Bacterial consortia inoculation mitigates the water shortage and salt stress in an avocado (*Persea americana* Mill.) nursery

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Abstract

Chile is among the largest avocado producers worldwide; however, currently, its production has diminished mainly attributed to abiotic stresses such as drought and soil salinity. Here, we evaluated the contribution of halotolerant bacterial consortia to water shortage and salt stress tolerance of avocado seedlings (*Persea americana* Mill.) under field conditions. Inoculation trials were conducted in a commercial nursery to study the effects of two endophytic (C1 and C2) and two rhizosphere (C3 and C4) halotolerant bacterial consortia on growth, biomass, superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) of avocado seedlings under salt (2% NaCl) stress and water shortage (50% less irrigation). Our results revealed that avocado inoculation with C4 significantly ($P \leq 0.05$) increased aerial and root length; aerial and root fresh weight and chlorophyll content of salt-stressed seedlings; and the aerial length and root fresh weight of seedlings under water shortage. Similarly, the C4 significantly ($P \leq 0.05$) increased SOD activity in leaves of both the control and seedlings grown under salt stress and water shortage and also decreased TBARS content in leaves of control plants and of seedlings grown under salt stress. Whereas, C3 increased significantly ($P \leq 0.05$) aerial and root length and root fresh weight of salt-stressed seedlings; and also increased the trunk diameter and chlorophyll content of seedlings under water shortage. Similarly, C3 significantly ($P \leq 0.05$) stimulated SOD activity of leaves as compared to the control seedlings and also reduced the TBARS content of leaves and roots of avocado seedlings under salt stress. In contrast, the endophytic consortia were less efficient than rhizosphere consortia. Thus, C1 only increased the trunk diameter and chlorophyll content of salt-stressed seedlings and C2 increased the chlorophyll content of avocado seedlings under water shortage. Our study showed the favorable effect of bacterial

inoculation on avocado nursey in nursery conditions under water shortage and salt stress, and identified consortia that potentially could be used as avocado biofertilizers.

Keywords: Bacterial consortia; avocado; water shortage; salinity; stress

1. Introduction

Water shortages and decreases in water quality are increasingly affecting crop production in world areas where crops are grown in arid and semi-arid regions in the globe. More than 800 million ha of land are now salt affected, corresponding to 20% of the world's irrigated lands producing an estimated 40% of the world's food supply (Munns and Tester, 2008; Zahir et al., 2009). In this context, avocado tree (*Persea americana* Mill.) is the most salt-sensitive of cultivated fruit trees, and has a high water requirement of some 4 acre feet of water per year for normal production (Bernstein and Meiri, 2004; Chartzoulakis et al., 2002). Chile is among the largest avocado producing and exporting countries worldwide. As a result of rapid market growth, the area planted with this crop has increased from 26,731 ha in 2005 to 31,727 h in 2014. However, despite this increased planting, Chilean avocado fruit yields have decreased during recent years, from 188,604 t in 2005 to 144,742 t in 2014 (Muñoz, 2015) due to drought conditions that have been affecting central Chile, and increased reliance on saline groundwater supplies. Water shortages in central Chile are predicted to become increasingly severe, with long-term climate estimations predicting a decline of 20-25% in rainfall by 2040 (Neuenchwander, 2010). Therefore, it is imperative to develop strategies to mitigate negative effects of environmental stresses on avocado plants to increase yields under changing climate conditions expected to occur with global warming.

Physiologically, both water shortage and salinity cause decrease in the soil water potential, leading to diminished root growth, water and nutrient uptake, and subsequent lower plant growth and crop yields (Munns and Tester, 2008; Khan et al., 2014). As a direct consequence of these and other environmental stresses, plants produce increased

amounts of ethylene that generates so-called 'stress-ethylene'. The increased ethylene levels cause root growth inhibition and initiation of senescence in a feedback loop that finally leads to plant death. Simultaneously, abiotic stress leads to oxidative stress due to the increasing levels of reactive oxygen species (ROS) as a direct result of the imbalance in electron transport rates and metabolic consumption activity of reducing equivalents in the plant cells (Kasim et al., 2012). These ROS react with several macromolecules including chlorophyll, proteins, DNA and lipids, leading to peroxidation of membrane lipids, which is used as a general indicator of stress-induced damage at the cellular levels (Kasim et al., 2012).

