemed y Kibret, 2014). Estos mecanismos aumentan, el vigor y crecimiento de los cultivos, la actividad de la clorofila, proteínas, enzimas antioxidantes y la biomasa total de las plantas (Hayat et al., 2010; Glick, 2012). Por lo tanto, las PGPR aumentan los rendimientos de los cultivos, la fertilidad de los suelos y poseen el potencial de contribuir en la agricultura sostenible, destacándose por la inducción de la tolerancia sistémica del estrés hídrico (Esquivel-Cote *et al.*, 2013; García-Fraile *et al.*, 2015). Dentro de los microorganismos del suelo que son benéficos para las plantas se destacan los pertenecientes a los géneros *Bacillus* sp. *y Pseudomonas* sp., debido a su especial importancia en la relación suelo-planta (Widnyana y Javandira, 2016). Sus efectos se destacan en la biosíntesis de fitohormonas importantes como es el caso del ácido indolacético (ácido indol-3-acético / IAA), siendo una auxina responsable en la promoción de la división celular y elongación radicular; los efectos de su actividad se incrementan durante todo ciclo de las plantas (Etesami *et al.*, 2014; Widnyana y Javandira, 2016). Por otro lado, el etileno es también una fitohormona de vital importancia, que desempeña funciones importantes en la germinación y en el desarrollo de las semillas (Jha *et al.*, 2012). Sin embargo, el etileno producido endógenamente por casi todas las plantas, aparte de ser un regulador de crecimiento, cuando es sintetizado en exceso provoca distintos efectos anormales en el desarrollo de las plantas, tal como el estrés hídrico, debido a su efecto inhibidor en la expansión y elongación radicular (Jha *et al.*, 2012). Estudios previos señalan que las PGPR de los géneros anteriormente mencionadas, poseen la capacidad de producción de la enzima ACC deaminasa (ácido 1-aminociclopropano-1-carboxílico), que es útil en la reducción de los niveles de etileno mediante la conversión de ACC en NH_4 y α-cetobutirato en plantas, proporcionando de esta forma, crecimiento e inducción a la tolerancia del estrés hídrico (Esquivel-Cote *et al.*, 2013; Ahmed y Kibret 2014). Así siendo, son microorganismos fundamentales para incrementar no solo el potencial exploratorio de las raíces y mejorar la absorción de nutrientes y agua, sino que también para alterar parámetros fisiológicos que confieren a la planta un aumento en la capacidad de resistencia a adversos factores ambientales y como beneficio incidir en el rendimiento y en la calidad de los cultivos (Lim y Kim, 2013). Así mismo, la correcta inoculación con PGPR que inducen a la tolerancia del estrés por sequía a los cultivos, pueden contribuir a superar los límites de la productividad en regiones con problemas de déficit hídrico (Lim y Kim, 2013).

HIPÓTESIS

El estrés hídrico, es uno de los principales factores limitantes del crecimiento y de la producción vegetal. De esta forma, la inoculación con PGPR a plantas sometidas a déficit hídrico, aumenta la tolerancia al estrés hídrico en genotipos de trigo.

OBJETIVO GENERAL

Estudiar los efectos que inducen en la tolerancia del estrés hídrico producido por dos rizobacterias promotoras de crecimiento vegetal en la rizósfera de un suelo volcánico (Andisol) utilizando dos genotipos de trigo (tolerante y susceptible al estrés hídrico).

OBJETIVOS ESPECÍFICOS

- Analizar los efectos de los metabolitos (ACC desaminasa y ácido indolacético), sintetizados por las PGPR, ante los parámetros morfo-fisiológicos y rendimiento por biomasa del cultivo en estudio.
- Evaluar los indicadores de calidad biológicas del suelo (biomasa microbiana, respiración microbiana, y actividad de la enzima ureasa), como inductores de resistencia al estrés hídrico.

LITERATURA CITADA

Ahemad, M., Kibret, M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Uni. Science. 26, 1-20.

Basu, S., Ramegowda, V., Kumar, A., & Pereira, A. 2016. Plant adaptation to drought stress. F1000Research, 5.

Budak, H., Kantar, M., Kurtoglu, K.Y. 2013. Drought Tolerance in Modern and Wild Wheat. Scientific World J. 2013 548246 10.1155/2013/548246.

