Universidad de Concepción
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INTERACCION DE LAS BACTERIAS PROMOTORAS DE CRECIMIENTO Y MICORRIZAS ARBUSCULARES ASOCIADAS AL PATRÓN MAXMA 60: EFECTO EN ENRAIZAMIENTO Y PROTECCIÓN FRENTE A ESTRÉS HIDRICO.

Tesis para optar al grado de Magíster en Ciencias Agronómicas

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CHILLÁN - CHILE
2024

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RESUMEN

El cambio climático es una importante amenaza para la agricultura, especialmente por los cambios en los patrones de precipitaciones y temperatura que intensifican el estrés hídrico en los cultivos. En este trabajo se estudiaron los efectos de la coinoculación con hongos micorrícicos arbusculares (HMA) y rizobacterias promotoras del crecimiento vegetal (PGPR) sobre la sobrevivencia de plantas, el crecimiento y la tolerancia al estrés hídrico del portainjerto micropropagado de cerezo Maxma 60 (*Prunus mahaleb* × *Prunus avium*). Los portainjertos se inocularon con *Pseudomonas koreensis* cepa AG-97, *Pseudomonas putida* cepa AG-30, *Claroideoglomus claroideum, Pseudomonas koreensis* cepa AG-97 + *Claroideoglomus claroideum y Funneliformis mosseae* durante la aclimatación, y después se sometieron a estrés hídrico. Los resultados mostraron que *Ps. koreensis* aumentó significativamente la supervivencia de las plantas y el peso seco de las hojas durante la aclimatación. Además, la inoculación con el hongo *F. mosseae* condujo a un mayor crecimiento de brotes y raíces en condiciones de buen riego, pero esto no se tradujo en una mejora del rendimiento en condiciones de sequía. Bajo déficit hídrico, los portainjertos inoculados con *Ps. koreensis* mantuvieron un mayor potencial hídrico del tallo y demostraron una mayor tolerancia al estrés hídrico en comparación con otros tratamientos. Además, la coinoculación de *Ps. koreensis* con *C. claroideum* dio lugar a una mayor conductancia estomática en condiciones de sequía, lo que indica un efecto sinérgico que podría beneficiar la resistencia de la planta durante la etapa de trasplante. Nuestros resultados sugieren que la inoculación microbiana, particularmente con la cepa AG-97 de *Ps. koreensis*, puede mejorar el establecimiento temprano y la tolerancia al estrés hídrico del portainjerto de *Prunus*, ofreciendo estrategias potenciales para mitigar los impactos del cambio climático en la producción de fruta. La investigación futura debe centrarse en los mecanismos subyacentes de la tolerancia al estrés inducida por PGPR y su aplicación en condiciones de campo.

SUMMARY

Climate change poses a significant threat to agriculture, particularly through shifts in precipitation and temperature patterns that intensify water stress on crops. Here, we studied the effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on the survival, growth, and water stress tolerance of the micropropagated cherry rootstock Maxma 60 (*Prunus mahaleb* × *Prunus avium*). Rootstocks were inoculated with *Pseudomonas koreensis* strain AG-97, *Pseudomonas putida* strain AG-30, *Claroideoglomus claroideum*, *Pseudomonas koreensis* strain AG-97 + *Claroideoglomus claroideum* and *Funneliformis mosseae* during acclimatization, and then subjected to water stress. Results showed that *Ps. koreensis* significantly increased plant survival and leaf dry weight during acclimatization. Moreover, inoculation with the fungus *F. mosseae* led to enhanced shoot and root growth under well-watered conditions, but this did not translate to improved performance under drought conditions. Under water deficit, rootstocks inoculated with *Ps. koreensis* maintained higher stem water potential and demonstrated greater water stress tolerance compared to other treatments. Additionally, the co-inoculation of *Ps. koreensis* with *C. claroideum* resulted in a higher stomatal conductance under drought conditions, indicating a synergistic effect that could benefit plant resilience during the transplanting stage. Our findings suggest that microbial inoculation, particularly with *Ps. koreensis* strain AG-97, can enhance the early establishment and water stress tolerance of *Prunus* rootstocks, offering potential strategies for mitigating the impacts of climate change on fruit production. Future research must be performed focused on the underlying mechanisms of PGPR-induced stress tolerance and their application in field conditions.

CAPÍTULO 1. **INTRODUCION GENERAL**

La agricultura y la seguridad alimentaria se han visto y seguirán siendo afectadas por el cambio climático, en particular por las variaciones en precipitaciones y temperaturas (Anderson et al., 2020), donde se estima que la temperatura media mundial aumentará 1,5°C entre 2030 y 2052 (Jiang, 2020). De acuerdo con Stoker *et al*. (2013) se plantea que a fines del siglo XXI habrá cambios en los patrones de aumento de temperaturas y en la intensidad y /o duración de la sequía a escala global. Es así como el déficit hídrico se ha convertido en uno de los principales estreses abióticos a los que están expuestas las plantas, produciendo cambios fisiológicos, fisicoquímicos y morfológicos en éstas, lo que afecta negativamente su crecimiento y productividad (Chandra et al., 2021; Pereira *et al*., 2020). Con relación a esto, Vurukonda *et al*. (2016) y Anderson *et al*. (2020) señalan que, dentro de los estreses abióticos, la sequía es una de las preocupaciones más urgentes, especialmente por el aumento de la población, lo que exige un alto rendimiento agrícola. Dentro de los rubros agrícolas, la industria frutícola, ha sido uno de los más afectados por el déficit hídrico a nivel mundial (Behrooz, 2019). El cerezo (*Prunus avium* L.) se ha convertido en uno de los cultivos frutales más atractivos y económicamente rentables del mundo y es uno de los frutales más importantes en Chile (Gonçalves et al., 2021; Tudela et al., 2023).

Los cerezos a su vez se establecen sobre portainjertos que les otorgan tolerancia a diferentes tipos de estrés entre otras ventajas. Los portainjertos inciden en la precocidad, la consistencia en el rendimiento y la calidad de la fruta y han otorgado beneficios como la resistencia a estrés abiótico por limitantes de agua (Hrotkó, 2016; Pedroso *et al*., 2014). Uno de los portainjertos más utilizado en Chile es Maxma 60 (*Prunus mahaleb* × *Prunus avium*) y se clasifica como tolerante a hipoxia en suelos de baja aeración y moderadamente resistente al déficit hídrico (Morales-Olmedo et al., 2021; Grau, 2007).

En la rizósfera de especies vegetales, se producen interacciones con microorganismos que son esenciales para estimular el crecimiento, absorción de nutrientes, tolerancia al estrés, bioncontrol de patógenos y desarrollo en general de las plantas (Trivedi *et al*., 2020). Dentro de estos actores rizosféricos están las bacterias promotoras del

crecimiento vegetal y los hongos micorrícicos (Raklami *et al*., 2019, Phour *et al*., 2020). Las micorrizas son hongos que pueden colonizar las raíces externa o internamente. Las ectomicorrizas se encuentran principalmente asociadas con árboles y arbustos; mientras que las endomicorrizas pueden ser de tipo arbuscular o helicoidal. Las micorrizas arbusculares forman propágulos, cómo esporas, hifas y rizomorfos, que son estructuras que sobreviven en la rizósfera. Cuando las esporas germinan y forman hifas, estas pueden entrar en contacto con las raíces y formar un manto externo, mientras que otras penetran entre las células de la epidermis radicular generando una red. Esta red aumenta el área de contacto entre las células radiculares y el suelo, facilitando la captación y transporte de nutrientes entre ambos (Santoyo *et al*., 2021).

Además de las micorrizas se ha reportado que existe un número considerable de especies de bacterias asociadas a la rizósfera que estimulan el crecimiento, rendimiento y calidad del cultivo (Esitken *et al*., 2006). Estas bacterias se llaman bacterias promotoras del crecimiento vegetal (PGPR; del inglés: *Plant Growth promoting rhizobacteria*), las cuales son un grupo diverso de bacterias del suelo de vida libre que colonizan la rizósfera y contribuyen a la promoción del crecimiento de las plantas, mediante la producción de diversas sustancias, lo que a su vez aumenta el rendimiento de los cultivos agrícolas (Kumar *et al*., 2016; Mhatre *et al*., 2019). Por otra parte, se ha investigado aspectos de las raíces, crecimiento, distribución, absorción de agua y autorregulación adaptativa (Li *et al*., 2019). Diversos estudios han demostrado un mejor crecimiento y desarrollo de las plantas en respuesta a la inoculación de semillas o raíces con inoculantes microbianos, capaces de producir reguladores del crecimiento (Zahir *et al.,* 2004).

