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Arbuscular mycorrhizal symbiosis and salicylic acid regulate aquaporins and root hydraulic properties in maize plants subjected to drought

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ABSTRACT

Climate change is leading to the intensification of drought effects worldwide, which considerably reduce crop production. A better understanding of the drought-tolerance mechanisms would lead into a more productive agriculture. The arbuscular mycorrhizal (AM) symbiosis has been shown to improve plant tolerance to drought. Salicylic acid (SA) is a phenolic compound involved in many aspects of plant growth and development. Apart from its role in biotic interactions, it is also involved in the regulation of important plant physiological processes, including plant water relations under stressful conditions. However, despite the importance of SA in plant physiology and in AM colonization, little is known about its effect on regulation of root water transport. Thus, the aim of this work was to study the combined effect of AM symbiosis and SA on root hydraulic properties under drought stress, with special focus on how these factors can alter radial root water transport pathways through aquaporin regulation. Also, the crosstalk between SA and other phytohormones was taken into account. Results showed that the AM symbiosis modifies root hydraulic responses to drought episodes. Under these conditions, AM plants showed increased Lpr and Lo. Exogenous SA application decreased Lpr and Lo under drought. SA modulation of water conductivity could be due to a fine-regulation of root aquaporins (as *ZmPIP2;4* or *ZmTIP1;1*). Furthermore, SA application differently modulated the percentage of water flowing by the apoplastic pathway, decreasing its contribution to total root water flow in AM plants and increasing it in non-AM plants. An intricate relationship between Lpr, aquaporins and endogenous levels of SA, ABA and jasmonic acid was observed. Future studies should explore more in detail the crosstalk mechanism between these hormones in the regulation of water transport in AM roots, in order to better understand the mechanism through which the AM symbiosis copes with drought stress.

1. Introduction

Climate change is leading to the intensification of drought effects and cultivable soils are progressively drying worldwide (Trenberth et al., 2014), with more often drought events that considerably reduce crop production (Lesk et al., 2016). Agricultural drought reduces plant growth and affects essential plant physiological and biochemical processes as stomatal conductance, transpiration, root water uptake, photosynthesis or membrane functions. It also increases the production of reactive oxygen species (ROS), producing oxidative stress that damages cells and even leads to plant death (Hasanuzzaman et al., 2014). Thus, a better understanding of the mechanisms that help plants to improve their water status during water stress would lead into a more productive agriculture. Phytohormones play essential roles and coordinate different signalling pathways during abiotic stress responses (Wani et al., 2016). Among these, salicylic acid (SA) is a phenolic compound involved in many aspects of growth and plant development as well as in the regulation of the response to different abiotic and biotic stresses (Khan et al., 2015; Miura and Tada, 2014). Salicylic acid has been studied mainly in relation to plant-pathogen interactions since it has the ability to induce systemic acquired resistance to different pathogens in plants (Gunes et al., 2007). Indeed, it coordinates the plant's defence against biotrophic pathogens (Lu, 2009)

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and Foo et al. (2013) suggested that SA might also have a role during arbuscular mycorrhizal (AM) colonization. Previous studies point in this direction, with a short-lived rise in SA levels during the early stages of AM colonization (Blilou et al., 1999). Herrera-Medina et al. (2003) showed that the rate of AM colonization was affected by the SA content. They found that transgenic plants with reduced SA levels exhibited a more rapid AM colonization while wild-type plants with constitutive SA biosynthesis retarded AM colonization of roots, although the final level of colonization was unaltered.

Apart from this role in biotic interactions, SA is also involved in the regulation of important plant physiological processes such as nitrogen metabolism, photosynthesis, antioxidant defense system and plant water relations under stress conditions and thereby provides protection in plants against abiotic stresses (Faried et al., 2017; Khan et al., 2015). SA has been found to improve plant tolerance to salt stress (Jini and Joseph, 2017; Miura and Tada, 2014) and to affect plant physiology in maize plants subjected to salinity (Gunes et al., 2007). Indeed, exogenous SA may induce stomatal closure (Miura and Tada, 2014), regulates biosynthesis of osmolytes (Li et al., 2016; Misra and Saxena, 2009) and increases antioxidative defenses in stressed tissues (Nazar et al., 2011). However, SA is thought to interact in a complex way with other hormonal compounds such as ethylene (Gharbi et al., 2016). Thus, its effects on plant physiology can be direct or indirect, through alteration of other plant hormones. Finally, SA influences plant functions in a dose dependent manner, where induced or inhibited plant functions can be possible with low and high SA concentrations, respectively (Khan et al., 2015).

