Environmental and Experimental Botany 131 (2016) 47–57

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato

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ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form 28 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Key-words:
Arbuscular mycorrhizal symbiosis
Drought stress
Plant growth promoting rhizobacteria
Recombinant inbred line

ABSTRACT

At a world scale, tomato is an important horticultural crop, but its productivity is highly reduced by drought stress. Combining the application of beneficial microbial inoculants with breeding and grafting techniques may be key to cope with reduced tomato yield under drought. This study aimed to investigate the growth responses and physiological mechanisms involved in the performance under drought stress of four tomato recombinant inbred lines (RIL) after inoculation with the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis* and the plant growth promoting rhizobacteria (PGPR) *Variovorax paradoxus* 5C-2. Results showed a variation in the efficiency of the different tomato RILs under drought stress and a differential effect of the microbial inoculants, depending on the RIL involved. The inoculants affected plant parameters such as net photosynthetic capacity, oxidative damage to lipids, osmolyte accumulation, root hydraulic conductivity or aquaporin abundance and phosphorylation status. RIL66 was the one obtaining maximum benefit from the microbial inoculants under drought stress conditions, due likely to improved CO_2-fixation capacity and root hydraulic conductivity. We propose that RIL66 could be selected as a good plant material to be used as rootstock to improve tomato growth and productivity under water limiting conditions. Since RIL66 is highly responsive to microbial inoculants, this grafting strategy should be combined with inoculation of *R. irregularis* and *V. paradoxus* in order to improve plant yield under conditions of drought stress.

1. Introduction

Drought stress has a major impact on plant growth and development, limiting crop production throughout the world. It has been estimated that nearly one third of soils are too dry to support normal plant development and productivity (Golldack et al., 2014). Moreover, global climate change is spreading this problem of water deficit to regions where drought impacts were negligible in the past (Trenberth et al., 2014).

To cope with environmental stresses, plants have developed a variety of strategies (Dobra et al., 2010). Under drought stress plants regulate the permeability of tissues to water movement, use osmotic adjustment and enhance their antioxidant systems. The first of these processes is based on modifying membrane water permeability, a process in which aquaporins are involved (Maurel et al., 2008; Chaumont and Tyerman, 2014). Aquaporins are water channel proteins that facilitate and regulate the passive movement of water molecules down a water potential gradient (Maurel et al., 2015), affecting directly the radial water flow through the cell-to-cell pathway. Under conditions of low transpiration, such as under drought stress, this pathway is predominant for water movement in plants (Steudle and Peterson, 1998). Among plant aquaporins, the plasma membrane intrinsic proteins subfamily (PIPs1 and

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http://dx.doi.org/10.1016/j.envexpbot.2016.06.015
0098-8472/

PIPs2) is critical for whole plant water transport (Javot and Maurel, 2002; Chaumont and Tyerman, 2014). Since plants undergo frequent environmental changes, the activity of PIPs must be regulated by mechanisms that allow rapid responses to these changes. Post-translational modifications are necessary to achieve such rapid regulation (Vandeleur et al., 2014), including phosphorylation/de-phosphorylation of specific serine residues, the first post-translational regulation mechanism found in aquaporins. This generates conformational changes allowing aquaporin gating (Johansson et al., 1998; Prado et al., 2013) or modifying the subcellular localization of PIPs in the membrane (Prak et al., 2008) and may be a mechanism to prevent water loss (Bárzana et al., 2015).

The accumulation of compounds such as soluble sugars, proline, glycine betaine, pinitol or mannitol allows plants to osmotically adjust to maintain cell turgor (Morgan, 1984; Bheemareddy and Lakshman, 2011). Proline, a non-protein amino acid that accumulates in most plant tissues subjected to water stress, is one of the most common osmolytes accumulated (Kishor and Sreenivasulu, 2014) and can be readily metabolized upon recovery from drought (Singh et al., 2000). Besides acting as an osmoregulatory compound, proline also serves as a sink for energy, regulating redox potentials, as a scavenger of hydroxyl radicals, as a means of reducing acidity in the cell, and as a solute that protects macromolecules against denaturation (Kishor and Sreenivasulu, 2014).

Under drought stress, several metabolic pathways are uncoupled and electrons are transferred to molecular oxygen to form reactive oxygen species (ROS) (Noctor et al., 2014). ROS are toxic molecules capable of causing oxidative damage to lipids, proteins and DNA (Miller et al., 2010). However, at low levels, ROS can act as signalling molecules for stress responses and its generation is an early plant stress response (Singh et al., 2011). Antioxidant systems aim to eliminate excessive ROS production under stress conditions (Gill and Tuteja, 2010). The scavenging of ROS is achieved through the action of non-enzymatic compounds and different enzymatic systems. Non-enzymatic mechanisms include compounds able to scavenge directly several ROS, such as ascorbic acid (AsA), glutathione (GSH), or α-tocopherol. Enzymatic antioxidants include superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), ascorbate- or thiol-dependent peroxidases, and the enzymes of the ascorbate-glutathione pathway (Scheibe and Beck, 2011).