Esquivel-Cote, R., Gavilanes-Ruiz, M., Cruz-Ortega, R., Huante, P. 2013. Importancia agrobiotecnológica de la enzima ACC desaminasa en rizobacterias, una revisión. Rev Fitotec Mex. 36, 251-258.

Etesami, H., Hosseini, M., Alikhani, H.A. 2014. In planta selection of plant growth promoting endophytic bacteria for rice (Oryza sative L.). J.Soil Sci. Plant Nut. 14, 491-503.

García-Fraile, P., Menéndez, E., Rivas, R. 2015. Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioengineering. 3, 183-205.

Glick, B.R. 2012. Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation, Scientifica. 2012. 10.6064/2012/963401.

Harrison, Paul. 2002. Agricultura mundial: hacia los años 2015/2030." Dirección de Información de la FAO.

Hassan, W., Hussain, M., Bashir, S., Shah, A.N., Bano, R., David, J. 2015. ACC-deaminase and/or nitrogen fixing rhizobacteria and growth of wheat (*Triticum Aestivum* L.). J. Soil Sci. Plant Nutr. 15, 232-248.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion. Ann Microbiol. 60, 579-598.

Jha, C.K., Annapurna, K., Saraf, M., 2012. Isolation of Rhizobacteria from Jatropha curcas and characterization of produced ACC deaminase. J. Basic Microbiol. 52, 85–95.

Lim, J.H., Kim, S.D. 2013. Induction of drought stress resistance by multi-functional PGPR Bacillus licheniformis K11 in Pepper. Plant Pathol J. 29, 201.

Lisar, S.Y., Motafakkerazad, R., Hossain, M.M., Rahman, I.M. 2012. Water stress in plants: causes, effects and responses. In Water stress. InTech, Croatia, pp: 1-14.

Ngumbi, E., Kloepper, J. 2016. Bacterial-mediated drought tolerance: Current and future prospects. Appl. Soil Ecol. 105, 109-125.

Widnyana, I.K., Javandira, C. 2016. Activities Pseudomonas spp. and Bacillus sp. to Stimulate Germination and Seedling Growth of Tomato Plants. Agriculture and Agricultural Science Procedia. 9, 419-423.

CAPÍTULO 2

PLANT GROWTH PROMOTING RHIZOBACTERIA FOR IMPROVED WATER STRESS TOLERANCE IN WHEAT GENOTYPES

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ABSTRACT

Water is a principal means of transport for metabolites and nutrients. Water deficit is one of the main sources of plant stress. We evaluated the effect of inoculation with the rhizobacterial strains, AG-70 (*Bacillus* sp.), AG-54 (*Pseudomonas* sp.), and the combination of both (AG-70 + AG-54), on the induction of water stress in two wheat genotype (*Triticum aestivum* L.), one water stress resistant (Fontagro 8) and another susceptible (Fontagro 98). Plants were irrigated at conditions 100% field capacity and at 45%. The AG 70 + AG-54 treatment provided a greater increase of shoot dry weight (88%) and root dry weight (211%) in relation to the control under drought conditions, on to the Fontagro 8 genotype, while the susceptible one increase the shoot and root dry weight 73% and 129% respectively. In addition, the inoculated plants showed significant increases in root length and volume, stomatal conductance and chlorophyll index. The AG-54 and AG-70 treatments increased NPK contents on the resistant genotype, while in the susceptible genotype the P content was increased by AG-54 and AG-70 + AG-54 treatments. The AG-54 and AG-70 + AG-54 treatments were the most effective in soil biological quality parameters (microbial biomass C and microbial respiration, urease enzyme activity). Therefore, the separate and combined inoculation of these strain promoted an increased tolerance to water stress in two wheat genotypes.

Key words: Inoculation, PGPR, tolerance, water stress.