Las PGPR producen varias fitohormonas o reguladores del crecimiento como: las auxinas (ácido indolacético, ácido indol butírico y ácido fenilacético); citoquininas (isopentenil adenosina, isopentenil adenina ribósido, trans-zeatina ribosa y zeatina); ácido giberélico; ácido abscísico; etileno; poliaminas; brasinoesteroides; jasmonatos; ácido salicílico; estrigolactonas; y otros compuestos reguladores, que influyen directamente en el crecimiento y el metabolismo de las plantas (Gopalakrishnan *et al*., 2015). Entre las fitohormonas el ácido indolacético es una de las auxinas más

estudiadas (Kasahara, 2016), la cual aumenta el enraizamiento lateral y adventicio, causando una mejor absorción de minerales y nutrientes (Yadav *et al*., 2021).

Las PGPR también pueden inducir la tolerancia a estrés en las plantas, al sintetizar la enzima 1-aminociclopropano-1-carboxilato (ACC) desaminasa al reducir las concentraciones de etileno (Glick, 2004). En tejidos vegetales, el ACC es un precursor del etileno, hormona que está relacionada, además de la maduración de frutos, con la senescencia y absición de las hojas ante condiciones de estrés. La presencia de PGPR con la habilidad de producir ACC-desaminasa, permite secuestrar y degradar el ACC, y de esta manera prevenir que concentraciones nocivas de etileno sean acumulados en los tejidos de la planta, mejorando de esta forma su respuesta ante estrés (Glick, 2004; Nascimento et al 2012).

La interacción de las PGPR con otros microorganismos rizósfericos cómo las micorrizas pueden generar un efecto sinérgico sobre la planta (Santoyo *et al*., 2021). El trabajo de Pereira *et al*. (2016) demostró que la interacción micorrizas y PGPR podría aliviar aún más el estrés en plantas. Ellos observaron que cepas de *Bacillus* endofítico co-inoculadas con hongos micorrícicos arbusculares, aumentaron la tolerancia a la sequía en *Lavandula dentata* al estimular el metabolismo oxidativo. Además, aumentaron el crecimiento y la nutrición de la planta. Otros estudios han demostrado que la interacción de las micorrizas arbusculares con PGPR en nogal (cv. Chandler) han permitido contener el estrés hídrico utilizando las bacterias *Azotobacter chroococcum* y *Azospirillium lipofrum* junto con las micorrizas *Glomus mosseae y G. etunicatum*. Los resultados mostraron que la coinoculación del consorcio microbiano, en comparación con las inoculaciones separadas de cada microorganismo mejoró los efectos negativos (crecimiento reducido y contenido de nutrientes de las hojas como N, P y Zn) del estrés por sequía en las plántulas de nogal (Behrooz *et al*., 2019). Además, la presencia de micorrizas favorece la colonización de las raíces por parte de las bacterias, ya que existe la posibilidad de formación de autopistas fúngicas o *fungal highways*, que son utilizadas por las bacterias para movilizarse a través de las hifas del hongo (Simón *et al*., 2015).

A la fecha, los antecedentes del efecto que puedan tener las PGPR y su interacción con las micorrizas frente al desarrollo radicular y protección al estrés hídrico en patrones de cerezo son limitadas. La cantidad de información disponible sobre interacciones entre hongos micorrícicos y bacterias y sus beneficios para las plantas es limitada y se centran en el efecto de las cepas bacterianas sobre el desarrollo fúngico (Santoyo *et al*., 2021). Por lo tanto, se considera esencial estudiar las interacciones de las bacterias rizosféricas y micorrizas para conocer cómo inciden en la promoción de crecimiento y la protección para la tolerancia al estrés hídrico en patrones y su utilización podría resultar beneficiosa para mitigarlo.

HIPÓTESIS

La interacción entre las rizobacterias promotoras del crecimiento vegetal y las micorrizas arbusculares: permiten una colonización efectiva del sistema radical del patrón Maxma 60 y le otorgan protección ante el déficit hídrico.

OBJETIVO GENERAL

Evaluar el efecto protector frente a estrés hídrico de la interacción de las micorrizas arbusculares y las rizobacterias promotoras del crecimiento vegetal y productoras de ACC deaminasa en el patrón de cerezo Maxma 60.

OBJETIVOS ESPECIFICOS

- Evaluar la compatibilidad y efecto sinérgico entre micorrizas arbusculares y bacterias promotoras de crecimiento y productoras de ACC deaminasa en el patrón Maxma 60.
- Determinar los parámetros fisiológicos que se ven beneficiados por la interacción de micorrizas y rizobacterias produtoras de ACC deaminasa y que impactan en el estrés hídrico en el patrón Maxma 60.
- Evaluar el efecto de las micorrizas arbusculares y las rizobacterias productoras de ACC deaminasa en el desarrollo radical y producción de biomasa del patrón Maxma 60.

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CAPÍTULO 2.

Manuscrito enviado al journal “Horticulture, Environment and Biotechnology”

Effects of the co-inoculation with arbuscular mycorrhizal fungi and rhizosphere bacteria on the early growth and water stress tolerance of *Prunus sp.* Rootstock

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Keywords: Climate Change; Abiotic Stress; Micropropagation; Drought Resistance; Symbiotic Relationships.

Abstract

Climate change poses a significant threat to agriculture, particularly through shifts in precipitation and temperature patterns that intensify water stress on crops. Here, we studied the effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on the survival, growth, and water stress tolerance of the micropropagated cherry rootstock Maxma 60 (*Prunus mahaleb* × *Prunus avium*). Rootstocks were inoculated with *Pseudomonas koreensis* strain AG-97, *Pseudomonas putida* strain AG-30, *Claroideoglomus claroideum*, *Pseudomonas koreensis* strain AG-97 + *Claroideoglomus claroideum* and *Funneliformis mosseae* during acclimatization, and then subjected to water stress. Results showed that *Ps. koreensis* significantly increased plant survival and leaf dry weight during acclimatization. Moreover, inoculation with the fungus *F. mosseae* led to enhanced shoot and root growth under well-watered conditions, but this did not translate to improved performance under drought conditions. Under water deficit, rootstocks inoculated with *Ps. koreensis* maintained higher stem water potential and demonstrated greater water stress tolerance compared to other treatments. Additionally, the co-inoculation of *Ps. koreensis* with *C. claroideum* resulted in a higher stomatal conductance under drought conditions, indicating a synergistic effect that could benefit plant resilience during the transplanting stage. Our findings suggest that microbial inoculation, particularly with *Ps. koreensis* strain AG-97, can enhance the early establishment and stress tolerance of *Prunus* rootstocks, offering potential strategies for mitigating the impacts of climate change on fruit production. Future research must be performed focused on the underlying mechanisms of PGPR-induced stress tolerance and their application in field conditions.

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Introduction

Agriculture and food security are under constant threat from climate change, particularly due to variations in precipitation and temperature (Stocker et al., 2013; Anderson et al., 2020). It is estimated that the global average temperature will increase by 1.5°C between 2030 and 2052, and by the end of the 21st century, there will be significant changes in temperature patterns and in the intensity and duration of drought events on a global scale (Jiang et al., 2020). Water deficit has become one of the major abiotic stresses faced by plants, resulting in physiological, physicochemical, and morphological changes that negatively affect plant growth and productivity (Vurukonda et al., 2016; Pereira et al., 2020; Chandra et al., 2021).

