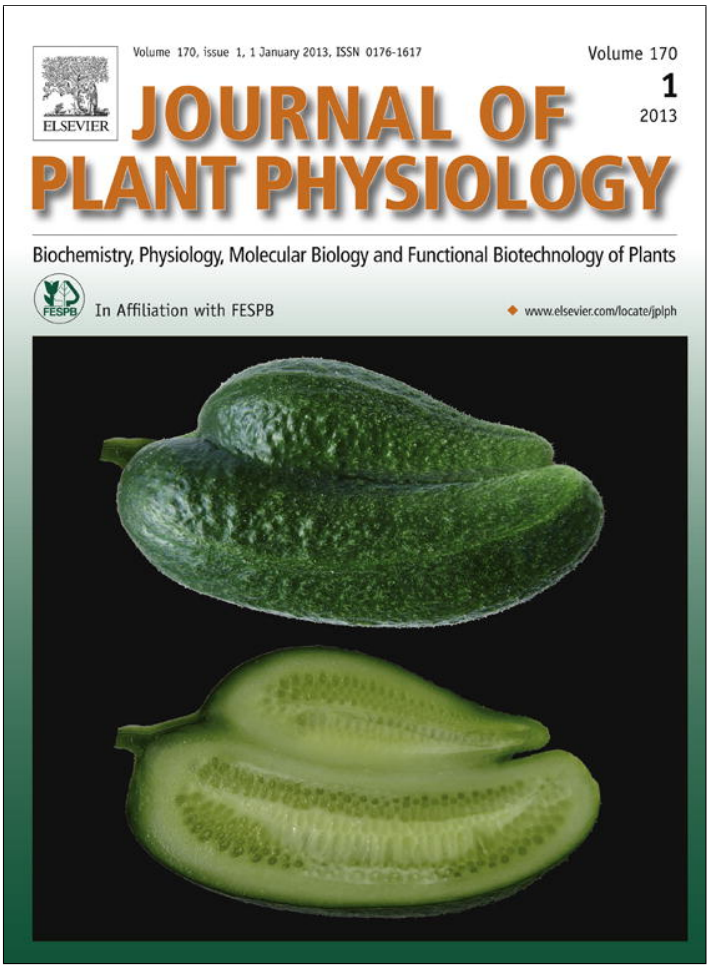

Journal of Plant Physiology 170 (2013) 47–55

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

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

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

Ricardo Aroca[a], Juan Manuel Ruiz-Lozano[a], Ángel María Zamarreño[b], José Antonio Paz[a], José María García-Mina[b], María José Pozo[a], Juan Antonio López-Ráez[a,*]

[a] Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas (EEZ-CSIC), Profesor Albareda 1, 18008 Granada, Spain
[b] R&D TAI Roullier Group, Polígono Arazuri-Orkoien, 31160 Orkoien, Spain

ARTICLE INFO

Article history:
Received 29 May 2012
Received in revised form 23 August 2012
Accepted 23 August 2012

Keywords:
Abscisic acid
Arbuscular mycorrhiza
Lettuce
Salt stress
Strigolactones

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

* Corresponding author. Tel.: +34 958 181600x223; fax: +34 958 181600.
E-mail address: juan.lopezraez@eez.csic.es (J.A. López-Ráez).

0176-1617/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2012.08.020

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 $\mu S\,cm^{-1}$ (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).

AM inoculation. Mycorrhizal inoculum was bulked in an open-pot culture of *Zea mays* and consisted of soil, spores, mycelia, and infected root fragments. The AM species used was *G. intraradices* (Schenck and Smith) strain EEZ 58 (Gi), obtained at the collection of Estación Experimental del Zaidín (CSIC, Granada). This strain has been recently reassigned to *Glomus irregulare* (Stockinger et al., 2009) and then to *Rhizophagus irregularis* (Błaszk, Wubet, Renker and Buscot) C. Walker, and A. Schüßler comb. nov. Ten grams of whole inoculum with about 70 infective propagules per gram (according to the most probable number test), were added to appropriate pots at transplanting time just below lettuce seedlings. Non-inoculated control plants received the same amount of autoclaved mycorrhizal inoculum, together with a 3 mL aliquot of a filtrate (<20 μm) of the AM inoculum, in order to provide a general microbial population free of AM propagules. This procedure has been largely used in order to mimic microbial population of the AM inoculum (Duan et al., 1996; Feng et al., 2002; Aroca et al., 2008; Jahromi et al., 2008; Kumar et al., 2010; Ruiz-Sánchez et al., 2010).