A sustainable strategy to alleviate the negative consequences of stress on crops is the inoculation with plant growth-promoting bacteria (PGPB). Most PGPB produce indole acetic acid (IAA), a phytohormone that can directly increase root growth, allowing enhanced access to water and nutrients required by the host plant (Patten and Glick, 2002). Many PGPB strains also synthesize the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD). The ACCD catalyzes the hydrolysis of the immediate ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) to α -ketobutyrate and ammonia. Therefore, ACCD-producing PGPB avoid the 'stress-ethylene' that otherwise would generate root growth inhibition of plants subjected to environmental stresses (Penrose and Glick, 2003). However, the beneficial effects of PGPB have generally been validated only in laboratory and/or greenhouse trials. In the field, results from studies examining the use of PGPB have been less consistent. This inconsistency in field results is likely associated with many factors including poor inoculum survival due to starvation, predation, and competition with indigenous bacteria, difficulty in obtaining good distribution of the inoculant in the soil profile, and variation in fitness in relation to variations in soil properties, among others. Altogether,

this has limited the widespread commercial adoption of PGPB biotechnologies. Inoculation trials with PGPB under field conditions are thus essential to assess inoculant performance and their potential ability to decrease the effects of environmental stresses on crop plants.

Previously, we isolated some endophytic and rhizosphere bacterial strains from avocado trees, and formulated four consortia with IAA- and ACCD- producing halotolerant bacteria (Table 1). Inoculation trials under greenhouse conditions with these bacterial consortia showed that inoculants improved the emergence, biomass, growth and the superoxide dismutase (SOD) activity of wheat seedlings subjected to salt stress (Barra et al., 2016). Therefore, the objective of the present study was to validate the contribution of these bacterial consortia on growth and antioxidant activity of avocado trees grown under salt stress and water shortage conditions in a commercial nursery from central Chile.

2. Materials and Methods

2.1. Halotolerant bacterial consortia and inocula preparation

Six endophytic and six rhizosphere bacterial strains were selected from a previous study performed by Barra et al. (2016). These strains were isolated from sterilized tissues and rhizosphere soil of avocado plants grown in a commercial orchard 'Jorge Schmidt & Company Ltd.' located in Region de Valparaiso, Chile (32° 47'S and 70° 47'W). The 16S rRNA gene of each strain was amplified by PCR with the universal primers set 27f (AGA GTT TGA TCC TGG CTC AG) and 1492r (TAC GGY TAC CTT GTT ACG ACT T) according to Peace et al. (1994). The resulting DNA fragments

were purified and sequenced by MacroGen Sequencing Service (MacroGen Inc., Seoul, Korea), using an ABI PRISM® 3730XL capillary sequencer (Applied Biosystems, Foster City, CA). Sequence data derived from this study were deposited in GenBank as Accession Nos. KR066642 - KR066653. The twelve selected bacterial strains were used to formulate four bacterial consortia (Table 1), with three IAA- and ACCD- producing halotolerant bacteria each, as follows: Consortium C1 was formulated with *Enterobacter* sp. st. 12, *Enterobacter* sp. st. 126 and *Serratia* sp. 73 isolated from endosphere of avocados and featured high IAA production and ACCD activity. Consortium C2 was formulated with *Serratia* sp. st. 16, *Pseudomonas* sp. st. 33 and *Microbacterium* sp. st. 35, all also isolated from avocado endosphere tissues. The C2 was characterized by lower IAA production and lower ACCD activity as compared to C1. Consortium C3 was formulated with strains from avocado rhizosphere and consisted of *Enterobacter* sp. st. 172, *Enterobacter* sp. st. 198 and *Enterobacter* sp. st. 206. Among all four consortia examined, C3 had the highest measured IAA production and ACCD activity. Consortium C4 was formulated with rhizosphere soil isolates *Achromobacter* sp. st. 249, *Serratia* sp. st. 343 and *Enterobacter* sp. st. 357 and had with lower IAA production and lower ACCD activity as compared to C3, the other rhizosphere consortium (Barra et al., 2016).

Inocula were prepared by growing the twelve bacterial strains separately in 2 L flasks containing 800 ml LB broth at 30°C for 16 h under shaking (120 rpm). The cells were collected by centrifugation ($6,000 \times g$ for 15 min at 4°C), washed twice with sterile saline solution (0.85%) and then resuspended in skim milk (as a cryoprotectant). Bacterial cells were freeze -dried separately with a Freeze Dry Systems (FreeZone, Labconco) and the lyophilized bacterial strains were stored at room temperature for further inoculation assays.