In the above scenario, the fruit industry is particularly vulnerable to water deficit, with cherry (*Prunus avium* L.) being a crop of high economic importance globally (Grau, 2007; Hrotkó, 2016; Gonçalves et al., 2021; Tudela et al., 2023). Cherry trees are typically established on rootstocks, which can influence earliness, yield consistency, and fruit quality, as well as confer benefits such as resistance to water stress (Pedroso et al., 2014). One of the most widely used rootstocks is Maxma 60 (*Prunus mahaleb* × *P. avium*), classified as tolerant to hypoxia and moderately resistant to water deficit (Morales-Olmedo et al., 2021).

To obtain cherry rootstocks, micropropagation is a widely used technique based on the rapid multiplication of plants. However, micropropagated plants often have poorly developed roots and are susceptible to water stress due to high transpiration losses during the acclimatization phase (Vettori et al., 2010; Sharma & Kumar, 2022). This vulnerability to drought stress can be mitigated through the inoculation with beneficial microorganisms (Vidal et al., 2022; Chávez et al., 2023; Santander et al., 2024).

In the rhizosphere, plants establish essential interactions with microorganisms that stimulate growth, nutrient uptake, stress tolerance, pest control, and overall

plant development (Glick, 2004; Trivedi et al., 2020). Among these microorganisms, arbuscular mycorrhizal fungi (AMF) are the most common plant mutualistic symbionts, which can confer plant stress tolerance, improve access to water and nutrients, and enhance soil structure and fertility (Pardo et al., 2003; Daynes et al., 2013; Chen et al., 2018; Ji et al., 2019; Santoyo et al., 2021). AMF produce a glycoprotein named glomalin, which contributes to the formation and stability of hydrophobic soil macroaggregates, positively affecting soil water retention and root distribution (Amerian et al., 2001; Fernández-Lizarazo & Moreno-Fonseca, 2016).

In addition, certain bacterial species in the rhizosphere stimulate crop growth, yield, and quality (Esitken et al., 2006; Kumar et al., 2016; Mhatre et al., 2019). These bacteria, known as plant growth-promoting rhizobacteria (PGPR), produce various metabolites that stimulate growth and stress tolerance, potentially increasing crop yield (Zahir et al., 2004; Gopalakrishnan et al., 2015; Kasahara, 2016; Yadav et al., 2021). Among the phytohormones produced by PGPR, indoleacetic acid is one of the most studied auxins due to its role in increasing lateral and adventitious rooting, which enhances mineral and nutrient uptake.

PGPR can also induce stress tolerance by reducing ethylene concentrations in plants through the synthesis of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick, 2004; Nascimento et al., 2012). In plant tissues, ACC is a precursor of ethylene, a hormone associated with leaf senescence and abscission under stress conditions. The presence of PGPR with ACC-deaminase activity allows for the sequestration and degradation of ACC, preventing harmful concentrations of ethylene from accumulating in plant tissues and thereby improving the plant's response to stress.

The interaction between plants, AMF, and PGPR is usually synergistic (Raklami et al., 2019), increasing biomass production and enhancing plant tolerance to various abiotic stresses (Gluszek et al., 2018; Luciani et al., 2019; Pan et al., 2020; Choudhury et al., 2021; Pan et al., 2022). In fruit trees, co-inoculation with

PGPR and AMF has been shown to improve tolerance to various types of stress and increase root development (Behrooz et al., 2019).

To date, there is limited information on the effects of PGPR and AMF on root development and water stress tolerance in cherry rootstocks. Therefore, the objective of this study was to evaluate the effects of AMF and PGPR co-inoculation on the water stress tolerance of micropropagated cherry rootstock Maxma 60 during the *ex-vitro* acclimatization phase and under water stress.

Materials and Methods

Biological material

The study was carried out on Maxma 60 rootstocks (*Prunus mahaleb* x *Prunus avium*) which were propagated under controlled *in vitro* conditions (23°C, 16 h light) for 15 days at the Agromillora nursery, Curicó Province, Maule Region, Chile (Lat. 35°20′ S; Long. 71°25′ W), using an agar-based propagation media, that includes macro and micronutrients, and phytohormones.

Two arbuscular mycorrhizal fungi were used: i) *Claroideoglomus claroideum* strain CC, which was isolated from agricultural Andisols from southern Chile, ii) and *Funneliformis mosseae* strain HCM7, which was isolated from the rhizosphere of *Baccharis scandens* plants growing in the Atacama Desert (northern Chile). Both fungal strains were provided by the Mycorrhizas laboratory, Universidad de La Frontera (Temuco, Chile).

The bacterial strains were provided by the Agricultural Microbiology Laboratory, Universidad de Concepción (Chillán, Chile). The strains used were *Bacillus* sp. AG-91, *Pseudomonas koreensis* AG-97, *B. subtilis* AG-92, and *Ps. putida* YSS6WT AG-30. The last strain was kindly provided by the University of Waterloo, Canada. Bacterial strains were chosen based in previous characterization of their ability to produce indole acetic acid (AG-97) and for their ACC-deaminase activity (AG-91, AG-92 and YSS6WT). The bacterial strains were obtained from stock cultures at -80°C and cultured in 200 mL of sterilized (121°C, 15 minutes at 1 atm)

standard nutrient broth in a rotary shaker (150rpm) at 25°C from 30 to 48 h, depending on the strain growth rate. Each bacterial cell suspension was standardized to an optical density (OD) of 0.1 at 600 nm wavelength in a spectrophotometer (MECASYS, POP Optizen Bio, Korea).

Experimental design for selection of microorganisms

For the selection of the microbial strains to be used during the acclimatization phase, four PGPR isolates, and one AMF were used in combinations in a completely randomized block design. Maxma 60 rooted plantlets were removed from the flasks containing the semi-solid media, roots were excised, and plantlets were transplanted to pots containing peat (50 %), perlite and coconut fiber, supplemented with 1000 mg L^{-1} indol-3-butyric acid (IBA). Plantlets were inoculated with 1 ml of cell suspensions at OD_{600} 0.1 for each bacterial strain, as single inoculum or in mixture with the fungus CC at a dose of 0.35 g of fungal inoculum per pot and were thoroughly mixed with the substrate using a sterile spatula. Inoculated plantlets were kept in a naturally lit greenhouse for 30 days, in which the amount of PPFD (Photosynthetic Photon Flux Density) was reduced by 20%, and air temperature and humidity were maintained at 25°C and 80%, respectively. After this period, root and shoot dry weight, plant height, percentage of survival and intrinsic efficiency of photosystem II (Fv/Fm) were evaluated.

Water stress tolerance experiment

For this experiment, rootstock plantlets were grown and inoculated as described above for the microbial selection trial, but only the microorganisms that showed the highest performance in plant survival and growth were used. Plants were kept under the same environmental conditions of the previous experiment for 90 days until they reached a size large enough to start the transplanting phase (approximately 20 cm height). Plants were then transferred to a shade house to avoid any potential impact of excessive solar radiation on young plants. Each plant was transplanted into 1.5 L pots, containing a mixture of sand, peat, and coconut fiber (7:2:1). Before the application of the irrigation treatments, all plants

were similarly managed for 20 days. Irrigation requirements were based on the substrate moisture content, aiming to satisfy 100% of the container capacity, which was determined before the experiment setup. Each irrigation event was applied when the substrate volumetric water content (θ) was 20% below the container capacity. While the irrigation frequency was maintained at 2 days until the end of the experiment, the amount of irrigation water varied from 60 to 150 mL because water requirements increased as plants grew.

The experimental design was a completely randomized block design in a split-plot arrangement. Twenty days after transplanting, two irrigation treatments (main plot) were applied for 15 consecutive days. The control treatment (WET) was irrigated to satisfy 100% of the container capacity, trying to maintain plants at optimum water conditions. In the Deficit Irrigation treatment (DRY), irrigation was completely ceased for 15 days to simulate a two-week period of water shortage during the time of year when seasonal atmospheric evaporative demand is highest (January to February).

The microbial treatments (subplot) were the following inoculants: *Pseudomonas koreensis* AG-97, *Pseudomonas putida* AG-30, *Clareidoglomus claroideum*, *Pseudomonas koreensis* AG-97 + *Clareidoglomus claroideum* and *Funneliformis mosseae*. An uninoculated control was also included.