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 (Lyse *et al.,* 2012). Drought reduces the water potential and turgor of plants, so then increasing difficulty to carry out their normal physiological functions (Lyse *et al.*, 2012). In fact, water is the main limiting factor for the growth and productivity of crops and play 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. 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 vigor, growth and chlorophyll activity, as well as the total biomass of crops (Hayat *et al.*, 2010; 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 (Ahemed and Kibret, 2014). Therefore, PGPR increase crop yields, soil fertility and have the potential to contribute to sustainable agriculture because they increase the systemic tolerance to stress (Esquivel-Cote *et al.*, 2013; 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 genres 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 (Ahmebd and Kibret, 2014; 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, water uptake, 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 increased stress tolerance may be a good tool to enhance production in water-deficit regions (Lim and Kim, 2013). However, further research is required to study the rhizospheric mechanisms that increase tolerance to water stress. 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).

MATERIALS AND METHODS

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 (Rovira, 1984; 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}.

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), in two environments, Cauquenes (35°58′ S, 72°17′ W; 177 masl), which was subjected to water stress (WS) as typically rainfed at this site; and in the Santa Rosa Experimental Station (36°32′ S, 71°55′ W; 220 masl), which was subjected 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.

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 masl), between October (2016) and January (2017). Harvest was carried out in stage 9 (physiological maturation), according to the Zadoks scale (1974). Plastic pots (20 Ø cm / 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 subjected 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.

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 Faculty of Agronomy of the University of Concepción. They were selected based on their production capacities of IAA and ACC deaminase. 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) and modified by Slack and Wheldon (1978) 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 twice in order to guarantee the survival and activity of the microorganisms during the study.

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.

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 and volume 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.

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, Faculty 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) 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; Green *et al.*, 2006). 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 was placed in a centrifuge tube and then placed in an incubation bottle. Bottles 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 was taken from the centrifuge tube and mixed with a volume of 2 mL of $BaCl_2$; 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 CO_2-C 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 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 2M 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 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 NH_3-N µg g^{-1} h^{-1} as described by Alef and Nannipieri (1995).

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).

RESULTS

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.). Shoot dry weight increase in range 91-88% in dry, with treatment (AG-70 + AG-54) under water stress and full irrigation respectively. While AG-54 increase this treatment in 67 and 58% under some water conditions 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 subjected to full irrigation and water stress, respectively. The second highest value 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. Regarding root volume, only Fontagro 98 showed significant differences between the treatments (Figure 4). The highest values were recorded under drought conditions in the AG-70+AG-54 treatment followed by the AG-54 treatment, resulting in increases of 172% and 70%, respectively.

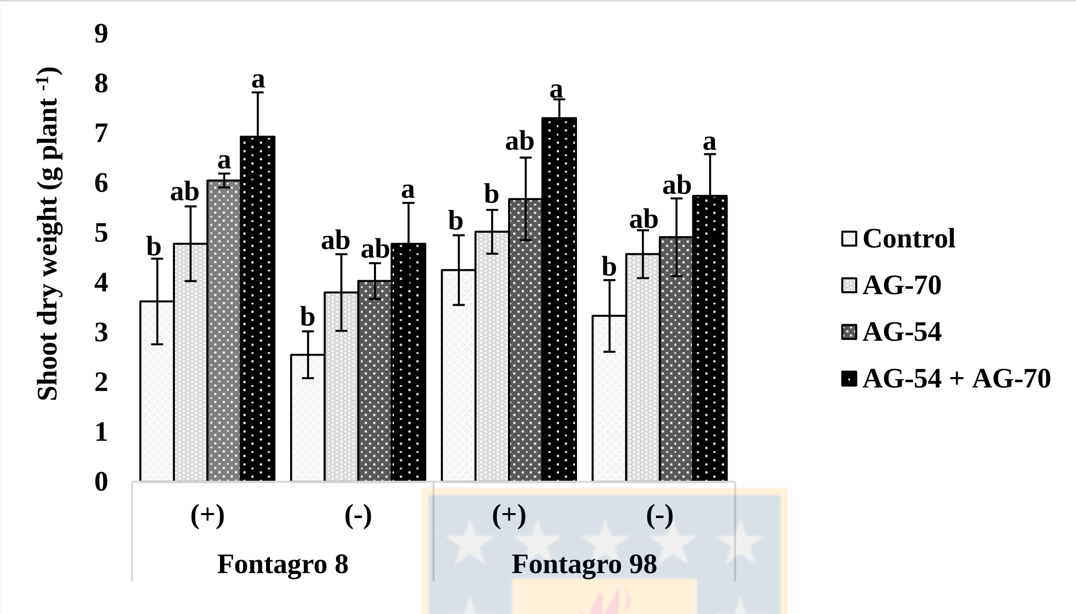

Figure 1. Shoot dry weight (g plant $^{-1}$) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. (+): Plants subjected to irrigation; (-): Plants subjected to drought conditions; Control: non-inoculated; AG-70: *Bacillus* 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 texture within the same genotype and water regime are not significantly different by the Duncan test (p> 0.05).