2.2. Avocado tree nursery trial

A field assay was conducted in an outdoor nursery of a commercial orchard (Jorge Schmidt & Company Ltd.) located near Llay Llay town, Region de Valparaiso, Chile (32°51'19.5"S and 71°00'22.0"W) (Figure 1). The company is currently the largest Chilean producer of avocados cv 'Hass', exporting about 85% of their production to the European and USA market and 15% remaining in the local market.

One hundred and fifty vigorous avocado seedlings cv 'Hass' recently grafted on 'Mexícola' rootstocks of ~30 cm were selected from the commercial nursery. The seedlings were randomly pooled in three groups of fifty seedlings each group. The seedlings were planted in plastic 5-L bags filled with sandy loam soil and irrigated according to company protocol. All seedlings were well irrigated to field capacity and maintained under the same regimen for one month before inoculation with the consortia. The salinity of the water used for irrigation measured as electric conductivity was 0.654 dS m⁻¹. Plant growth was restricted to the period between March of 2014 and March of 2015 with annual average temperature and total rainfall of 14.4 °C and 236.8 mm in the region.

For the inoculation process, the twelve lyophilized strains were resuspended in sterile distilled water to reach a final bacterial load of ~10⁸ CFU ml⁻¹ according to agar plate-counting methods and the three strains belonging to each of the four consortia (C1; C2; C3; and C4) were proportionally mixed (1:1:1). Roots of ten avocado seedlings of each group (treatment) were inoculated with freshly prepared inoculum once a month by drench application of 100 mL of each bacterial consortia. Responses to the inoculation treatments were compared to a control group consisting of ten

uninoculated plants. Each group was subjected to different treatments for one year as follows: i) Control; seedlings irrigated according to company protocol (irrigated to field capacity twice a week); ii) Salt stress treatment; seedlings irrigated once a month with 340 mM (2%) NaCl solution for one year (Castro et al., 2009) and iii) Water shortage treatment; the seedlings were irrigated at half normal irrigation rates.

The trees were arranged in the nursery using a completely randomized factorial design. The experimental treatments were: i) four bacterial consortia (C1, C2, C3 and C4) plus an uninoculated control (with 10 replicates each); ii) one salt stress treatment, one water shortage treatment plus well-irrigated control without added salt.

2.3. Growth parameters determination

After one year, leaf chlorophyll contents were measured and data were collected on the trunk diameter, shoot and root length, and shoot, and root biomass. Immediately prior to harvest, leaf chlorophyll contents were quantified for the first fully expanded leaves by nondestructive sampling technique using a hand-held chlorophyll meter SPAD-502 (Minolta, K. Arano & Co. Ltd, Tokyo, Japan). This instrument provides a relative measurement of leaf chlorophyll through the evaluation of the changes of the transmittance in the 600–700 nm region of the visible spectra and in the near infrared region (San-Francisco et al., 2005). Mean leaf chlorophyll content for each treatment was derived from three readings taken at the base, middle and tip of leaf 1, 2 and 3.

Trunk diameters were measured 2 cm above the graft union of each tree using dial calipers according Mickelbart & Arpaia (2002). For biomass determinations, plants were carefully removed from the pots and the root surfaces were carefully cleaned several times first with tap water and then with distilled water. Each plant was divided

into root and shoot portions, and growth parameters including root and shoot length and biomass were measured for the harvested avocado plants. Fine roots (<1 mm) and fully expanded leaves were carefully separated, frozen in liquid nitrogen and kept at 80°C to determine superoxide dismutase (SOD) activity and lipid peroxidation.

2.4. Superoxide dismutase activity

To quantify SOD activity, fresh samples of both roots and leaves of avocado seedlings were pulverized by means of a pestle and mortar in liquid nitrogen. The samples were resuspended with 50 mM potassium phosphate buffer (pH 7.0), then were centrifuged for 15 min at 11,000 g (4°C), and posteriorly the supernatant was collected as a crude protein extract. The SOD activity was measured by its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Donahue et al. 1997). One enzyme unit was defined as the enzyme quantity that inhibited the reduction of NBT by 50%. The SOD activity was expressed on a protein basis. Protein content was determined in the crude extract using the Bradford's method (Bradford, 1976).