Measurements

Plant height and leaf number were measured before and at the end of the water stress period. Shoot dry weight was measured at the end of the trial by separating the aerial part of the plant from the root and placing it in an oven at 60°C during 72 h. Volumetric water content and electrical conductivity in the substrate were measured at two depths (depth 1: between 0 and 5; depth 2: between 5 and10 cm) using a capacitance sensor (5TE, Decagon instruments, Portland, WA, USA). Soil moisture data was stored in a portable datalogger (Procheck, Decagon instruments, Portland, WA, USA). The relative chlorophyll content was measured on a well-exposed mature leaf using a portable chlorophyll meter (SPAD502 Plus,

Konica Minolta, Tokyo, Japan). Stomatal conductance was measured in one sun-exposed leaf per plant, using a steady-state porometer (Li-cor 1600, Li-Cor, Nebraska, USA). Maximum quantum efficiency of photosystem II (Fv/Fm) was measured on the same leaf where stomatal conductance was measured using a chlorophyll fluorimeter (Pocket PEA, Hansatech Instruments, England, UK). For this procedure, the plants were adapted to darkness for 30 min before the minimum fluorescence (Fo) and maximum fluorescence (Fm) measurements (Liang et al., 2019). Fv/Fm was calculated with the following formula: Fv/Fm = (Fm - Fo) Fm, were Fv = [Fm − Fo], and Fm = maximum fluorescence performance. The stem water potential (Ψs) was evaluated as the physiological indicator of plant water stress only at the end of experiment to minimize plant defoliation, as this is a destructive technique. Plant water stress was measured in one leaf per plant at midday (between 12:00 and 15:30 h) using a pressure chamber (PMS 615, PMS Instruments, Washington, USA). The sampled leaves were previously covered for 40 min with an opaque airtight bag, based on the methodology proposed by McCutchan and Shackel (1992).

Several samples of 1 cm of roots were taken from each experimental unit and the placed in a 1.5 mL Eppendorf flask with 50% glycerol solution. All samples were refrigerated at 5°C. Subsequently, root AMF were stained with a solution of 0.05% w/v trypan blue in lactic acid using the methodology of Phillips and Hayman (1970) and the frequency of colonization was quantified according to the Trouvelot Morphometric method (Ho-Plágaro et al., 2020).

Statistical analysis

In the selection experiment, data sets were subject to cluster analysis using Euclidean metric as a distance measure and a non-parametric analysis of variance was performed using the Kruskal Wallis test ($P<0.05$) to compare clusters. For the water stress experiment data, factorial ANOVA was carried out after checking for normality and homoscedasticity. The statistical differences between treatments were analyzed using Tukey's multiple range test at a level of

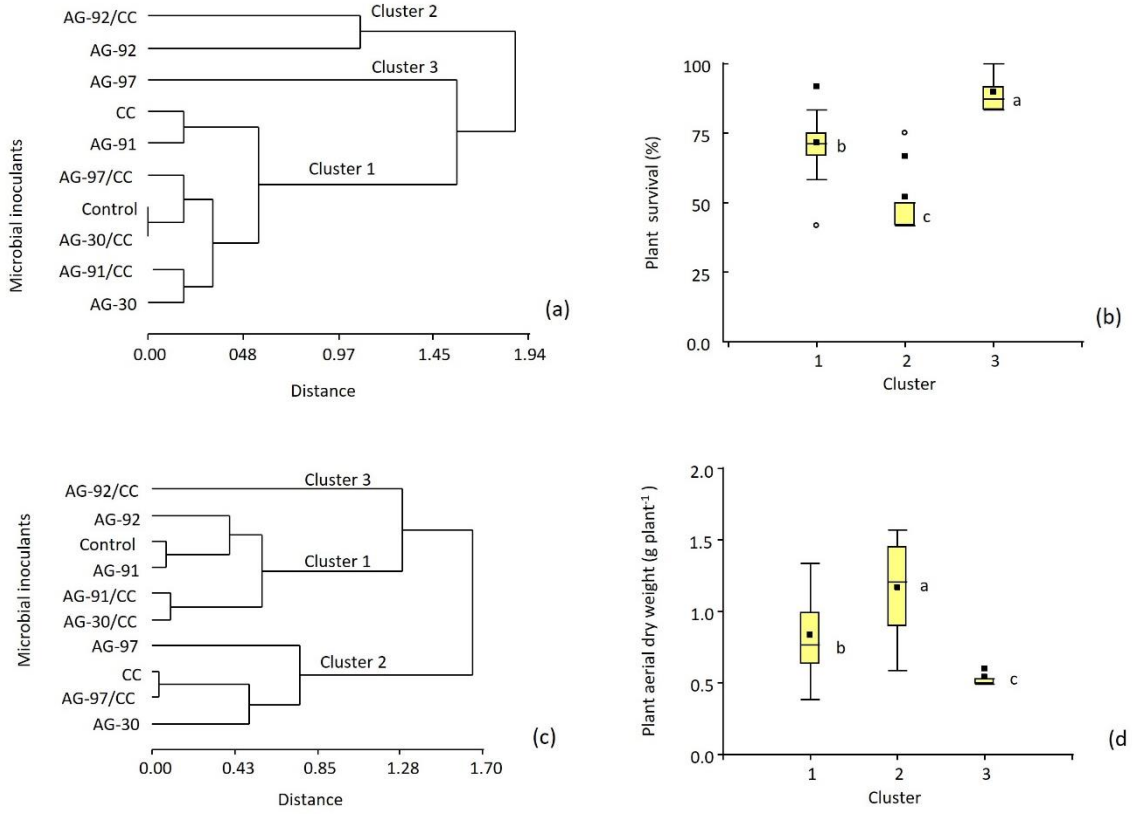
significance of P<0.05. For variables that did not meet the assumptions, transformations and non-parametric tests were performed using Friedman's non-parametric analysis and the Conover test. Data were analyzed using the R and Infostat statistical software.

Results

Experiment for the Selection of Microorganisms

Inoculating Maxma 60 rootstock during the acclimatization stage with various combinations of microbial inoculants significantly affected plant survival and leaf dry weight ($P < 0.05$). Cluster analysis using Euclidean distance for both parameters grouped the inoculation treatments into three clusters (Fig. 1). Cluster 3 was significantly superior to Clusters 1 and 2 in terms of leaf dry weight (Fig. 1b) and included only *Pseudomonas koreensis* strain AG-97. For plant survival, Cluster 2 outperformed Clusters 1 and 3 (Fig. 1d). The inoculation treatments that improved plant survival included *P. koreensis* AG-97, *Claroideoglomus claroideum* (CC), and *Pseudomonas putida* AG-30, either individually or in combination. No significant differences were observed among the inoculation treatments for plant height (cm), number of leaves, root dry weight (g), and internode length ($P > 0.05$) (Supplementary Data).

Fig. 1. Phenogram of the cluster analysis and comparison between clusters of the effect of inoculation treatments on micropropagated Maxma 60 cherry rootstocks: (a) and (b) Plant survival (%) (c) and (d) Plant aerial dry weigh (g plant^{-1}). Means sharing a letter are not significantly different according to the Kruskal-Wallis test ($P < 0.05$). Inoculation treatments: AG-91: *Bacillus sp*.; AG-92: *Bacillus subtilis*; AG-97: *Pseudomonas koreensis*; AG-30: *Pseudomonas putida*; CC: *Claroideoglomus claroideum.*



Plant growth promotion and stress tolerance experiment

No significant differences in volumetric water content were detected between microbial treatments at either 0-5 cm or 5-10 cm depth throughout the experiment, prior to application of water deficit. Seven days after the application of water deficit, significant differences between DRY and WET treatments were detected, persisting until the experiment's conclusion (Table 1). Electrical conductivity was monitored every two days, with no significant differences found between inoculant and irrigation treatments, consistently showing values below 1 (Table 2).

Table 1. Soil water content for the different inoculation treatments throughout the water stress experiment.

