Plant Physiology and Biochemistry 90 (2015) 64–74

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Autochthonous arbuscular mycorrhizal fungi and *Bacillus thuringiensis* from a degraded Mediterranean area can be used to improve physiological traits and performance of a plant of agronomic interest under drought conditions

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ARTICLE INFO

Article history:
Received 29 January 2015
Accepted 17 March 2015
Available online 18 March 2015

Keywords:
AM fungi
Aquaporin
Bacillus thuringiensis
Drought
Maize
Plant growth promoting microorganism

ABSTRACT

Studies have shown that some microorganisms autochthonous from stressful environments are beneficial when used with autochthonous plants, but these microorganisms rarely have been tested with allochthonous plants of agronomic interest. This study investigates the effectiveness of drought-adapted autochthonous microorganisms [*Bacillus thuringiensis* (Bt) and a consortium of arbuscular mycorrhizal (AM) fungi] from a degraded Mediterranean area to improve plant growth and physiology in *Zea mays* under drought stress. Maize plants were inoculated or not with *B. thuringiensis*, a consortium of AM fungi or a combination of both microorganisms. Plants were cultivated under well-watered conditions or subjected to drought stress. Several physiological parameters were measured, including among others, plant growth, photosynthetic efficiency, nutrients content, oxidative damage to lipids, accumulation of proline and antioxidant compounds, root hydraulic conductivity and the expression of plant aquaporin genes. Under drought conditions, the inoculation of Bt increased significantly the accumulation of nutrients. The combined inoculation of both microorganisms decreased the oxidative damage to lipids and accumulation of proline induced by drought. Several maize aquaporins able to transport water, CO_2 and other compounds were regulated by the microbial inoculants. The impact of these microorganisms on plant drought tolerance was complementary, since Bt increased mainly plant nutrition and AM fungi were more active improving stress tolerance/homeostatic mechanisms, including regulation of plant aquaporins with several putative physiological functions. Thus, the use of autochthonous beneficial microorganisms from a degraded Mediterranean area is useful to protect not only native plants against drought, but also an agronomically important plant such as maize.

1. Introduction

Plants are constantly confronted with environmental constraints of both biotic and abiotic origin. In particular, drought one of the most common environmental stresses experienced by soil plants (Shinozaki et al., 2003). Drought stress affects plant–water relations, as well as, specific and nonspecific physiological responses (Beck et al., 2007), causing an important detrimental effect on plant growth and nutrition and, thus, limiting crop production. In fact, drought is considered as major cause of declining crop productivity worldwide (Vinocur and Altman, 2005). There is consensus that global climate change is actually occurring and that its negative effects will probably increase in the coming years, imposing significant difficulties to plant and crop development in many areas of the world. These difficulties will be particularly important in current semi-arid agricultural zones (Denby and Gehring, 2005).

Plants usually interact with soil microorganisms that make them more efficient in coping with environmental limitations such as drought. Several strategies have been suggested to overcome the negative effects of drought (Warren, 1998). The most explored approaches have been the breeding for tolerant varieties and the use

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http://dx.doi.org/10.1016/j.plaphy.2015.03.004
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of genetic engineering. However, an alternative strategy is to induce drought stress tolerance by using beneficial microorganisms such as arbuscular mycorrhizal (AM) fungi and plant growth promoting rhizobacteria (PGPR). There is ample information about the interactions occurring among AM fungi and PGPR, resulting in the promotion of key processes for plant nutrition, growth and health, particularly in stressed environment (Armada et al., 2014b; Marulanda-Aguirre et al., 2008; Vivas et al., 2006). Moreover, several studies have shown that using native AMF and PGPR, which appear to be physiologically and genetically adapted to the stress conditions of the environment of origin, provides a higher benefit for plant performance than non-native isolates (Armada et al., 2014b; Oliveira et al., 2005; Querejeta et al., 2006).

Plants can tolerate severe environmental conditions such as drought, and for that they need to adapt several physiological, biochemical and cellular/molecular processes in order to maintain cell homeostasis (Urano et al., 2010). Osmotic stress is a frequent consequence of plant tissues exposed to drought that induces plant water imbalance (Beck et al., 2007). The accumulation of some metabolites in plant tissues is an important mechanism to overcome the osmotic stress (Armada et al., 2014b; Bárzana et al., 2014). Several authors have reported that PGPR inoculation provides a better plant water balance under osmotic stress (Pereyra et al., 2012). Bacteria have developed mechanisms to cope with drought stress such as the ability to enhance indole-3-acetic acid (IAA) synthesis (Marulanda et al., 2009). Moreover, activities of several bacterial enzymes involved in the ascorbate–glutathione cycle are related with the severity of the stress (Kasim et al., 2013). In many cases, inoculated drought-stressed plants showed lower antioxidant activities than non-inoculated plants. These results are indicative of the bacterial capacity to reduce reactive oxygen species (ROS) levels in drought stressed plants and were also correlated with increased physiological parameters such as photosynthesis (Armada et al., 2014a, 2014b; Kasim et al., 2013; Rueda-Puente et al., 2010).