2.5. Lipid peroxidation measurements

Lipid peroxidation was quantified by monitoring the thiobarbituric acid reactive substances (TBARS) in shoot tissues following the modified protocol of Du and Bramlage, (1992). For this purpose, 30 mg of root and leaf tissues were homogenized and macerated with 500 ml 0.2% trichloroacetic acid (TCA) and then centrifuged for 5 min at 10,000 g. Later, 200 μ l of supernatant was mixed with 800 μ l 0.5% thiobarbituric acid (TBA) in 20% TCA, the mixture was incubated at 95 °C for 30 min, and then, it

was rapidly cooled. The absorbance was measured at 440, 532 and 600 nm, to correct the interference generated by TBARS-sugar complexes, where the absorbance at 532 nm is the maximum absorbance of the TBARS complex, at 440 nm corresponds to the absorbance of sucrose, and absorbance at 600 nm represents the correction for nonspecific turbidity. Finally, the malondialdehyde (MDA) concentration was estimated by using an extinction coefficient of $84.152 \text{ M}^{-1}\text{cm}^{-1}$.

2.6. Statistical analysis

The analysis of variance was performed with two factors (3×5): stress treatment (salt, water shortage and control) and consortia inoculation (C1, C2, C3, C4 and control), using ten replications per treatment. Data obtained from each treatments were analyzed statistically using the one-way ANOVA followed by post hoc Duncan's test for multiple comparisons. Difference at $P \leq 0.05$ was considered as significant between treatments. The analyses were conducted using the IBM SPSS 21 software.

3. Results

3.1. Vegetative growth

The effects of the consortia inoculation on growth and biomass accumulation for roots and aerial parts of avocado seedlings that were subjected to salt stress for one year are presented in Figure 2. In general, aerial and root lengths of seedlings under salt stress were significantly ($P \leq 0.05$) decreased by 23.0% and 27.9%, respectively, compared with the well-irrigated seedlings in the control treatment. Avocado seedlings grown

under water shortage had significantly ($P \leq 0.05$) 28.2% and 32.0% shorter aerial part and root lengths, respectively, than control seedlings grown in nonsalinized soil (Figure 2a). With respect to biomass accumulation, only the fresh weights of aerial part were significantly ($P \leq 0.05$) lower by 25.5% in salt stress treatment as compared with the control. Whereas, avocado seedlings grown under water shortage, showed significantly ($P \leq 0.05$) lower fresh weights of aerial part and roots by 36.4% and 34.0%, respectively, compared with the well-irrigated seedlings in the control treatment (Figure 2b).

With respect to the inoculation of the trees with bacterial consortia, the results were variable depending on the compositions of the individual consortia for all of the growth parameters that were measured. Of the four consortia, the C3 and C4 were the more efficient increasing the growth and biomass accumulation of avocado plants under both treatment (Figure 2a and 2b). In general, inoculated seedlings showed greater root lengths than uninoculated avocado seedlings. Therefore, C3 and C4 increased significantly ($P \leq 0.05$) by 34.4% and 64.4% the root lengths and by 19.8% and 20.6% the aerial parts of avocado seedlings under salt stress in comparison to the uninoculated control trees. In water shortage treatment, only C4 increased significantly ($P \leq 0.05$) by 26.2% the aerial lengths of avocado seedlings in comparison to the uninoculated control trees. In relation to biomass, the inoculated seedlings subjected to salt stress and water shortage conditions also had greater aerial and root fresh weights than uninoculated seedlings. However, significant differences were only found for some bacterial consortia, especially C4. Thus, compared to the inoculated control, the consortium C4 significantly increased ($P \leq 0.05$) the aerial and root fresh weights of seedlings under salt stress by 43.1% and 59.4%, respectively, and the root fresh weights of seedlings under water shortage by 68.4%. Similarly, the C3 significantly increased ($P \leq 0.05$) the root fresh weights of seedlings under salt stress by 54.6%.

On the other hand, the results illustrated in Figure 2c shows that trunk diameters of well-irrigated control and seedlings growing under salt stress and water shortage were not significantly different ($P \leq 0.05$). Although in general trees inoculated with the bacterial consortia had greater trunk diameters than the uninoculated seedlings, only C1 and C3 were able to significantly increase ($P \leq 0.05$) the trunk diameters of seedlings growing under salt stress and water shortage by 15.9% and 16.1%, respectively, in comparison to the control treatment.