Soil water content (m^3 m^{-3})												
Days after water stress application	-4	-2	1	3	7		10		12		14	
Soil Depth (cm)	0 - 5	0 - 5	0 - 5	0 - 5	0 - 5	5 - 10	0 - 5	5 - 10	0 - 5	5 - 10	0 – 5	5 - 10
Inoculants treatments												
AG-97	0.19	0.21	0.22	0.23 a	0.16 b	0.24	0.14 ab	0.19	0.16	0.24	0.1	0.17
AG-30	0.18	0.21	0.22	0.20 b	0.14 b	0.23	0.13 b	0.18	0.14	0.22	0.1	0.17
CC	0.19	0.21	0.22	0.21 ab	0.15 b	0.25	0.14 ab	0.19	0.14	0.23	0.11	0.17
CC+AG-97	0.2	0.22	0.23	0.22 ab	0.16 b	0.25	0.14 ab	0.19	0.14	0.23	0.12	0.17
HMC7	0.18	0.21	0.22	0.21 ab	0.15 b	0.23	0.13 b	0.17	0.15	0.23	0.1	0.16
Control	0.19	0.22	0.23	0.22 ab	0.21 a	0.25	0.15 a	0.19	0.17	0.24	0.11	0.17
p value	0.37	0.513	0.95	0.046	0.003	<0.01	<0.01	<0.01	0.434	0.685	0.141	0.597
Irrigation												
Dry	0.19	0.22	0.23	0.28	0.24 a	0.35 a	0.22 a	0.27	0.25	0.37 a	0.18 a	0.26 a
Wet	0.19	0.22	0.23	0.16	0.1 b	0.14 b	0.07 b	0.11	0.06	0.1 b	0.05 b	0.09 b
p value	0.253	0.849	0.494	0.737	<0.01	<0.01	<0.01	0.293	0.275	<0.01	<0.01	<0.01
Interaction Irrigation x Inoculants	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Different letters represent statistically significant differences (P ≤ 0.05) Tukey test

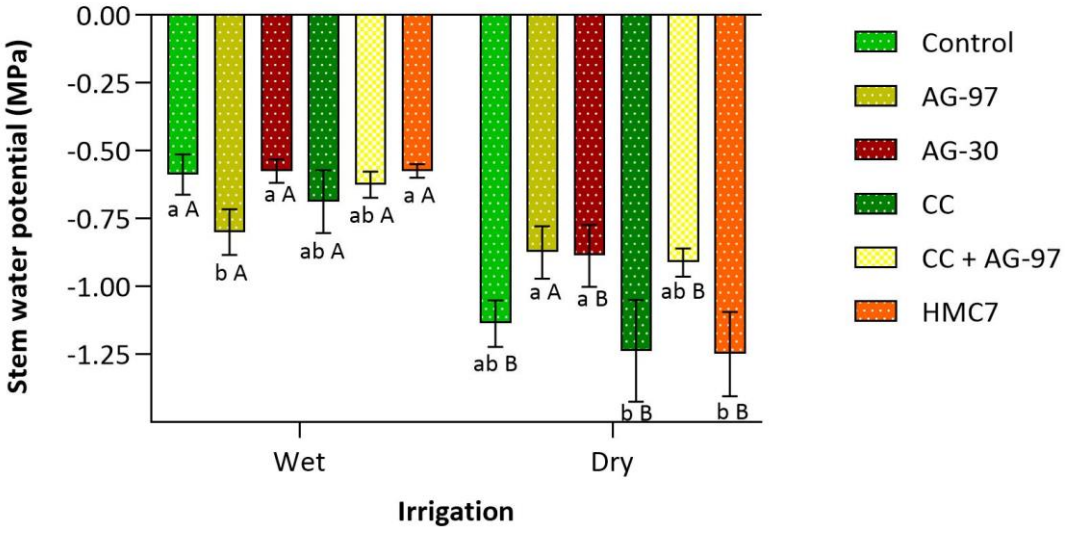
Table 2. Soil electrical conductivity (dS m^{-1}) for the different inoculation treatments throughout the water stress experiment.

Soil electrical conductivity (dS m^{-1})												
Days after water stress application	-4	-2	1	3	7		10		12		14	
Soil Depth (cm)	0 - 5	0 - 5	0 - 5	0 - 5	0 - 5	5 - 10	0 - 5	5 - 10	0 - 5	5 - 10	0 – 5	5 - 10
Inoculants treatments												
AG-97	0.04 a	0.05	0.06	0.07	0.03	0.04	0.01	0.03	0.03	0.05	0.008	0.02
AG-30	0.02 b	0.03	0.05	0.04	0.02	0.03	0.01	0.01	0.02	0.03	0.007	0.01
CC	0.03 ab	0.04	0.04	0.05	0.02	0.04	0.02	0.02	0.04	0.05	0.006	0.02
CC+AG-97	0.03 ab	0.04	0.05	0.05	0.02	0.05	0.01	0.02	0.03	0.03	0.008	0.02
HMC7	0.02 b	0.03	0.05	0.06	0.03	0.04	0.02	0.02	0.03	0.05	0.006	0.02
Control	0.02 b	0.04	0.06	0.07	0.03	0.04	0.01	0.02	0.03	0.05	0.009	0.02
p value	<0.01	0.339	0.578	0.839	0.332	0.289	0.27	0.105	0.209	0.186	0.75	0.771
Irrigation												
Dry	0.03	0.04	0.04 b	0.02 b	0.00 b	0.01 b	0.00 b	0.01 b	0.00 b	0.00 b	0.00 b	0.00 b
Wet	0.418	0.692	0.021	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
p value	0.418	0.692	0.021	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Interaction												
Irrigation x Inoculants	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Different letters represent statistically significant differences (P ≤ 0.05) Tukey test.

Stem water potential was influenced by the water regime, varying between -0.5 and -0.75 MPa under WET conditions and between -1.0 and -1.25 MPa under DRY conditions (Fig. 2). Significant differences among inoculation treatments were observed under both water regimes ($P < 0.05$). Under irrigated conditions, Maxma 60 rootstocks inoculated with *Ps. koreensis* AG-97 showed lower stem water potential compared to the control, *Pseudomonas* AG-30, and rootstocks colonized by HMC7 ($P < 0.05$) (Fig. 2). Under drought conditions, rootstocks inoculated with bacterial strains AG-97 and AG-30 exhibited higher stem water potential than those inoculated with AMF alone, but they were similar to the uninoculated control and to rootstocks co-inoculated with CC and *Pseudomonas* AG-97. Stem water potential was significantly lower under DRY conditions for most treatments when compared within each inoculation treatment (Fig. 2), except for rootstocks inoculated with AG-97, which did not show significant variations in stem water potential between contrasting watering regimes.

Fig. 2. Maxma 60 rootstock stem water potential (MPa) with different microbial inoculation treatments (Uninoculated; *Pseudomonas koreensis* strain AG-97; *Pseudomonas putida* AG-30; *Claroideoglomus claroideum* CC; and *Funneliformis mosseae* HMC7) under two water regimes: WET and DRY. (a) Comparison between inoculant treatments within each water regime; (b) Comparison between DRY and WET conditions for each inoculation treatment. Vertical bars correspond to the standard error of the mean. Inoculation treatments that share a lower-case letter within each water condition are not significantly different according to Tukey's test ($P < 0.05$). Water treatments that share a capital letter within each inoculant are not significantly different according to Tukey's test ($P < 0.05$).