Growth conditions. The experiment was carried out under greenhouse conditions with temperatures ranging from 19 to 25 °C, 16/8 light/dark period, a relative humidity of 50–70%, and an average photosynthetic photon flux density of 800 μmol m^{-2} s^{-1}, as measured with a light meter (LICOR, Lincoln, NE, USA, model LI-188B). After transplantation of the lettuce seedlings to the pots containing the growing substrate, plants were watered three times per week with 25 mL of aqueous saline solutions containing 0 mM, 40 mM, and 80 mM NaCl. The same amount of tap water was added on alternative days in order to avoid excessive salt accumulation in the soil. Plants were maintained under these conditions until harvest at 3, 5, and 7 weeks, and then frozen in liquid nitrogen and stored at −80 °C until use.

Parameters measured

Biomass production. At harvest (3, 5, or 7 weeks after planting), the shoot and root system were separated and the shoot dry weight (DW) was measured after drying in a forced hot-air oven at 70 °C for 2 days. The mycorrhizal dependency (MD), or response to mycorrhizal colonization, was calculated for each salinity treatment by using the following formula provided by Kumar et al. (2010): MD (%) = (DW of mycorrhizal plant − DW of non-inoculated plant)/DW of mycorrhizal plant × 100.

Symbiotic development. At each harvest, the percentage of mycorrhizal root colonization was estimated by visual observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactic acid (v/v), according to Phillips and Hayman (1970). The extent of mycorrhizal colonization was calculated according to the gridline intersect method (Giovannetti and Mosse, 1980).

Photosynthetic efficiency. The efficiency of photosystem II was measured with FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic), which allows a non-invasive assessment of plant photosynthetic performance by measuring chlorophyll fluorescence. FluorPen quantifies the quantum yield of photo system II as the ratio between the actual fluorescence yield in the light-adapted state (FV′) and the maximum fluorescence yield in the light-adapted state (FM′), according to Oxborough and Baker (1997). Measurements were taken in the second youngest leaf of 5 different plants of each treatment after 7 weeks of plant transplantation.

Stomatal conductance. Stomatal conductance was measured 2 h after light turned on by using a porometer system (Porometer AP4, Delta-T Devices Ltd, Cambridge, UK) following the user manual instructions. Measurements were taken in the second youngest leaf from 5 independent plants from each treatment after 7 weeks of plant transplantation.

ABA content. ABA extraction, purification, and quantification were carried out in plants after 7 weeks of plant transplantation, using the method described by Bacaicoa et al. (2009), but using 0.25 g of frozen root tissue (previously ground to a powder in a mortar with liquid nitrogen) instead of 0.5 g. ABA was quantified by liquid chromatography coupled to a 3200 Q TRAP (HPLC/MS/MS) system (Applied Biosystems/MDS Sciex, Ontario, Canada), equipped with an electrospray interface, using an reverse-phase column (Synergi 4 mm Hydro-RP 80A, 150 mm × 2 mm, Phenomenex, Torrance, CA). A linear gradient of methanol (A) and 0.5% acetic acid in water (B) was used: 35% A for 1 min, 35–95% A in 9 min, 95% A for 4 min, and 95–35% A in 1 min, followed by a stabilization time of 5 min. The flow rate was 0.20 mL/min, the injection volume was 40 μL, and the column and sample temperatures were 30 and 20 °C, respectively. Detection and quantification were performed by multiple reaction monitoring (MRM) in the negative-ion mode, employing a multi-level calibration graph with deuterated hormones as internal standards. Compound-dependent parameters are described in Bacaicoa et al. (2009). The source parameters were: curtain gas 25 psi, GS1 50 psi, GS2 60 psi, ion spray voltage −4000 V, CAD gas medium, and temperature 600 °C.

