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Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants

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ABSTRACT

Arbuscular mycorrhizal (AM) symbiosis can alleviate salt stress in plants. However the intimate mechanisms involved, as well as the effect of salinity on the production of signalling molecules associated to the host plant-AM fungus interaction remains largely unknown. In the present work, we have investigated the effects of salinity on lettuce plant performance and production of strigolactones, and assessed its influence on mycorrhizal root colonization. Three different salt concentrations were applied to mycorrhizal and non-mycorrhizal plants, and their effects, over time, analyzed. Plant biomass, stomatal conductance, efficiency of photosystem II, as well as ABA content and strigolactone production were assessed. The expression of ABA biosynthesis genes was also analyzed.

AM plants showed improved growth rates and a better performance of physiological parameters such as stomatal conductance and efficiency of photosystem II than non-mycorrhizal plants under salt stress since very early stages – 3 weeks – of plant colonization. Moreover, ABA levels were lower in those plants, suggesting that they were less stressed than non-colonized plants. On the other hand, we show that both AM symbiosis and salinity influence strigolactone production, although in a different way in AM and non-AM plants. The results suggest that AM symbiosis alleviates salt stress by altering the hormonal profiles and affecting plant physiology in the host plant. Moreover, a correlation between strigolactone production, ABA content, AM root colonization and salinity level is shown. We propose here that under these unfavourable conditions, plants increase strigolactone production in order to promote symbiosis establishment to cope with salt stress.

Introduction

Plants are exposed to ever changing and often unfavourable environmental conditions, which cause both biotic and abiotic stresses such as extreme temperatures, flood, drought, and salinity. Overexploitation of available water resources, as well as environmental factors such as low precipitations, high temperatures and contamination from parental rocks are leading to an increase in soil salinization. Actually, salinization is one of the most important agricultural and eco-environmental problems nowadays, which is increasing steadily in many parts of the world (Evelin et al., 2009; Porcel et al., 2012). Saline soils have been estimated to occupy more than 7% of the Earth's land surface and it is expected to be increased by up to 50% by the middle of the twenty-first century (Ruiz-Lozano et al., 2012). Soil salinity is a major constraint to food production because it restricts the use of previously cultivated lands. Moreover, it dramatically limits agricultural yield as it negatively affects plant growth and development, decreasing crop production over 20% (Porcel et al., 2012). Plants growing in saline soils are subjected to different physiological stresses that induce nutrient imbalance, damage cell organelles, and disrupt photosynthesis and respiration (Juniper and Abbott, 1993; Evelin et al., 2012). High salinity also results in a considerable increase in the levels of abscisic acid (ABA), which are accompanied by major changes in gene expression and in adaptive physiological responses (Raghavendra et al., 2010). ABA is a phytohormone, well-known for its important signalling role in the regulation of plant growth and development, but it can also promote plant defense and enable plants to survive under a variety of abiotic stresses such as cold, drought, and salinity (Christmann et al., 2006). ABA promotes stomatal closure to reduce water loss and induces the expression of stress-related genes, diminishing the damage it has caused (Evelin et al., 2009). Understanding the mechanisms that enable the plants to grow and develop under these

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unfavourable conditions is crucial. Current strategies to cope with soil salinity and minimize crop losses are focused on traditional breeding for salt-tolerant crops and the use of genetic engineering to develop resistant cultivars by targeting molecular markers (Sanan-Mishra et al., 2005; Cuartero et al., 2006). However, implementation of these approaches is costly and requires extensive knowledge. Therefore, there is an urgent need to find cheaper and environmentally-friendly alternatives to overcome salt stress.

Plants continuously interact with other microorganisms present in their environment (Raaijmakers et al., 2009; López-Ráez et al., 2011b). Moreover, they are able to establish mutually beneficial associations with some of these microorganisms present in the rhizosphere. One of the most well-studied beneficial plant-microorganism associations is that established with certain soil fungi known as arbuscular mycorrhizal (AM) fungi (Smith and Read, 2008). Interestingly, the vast majority of land plants, including most agricultural crop species, are able to establish AM symbiosis (Smith and Read, 2008). It positively affects plant growth and provides tolerance against biotic and abiotic stresses (Pozo and Azcón-Aguilar, 2007). Nevertheless, negative growth responses of host plants to AM colonization have also been described (Smith et al., 2009), which have been frequently related to unbalance in C cost and P benefit between the plant and the fungus. AM fungi are ubiquitous and are known to exist in saline environments (Giri et al., 2003), and there is evidence demonstrating that AM symbiosis can alleviate the negative effects induced by soil salinity (Evelin et al., 2009; Porcel et al., 2012). Indeed, AM symbiosis has been demonstrated to increase resistance to soil salinity in a variety of host plants such as maize, tomato and lettuce (Al-Karaki, 2000; Feng et al., 2002; Jahromi et al., 2008), although the intimate mechanisms are not well understood (Ruiz-Lozano et al., 2012).

