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Promotion of sunflower growth under saline water irrigation by the inoculation of beneficial microorganisms

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ABSTRACT

Soil salinization and fresh water scarcity are amongst the main environmental/agricultural problems, with serious consequences to plant productivity. Amelioration with microorganisms can enhance plant performance under salt conditions. The aim of this work was to evaluate the role of beneficial rhizospheric microorganisms on the growth of sunflower plants irrigated with salinized water with particular attention to nutrient balance and biochemical responses. Sunflower seedlings were inoculated with the arbuscular mycorrhizal fungi *Rhizophagus irregularis*, the rhizobacteria *Chryseobacterium humi* ECP37^T, or the bacterial endophyte *Ochrobacterium haematophilum* ZR3-5, and with a mixed inocula of those microorganisms. Plant growth, nutrient accumulation and lipid peroxidation in plant tissues, and the activity of soil enzymes, were evaluated. Irrigating sunflower plants with saline water resulted in decreases in growth and negative effects in salt stress markers, however the application of bioinoculants enhanced biomass production and accumulation of K^+, Mg^{2+}, Ca^{2+}, N and P, reduced Na^+ levels in tissues and increased plant antioxidative response.

This study contributes to devise inoculation strategies for sunflower cultivation in areas prone to salinization.

1. Introduction

One of the major developing environmental/agricultural threats is the increase of soil salinization and sodification (GFSCC, 2010). According to the FAO Land and Plant Nutrition Management Service, more than 6% of the world's land is salt-affected, which has negatively impacted global agricultural productivity (Munns and Tester, 2008). Additionally, the scarcity of fresh water is becoming a constraint all over the globe (Ridoutt and Pfister, 2010). The quality of water for irrigation affects soil physicochemical conditions and consequently crop yield, causing loss of fertility, as plants facing salt stress suffer alterations in their physiology that adversely affect their growth (Parihar et al., 2015). High salt levels in soil and in the irrigation water induce osmotic stress, by decreasing water absorption capacity of root systems and also cause ionic stress due to the high accumulation of salt ions in plant cells (Kohler et al., 2009). The entry of Na^+ and Cl^- into the cells causes severe ion imbalance and excess uptake might cause injury of several significant life-supporting processes including photosynthesis, protein synthesis, energy and lipid metabolism (Parida and Das, 2005). In addition, high Na^+ concentration inhibits uptake of K^+ and Ca^{2+}, rendering the plants more prone to nutritional deficits and ionic disorder lowering its growth and survival yields (Sivritepe et al., 2003).

However, plants have several salt tolerance strategies which are linked to regulation and compartmentalization of ions (e.g. ion exclusion and/or accumulation in vacuoles), synthesis of osmolytes, induction of antioxidant enzymes and plant hormones and changes in photosynthetic pathways (Parida and Das, 2005; Parihar et al., 2015).

The most produced and widely used crops for human/animal nutrition, such as cereals, forages or horticultural crops, are relatively susceptible to high concentrations of salts, either dissolved in irrigation water or present in rhizosphere (Ondrasek et al., 2011). Sunflower has shown to be moderately tolerant to

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salinity (Katerji et al., 2000), although with differences among genotypes (Wahid et al., 1999). This plant species has a great value for food production, but also for biomass and biofuels, which are considered some of the most promising renewable energy options.

A possible strategy to decrease the detrimental effects of salinity on crop yield and productivity may be the inoculation of crops with beneficial microorganisms that can enhance plant productivity through lowering stress caused by salinity. These microorganisms increase plant growth through various forms, such as modulating the level of ethylene, raising the solubilisation of nutrients for plant uptake and increasing its absorption, inducing root system modifications, production of phytohormones and synthesis of antibiotics (Vessey, 2003). Amongst these organisms are the plant growth promoting rhizobacteria (PGPR) and the endophytic bacteria (EB). Several reports concerning PGPR (Marques et al., 2013; Moreira et al., 2014; Pereira et al., 2015a) and EB (Ma et al., 2011; Pereira and Castro, 2014) showed positive effects on plant growth and stress reduction. These microorganisms have also been pointed out as growth promoter agents in salinized soils (Han et al., 2014; Rojas-Tapias et al., 2012). Ali et al. (2014) reported that EB were able to increase the resistance of tomato plants under salt stress conditions. In addition, the arbuscular mycorrhizal fungi (AMF) which form symbiotic associations with the majority of land plants can hamper plant survival and growth in disturbed ecosystems (Nadeem et al., 2014). It has been reported that AMF can have a positive effect on plants facing high salinity environments (Cho et al., 2006; Estrada et al., 2013a) and when combined with PGPR enhanced growth and nutrient uptake of lettuce growing in salinized soil (up to 0.920 dS m^{-1}) (Kohler et al., 2009). Despite their beneficial effects when single or dual inoculated, to the best of our knowledge this is the first report focusing on the effects of mix inoculation with PGPR, EB and AMF on sunflower plants irrigated with saline water.

Biochemical properties of soil are useful indicators of stress and environmental changes, and usually are related to the presence of enzymes (Silva and Fay, 2012), so an evaluation of their activities could be useful to assess the quality of soils when irrigated with saline water. However, very few studies have focused on the effects of salinization on soil enzymatic activities.

