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Combined use of beneficial soil microorganism and agrowaste residue to cope with plant water limitation under semiarid conditions

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ABSTRACT

This study investigated the effectiveness of several microorganisms, such as a *Bacillus megaterium* strain and/or an autochthonous consortium of arbuscular mycorrhizal fungi (AMF) on plant growth and drought tolerance in a natural semiarid soil. The effect of treated *Aspergillus niger* residue from sugar beet was also evaluated in non-inoculated and inoculated plants. Results from three successive harvests allowed us to determine the persistence along the time of beneficial effects of these treatments under natural drought conditions. Biomass production and nutrition were more increased by the transformed residue than concomitantly decreased antioxidant enzymatic activities under drought. The microbial inoculants assayed contributed to plant drought tolerance through strategies such as increased nutrition (particularly K^+), hydric content and by decreasing stomatal conductance and antioxidant enzymatic activities. Similar microbial-mediated effects were confirmed at each harvest. The effectiveness of bacterial inoculation under drought conditions in natural soil has been almost unexplored. Here, the interactive effect of these bacteria with an AMF consortium maximized plant growth, water content and C, K, Ca and Mg content. A relevant result is the greater effectiveness of the bacteria when inoculated in residue amended soil that promoted plant growth and hydric content and decreased most antioxidant activities to a greater extent than AMF inoculation. *B. megaterium* (without compost) also affected root growth, physiological and biochemical plant values involved in the adaptative plant drought response. The ability of *B. megaterium* in axenic medium to maintain indole acetic acid (IAA) like molecules and to increase proline production under osmotic stress conditions indicated the drought tolerance of this strain. In this study the management of natural resources, such as selected and drought adapted soil microorganisms and *A. niger* treated agrowaste resulted determinant for enhancing plant performance in an arid degraded soil.

1. Introduction

Desertification processes disturb and limit the re-establishment of the natural plant cover. Nevertheless, the plant association with beneficial soil microorganisms may be critical for plant growth in stressed degraded ecosystems (Bashan and de-Bashan, 2010b; Jeffries and Barea, 2001). The management of selected and appropriate plant growth promoting microorganisms (PGPM) can help plants to grow and to promote the stabilization of a self-sustaining ecosystem under stress conditions. Arbuscular mycorrhizal (AM) fungi enable plants to cope with drought stress not only by alleviating nutrient deficiencies but also by improving drought tolerance overcoming the detrimental effect of water and nutrient limitations (Augé, 2000, 2004; Bashan et al., 2009; Medina et al., 2003). Plant nutrients and water deficit are common stresses affecting plant survival and development in arid and semiarid areas. Thus, the improvement of nutrients and water uptake is important for plants growing under stressed conditions. Detrimental environmental conditions also negatively affect the survival and activity of rhizosphere microorganisms but those autochthonous adapted to adverse conditions (such as water limitation and nutrient deficiencies) may be the best candidates to be used as inocula to compensate in inoculated plants such stress conditions (Bashan et al., 2009; Marulanda et al., 2007; Marulanda-Aguirre et al., 2008).

In fact, ecosystem functioning is largely governed by microbial activity and both soil bacteria and arbuscular mycorrhizal (AM) fungi, particularly autochthonous strains adapted to specific environmental conditions, may be appropriate inoculants since they are able to develop plant-tolerance to cope with these stressful environments (Azcón et al., 2010; Dimkpa et al., 2009; Kim et al., 2012). The role of these microorganisms in alleviating plant drought stress has been previously studied under sterile soil conditions and provided a biological understanding about the plant adaptation to stressed environments (Galleguillos et al., 2000; Marulanda et al., 2009; Valdenegro et al., 2001). The use of these selected microorganisms may result relevant regarding the sustainability of stressed environments (Bowen and Rovira, 1999).

Different effectiveness was found when the contribution of six AM fungi (all of them *Glomus* sp.) to water uptake by colonized plants

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under drought stress conditions was analyzed (Marulanda et al., 2003). Similarly, the particular drought tolerance and plant responses of three indigenous bacterial strains isolated from drought soil were tested, and also the biochemical mechanisms related to bacterial effectiveness in water limited soil were studied (Marulanda et al., 2009). From this previous study under sterile conditions *Bacillus megaterium* resulted the most efficient bacteria in alleviating plant drought symptoms when applied alone or associated with each one of the three different autochthonous AM fungi (Marulanda et al., 2009). Authors concluded that microbial activities of adapted bacterial and fungal strains may exert a beneficial interactive effect on plant growth under drought conditions as also reported Bashan et al. (2009). In fact autochthonous AM fungi and bacteria populations naturally growing in arid soils have developed the ability to survive in water limited soils and are adapted to drought conditions (Bashan et al., 2012).

Arid soils are generally characterized by poor structure, lack of organic matter and low water-holding capacity. The most important factor making the rhizosphere an attractive habitat for saprophyte microorganisms, like many bacteria, is the organic carbon provided by plant roots. Thus, the limited plant growth and C exudation under arid conditions may cause the poor surviving of saprophyte microbial inoculum and it is necessary to assure their establishment to be effective on plant growth in arid soils. In fact, the deterioration of biological properties of arid soils is in part due to their progressive decrease in organic matter content (Bashan and de-Bashan, 2010b). In this respect the application of organic amendments to desertified soil, prior to the microbial inoculation has been recommended (Medina et al., 2004a,b; Trejo et al., 2012). In previous studies the most important effects of organic amendments included not only the improvement of soil quality (nutrients, humus, water-holding capacity) but also an increase of microbial activities (Caravaca et al., 2005b, 2006; Kloepper et al., 1999; López et al., 2013; Trejo et al., 2012).