Gene expression analysis by real-time RT-PCR (qPCR)

After 7 weeks of plant transplantation, total RNA was isolated from lettuce roots by phenol/chloroform extraction according to the method described by Kay et al. (1987). The RNA was treated with RQ1 DNase (Promega, Madrid, Spain), purified through a silica column using the NucleoSpin RNA Clean-up kit (Macherey-Nagel, Hoerd, France), and stored at −80 °C until use. Real-time quantitative RT-PCR (qPCR) was performed using the iCycler iQ5 system (Bio-Rad, Hercules, CA, USA) and gene-specific primers (Table 1). The first strand cDNA was synthesized with 1 μg of purified total RNA using the iScript cDNA Synthesis kit (Bio-Rad) according to the manufacturer's instructions. Three independent biological replicates were analyzed per treatment. Relative quantification of specific mRNA levels was performed using the comparative $2^{-\Delta(\Delta Ct)}$ method (Livak and Schmittgen, 2001). Expression values were normalized using the housekeeping gene *LsBtub3*, encoding a beta-tubulin 3.

Extraction of strigolactones from roots and quantification by germination bioassay

As mentioned above, strigolactones are germination stimulants of root parasitic plant seeds. Because of this germinating activity, bioassays based on seed germination of root parasitic plants can be used as a reliable indirect way to quantify the levels of strigolactones produced by plant roots (Matusova et al., 2005; López-Ráez et al., 2008; 2011a).

For strigolactone analysis in root extracts, 0.5 g of roots from each treatment cultivated for 7 weeks, were ground in a mortar with liquid nitrogen and extracted twice with 0.5 mL of 50% acetone in a 2 mL eppendorf tube. Tubes were vortexed for 2 min and centrifuged at 4 °C for 5 min at 8000 × *g* in a table top centrifuge. The organic phase was carefully transferred to 2 mL glass vials and stored at −20 °C until use. Before germination, bioassays were performed. The acetone was removed from the samples by first adding a corresponding volume of demineralised water and then evaporating the solvent *in vacuo* in a SpeedVacuum SC100 (Savant Instruments, Holbrook, NY, USA). Germination bioassays with *P. ramosa* seeds were performed as described in López-Ráez et al. (2008). Briefly, seeds of *P. ramosa* were preconditioned for 12 days at 21 °C. Then, aliquots of 50 μl of root extracts were added to triplicate discs bearing approximately 100 pre-conditioned seeds and incubated at 25 °C. The synthetic germination stimulant GR24

Table 1
Primer sequences used in the real time qPCR analysis.

ID	Gene	Primers (5′-3′)	Reference
AB120107	*LsNCED1*[1]	AAACCCTACAATCCGACTATTCG GGCCGCAGCTCTTTGTAAG	Sawada et al. (2008)
AB120108	*LsNCED2*[1]	CTTCAGTTTCCTAAACAGTCTGTTGGTA TGCTTTCAATCCATCTTCAACG	Sawada et al. (2008)
AB120109	*LsNCED3*[1]	CAGTCGTCGGAGAAATTCCA GCCTTTGTGTCTCCGTATGG	Sawada et al. (2008)
AB120110	*LsNCED4*[1]	GGACACGGCTCATGGAATC GCGAGATCACCGTCACGTT	Sawada et al. (2008)
AB232706	*LsBtub3*	CAGGATCAGGAATGGGAACTC CCTTGGGAGAAGGGAATACAG	This work

and demineralised water were included as positive and negative controls in each bioassay. After 7 days, the germinated and non-germinated seeds were counted using a binocular microscope. Seeds were considered germinated when the radical had protruded through the seed coat. To avoid saturation of the germination response, a series of dilutions of the extracts (1:100, 1:200, 1:400 and 1:800) with demineralised water were used.

Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) using the software SPSS Statistics v. 20 for Windows and with AM fungus inoculation, salt level and AM fungus inoculation-salt level interaction as sources of variation, followed by Duncan's multiple range test. Percentage values were arcsine [squareroot(X)] transformed before statistical analysis.

Results

Root colonization by G. intraradices under salt stress conditions

In order to assess the effect of salinity on the establishment of AM symbiosis, lettuce plants were inoculated with the AM fungus *Glomus intraradices* and treated with three different NaCl concentrations (0, 40 and 80 mM) during the whole growth period. The levels of root colonization for the different treatments were analyzed at three different time points – 3, 5 and 7 weeks – after inoculation. Non-inoculated plants did not show any colonization. After 3 weeks the colonization rate was low (about 8%) and no differences between the treatments were observed (Fig. 1). However, after 5 weeks a significant ($P < 0.05$) reduction in mycorrhizal colonization was observed in the 40 and 80 mM NaCl-treated compared with non-treated plants (0 mM), 17% and 30%, respectively. This trend was more pronounced at 7 weeks (Fig. 1). Hereto, a decrease on colonization intensity of about 20% was observed in plants treated with 40 mM NaCl, while a reduction of about 40% was detected after treatment with 80 mM NaCl.