3.2. Chlorophyll content of avocado seedlings

The chlorophyll content of inoculated avocado seedlings grown under salt stress and water shortage are illustrated in Figure 3a. Thus, the results show that chlorophyll contents were significantly ($P \leq 0.05$) decreased in avocado seedlings grown under salt and water shortage by 22.6% and 21.1%, respectively, as compared with uninoculated seedlings. All inoculated seedlings with the bacterial consortia had greater chlorophyll contents than the uninoculated seedlings under both salt stress and water shortage. However, in salt stress, only C1 and C4 were able to increase significantly ($P \leq 0.05$) the chlorophyll content by 23.4% and 25.4%, respectively. In contrast, in water shortage, only the C2 and C3 increased chlorophyll content significantly ($P \leq 0.05$) by 22.1% and 19.6%, respectively, when compared to the uninoculated control treatment.

3.3. Superoxide dismutase (SOD) activity

The effects of salt stress and water shortage on SOD activity of root and leaves of inoculated avocado seedlings are illustrated in Figure 3b. The SOD activities of avocado

leaves were not significantly different ($P \leq 0.05$) between the well-irrigated control treatment and avocado seedlings that were subjected to salt and water shortage. In contrast to results with leaf tissues, root SOD activities of uninoculated avocado seedlings subjected to salt stress were significantly ($P \leq 0.05$) greater than the control by 48%. Inoculation of avocado plants with the selected bacterial consortia showed variable results. Inoculation with C4 significantly ($P \leq 0.05$) increased SOD activity in leaves by 68%, 95% and 43% of both the control and seedlings grown under salt stress and water shortage, respectively. Similarly, C3 significantly stimulated SOD activity ($P \leq 0.05$) as compared to the control seedlings by 70%. The other tested consortia also appeared to increase SOD activities of the leaves, as compared to control seedlings and seedlings grown under water shortage conditions, although no significant differences were found with respect to the uninoculated control. Curiously, the root SOD activities of avocado seedlings under stress were not significantly affected ($P \leq 0.05$) by bacterial inoculation. Only C1 increased the SOD activity of avocado seedling in the control treatment by 93%.

3.4. Lipid peroxidation

The effects of salt stress and water shortage treatments on lipid peroxidation of leaves and roots of avocado seedlings were determined by measuring changes in the content of thiobarbituric acid reactive substances (TBARS). The results illustrated in Figure 3c show that TBARS in both leaves and roots of avocado seedlings subjected to salt stress were significantly ($P \leq 0.05$) increased by 482% and 101% as compared with the well-irrigated control. Although TBARS contents in leaves and roots of avocado seedlings under water shortage stress were increased by 54%, there were no significant

differences ($P \leq 0.05$) with seedlings in the well-irrigated control treatment. With respect to bacterial inoculation effects, C4 was again the most efficient bacterial consortia for decreasing the effects of stress as determined by TBARS content (Figure 3c). In this context, C4 decreased significantly ($P \leq 0.05$) TBARS content in leaves of control plants and of seedlings grown under salt stress by 62% and 65%, respectively. Similarly, C3 significantly reduced ($P \leq 0.05$) the TBARS content of leaves and roots of avocado seedlings under salt stress by 31% and 53%.

4. Discussion

Plants are broadly variable in their sensitivity to environmental stresses with avocado trees displaying the highest sensitivity to salinity stress of cultivated fruit trees (Oster et al., 2007). Our results confirmed that both water shortage and salt stress significantly affected root and aerial length, aerial fresh weight and chlorophyll content of avocado seedlings. In contrast, under the conditions of this experiment, only water shortage reduced the root fresh weights. In agreement with these findings, Bernstein and Meiri (2004) determined that root and shoot growth of avocado plants decreased by 43% and 10%, respectively, when irrigated with 15 mM NaCl. With respect to water shortage, there are conflicting results in the literature. Thus, a field experiment in avocado plants carried out by Oster et al. (2007) determined that water shortage had no significant effects on the growth and biomass accumulation of mature avocado trees: while, yield was significantly ($P \leq 0.05$) decreased. Whereas, Chartzoulakis et al. (2002) determined in a greenhouse assay that moderate water stress reduced significantly ($P \leq 0.05$) the total plant leaf area by 69% and the total plant dry weight by 80% of avocado plants cv. 'Hass'. On the other hand, it is well documented that plants exposed

to stressful environments often have decreased chlorophyll contents, such that this parameter is widely used as an index to indicate the abiotic sensitivity level in plants (Nadeem et al. 2006; Zahiret al. 2009; Qiu et al. 2014). Similar to our findings, decreases in the chlorophyll contents of avocado plants under salt stress has previously been reported by Mickelbart and Arpaia (2002). With respect to trunk diameter, our results showed that trunk diameters were not significantly ($P \leq 0.05$) affected for trees subjected to salinity and water shortage. Similar effects were described by Silber et al. (2013), who determined in a field study that the trunk diameters of avocado plants were not affected by irrigation water shortage.