Under drought conditions, plants have to face with the problem of acquiring sufficient amount of water from the soil (Ouziad et al., 2006), and aquaporins participate in this process (Maurel et al., 2008). Aquaporins are water channel proteins that facilitate and regulate the passive movement of water molecules down a water potential gradient (Maurel et al., 2008). These proteins are present in all kingdoms and belong to the major intrinsic protein (MIP) family of transmembrane proteins. In maize two major classes of plant aquaporins are located in the plasma membrane (PIPs) and in the tonoplast (TIPs). PIPs and TIPs isoforms have been recognized as central pathways for transcellular and intracellular water transport (Maurel et al., 2008).

In the last few years, much effort has been concentrated on investigating the function and regulation of aquaporins. High levels of aquaporin expression were shown in tissues with high water fluxes across membranes (Maurel et al., 2008; Otto and Kaldenhoff, 2000). Thus, aquaporins seem to play a specifically important role in controlling transcellular water transport in plant tissues (Javot and Maurel, 2002). In any case, the relationship between aquaporins and plant responses to water deficit is still elusive and with contradictory results (Aharon et al., 2003; Lian et al., 2004). In addition, although many aquaporins are highly selective for water, uptake experiments with *Xenopus laevis* oocytes clearly showed that certain aquaporins are permeable to small solutes such as glycerol, urea, amino acids, CO_2 and/or NH_3/NH_4 or even small peptides and ions (Kaldenhoff et al., 2007; Uehlein et al., 2007), which opens many questions about the physiological roles of aquaporins, especially in AM plants (Maurel and Plassard, 2011). Interestingly, several maize aquaporins have been shown to be regulated by the AM symbiosis under different drought scenarios, and their regulation has been related with the exchange of water and other molecules of physiological importance between the host plant and the AM fungus (Bárzana et al., 2014).

In a previous study we have shown that several native PGPRs from an arid and degraded Mediterranean area were effective in promoting plant growth and development in *Lavandula dentata* and *Salvia officinalis* growing under drought conditions in a natural soil containing also the native AM fungal population (Armada et al., 2014b). However, the question remains if these microorganisms can be also used to promote plant growth in a non-native plant of agronomic interest such as maize (*Zea mays* L.).

Maize is one of the most important crops both for human and animal consumption. According to the Maize CRP Annual Report (2013) (http://maize.org/wp-content/uploads/sites/5/2014/07/MAIZE-CRP-Annual-Report-2013-web.pdf), maize is cultivated on more than 142 million ha worldwide and it is estimated to produce around 913 million tonnes of grain per year, accounting for one third of the total global grain production. Although maize is originally from Mesoamerica, nowadays it is the third most important cereal crop and ranks first in countries with developing economies (Mejía, 2003). However, in arid and semi-arid regions and, particularly in Mediterranean areas, maize is vulnerable to adverse environmental conditions due to limited rainfall, high evapotranspiration, and high temperature (Azevedo Neto et al., 2006).

Thus, the aim of the present study was to analyse the effectiveness of drought-adapted autochthonous microorganisms (*Bacillus thuringiensis* and a consortium of AM fungi) to improve plant growth and physiology under two watering conditions of a non-native plant species which is an important cereal crop. The bacterium *B. thuringiensis* was selected as it was the most effective bacterial strain in the previous study (Armada et al., 2014b).

2. Materials and methods

2.1. Experimental design

The experiment had a 3×2 factorial design with four inoculation treatments: (1) non-inoculated control plants (C), (2) plants inoculated with *B. thuringiensis* (Bt), (3) plants inoculated with a consortium of AM fungi (AMF) and (4) plants dually inoculated with AMF + Bt. In addition, plants were cultivated either under well-watered conditions throughout the entire experiment, or were subjected to drought stress for 8 weeks. Each treatment had ten replicates to give a total of 80 pots.

2.2. Molecular identification of the bacterial strain

The autochthonous bacterium, identified as *B. thuringiensis*, was isolated from a semiarid soil at the Natural Ecological Park “Vicente Blanes” in Molina de Segura, (Murcia, Spain) (Armada et al., 2014b). This area suffers from drought and low nutrients availability and, as a result, desertification. Bt was the most abundant cultivable bacterial type in such arid soil. The bacterium was isolated from the above-mentioned soil (a mixture of rhizospheres from several autochthonous plant species). A homogenate of 1 g soil in 9 mL sterile water was diluted (10^{-2} to 10^{-4}), plated on three different media [Agar Yeast Mannitol, Dextrose Potato agar or Luria–Bertani agar (LB)] and then incubated at 28 °C for 48 h, to isolate bacteria from different taxonomic groups.

Identification of isolated bacteria was done by sequencing the 16S rDNA gene. Bacterial cells were collected, diluted, lysed and their DNA used as a template in the PCR reactions. All reactions were conducted in 25 μL volume containing PCR buffer 10X, 50 mM $MgCl_2$, 10 μM each primers: 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT), (Rees et al., 2004) and 5 U/μL of