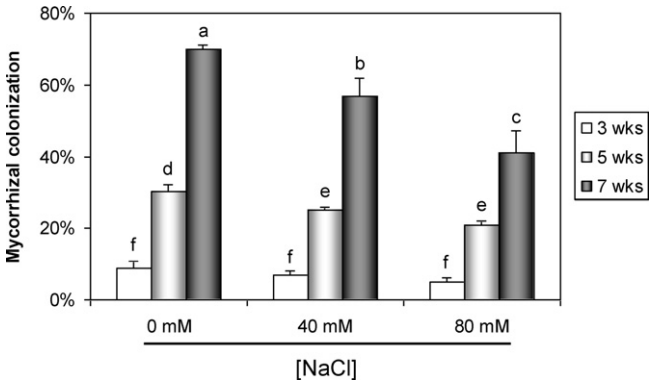

Fig. 1. Effect of salinity and time on the percentage of AM root colonization in lettuce (*Lactuca sativa*) plants. Intensity of mycorrhizal colonization by *G. intraradices* in the roots. Plants were subjected to 0, 40 or 80 mM NaCl. Data points represent the means of five replicates (±SE). Data not sharing a letter in common differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Influence of salinity on physiological status of lettuce plants

Plant growth. The effect of salt stress on plant development was investigated along the time. Salinity negatively affected shoot dry weight (SDW) in non-mycorrhizal plants in a dose-dependent manner, even after only 3 weeks of transplantation (Table 2 and Table S1). This decrease in SDW was more evident in later stages of development, where a reduction of 70% and 80% was observed at 5 weeks after treatment with 40 mM and 80 mM NaCl, respectively, compared with non-saline conditions (Table 2). After 7 weeks, salinity clearly depressed growth of lettuce (Fig. 2). Here, about 55% decrease in SDW was detected after 40 mM NaCl treatment, whereas the reduction was almost 70% with 80 mM NaCl (Table 2).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jplph.2012.08.020.

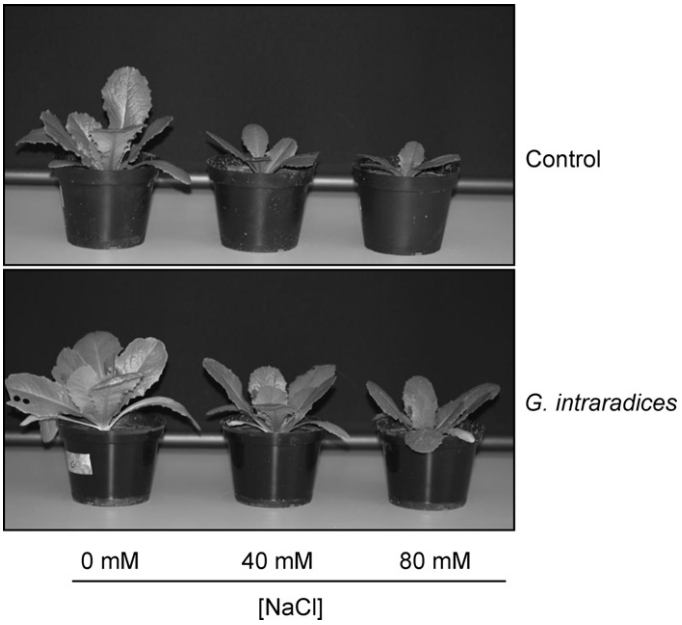

Fig. 2. Phenotypic comparison of non-mycorrhizal (Control) and mycorrhizal (*G. intraradices*) lettuce plants growing for 7 weeks under increasing salinity levels ranging from 0 to 80 mM NaCl.

Table 2
Influence of salinity and AM symbiosis on growth in lettuce plants.