AM symbiosis establishment require a high degree of coordination between the two partners based on a finely regulated molecular dialogue (Hause et al., 2007). This communication starts in the rhizosphere with the production and exudation of signalling molecules by the host plants (under nutrient deficient conditions) that are recognized by AM fungi and stimulate hyphal growth. Among these signals, the strigolactones have arisen as essential cues acting as a 'cry for help' under unfavourable conditions (López-Ráez et al., 2011b). Strigolactones are multifunctional molecules that have been recently classified as a new class of plant hormones regulating above- and below-ground plant architecture, and reproductive development (Gómez-Roldán et al., 2008; Kapulnik et al., 2011; Ruyter-Spira et al., 2011; Kohlen et al., 2012). Originally, they were identified as signalling molecules playing a dual role in the rhizosphere, where not only favour AM symbiosis establishment, but also act as host detection cues for root parasitic plants of the family Orobanchaceae, including *Striga*, *Orobanche* and *Phelipanche* species, by stimulating the germination of seeds of these parasitic weeds (Bouwmeester et al., 2007; López-Ráez et al., 2011b). These root parasitic weeds are some of the most damaging agricultural pest worldwide, causing large crop losses (Parker, 2009).

Strigolactones are mainly produced in the roots and have been detected in the root extracts and exudates of monocot and dicot plants (Xie et al., 2010). They are derived from the carotenoids by oxidative cleavage (Matusova et al., 2005; López-Ráez et al., 2008), thus belonging to the apocarotenoid class as the phytohormone ABA (Ohmiya, 2009). ABA also plays an essential role in AM symbiosis. Besides its stress protection function in mycorrhizal roots, recent research has shown that ABA is important in the establishment of symbiosis, being necessary to complete arbuscule formation and promoting sustained colonization of the plant root (reviewed in López-Ráez et al., 2011b). Interestingly, a regulatory role of ABA in strigolactone biosynthesis has also been proposed since a correlation between ABA levels and strigolactones was observed (López-Ráez et al., 2010).

Although the occurrence of AM fungi has been described in salt-marshes environments (Wilde et al., 2009), salinity not only negatively affects the host plant but also the AM fungus. It can reduce colonization capacity, spore germination and growth of fungal hyphae (Juniper and Abbott, 2006; Jahromi et al., 2008). However, few studies have shown an increased AM fungal sporulation and colonization under salt stress conditions, or even no effect in colonization rates (Aliasgharzadeh et al., 2001; Yamato et al., 2008). Therefore, the effects of salinity on the fungal colonization capacity are not well established and seem to depend on the host plant and fungal species, as well as to the growing conditions (Evelin et al., 2009). In the same way, the effects of salinity on the production of signalling molecules such as strigolactones have not been studied so far. Since strigolactones are induced by nutrient deficiency and have been proposed as mediators of plant responses to the environmental conditions, it might be that they are regulated by other adverse environmental conditions, thus affecting the AM fungal colonization (García-Garrido et al., 2009).

In the present investigation, a study covering the effects of salinity on both the mycorrhizal root colonization and the production of strigolactones was carried out in an agronomical important plant such as lettuce. Two different salt concentrations were used and their effects investigated at early, middle, and well-established mycorrhization stages. Plant biomass production and some physiological parameters associated to this kind of stress such as stomatal conductance, efficiency of photosystem II and ABA levels were analyzed, and correlated with expression of ABA biosynthesis genes. Strigolactone production, according to the germinating activity of root extracts for *Phelipanche ramosa* seeds under saline conditions in both mycorrhizal and non-mycorrhizal plants, was also assessed.

Materials and methods

Experimental design

The experiment consisted of a randomized complete block design with two inoculation treatments: (1) non-inoculated control plants (Control) and (2) plants inoculated with the AM fungus *Glomus intraradices* (Gi), and three salt stress treatments: (i) plants treated with 0 mM NaCl, (ii) plants treated with 40 mM NaCl and (iii) plants treated with 80 mM NaCl. Fifteen replicates of each of these treatments were used totaling 90 pots (one plant per pot), so that five individual plants of each treatment (30 in total) were harvested after 3 weeks of transplantation, another set after 5 weeks, and the last set after 7 weeks.

Soil, biological material and growth conditions

Soil. Loamy soil was collected from Granada province (Spain), sieved (5 mm), diluted with quartz-sand (<2 mm) (1:1, soil:sand, v/v) to avoid excessive compaction and sterilized by steaming (100 °C for 1 h on 3 consecutive days). The original soil had a pH of 8.2 [measured in water 1:5 (w/v)]; 1.5% organic matter, nutrient concentrations (g kg^{-1}): N, 1.9; P, 1.0; K, 6.9. The electrical conductivity of the soil was 200 μS cm^{-1} (1:5, w/v).

Plant material. Seeds of lettuce (*Lactuca sativa* cv 'Romana') were provided by Semillas Fitó SA (Barcelona, Spain). *L. sativa* is a glycophyte species with a moderate tolerance to salinity (Ünlükara et al., 2008). Seeds were sown in trays containing sterile moist sand for germination during 1 week and then individual seedlings were transferred to pots containing 900 grams of the soil/sand mixture described above. Seeds of the parasitic plant *Phelipanche ramosa* were kindly provided by Maurizio Vurro and Angela Boari (Instituto di Scienze delle Produzioni Alimentari, Bari, Italy).