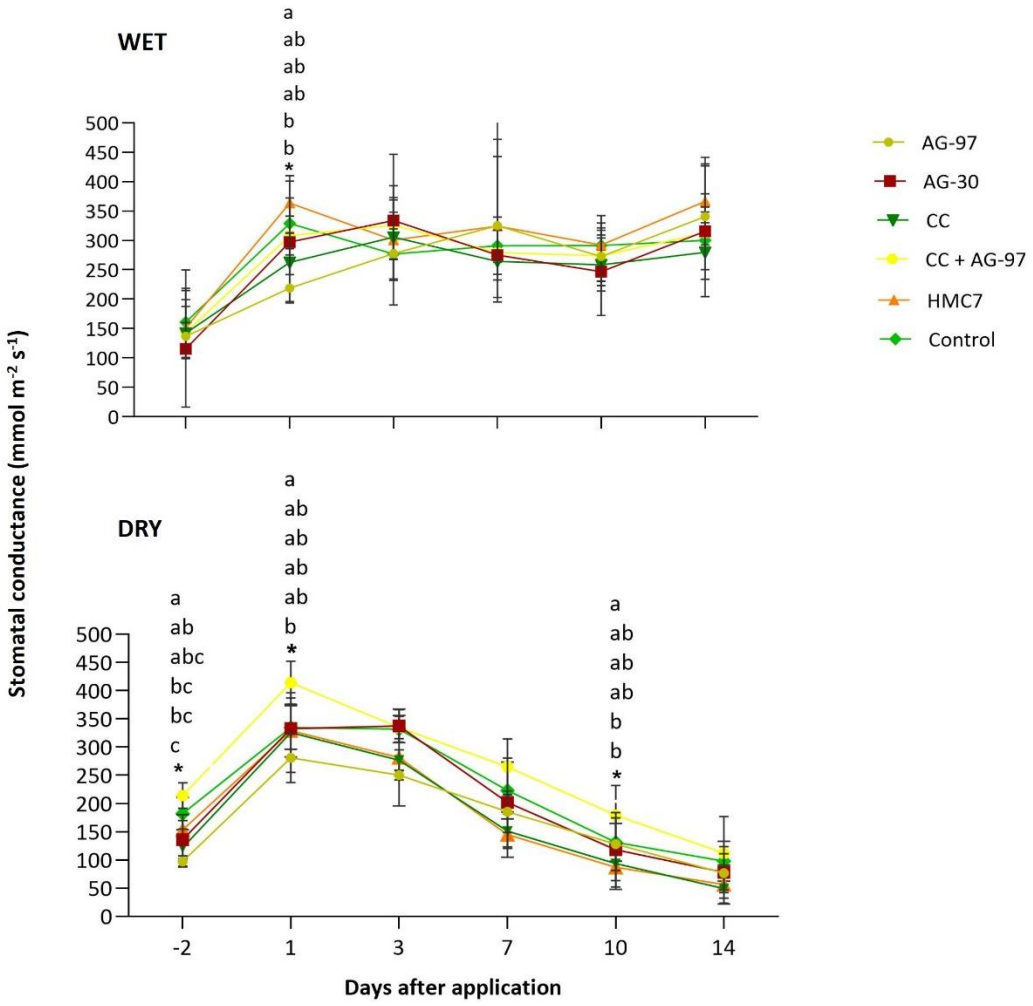


No significant differences were found in the Fv/Fm or the relative chlorophyll content between the various inoculants used under both water regimes throughout the experiment ($P > 0.05$) (Supplementary Material 2). Root colonization was confirmed in rootstocks inoculated with CC, HMC7, and CC + AG-97, with average colonization percentages of 8.34%, 12.88%, and 18.78%, respectively.

Stomatal conductance was homogeneous across water regimes before the application of the stress treatment, with values ranging from 100 to 214 mmol m^2 s^{-1} ($P > 0.05$). However, differences between inoculation treatments were detected within each water regime. In plants subjected to stress, treatments inoculated with CC + AG-97 exhibited higher stomatal conductance than those inoculated with CC, AG-97, and AG-30 alone (Fig. 3b). Under well-watered conditions (WET), stomatal conductance ranged from 200 to 350 mmol m^2 s^{-1} on day 1, where microbial inoculants induced significant differences ($P < 0.05$). Plants inoculated with HMC7 had higher stomatal conductance compared to the uninoculated control (Fig. 3). In the DRY treatments (Fig. 3), significant differences ($P < 0.05$) were observed on days 1 and 10 after stress application, with plants of

treatments inoculated with CC + AG-97 showing the highest stomatal conductance, and those inoculated with HMC7 the lowest.

Fig. 3. Maxma 60 rootstock stomatal conductance with different microbial inoculation treatments (Uninoculated; *Pseudomonas koreensis* strain AG-97; *Pseudomonas putida* AG-30; *Claroideoglomus claroideum* CC; and *Funneliformis mosseae* HMC7) under two water regimes: WET and DRY. Vertical bars correspond to the standard error of the mean. Treatments that share a letter within each day and water condition are not significantly different according to Tukey's test ($P < 0.05$).



Significant differences in shoot and root dry biomass were observed among the different inoculation treatments ($P < 0.05$). For root dry weight under well-watered conditions (WET), rootstocks inoculated with HMC7 achieved the highest dry weight (1.14 g), significantly greater than the uninoculated control (0.78 g) and other microbial inoculants ($P < 0.05$) (Fig. 4). Rootstocks inoculated with *Pseudomonas koreensis* AG-97 and *Pseudomonas putida* AG-30 also showed superior root growth compared to the control. However, under drought conditions (DRY), inoculation treatments did not show significant differences compared to the uninoculated control ($P > 0.05$). For shoot dry weight, there was no significant interaction between irrigation and microbial inoculants, so the main effects of inoculation are presented in Figure 5. Rootstocks inoculated with the mycorrhizal fungi HMC7 and CC exhibited higher shoot dry weights than the uninoculated control, with HMC7 also outperforming inoculation treatments that included bacteria (AG-30, AG-97, and CC + AG-97) ($P < 0.05$) (Fig. 5).

Fig. 4. Effect of microbial inoculants (Uninoculated; *Pseudomonas koreensis* strain AG-97; *Pseudomonas putida* AG-30; *Claroideoglomus claroideum* CC; and *Funneliformis mosseae* HMC7) applied under two water regimes (DRY and WET) on root dry weight of Maxma 60 rootstock. Vertical bars correspond to the standard errors of the means. Treatments within each water regime that share a letter are not significantly different according to Tukey's test ($P < 0.05$).

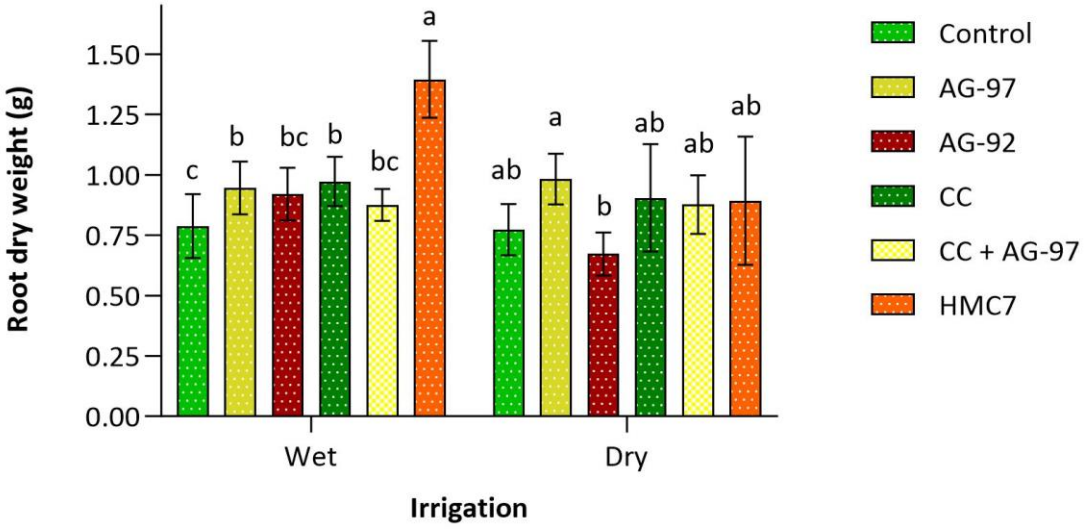
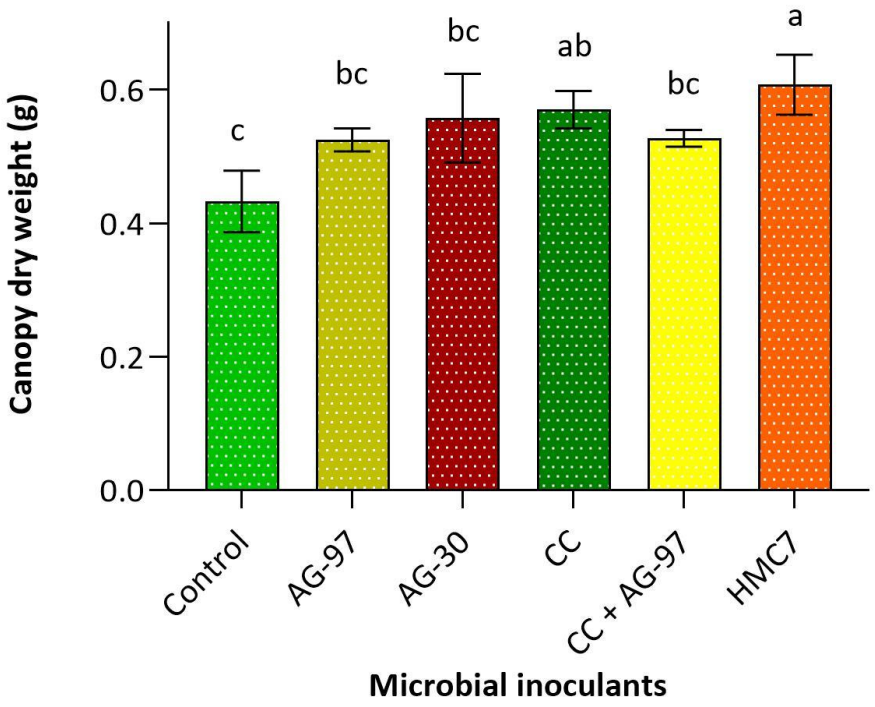