Treatment	0 mM NaCl		40 mM NaCl		80 mM NaCl	
	NM	Gi	NM	Gi	NM	Gi
Week 3						
SDW (g per plant)	0.15 ± 0.02d	0.75 ± 0.07a	0.17 ± 0.03d	0.57 ± 0.08b	0.05 ± 0.02e	0.35 ± 0.05c
MD (%)	–	79.5	–	69.8	–	84.5
Week 5						
SDW (g per plant)	0.83 ± 0.41d	1.88 ± 0.14a	0.25 ± 0.11ef	1.41 ± 0.12bc	0.17 ± 0.02f	1.22 ± 0.34c
MD (%)	–	55.8	–	82.1	–	86.0
Week 7						
SDW (g per plant)	1.34 ± 0.20d	2.54 ± 0.11a	0.57 ± 0.25ef	1.77 ± 0.15b	0.42 ± 0.12f	1.51 ± 0.12cd
MD (%)	–	47.3	–	67.8	–	72.5

Shoot dry weight (SDW) of lettuce plants and mycorrhizal dependency (MD, %) of plants subjected to salt stress. Plants were cultivated under increasing salinity levels ranging from 0 to 80 mM NaCl and inoculated with the AM fungus *G. intraradices* (Gi) or remained as non-mycorrhizal controls (NM). Within each harvest time, data represent mean ± SD. Data with different letters differ significantly ($P < 0.05$), as determined by the Duncan's multiple range test ($n = 5$).

Colonization by *G. intraradices* significantly promoted plant growth in lettuce under non-saline conditions (Fig. 2 and Table S1). An increase in SDW of 5-, 2.3-, and 1.9-fold was observed after 3, 5, and 7 weeks of growing, respectively, indicating a high mycorrhizal dependency (Table 2). AM symbiosis also positively affected plant growth under salt stress (Table 2 and Fig. 2). After 40 mM NaCl treatment, an increase in SDW of 3.4-, 5.6-, and 3.1-fold compared to non-mycorrhizal plants was detected in *G. intraradices*-colonized plants at 3, 5, and 7 weeks, respectively. The effect of the mycorrhiza was clearer in the 80 mM NaCl-treated plants, where the SDW was increased 7-, 7-, and 3.7-fold at 3, 5, and 7 weeks, with values of mycorrhizal dependency ranged from 72 to 86%. Since the effect of salt stress on plant development showed a similar trend for all the three time points investigated, only the plants harvested after 7 weeks were used in the following analyses.

Efficiency of photo system II. The efficiency of photo system II was negatively affected by the higher salt level, decreasing in both colonized and non-colonized plants as compared to non-saline conditions (Fig. 3A). In any case, except at 40 mM NaCl, this parameter was always higher in mycorrhizal plants as compared to non-mycorrhizal plants.

Stomatal conductance. Salinity significantly ($P < 0.05$) reduced stomatal conductance in non-mycorrhizal plants, obtaining the lowest level after treatment with 80 mM NaCl (Fig. 3B and Table S1). Under non-saline conditions, plants colonized by *G. intraradices* showed an increase in the stomatal conductance of about 1.5-fold. Mycorrhizal colonization also increased stomatal conductance under salt stress, remarkably after 40 mM NaCl where an increase of more than 2 times was observed compared to non-mycorrhizal plants (Fig. 3B).

Salt stress effect on ABA content in colonized roots

ABA is a phytohormone normally associated to plant responses against abiotic stresses, including salinity, which has also been related to AM symbiosis. When ABA content in mycorrhizal roots without salt stress was analyzed after 7 weeks, a reduction of 4.4-fold was detected respect to non-colonized plants (Fig. 4A). Salinity induced an increase in ABA content after treatment with 40 mM NaCl in non-mycorrhizal plants. In these plants, the ABA levels were restored to control values at 80 mM NaCl (Fig. 4A). Salt stress also induced ABA content in roots of mycorrhizal plants in a dose-dependent manner, showing an interaction between the two treatments (Table S1). Here, after treatment with 80 mM NaCl ABA levels reached those of non-mycorrhizal plants (Fig. 4A). Under these conditions, a 5.6-fold increase in ABA was detected compared to non-saline conditions.