The aim of this work was to evaluate the contribution of AMF, PGPR and EB on sunflower plants growing in soils irrigated with saline water. The effects of different inocula on plant growth and nutrient balance and on the soil enzymes and plant biochemical response were also assessed.

2. Material and methods

2.1. Characterization of beneficial microorganisms

Selected bacterial strains were isolated from a degraded site (Esteiro de Estarreja) with long history of contamination from agricultural and industrial sources (Marques et al., 2007; Pereira et al., 2015b). The strains selected for the present work were identified through 16S rRNA as *Chryseobacterium humi* ECP37^T (B—rhizobacteria) (Pires et al., 2010) and *Ochrobacterium haematophilum* ZR3-5 (E—endophytic bacteria) (Pereira and Castro, 2014) and were isolated from sediments and from the tissues of maize plants growing in a contaminated agricultural soil, respectively. These strains revealed *in vitro* plant growth promoting (PGP) traits, and were reported to enhance plant growth *in vivo* (Marques et al., 2010; Moreira et al., 2014; Pereira and Castro, 2014) and therefore were selected to carry out the present study.

IAA production by the bacterial isolates under salt exposure was measured by the method of Gordon and Weber (1951). Bacteria were grown overnight in trypticase soy broth (TSB) and then aseptically collected by centrifugation at 9000 rpm for 10 min. The bacterial pellet was then incubated at 30 °C for 48 h with 3 ml of phosphate buffer (pH 7.5) with glucose (1%) and different NaCl concentrations (0, 1, 2, 4 g l^{-1}) and 2 ml of L-tryptophan (1%). After incubation, 2 ml of 5% trichloroacetic acid and 1 ml of 0.5 M $CaCl_2$ were added. The solution was centrifuged at 9000 rpm and the supernatant (500 μl) was mixed with 300 μl of Salper solution (2 ml of 0.5 M $FeCl_3$ and 98 ml of 35% perchloric acid). The absorbance of pink color developed after 30 min of incubation in the dark was read at 535 nm with a Shimadzu UV-1603 spectrophotometer.

For assessing the ability to produce NH_3, fresh cultures were inoculated into 10 ml peptone water supplemented with different NaCl concentrations (0, 1, 2, 4 g l^{-1}) and incubated for 48 h at 30 °C; following this, 0.5 ml of Nessler's reagent were added to each tube and development of yellow to brown color was considered as a positive result for ammonia production (Cappuccino and Sherman, 1992).

The screening of hydrogen cyanide production by the bacterial isolates was made by amending nutrient agar with 4.4 g glycine l^{-1} and with different NaCl concentrations (0, 1, 2, 4 g l^{-1}) and streaking the isolates on this modified agar plates; a Whatman no.1 filter paper soaked in a 2% sodium carbonate in 0.5% picric acid solution was placed on top of each plate and plates were sealed and incubated at 30 °C for 4 d after which development of orange to red color indicated HCN production by the isolates (Ahmad et al., 2008).

Bacterial isolates were assayed for siderophores production by inoculating the isolates on Chrome azurol S agar medium supplemented with different NaCl concentrations (0, 1, 2, 4 g l^{-1}); development of a yellow to orange halo around the bacterial growth after incubation at 30 °C for 72 h indicated a positive result for siderophore production (Schwyn and Neilands, 1987).

Phosphate solubilizing activity was assessed in modified National Botanical Research Institute's phosphate (NBRIP) medium amended with 0.5% tricalcium phosphate (Nautiyal, 1999) and different NaCl concentrations (0, 1, 2, 4 g l^{-1}). The presence of a clearing halo around bacterial colonies is indicative for positive phosphate solubilization. For all the above mentioned tests, sterile nutrient broth or agar were used as a control for bacterial growth, and on the control samples no growth was observed.

A commercial AMF product of *Rhizophagus irregularis* purchased from INOQ, GmbH (Germany) was used, consisting of 210 mycorrhizal units per cm^3 of vermiculite (1–2 mm).

2.2. Experimental design

The soil used in this study was an agricultural soil from the North of Portugal (soil properties are presented in Table 1). Soil was collected randomly in the selected area, to a 20 cm depth and milled to <2 mm.

The experiment consisted of a factorial design with 5 microbial treatments (soil only; non-inoculated soil with sunflower (C); soil with *H. annuus* inoculated with the bacteria *C. humi* (B); F—soil with *H. annuus* inoculated with the AMF *R. irregularis* (F); soil with *H. annuus* inoculated with the endophytic bacteria *O. haematophilum* (E) and soil with *H. annuus* inoculated with a mixture of *C. humi*, *O. haematophilum* and *R. irregularis* (MIX) and 3 levels of saline water irrigation (0, 1 and 2 g NaCl l^{-1}). Each treatment was replicated 6 times.

R. irregularis was mixed in the soils according to the manufacturer's recommendations (100 ml kg^{-1}) one day before seedling transference.

C. humi and *O. haematophilum* strains were grown overnight at 150 rpm and 30 °C in Luria-Bertani's (LB) medium. The pellets were then washed twice and ressuspended in 10 mM phosphate buffer pH 8.0 to get an inoculum density of ca. 10^8 CFU ml^{-1}.