Fig. 5. Effect of microbial inoculants (Uninoculated; *Pseudomonas koreensis* strain AG-97; *Pseudomonas putida* AG-30; *Claroideoglomus claroideum* CC; and *Funneliformis mosseae* HMC7) on canopy dry weight of Maxma 60 rootstock. Vertical bars correspond to the standard error of the mean. Treatments that share a letter are not significantly different according to Tukey's test (P < 0.05).



Plant height was affected by microbial inoculation and irrigation regime (Table 3). Before the stress period, rootstocks inoculated with HMC7 were significantly taller than the uninoculated control (P < 0.05). However, after 14 days of water stress, plant height did not differ significantly among inoculation treatments. Inoculation with HMC7 also induced a significantly higher number of leaves per plant (P < 0.05) before and after the rootstocks were subjected to water stress (Table 3).

Table 3. Effect of microbial inoculants (Uninoculated; *Pseudomonas koreensis* strain AG-97; *Pseudomonas putida* AG-30; *Claroideoglomus claroideum* CC and *Funneliformis mosseae* HMC7) applied under two water regimes (DRY and WET) on plant height (cm), internode length (cm)and leaf number in Maxma 60 rootstock.

	Days after water deficit application					
	-2	14	-2	14	-2	14
Inoculants	Plant height (cm)		Leaf number per plant		Internode length (cm)	
Control	6.93 b	7.97	7.22 b	7.52 b	0.97 a	1.05
AG-97	8.05 ab	8.30	7.35 b	7.32 b	1.11 ab	1.14
AG-30	7.31 ab	7.97	7.60 b	7.55 b	0.97 ab	1.08
CC	8.30 ab	8.90	8.20 ab	8.02 ab	1.02 ab	1.12
CC+AG-97	8.05 ab	9.20	7.97 ab	7.97 ab	1.02 ab	1.17
HMC7	8.31 a	9.55	9.60 a	9.40 a	0.86 b	1.01
p values	0.018	0.13	<0.0001	0.0006	0.04	0.55
Irrigation						
Wet	7.40 a	8.50	7.78	7.73	0.97	1.11
Dry	8.30 b	8.80	8.21	8.21	1.02	1.09
p value	0.001	0.445	0.337	0.325	0.22	0.65
Interaction						
Irrigation x Inoculants	0.016	ns	ns	ns	ns	ns

Different letters represent statistically significant differences (P≤0.05) Tukey test.

Discussion

One of the major challenges in plant micropropagation is the survival of plants when they are transferred from *in vitro* to *ex vitro* conditions and then to the field, where they are exposed to environmental changes and various biotic and abiotic stresses (Chandra et al., 2010). Here, we demonstrate the importance of microbial inoculation of the cherry rootstock Maxma 60 during the acclimation phase, not only to reduce plantlet mortality upon transfer to *ex vitro* conditions, but also to enhance growth and induce tolerance to water stress.

At the early stages of rootstock development, the bacterial strains AG-97 (*Ps. koreensis*), AG-30 (*Ps. putida*), the mycorrhizal fungus *C. claroideum* strain CC, and the combined inoculation of AG-97 and CC, all contributed to higher plantlet survival. Notably, strain AG-97 also increased leaf dry weight during this critical acclimation phase. Bacteria of the genus *Pseudomonas* can enhance plant growth by regulating plant hormones production such as indoleacetic acid (IAA) and cytokinin, and by modulating ACC catabolism (Patten & Glick, 2002; Glick et al., 2023). The *Ps. koreensis* AG-97 strain used in this study produces IAA, which specifically stimulates root development (Sepúlveda-Caamaño et al., 2018). Bacterial IAA promotes root elongation, cell division, and differentiation (Tabassum et al., 2017), as well as the formation of lateral roots and root hairs (Etesami & Glick, 2024), thereby enhancing mineral and water uptake (Ona et al., 2003; Won, 2011). This strain induced higher plant survival when co-inoculated with the CC fungal strain, consistent with findings in other studies (Yu et al., 2022; Gamalero et al., 2004; Gamalero et al., 2009; Raklami et al., 2019). The interaction between bacteria and AMF, and their synergistic effects, have been extensively studied. Inoculation with AMF + PGPB can enhance plant growth and yield (Gamalero et al., 2004; Gamalero et al., 2009; Raklami et al., 2019; Carvalho Neta et al., 2024; Pérez-Moncada et al., 2024), making the root system more efficient in nutrient uptake and soil exploration (Santoyo et al., 2021a).

It is suggested that bacteria can stimulate the root colonization by AMF through the production of metabolites and hormones. The production of ACC-deaminase by bacteria may be particularly important in this context (Carvalho Neta et al., 2024; Gamalero et al., 2008), as it regulates ethylene levels, which are known to inhibit AMF root colonization (Zsögön et al., 2008). Additionally, ACC-deaminase mitigates the deleterious effects of ethylene on plant growth under stress conditions (Glick & Nascimento, 2021). The ACC-deaminase-producing bacterium *Ps. putida* strain AG-30 did not show beneficial effects when co-inoculated with CC during the acclimatization phase, although it did increase plant

survival when inoculated alone. In the water stress experiment, AG-30 was expected to outperform other microbial inoculants, given its previously demonstrated growth-promoting effects in alfalfa under water stress (Cedeño-Garcia et al., 2018). However, it did not significantly affect rootstock growth or stress tolerance. A possible explanation is that this strain may not have survived in the rootstock rhizosphere after the acclimatization phase, as plants can selectively shape the rhizosphere microbiome through their root exudates (Korenblum et al., 2022; Santoyo et al., 2021b).

At later stages of rootstock development, AMF alone produced better results in terms of leaf weight, plant height, and root development under irrigation conditions, with *F. mosseae* strain HMC7 showing particularly high stomatal conductance over time. This species has shown positive effects on other crops, such as walnut (Behrooz et al., 2019), tomato (Cesaro et al., 2020) and potato (Cayún et al., 2023). The growth promotion by AMF is primarily attributed to the transport of assimilable sources of nutrients, facilitating nutrient uptake (Hodge & Storer, 2014; Bowles et al., 2018; Santoyo et al., 2021a).

Under water deficit conditions, both AMF colonized plants and non-inoculated treatments showed a drastic reduction in stem water potential. The higher leaf dry weight of rootstocks inoculated with AMF likely led to increased transpiration and rapid dehydration due to higher water demand (Cheng et al., 2014), or the AMF hyphae may have been disadvantaged by the low water content (Lekberg & Koide, 2008).

The ability of AMF to produce glomalin, a fungal glycoprotein with hydrophobic properties (Rillig & Mummey, 2006; Rillig, 2004), contributes to the stability of hydro-stable soil macroaggregates even under water deficit conditions (Ji et al., 2019), improving soil water retention and increasing plant water availability (Bowles et al., 2018; Pardo et al., 2003). In this study, rootstocks were transferred to larger pots containing a peat and sand-based substrate, so the effect of glomalin may have been less pronounced than it would be in soil.