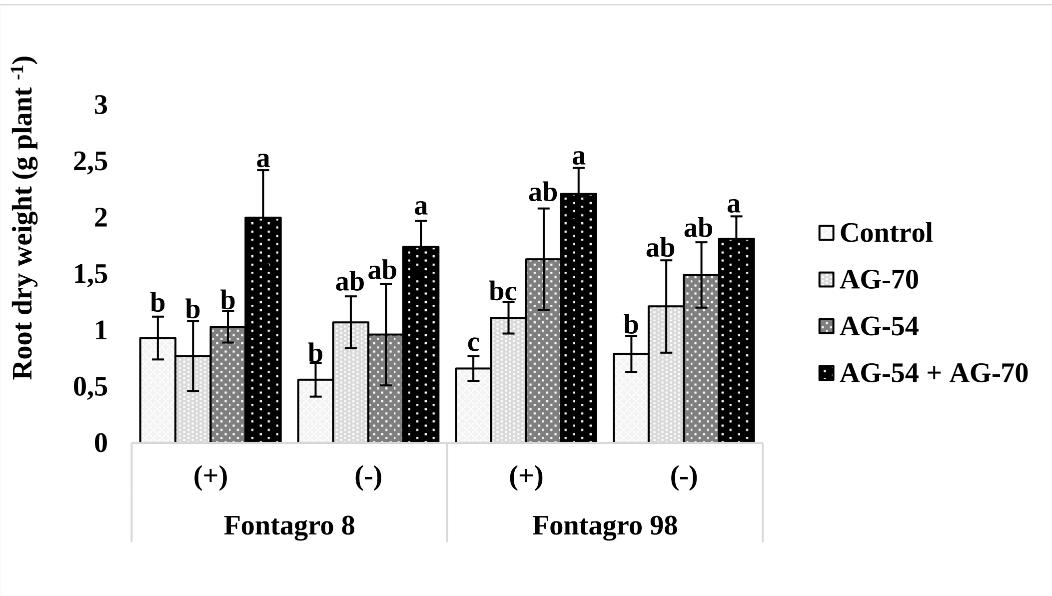

Figure 2. Root dry weight (g plant $^{-1}$) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. (+): Plants subjected to irrigation; (-): Plants subjected to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. 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 texture within the same genotype and water regime are not significantly different by the Duncan test (p> 0.05).