At a world scale, tomato is the most important horticultural crop, and the second most important vegetable consumed after potato. Tomato is a major dietary component in many countries and constitutes an important source of vitamins, sugars, minerals, and antioxidant compounds. However, its productivity is highly reduced by abiotic stresses, including drought (Schwarz et al., 2010). While climate change is reducing crop productivity, world agriculture must increase its productivity by 60% to feed the expected population of 9.6 billion people in 2050 (Cabot et al., 2014). Therefore, drought tolerance is a target trait in breeding programs, particularly for rootstocks. Combining breeding techniques with grafting techniques and the application of beneficial microbial inoculants will play a key role in developing a more profitable horticulture to address this challenge (Asins et al., 2010; Albacete et al., 2015b). The rootstock effect to ameliorate abiotic stress tolerance in tomato was previously tested in a population of recombinant inbred lines (P-RILs) (Albacete et al., 2015a,c).

Grafting is a biotechnological tool to improve not only the amount and uniformity of crop yield, but also stress tolerance (reviewed by Albacete et al., 2015b). Nowadays, most fruit crops and many horticultural species are grown as scion-rootstock combinations. This strategy allows desired features such as stress tolerance to be conferred by a suitable rootstock, while retaining excellent fruit yield and quality traits of a given scion (Asins et al., 2010). Thus, to start a grafting program to improve tomato drought tolerance, the selection of suitable genotypes to be used as rootstocks is the first necessary step.

Many studies have shown that the arbuscular mycorrhizal (AM) symbiosis and plant growth-promoting rhizobacteria (PGPR) may enhance host plant stress tolerance, including to drought (Azcón et al., 2013; Malusá et al., 2013; Zoppellari et al., 2014). Indeed, plant symbiotic relationships with mycorrhizal fungi greatly increase the surface area over which plant root systems take up water and nutrients. Soil bacteria on the root surface alter root phytohormone status thereby increasing growth, and can make nutrients more available to the plant. Studies have also shown that these beneficial microorganisms improve plant osmotic adjustment and antioxidant responses, as well as, water status throughout regulation of plant aquaporins (Marulanda et al., 2010; Dodd and Ruiz-Lozano, 2012; Azcón et al., 2013; Ruzzi and Aroca, 2015; Kaushal and Wani, 2016). Combining these two groups of microorganisms can increases crop resource use efficiency and productivity under stressful environmental conditions (Dodd and Ruiz-Lozano, 2012).

In spite of the positive effects of AM fungi and PGPR on plant productivity, no studies have dealt with the use of breeding techniques in combination with these microorganisms to improve plant productivity under stressful conditions. Thus, we hypothesize that combining the use of a selected group of RILs having specific traits with microbial inoculants with a proved ability to improve drought tolerance will be useful to combine drought tolerance features coming from both the plant genotype and its interaction with AMF and PGPR and, thus, will improve tomato performance under drought. This study aimed to investigate the growth and physiological response of four P-RILs after root colonization by AMF and PGPR. The tomato lines obtaining maximum benefit from the microbial inoculants and performing best under drought stress conditions will be identified and selected as the most suitable in grafting programs directed toward improved tomato productivity under drought. The study also aims to understand the underlying physiological mechanisms involved in the improved plant performance.

The RILs used represent a valuable resource that has already been used to identify a specific QTL conferring salinity resistance (Asins et al., 2010, 2015). The AM fungus *Rhizophagus irregularis* DAOM 197198 was formerly known as *Glomus intraradices*. It was reassigned to *G. irregulare* by Stockinger et al. (2009) and then as *Rhizophagus irregularis* (Kruger et al., 2012). This fungus is widely used in abiotic stress studies, being one of the most effective in drought stress alleviation (Ruiz-Lozano et al., 2012; Azcón et al., 2013). *Variovorax paradoxus* 5C-2 is a PGPR that promotes tomato root length in vitro irrespective of bacterial load (Belimov et al., 2007) and stimulates root and shoot growth of another Solanaceae (potato) grown in both well-watered and drying soils (Belimov et al., 2015).

2. Materials and methods

2.1. Plant materials and experimental design

The experiment consisted of a complete randomized factorial design with four tomato recombinant inbred lines (RIL20, RIL40, RIL66, RIL100) plus one commercial cultivar (Boludo) used as reference (*Solanum lycopersicum* L. cv. Boludo F1, Monsanto). These RILs belong to a population of F10 lines (P population) derived by single seed descendent from a cross between a salt sensitive genotype of *Solanum lycopersicum* var. Cerasiforme (formerly *L. esculentum*) and a salt tolerant line from *S. pimpinellifolium* L. (formerly *L. pimpinellifolium*) (Monforte et al., 1997). P population