In plants inoculated with the strain HMC7 and subjected to water stress, a significant decrease in stomatal conductance was observed, which could affect CO_2 absorption, photosynthesis, and plant growth. Typically, in response to water deficit stress, ion and water transport systems reduce turgor pressure in guard cells and stimulate stomatal closure (Osakabe et al., 2014). This finding contrasts with literature reporting that AMF can enhance stomatal conductance, CO_2 assimilation, and osmotic adjustment under water stress conditions (Amerian et al., 2001; Fernandez-Lizarazo & Moreno-Fonseca, 2016; Chitarra et al., 2016; Tereucán et al., 2022), and even increase root hydraulic conductivity and/or reduce water loss under stress conditions through aquaporin production (Wang et al., 2023; Cheng et al., 2020; Santander et al., 2021).

Moreover, results showed that water deficit reduced stem water potential in all treatments except for AG97-inoculated plants. Although irrigation was ceased for 14 days in the water deficit treatment, AG97-inoculated plants maintained a midday SWP of -0.8 MPa under both wet and dry conditions. These results clearly demonstrate that AG-97-inoculated plants exhibited the best tolerance to water deprivation among the inoculation treatments. The lack of irrigation effects on SWP in AG-97-inoculated plants was not due to water stress-induced reductions in stomatal conductance, shoot or root biomass production, as all these variables were similar across all inoculation treatments under dry conditions. In this study, the reduction of substrate water content (SWC) at 0-5 and 5-10 cm depth in water-stressed plants was nearly identical across all inoculation treatments. However, the sampling protocol used for SWC measurements did not capture data below 10 cm depth, which may have been masking differences in substrate desiccation among inoculation treatments in deeper substrate layers. Therefore, AG-97 inoculation might have stimulated hair root growth below 10 cm depth, enhancing soil exploration for water with minimal changes in root dry weight. Several studies have reported increased root hair growth in plants inoculated with *Pseudomonas* sp. due to the stimulation of osmotic adjustment (Arkhipova et al., 2020;

Chakraborty et al., 2013) or the synthesis of growth-promoting hormones in root cells (Sepúlveda-Caamaño et al., 2018; Kang et al., 2014).

Co-inoculation with CC + AG97 increased stomatal conductance under water deficit conditions. Bacteria, particularly *Ps. koreensis*, can regulate stomatal aperture by decreasing ABA content in leaves and enhancing auxin and polyphenol-related pathways, thus improving plant responses to stress (Qiao et al., 2021; Bent et al., 2001). Although co-inoculation did not result in improved growth or survival of rootstocks compared to individual inoculation, it did lead to a higher percentage of AMF root colonization. Bacterial metabolites can increase root cell permeability (e.g., IAA) or regulate ethylene levels in the case of ACC-deaminase-producing bacteria (Gamalero et al., 2008; Ma et al., 2003; Sepúlveda-Caamaño et al., 2018; Cedeño-García et al., 2018).

The results obtained in this study highlight the positive role of microorganisms in early plant growth and survival. The fungus HMC7 shows promise for inducing greater shoot and root growth in cherry rootstocks under nursery conditions, which could translate to enhanced soil exploration capacity under field conditions. It is noteworthy that although *Ps. koreensis* did not promote plant growth at the nursery stage, it did enhance survival during the acclimatization stage and improve water stress tolerance. Future research should aim to elucidate the mechanisms employed by this strain for osmotic regulation and water stress management.

Conclusions

Micropropagated cherry rootstocks exhibited higher survival rates and increased leaf dry weight during the acclimation stage when inoculated with Indole-3-acetic acid (IAA) and ACC-deaminase-producing bacterial strains, both alone and in combination with arbuscular mycorrhizal fung (AMF). In the nursery, inoculation with AMF significantly enhanced shoot and root development. However, this enhanced root development did not mitigate the increased dehydration observed

under water stress conditions. At this stage, rootstocks inoculated with *Pseudomonas koreensis* AG-97 demonstrated the highest tolerance to water shortage. Additionally, co-inoculation with AMF and *Ps. koreensis* AG-97 resulted in a greater synergistic effect on stomatal conductance under water stress conditions, an outcome not observed when AMF were applied alone. This finding is particularly promising for the transplanting management of cherry rootstocks in regions where climate change has led to reduced rainfall and water availability, exposing plants to potential drought stress.

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Supplementary material

Supplementary material 1. Morphophysiological responses of MAXMA 60 micropropagated rootstocks to inoculation with growth promoting bacteria and mycorrhizal fungi. Control: Uninoculated; AG-91: *Bacillus* sp; AG-92: *Bacillus subtilis*; AG-97: *Pseudomonas koreensis*; AG-30: *Pseudomonas putida*; CC: *Claroideoglomus claroideum.*

Inoculants	Plant height (cm)	Leaf number per plant	Root dry weight (g planta^{-1})	Internode length (cm)	Fv/Fm
Control	2.46 ± 0.326	9.38 ± 0.694	0.22 ± 0.025	3.68 ± 0.340	0.82 ± 0.003 d
AG-91	3.14 ± 0.311	8.41 ± 0.436	0.23 ± 0.032	2.84 ± 0.199	0.80 ± 0.003 abc
AG-92	3.69 ± 0.571	8.78 ± 0.704	0.23 ± 0.046	3.04 ± 0.232	0.81 ± 0.004 cd
AG-97	3.39 ± 0.445	8.94 ± 0.610	0.28 ± 0.050	2.77 ± 0.223	0.82 ± 0.005 d
AG-30	3.91 ± 0.596	9.40 ± 0.791	0.26 ± 0.059	2.91 ± 0.490	0.81 ± 0.006 bcd
CC	3.43 ± 0.423	10.30 ± 0.718	0.30 ± 0.059	3.03 ± 0.213	0.81 ± 0.005 bcd
CC + AG-91	3.37 ± 0.307	8.49 ± 0.796	0.24 ± 0.037	2.89 ± 0.247	0.75 ± 0.042 ab
CC + AG-92	2.99 ± 0.151	8.35 ± 0.539	0.15 ± 0.027	2.90 ± 0.114	0.79 ± 0.004 a
CC + AG-97	3.82 ± 0.301	10.38 ± 0.630	0.36 ± 0.049	3.02 ± 0.277	0.80 ± 0.012 bcd
CC + AG-30	3.27 ± 0.460	8.69 ± 1.028	0.21 ± 0.019	2.86 ± 0.204	0.21 ± 0.003 bcd
Anova p values	0.1027	0.4028	0.1079	0.2548	0.0011

*Different lowercase letters indicate significant differences according to the Tukey test ($P < 0.05$).

Supplementary material 2. Effect of microbial inoculants (Uninoculated; *Pseudomonas koreensis* strain AG-97; *Pseudomonas putida* AG-30; *Claroideoglomus claroideum* CC and *Funneliformis mosseae* HMC7) applied under two water regimes (DRY and WET) on maximum efficiency of photosystem II and chlorophyll content of Maxma 60 rootstocks on different days after water stress application.

	Days after water stress application						
	-2	1	3	5	8	12	
Inoculants	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	SPAD
AG-97	0.78	0.81	0.78 a	0.77	0.78	0.79	25.88
AG-30	0.76	0.78	0.79 a	0.77	0.78	0.79	21.58
CC	0.75	0.79	0.77 ab	0.76	0.76	0.78	18.9
CC+AG-97	0.77	0.8	0.74 b	0.75	0.76	0.75	24.59
MAF	0.76	0.81	0.77 ab	0.77	0.78	0.78	22.51
Control	0.77	0.8	0.76 ab	0.77	0.77	0.78	24.59
p value	0.059	0.517	0.010	0.130	0.335	0.093	0.363
Irrigation							
Wet	0.77	0.81	0.78	0.77	0.78 a	0.79	24.77
Dry	0.77	0.80	0.77	0.77	0.77 b	0.78	21.21
p value	0.945	0.45	0.144	0.941	0.030	0.356	0.076
Interaction							
Irrigation x Inoculants	ns	ns	ns	ns	ns	ns	ns

*Different lowercase letters indicate significant differences according to the Tukey test ($P < 0.05$).