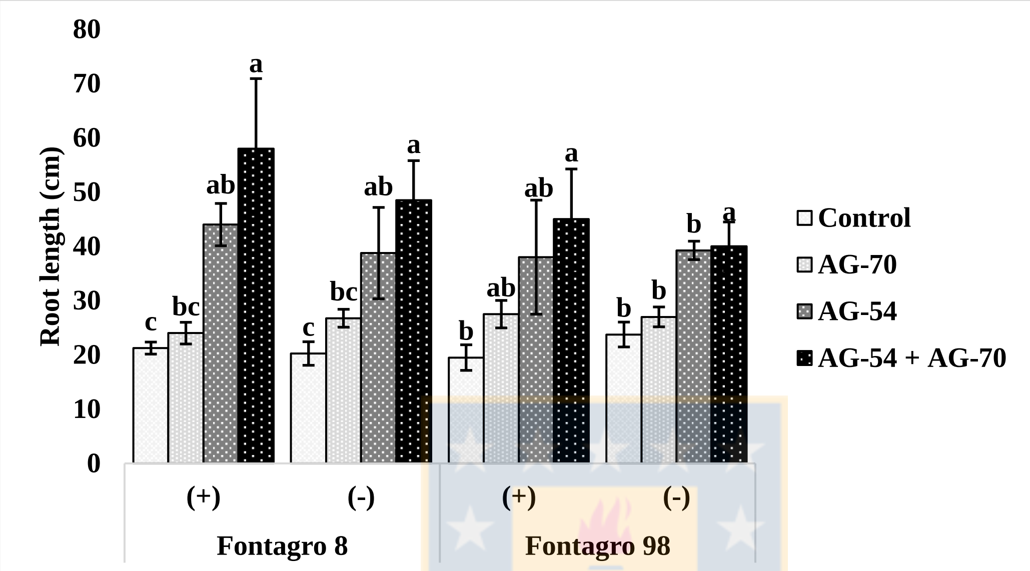

Figure 3. Root length (cm) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. (+): Plants subjected to irrigation; (-): Plants subjected to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. Strain; AG-54: *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 texture within the same genotype and water regime are not significantly different by the Duncan test (p> 0.05).

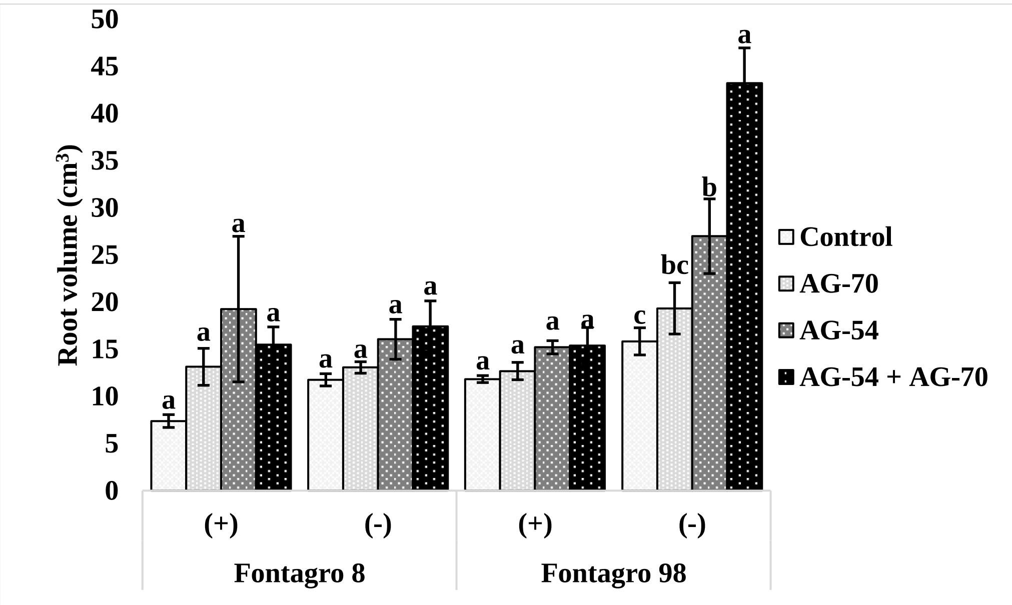

Figure 4. Root volume (cm^3) of two wheat genotypes in response to two water regimes before the inoculation with two bacterial strains and the mixture of both. (+): Plants subjected to irrigation; (-): Plants subjected to drought conditions; Control: non-inoculated; AG-70: *Bacillus* sp. strain; AG-54: *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 texture within the same genotype and water regime are not significantly different by the Duncan test ($p > 0.05$).

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 subjected 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 subjected to full irrigation, followed by AG-54 (*Pseudomonas* sp.) (141%) under the same water regime. Plants subjected 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 both genotypes (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	
	Control	316.00±64.00 a	90.71±11.80 a	
Fontagro 8	AG-70	367.11±55.50 a	77.04±21.33 a	0.5615
	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	
	Control	99.99±12.33 c	126.65±20.73 a	
Fontagro 98	AG-70	191.52±18.12 b	148.43±23.08 a	0.0158
	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	
	Control	42.42±1.83 b	41.88±1.96 c	
Fontagro 8	AG-70	51.79±0.86 a	52.35±2.17 b	0.0267
	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	
	Control	45.78±1.22 c	37.58±2.83 d	
Fontagro 98	AG-70	49.06±0.91 bc	51.77±2.31 abc	0.0272
	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.