Our results also revealed that root and leaf TBARS were increased under salt stress by two and five fold, respectively, as compared with the well-irrigated control, demonstrating that the integrity of the cell membranes was seriously damaged. It should be noted although the root and leaf TBARS contents of seedlings under water shortage were over 54% greater than the control, this difference was not significant. Increased SOD activity is correlated with improved protection from injury associated with oxidative stress (Qiu et al., 2014). Contrary to expectations, in the present study no significant differences were found in the SOD activity between control and stressed seedlings. Only the root SOD activities of salt-stressed plants were significantly increased. These results could be due to direct damage of proteins integrity, and therefore SOD integrity, produced by ions. However, variation in antioxidant concentrations are dependent on the severity and duration of the stress and the species and age of the plant. Differences in protective enzyme activities are known for a number of species (Abogadallah, 2010; Hernandez and Almansa, 2002). For example, overexpression of SOD occurs in stressed wheat seedlings (Barra et al., 2016; Ruan, 2002; Sairam and Srivastava, 2002; Sairam et al., 2005). Whereas, others researchers

have determined a decrease in SOD activity in wheat seedlings under salt and drought stresses (Gallé et al., 2013; Qiu et al., 2014). In contrast, Yu and Rengel (1999) described that SOD activity in lupin was not affected by salt stress, whereas it was increased by 17% in plants subjected to drought stress, indicating that different mechanisms may be involved in oxidative stress injury caused by drought and salt.

Inoculation of avocado seedlings with the bacterial consortia had variable but several significant effects on growth and biochemical markers of stress in the plant tissues. Some of the selected consortia were able to enhance growth, biomass accumulation and chlorophyll content of stressed seedlings. Thus, the consortia formulated with rhizobacteria, C4 and C3, were the more efficient alleviating the stress effects. Thereby, C4 significantly increased aerial and root length; aerial and root fresh weight and chlorophyll content of salt-stressed seedlings. The C4 also increased aerial length and root fresh weight of seedlings under water shortage. Whereas, C3 increased aerial and root length and root fresh weight of salt-stressed seedlings; and also increased the trunk diameter and chlorophyll content of seedlings under water shortage. In contrast, the endophytic were less efficient than rhizosphere consortia. Thus, C1 only increased the trunk diameter and chlorophyll content of salt-stressed seedlings and C2 increased the chlorophyll content of avocado seedlings under water shortage.

The observed growth promotion of avocados seedlings by these bacteria in the present study can be attributed to two main reasons. First, the IAA released by the isolates may directly stimulate root cell elongation and lateral root growth, increasing the root surface area, and consequently, the ability to acquire water and nutrients (Marques et al., 2010; Vessey, 2003). In addition, previous studies have also shown that application of exogenous IAA to plants directly stimulates chlorophyll production (Hayat et al., 2001), probably by improved iron acquisition. Siderophore production

may also contribute to iron mobilization in the rhizosphere. Secondly, in accord with general models, the avocado plants subjected to stress conditions will accumulate and cycle ACC in the rhizosphere, which is released in the root exudates and reabsorbed as with organic acids. This temporary extracellular ACC provides a nitrogen source for proliferation of both inoculated and native ACCD-producing bacteria. The ACCD-producing bacteria act as a sink for ACC, the immediate biosynthetic precursor of ethylene, thereby decreasing plant ethylene levels and its detrimental effects on plant development (Glick et al., 2007).

Our findings also suggest that bioaugmentation with certain PGPB introduced through the irrigation water may enhance the oxidative stress tolerance of avocado seedlings. Here, inoculation with C4 significantly increased SOD activity and decreased TBARS content in leaves of salt-stressed seedlings; increased the leaf SOD activity of plants under water shortage; and decreased the TBARS content of leaves from nonstressed seedlings. These results clearly demonstrated ability of C4 to ameliorate stress by inducing physiological protection of plants against oxidative damage, being able to decrease by over 60% the lipid peroxidation both in control and salt-stressed plants. This effect is attributed to SOD activity, which was widely increased in both treatments. In addition, lower cellular damage would have induced the higher growth and biomass accumulation observed in this study.