Nine-*cis*-epoxycarotenoid dioxygenases (NCEDs) catalyse the rate-limiting step in ABA biosynthesis (Taylor et al., 2005). The expression levels of all the NCEDs described in lettuce were analyzed by quantitative real time PCR (qPCR) using specific primers (Table 1). The gene *LsNCED2* was induced at 40 mM NaCl in roots from non-mycorrhizal plants, with no changes in gene expression at 80 mM (Fig. 4B). In roots from *G. intraradices*-colonized plants, an induction in the expression of *LsNCED2* of 3.7 and 4.2 times at 40 and 80 mM NaCl, respectively, was observed (Fig. 4B). The expression of the other *LsNCED* genes (*LsNCED1*, *LsNCED3* and *LsNCED4*), although

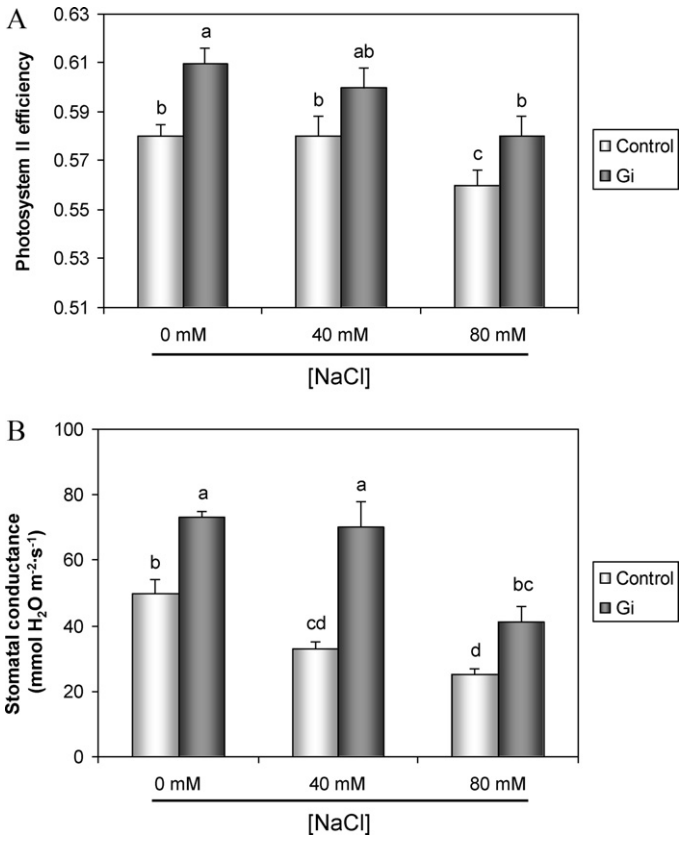

Fig. 3. Influence of arbuscular mycorrhizal (AM) symbiosis and salt stress on the physiological status of lettuce plants. (A) Effect on the efficiency of photosystem II after 7 weeks. (B) Effect on the stomatal conductance after 7 weeks. Grey bars represent non-inoculated control plants (Control) and dark grey bars represent plants inoculated with *G. intraradices*. Bars represent the means of five replicates (±SE). Bars with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

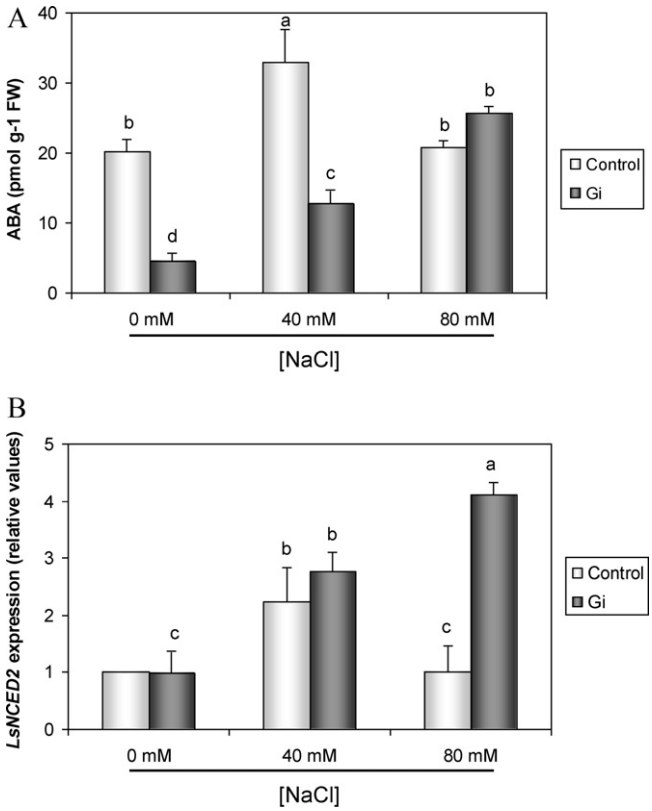

Fig. 4. Effect of salt stress and AM symbiosis in abscisic acid (ABA) pathway. (A) ABA content in lettuce roots after 7 weeks. (B) Gene expression analysis by real time qPCR for the ABA biosynthesis gene *LsNCED2* after 7 weeks. See legend of Fig. 3. Bars represent the means of five plants (A) or three (B) replicates (±SE).