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 subjected to full irrigation. However, plants of the same genotype but subjected 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 subjected to full irrigation did not show significant differences in terms of NPK (Table 3). However, significant differences were found in those subjected 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 indicated 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 subjected to drought conditions. The plants subjected to full irrigation did not show significant differences compared to the control. In addition, 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%) subjected 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 the mixture of both in two wheat genotypes subjected to two water regimes.

Genotype	Water regime	Variables (mg kg^{-1})	Treatments: 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 subjected to irrigation; (-): Plants subjected 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. Microbial activity (FDA) in the soil inoculated with two strains of PGPR and the mixture of both in two wheat genotypes subjected to two water regimes.

Genotype	Treatments	Water regime: Irrigation	Drought	Interaction Tr x Wr (p-value)
Fontagro 8	Control	22.46±1.16 ab	25.70±1.08 a	0.2967
	AG-70	27.71±1.16 a	32.11±2.21 a	
	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	
Fontagro 98	Control	33.41±3.92 a	26.00±6.38 b	<0.0001
	AG-70	33.07±0.70 a	45.66±0.74 a	
	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. Test. 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. Microbial respiration (µg CO_2-C g^{-1}) in the soil inoculated with two strains of PGPR and the mixture of both in two wheat genotypes subjected to two water regimes.

Genotype	Treatments	Water regime: Irrigation	Water regime: Drought	Interaction Tr x Wr (p-value)
Fontagro 8	Control	3.39±0.42 b	1.98±0.21 c	0.0149
	AG-70	3.06±0.46 b	3.42±0.31 a	
	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	
Fontagro 98	Control	1.10±0.05 b	1.51±0.05 b	0.0872
	AG-70	2.72±0.34 a	2.34±0.25 a	
	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 microbial respiration (µg CO_2-C 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: Combination 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. Soil urease activity (µg NH_3-N g^{-1} h^{-1}) in the soil inoculated with two strains of PGPR and by the mixture of both in two wheat genotypes subjected to two water regimes.

Genotype	Treatments	Water regime: Irrigation	Water regime: Drought	Interaction Tr x Wr (p-value)
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 NH_3-N 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.

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. I 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 N and α-ketobutyrate available for the plant. Furthermore, Sánchez Lopez (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, the 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 Dimkpa et al. (2009) and 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 and volume (Figure 3-4); this indicate that the inoculation with these strains can be a morphological strategy to increase tolerance to water stress. Patten and Glick (2002) explained that this occurred because low levels of IAA stimulate root elongation, whereas elevated levels of this hormone stimulate lateral and adventitious root growth. 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 subjected 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 (Tanaka *et al.*, 2013; 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 content. 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 subjected 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 subjected 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. Turan et al. (2011) also reported increases in the urease activity and other chemical soil parameters with inoculations with different strains. 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.

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.

REFERENCES

Ahmadi, J., Asgharzadeh, A., Bakhtiari, S. 2013. The effect of microbial inoculants on physiological responses of two wheat cultivars under salt stress. IJABBR. 4, 421-431.

Ahemad, M., Kibret, M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Uni. Science. 26, 1-20.

Alef, K., Nannipieri, P. (Eds.). 1995. Methods in applied soil microbiology and biochemistry (Vol. 576). Academic press, London, 576 p.

Aparna, K., Rao, D.L.N., Manna, M.C. 2014. Microbial inoculation of chickpea (*Cicer arietinum* L.) enhances rhizosphere effects on soil biological quality. Agrochimica. 58, 114-125.

Banerjee, A., Bareh, D.A., Joshi, S.R. 2017. Native microorganisms as potent bioinoculants for plant growth promotion in shifting agriculture (Jhum) systems. J. Soil Sci. Plant Nutr. 17, 127-140.

Budak, H., Kantar, M., Kurtoglu, K.Y. 2013. Drought Tolerance in Modern and Wild Wheat. Scientific World J. 2013 548246 10.1155/2013/548246.

Chen, L., Xu, M., Zheng, Y., Men, Y., Sheng, J., Shen, L. 2014. Growth promotion and induction of antioxidant system of tomato seedlings (*Solanum lycopersicum* L.) by endophyte TPs-04 under low night temperature. Sci Hort. 176, 143-150.