Despite these results, we cannot conclude the degree to which the increase in SOD activity is a consequence of the improvement in stress tolerance (led by stress ethylene reduction), or vice versa, whether the improvement in salt stress tolerance is a consequence of the higher SOD activity. A third option would be that the bacterial consortia independently stimulate both mechanisms. There is contradictory evidence regarding the mechanism by which the bacterial inoculation improve the antioxidant

enzymes activity, such as SOD. In this context, Wang et al. (2012) determined that inoculation with bacterial consortia (formulated with *Bacillus cereus*, *B. subtilis* and *Serratia* sp.) conferred induced systemic tolerance to drought stress in cucumber plants, by protecting plant cells, maintaining photosynthetic efficiency and root vigor and increasing some of antioxidant activities, without involving the action of ACCD to lower plant ethylene levels. Whereas, Gururani et al. (2012) showed by quantitative PCR approach that *Solanum tuberosum* inoculated with *Bacillus* spp. enhanced the gene expression of different ROS-scavenging enzymes of plants exposed to heavy-metal and salt stress. Whereas, Li et al., 2013 determined by proteomic approach that inoculation of *Cucumis sativus* with ACCD-producing bacteria *P. putida* UW4 generated downregulation of SOD enzyme under anoxic conditions. Despite this, our results are consistent with previous research showing that PGPB inoculation increase the SOD activity and decrease TBARS of cotton plants under salt stress conditions inoculated with *Klebsiella oxytoca* (Wu et al., 2014) and soybean growing under drought stress inoculated with *Azospirillum brasilense* and *Azotobacter chroococcum*.

It is noteworthy that in a prior research, the bacterial consortia used here significantly ($P \geq 0.05$) increase the emergence, biomass, growth and SOD activity of wheat seedlings under salt stress (Barra et al., 2016). In contrast to present study, the previous results obtained in wheat plants showed that endophytic consortia and C3 were more efficient decreasing the salt stress effects than C4 (Barra et al., 2016). The lower effects of endophytic consortia observed in avocado plants would be because were inoculated in the rhizosphere soil where endophytic bacteria were less competitive than rhizobacteria. Further studies using different inoculation methods are necessary to confirm this hypothesis.

Consortium C4, the most effective bacterial consortium, was formulated with halotolerant rhizobacterial strains identified as *Enterobacter* sp., *Achromobacter* sp., and *Serratia* sp. Some earlier studies have described the occurrence of *Enterobacter* spp. producers of IAA and ACCD associated with numerous plant species, such as *Piper nigrum*, *Citrus reticulata* and *Populus* spp. showing also plant growth-promoting properties (Jasim et al., 2013; Thokchom et al., 2014; Taghavi et al., 2009). Thus, Thokchom et al. (2014), in a greenhouse experiment determined significantly increased aerial length, and aerial and root dry biomass of *C. reticulata* seedlings treated with *Enterobacter* spp. compared with untreated seedlings. Similarly, previous investigations have reported the association of *Achromobacter* spp. producer of IAA and ACCD with *C. reticulata* and *Catharanthus roseus* (Thokchom et al., 2014; Karthikeyan et al., 2012). Thus, Barnawal et al., (2012) reported that *Achromobacter xylosoxidans* producer of IAA- and ACCD improved the growth and biomass accumulation of *Ocimum sanctum* subjected to waterlogging stress. In addition, some studies have described both endophytic and rhizosphere *Serratia* spp., with PGP capacity, associated with some plants such as tomato and peppers (Amaresan et al., 2011), bean (Saïdi et al., 2013), poplar trees (Taghavi et al., 2009), and rice (Gyaneshwar et al., 2001). Furthermore, Zahir et al., (2009) determined that ACCD-producing halotolerant *Serratia proteamaculans* significantly improved the growth and yield of wheat plants under salt-stress. Independently of IAA production and ACCD activity, the taxonomic affiliations of our isolates have been frequently classified as PGPB.