Large amounts of agrowastes are produced during the extraction of sugar from the sugar beet, but this product only can be used as organic amendment after biological transformation processes. Sugar beet residue, because of its lignocellulosic composition, may be mineralized by specific lignocellulosic microorganisms such as *Aspergillus niger*, resulting in a product rich in minerals for plant growth and also in sugars that can be used as energy sources for heterotrophic microorganisms such as plant growth promoting bacteria (PGPB) as suggested by Bashan and Holguin (1998). Nevertheless, the fertilizer ability of this agrowaste can be increased when rock-phosphate (RP) is added to the fermentation medium (Medina et al., 2005). The rock-phosphate solubilization was carried out by the citric acid production by *A. niger* growing on the agrowaste residue. The application of this *A. niger* + RP treated product as amendment improved soil fertility and in previous studies this amendment was used in reclamation strategies of degraded systems particularly associated with AM fungi or yeast (Medina and Azcón, 2010). The application of this amendment, in addition to the nutritional abilities for plants and microorganisms, may affect water uptake by plants (Caravaca et al., 2006). This amendment could be considered as an interesting product to be used for revegetation in water-limited environments improving plant/soil quality and PGPB inocula survival.

We hypothesized that plant responses to drought in an arid soil is a consequence of variations in the level of plant tolerance according to the root microorganisms associated and the amendment applied as Bashan et al. (2012) also suggested. Normally, the combined inoculation of bacterial strains with AM fungi produced growth stimulating effect that surpassed those of individual inoculations (Marulanda-Aguirre et al., 2008). In this study as a next step, we investigate under natural soil conditions the importance of these treatments (amendment, AMF and bacteria) on plant growth, nutrition and drought stress tolerance over time (three harvest) to evaluate the benefits and persistence of these treatments.

In previous studies the effectiveness of this *B. megaterium* strain was assayed in a sterilized Mediterranean soil underlying in this bacteria mechanisms of osmotic stress tolerance (Marulanda et al., 2009). But the persistence in the efficiency of this amendment and/or microbial inocula activity over time under arid natural conditions needs to be evaluated. The present study was carried out under natural (non-sterile) conditions and we determined how the single or combined inoculation of drought adapted selected microorganisms (*B. megaterium* and/or autochthonous consortium of mycorrhizal fungi) in soil amended or not with *A. niger* treated sugar beet residue attenuated the negative effect of water limitation over time. For that three successive harvests were done. As drought stress markers, we analyzed physiological and biochemical plant parameters such as the stomatal conductance and shoot enzymatic antioxidant activities [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR)]. All these values are involved in the plant responses for survival in drought stressed soil.

In addition, the production of proline and IAA-like molecules by *B. megaterium* growing in axenic culture under increasing osmotic stress levels [induced by polyethylene glycol (PEG)] was also evaluated over time to determine the ability of this strain to thrive under drought conditions.

2. Materials and methods

Two independent experiments were carried out in this study. Firstly, in a microcosm experiment we determined both the microbial inocula and *A. niger* treated residue abilities to increase plant growth, nutrition, physiological and biochemical values under natural drought conditions.

In a second experiment the bacteria *B. megaterium* was assayed in axenic medium to test indole acetic acid (IAA-like molecules) and proline accumulation over time under increasing levels of polyethylene glycol (PEG) (0%, 5% and 10%) in the culture medium to induce osmotic stress.

2.1. Experiment I

2.1.1. Fermentation process

The strain of *A. niger* NB2 used in this study was maintained on potato–dextrose agar slants at 4 °C. It was shown to produce only citric acid on complex substrates (Vassilev et al., 1986) and to mineralize lignocellulosic materials (Vassilev et al., 1998). For inoculum preparation, *A. niger* was grown on a slant at 30 °C for 7 days and spores were scraped in sterile distilled water.

Sugar beet waste (SBW) was used as substrate in the fermentation trials. Its characteristics were: cellulose 29%, hemicellulose 23%, lignin 5%, total C 55% and total N 1.7%. This solid residue was dried in a 60 °C oven and ground to pass a 2-mm-pore screen. It was mixed at a concentration of 10% in 40 mL and placed in 250-mL Erlenmeyer flasks with Czape-Dox mineral salt solution. Rock phosphate (Morocco fluorapatite, 12.8% soluble P, 1 mm mesh), was added at a rate of 1.5 g L^{-1}. This culture medium were sterilized by autoclaving at 120 °C for 30 min. Spore suspension of *A. niger* (1.2×10^7), 3 mL, was spread carefully over the surface of the medium. The experiment was carried out in 250-mL Erlenmeyer flasks (in triplicate) in conditions of solid-state fermentations, at 30 °C for 20 days. This amendment was added (5%) to the growing substrate (Medina et al., 2006).

2.1.2. Inocula isolation and production

Rhizosphere soil samples for mycorrhizal inoculum isolation were taken from a natural semiarid soil in the east of Spain (Murcia province). This soil containing colonized roots, spores and mycelia belonging to the native adapted AM fungi was cultivated for inoculum production.

Indigenous AM spores were isolated by wet-sieving and decanting as described by Ruíz-Lozano and Azcón (1995). All the spores obtained were morphologically similar to *Septoglomus constrictum* (EEZ 198), *Diversispora aunantia* (EEZ 199), *Archaeospora trappei* (EEZ 200), *Glomus versiforme* (EEZ 201), and *Paraglomus ocultum* (EEZ 202) compared to those from our current EEZ collection.