Chen, C., Xin, K., Liu, H., Cheng, J., Shen, X., Wang, Y., Zhang, L. 2017. Pantoea alhagi, a novel endophytic bacterium with ability to improve growth and drought tolerance in wheat. Sci. Rep. 7, 41564.

Di Rienzo J. A., Casanoves F., Balzarini M. G., Gonzalez L., Tablada M., Robledo C. W. InfoStat versión 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

Dimkpa, C., Weinand, T., Asch, F. 2009. Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant, cell & environment. 32, 1682-1694.

Durán, P., Acuña, J.J., Armada, E., López-Castillo, O. M., Cornejo, P., Mora, M. L., Azcón, R. 2016. Inoculation with selenobacteria and arbuscular mycorrhizal fungi to enhance selenium content in lettuce plants and improve tolerance against drought stress. J. Soil Sci. Plant Nutr. 16, 211-225.

Esquivel-Cote, R., Gavilanes-Ruiz, M., Cruz-Ortega, R., Huante, P. 2013. Importancia agrobiotecnológica de la enzima ACC desaminasa en rizobacterias, una revisión. Rev Fitotec Mex. 36, 251-258.

Galland, M., Gamet, L., Varoquaux, F., Touraine, B., Touraine, B., Desbrosses, G. 2012. The ethylene pathway contributes to root hair elongation induced by the beneficial bacteria Phyllobacterium brassicacearum STM196. Plant Sci. 190, 74-81.

García-Fraile, P., Menéndez, E., Rivas, R. 2015. Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioengineering. 3, 183-205.

Gianfreda, L. 2015. Enzymes of importance to rhizosphere processes. J. Soil Sci. Plant Nutr. 15, 283-306.

Glick, B.R., Cheng Z., Czarny J., Duan, J. 2007. Promotion of plant growth by ACC deaminase containing soil bacteria. Eur J Plant Pathol. 119, 329–39.

Glick, B.R. 2012. Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation, Scientifica. 2012. 10.6064/2012/963401.

Green, V.S., Stott, D.E., Diack, M. 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biol Biochem. 38, 693-701.

Hassan, W., Hussain, M., Bashir, S., Shah, A.N., Bano, R., David, J. 2015. ACC-deaminase and/or nitrogen fixing rhizobacteria and growth of wheat (*Triticum Aestivum* L.). J. Soil Sci. Plant Nutr. 15, 232-248.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion. Ann Microbiol. 60, 579-598.

Hou, J., Liu, W., Wang, B., Wang, Q., Luo, Y., Franks, A.E. 2015. PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. Chemosphere. 138, 592-598.

Jha, C.K., Annapurna, K., Saraf, M., 2012. Isolation of Rhizobacteria from Jatropha curcas and characterization of produced ACC deaminase. J. Basic Microbiol. 52, 85–95.

Lim, J.H., Kim, S.D. 2013. Induction of drought stress resistance by multi-functional PGPR Bacillus licheniformis K11 in Pepper. Plant Pathol J. 29, 201.

Lisar, S.Y., Motafakkerazad, R., Hossain, M.M., Rahman, I.M. 2012. Water stress in plants: causes, effects and responses. In Water stress. InTech, Croatia, pp: 1-14.

Manivannan. P., Abdul Jaleel. C., Sankar. B., Kishorekumar. A., Somasundaram. R., Lakshmanan. G.A., Panneerselvam, R. 2007. Growth, biochemical modifications and proline metabolism in Helianthus annuus L. as induced by drought stress. Colloids Surf. B. 59, 141–149.

Mengual, C., Schoebitz, M., Azcón, R., Roldán, A. 2014. Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. J. Environ. Manage. 134, 1-7.

Miles, A.A., Misra, S.S., Irwin, J.O. 1938. The estimation of the bactericidal power of the blood. J Hyg (Lond). 38, 732–749.

Mishra, P.K., Bisht, S.C., Bisht, J.K., Bhatt, J.C. 2012. Cold-tolerant PGPRs as bioinoculants for stress management. In Bacteria in Agrobiology: Stress Management. Springer Berlin Heidelberg, pp: 95-118.