In general plant inoculation studies with bacterial consortia have been carried out under greenhouse conditions. In this way, it is important to note that isolates used in this study have been tested in avocado seedlings grown in a nursery located outdoors and within the commercial avocado orchard, and therefore, with the same

environmental conditions of avocado crops. For these reasons, the results of our study are a close approximation to actual field conditions. The present study is the first showing the positive effects of bacterial inoculation on growth of any tree fruit growing in a nursery under stress conditions. However, with the final objective of formulating a biofertilizers product, the next step would be to produce inoculants testing different vehicles for soil inoculation. Finally, the selected consortia still need to be tested in actual field trials with bearing trees to determine the survival of the isolates in the rhizosphere, and their economic effects on avocado growth under stress conditions.

In conclusion, our study shows that halotolerant IAA- and ACCD-producing bacteria, isolated from adult avocado trees, are able to mitigate the effects of water shortage and salt stress on avocado tree seedlings grown in a commercial nursery under field conditions. In this way, the selected IAA- and ACCD-producing consortia increased SOD activity, which resulted in lower oxidative damage and consequently, higher growth, biomass accumulation and chlorophyll contents. Therefore, our isolates could be used as suitable bioinoculants for avocado plants subjected to water scarcity or grown under salt affected area. To our knowledge, this is the first study showing the beneficial effect of bacterial inoculation on growth of avocado plants under both water shortage and salt stress conditions. Our findings in nursery conditions endorse the need for further field studies on avocado yields of with mature orchards, and hold promise for enhancing avocado trees tolerance under increasingly stressful conditions expected from global warming.

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Figure 1. Field nursery assay carried out on commercial orchard of avocado ‘Jorge Schmidt & Company Limited’ located in the vicinity of the Llay Llay town, Valparaíso Region, Chile (32°51'19.5"S and 71°00'22.0"W).

Figure 2. Effects of water shortage and salt stress treatments on (a) growth (length); (b) biomass accumulation (fresh weight) of both root and shoot and (c) trunk diameter of avocado seedlings inoculated with four selected bacterial consortia. Vertical bars represent average ($n=10$) $\pm$ standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

Figure 3. Effects of water shortage and salt stress treatments on (a) leaf chlorophyll content (b) root and shoot superoxide dismutase (SOD) activity and (c) root and shoot TBARS content of avocado seedlings inoculated with four selected bacterial consortia. Vertical bars represent average ($n=10$) $\pm$ standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

Figure 1. Barra *et al*

Figure 2. Barra *et al.*

Figure 3. Barra *et al.*

Table 1. Characteristics of selected halotolerant IAA- and ACCD-producing strains (Barra et al., 2016).

Consortia	Isolate	Accession no.	IAA ^{ad}	Salt tolerance ^b	ACCD activity ^{cd}
Endophytic consortia					
Consortium 1 (C1)	<i>Enterobacter</i> sp. 12	KR066645	8.4 ± 1.6	5.0	2.70 ± 0.18
	<i>Enterobacter</i> sp. 126	KR066643	13.5 ± 0.9	10.0	2.60 ± 0.42
	<i>Serratia</i> sp. 73	KR066653	14.6 ± 2.1	5.0	2.06 ± 0.18
Consortium 2 (C2)	<i>Microbacterium</i> sp. 35	KR066649	3.8 ± 0.3	10.0	0.91 ± 0.08
	<i>Pseudomonas</i> sp. 33	KR066650	4.7 ± 1.1	7.5	0.75 ± 0.04
	<i>Serratia</i> sp. 16	KR066652	6.1 ± 1.4	5.0	0.65 ± 0.05
Rhizosphere consortia					
Consortium 3 (C3)	<i>Enterobacter</i> sp. 172	KR066644	63.2 ± 0.9	7.5	3.63 ± 0.11
	<i>Enterobacter</i> sp. 206	KR066647	17.3 ± 2.3	7.5	3.54 ± 0.75
	<i>Enterobacter</i> sp. 198	KR066646	21.8 ± 2.7	7.5	3.44 ± 0.37
Consortium 4 (C4)	<i>Enterobacter</i> sp. 357	KR066648	9.6 ± 0.4	5.0	0.78 ± 0.18
	<i>Serratia</i> sp. 343	KR066651	10.8 ± 0.5	5.0	0.25 ± 0.03
	<i>Achromobacter</i> sp. 249	KR066642	9.2 ± 0.5	5.0	0.21 ± 0.03

^a expressed as µg ml⁻¹^b expressed as percentage (%) of NaCl, which did not affect bacteria growth rate^c expressed as µmol α-ketobutyrate mg⁻¹ protein h⁻¹. Values are means ± SE of three experiments^d values are means ± SE of three experiments