There are increasing evidences of enhanced drought tolerance when exogenous SA is applied (Alam et al., 2013; Miura and Tada, 2014; Li et al., 2016). However, this regulation is orchestrated in a complex cross-talk between different phytohormones (auxins, cytokinins, ABA, gibberellins) under optimal and stressful conditions (Munné-Bosch and Müller, 2013). On the other hand, AM fungi (which establish a mutualistic relationship with most crop plants) have been described to improve water and nutrient uptake, enhancing tolerance to abiotic stresses such as drought (Ruiz-Lozano et al., 2012) being a possible alternative to the use of inorganic fertilizers (Zoppellari et al., 2014). This amelioration is achieved by allowing plants the access to distant water from the soil, and by altering root hydraulic properties (Bárzana et al., 2012). Water transport in roots, according to the composite model (Steudle and Peterson, 1998) occurs as the sum of three pathways: apoplastic (via the cell wall continuum), symplastic (via plasmodesmata) and transcellular (across the cell membranes). The last two pathways cannot be differentiated empirically, being reduced to the so-called cell-to-cell pathway. Aquaporins play an important regulatory role in this last pathway, and within this protein family, water channel activity is mainly found in the PIP2 subfamily (Maurel et al., 2008). By measuring root hydraulic conductivity (Lpr), root water transport capacity can be estimated, providing information on plant water status and water mobilization capacity of roots.

It is known that under non-stressful conditions the radial water flow is mainly apoplastic, following the hydrostatic gradient created by transpiration. However, when transpiration is restricted (as under drought), water goes mainly by the cell-to-cell pathway following an osmotic gradient between soil solution and xylem sap. Thus, relative contribution of these two pathway to overall water uptake or hydraulic conductivity may change substantially (Hachez et al., 2006; Martínez-Ballesta et al., 2003; Vadez et al., 2013) and, under drought conditions, root hydraulics is adjusted by switching between both pathways (Ranathunge et al., 2004). It is expected that aquaporins play a key role in the regulation of water flow in plants under conditions of water limitation, affecting important parameters such as the root hydraulic conductivity (Hachez et al., 2006; Zarrouk et al., 2016). Moreover, there is growing evidence that the contribution of aquaporin-mediated water transport to root water uptake is much larger than previously thought, even under conditions of high transpiration (Knipfer and Fricke, 2010, 2011).

Previous studies have investigated the effects of the AM symbiosis on water pathways in the roots of host plants, combined with the use of an inhibitor of aquaporins activity (Bárzana et al., 2012). Results showed that roots of AM plants enhanced significantly the water circulating by apoplastic pathway as compared to non-AM plants, both under well-watered and under drought stress conditions. Data also showed that the presence of the AM fungus in the roots of the host plants could modulate the switching between cell-to-cell and apoplastic water transport pathways. This was interpreted as a way to provide higher flexibility in the response of AM plants to water shortage according to the demands from the shoot (Bárzana et al., 2012). Other recent evidences suggest that the modulation of ABA, auxins and/or SA levels in the host plant may contribute to this switching between water pathways mediated by the AM fungus (Calvo-Polanco et al., 2014; Sánchez-Romera et al., 2016). Indeed, ABA was found to increased Lpr at root cortical cell and organ levels in maize, facilitating water uptake under water limiting conditions (Hose et al., 2000) and ABA was identified as a possible aquaporin regulator (Boursiac et al., 2008; Wan et al., 2004). Studies in Arabidopsis indicated that indole acetic acid (IAA) acts through an Auxin Response Factor 7 (ARF7)-dependent path to inhibit the expression of most PIPs at both transcriptional and translational levels (Péret et al., 2012). Similarly, SA down regulates PIP aquaporins and root hydraulic conductivity by a ROS-mediated mechanism which provoked membrane internalization of PIP aquaporins (Boursiac et al., 2008).

Despite the importance of SA in plant physiology and in AM colonization, as well as its putative role under drought conditions, little is known about its effect on root hydraulic conductivity and regulation of water transport in roots, and to the best of our knowledge, studies about the combined effect of exogenous SA application and AM symbiosis are lacking. Thus, the aim of this research was to study the combined effect of AM symbiosis and SA on root hydraulic properties under drought stress, being specially focused on how these factors can alter radial root water transport pathways through aquaporin regulation. For that, we applied exogenous SA or an inhibitor of its biosynthesis (2-aminoindan-2-phosphonic acid, AIP; Pan et al., 2006). Also, the crosstalk between SA and other plant hormones under the former conditions will be discussed. The results of this study could lead to a better understanding of water uptake mechanisms and plant tolerance to drought when the AM fungus is present, increasing our knowledge of its effect on plant water balance.

2. Material and methods

2.1. Experimental design

The experiment consisted of a factorial design with three factors: (1) inoculation treatment, with non-inoculated control plants (C) and plants inoculated with the AM fungus *Rhizophagus irregularis*, strain EEZ 58 (Ri); (2) chemical treatment, so that one group of each inoculation treatment was maintained without hormone (untreated), another group of plants was treated with salicylic acid (SA), and the last group was treated with 2-aminoindan-2-phosphonic acid (AIP), as inhibitor of SA biosynthesis; (3) watering treatment so that half of the plants were grown under well-watered (WW) conditions throughout the entire experiment and the other half was subjected to drought stress for 15 days before harvest (DS). The different combination of these factors gave a total of 12