Naiman, A.D., Latrónico, A., de Salamone, I.E.G. 2009. Inoculation of wheat with Azospirillum brasilense and Pseudomonas fluorescens: impact on the production and culturable rhizosphere microflora. Eur. J. Cell Biol. 45, 44-51.

Ngumbi, E., Kloepper, J. 2016. Bacterial-mediated drought tolerance: Current and future prospects. Appl. Soil Ecol. 105, 109-125.

Patten, C., Glick, B. 2002. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68, 3795-3801.

Romheld, V., Kirkby, E.A. 2010. Research on potassium in agriculture: needs and prospects. Plant Soil. 335, 155-180.

Rovira, A. 1984. Geografía de los suelos. Tomo V Geografía de Chile, Instituto Geográfico Militar, Santiago, 180 p.

Sadzawka, A., Carrasco, M.A., Grez, R., Mora, M.D.L.L., Flores, H., Neaman, A. 2006. Métodos de análisis recomendados para los suelos de Chile. Serie Actas N° 34. INIA La Platina, Santiago, Chile, 164 p.

Sánchez López, D. B. 2011. Efecto de la inoculación con bacterias promotoras de crecimiento vegetal sobre el cultivo de tomate *(Solanum Lycopersicum var. Sofía*) bajo invernadero. Tesis de magister, Pontificia Universidad Javeriana, Bogotá,

Sepulveda, M.R. 2013. Aislamiento, selección y evaluación de bacterias promotoras de crecimiento (PGPR) en lechuga (*Lactuca sativa* L.) var. Capitata. Tesis pregrado. Universidad de Concepcion Bío Bío, Chillán, 6 p.

Slack, M.D.B., Wheldon B. 1978. A Simple and Safe Volumetric Alternative to the Method of Miles, Misra and Irwin for Counting Viable Bacteria. J. Med. Microbiol. 4, 541-545.

Stolpe, N.B. 2006. Descripciones de los principales suelos de la VIII región de Chile. Chillán, Udec, pp: 15-17.

Tanaka, Y., Sugano, S.S., Shimada, T., Hara-Nishimura, I. 2013. Enhancement of leaf photosynthetic capacity through increased stomatal density in Arabidopsis. New Phytol. 198, 757-764

Turan, M., Gulluce, M., Karadayi, M., Baris, O., Sahin, F. 2011. Role of soil enzymes produced by PGPR strains in wheat growth and nutrient uptake parameters in the field conditions. Curr. Opin. Biotechnol. 22, 133.

Widnyana, I.K., Javandira, C. 2016. Activities Pseudomonas spp. and Bacillus sp. to Stimulate Germination and Seedling Growth of Tomato Plants. Agriculture and Agricultural Science Procedia. 9, 419-423.

Zadoks, J.C., T.T. Chang, C.F. Konzak. 1974. A Decimal Code for the Growth Stages of Cereals. Weed Res. 14, 415-421.

Zhang C, Liu G, Xue S, Song Z. 2011. Rhizosphere soil microbial activity under different vegetation types on the Loess Plateau, China. Geoderma. 161, 115–125.

CAPÍTULO 3

CONCLUSIONES GENERALES

- Las rizobacterias inoculadas promovieron tolerancia del estrés hídrico en los dos genotipos estudiados, bajo plantas sometidas al estrés, mediante la síntesis de ACC desaminasa y del ácido indolacético.

- La inoculación combinada entre las dos cepas resultó ser la más eficiente en la inducción de tolerancia al estrés hídrico en los dos genotipos, mediante el incremento de la biomasa vegetal y de otros parámetros morfo-fisiológicos analizados.

- La inoculación separada entre las cepas AG-70 y AG-54 (*Bacillus* sp. y *Pseudomonas* sp.), ha proporcionado efectos benéficos en los dos genotipos de trigo, mediante su efectividad en las propiedades químicas y biológicas del suelo.

- Basándose en estos datos, la aplicación separada y combinada de estas PGPR, puede ser una herramienta biotecnológica para la promoción de crecimiento de cultivos en ecosistemas que presentan problemas de déficit hídrico, mediante la inducción de tolerancia al estrés hídrico en una agricultura sostenible.