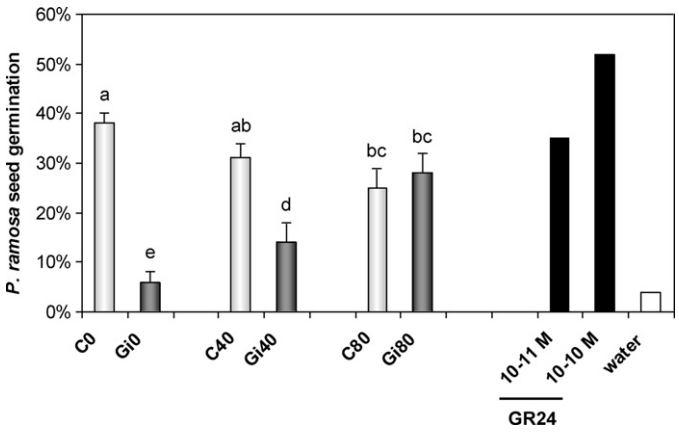

Fig. 5. Influence of salinity and mycorrhizal colonization on strigolactone production. Germination of *Phelipanche ramosa* seeds induced by root extracts of lettuce (*Lactuca sativa* L. cv. Romana) plants after 7 weeks. Dilution 1:400 in demineralised water of each root extract was used. GR24 (10^{-10} and 10^{-11} M) and demineralised water were used as positive and negative controls, respectively. See legend of Fig. 3.

detected in roots, did not change significantly neither under salt stress nor mycorrhization (data not shown).

Influence of salinity and mycorrhizal colonization on strigolactone production

To assess the influence of salt stress and mycorrhization on strigolactone production in lettuce, we performed a germination bioassay with seeds of *P. ramosa* using root extracts from plants at 7 weeks. The synthetic germination stimulant GR24 (10^{-10} and 10^{-11} M), used as a positive control, always induced germination of pre-conditioned *P. ramosa* seeds (Fig. 5). Water alone, used as a negative control, induced a basal germination. Germination induced by the lettuce root extracts was always lower than that induced by GR24, indicating that saturation of the germination response did not occur at the root extract dilutions used in the bioassays. Germination induced by the root extracts from *G. intraradices*-colonized plants under non-saline conditions was 6.3 times lower than that induced by non-mycorrhizal plant (Fig. 5). In the absence of mycorrhization, the germination stimulatory activity of extracts from NaCl-treated plants was lower than that induced by the control plants (Fig. 5), suggesting a negative effect of this stress on strigolactone biosynthesis. Conversely, in mycorrhizal plants salinity significantly ($P < 0.05$) promoted strigolactone production in a dose-dependent manner, showing an induction of about 5-fold at 80 mM compared to 0 mM NaCl (Fig. 5 and Table S1).

Discussion

It is widely accepted that AM symbiosis is a key component in helping plants to cope with adverse environmental conditions, including salt stress (Pozo and Azcón-Aguilar, 2007; Evelin et al., 2009; Porcel et al., 2012). The beneficial effects of different AM fungi on plant growth and development under saline conditions have been shown in a number of plant species (Al-Karaki, 2000; Feng et al., 2002; Jahromi et al., 2008; Fan et al., 2011). We previously showed that in lettuce, mycorrhizal plants grew more than non-mycorrhizal plants under salt stress conditions when AM symbiosis was well established (after 7 weeks) (Jahromi et al., 2008). In that experiment, plants were established prior to salinization to allow a proper mycorrhizal colonization. In contrast, in the present study we show that the positive effect of AM symbiosis on plant growth also takes place when salt stress and AM fungus were applied together from the beginning of the growing period, which resembles more to natural conditions. The promotion of plant growth started from early stages of mycorrhizal colonization (after 3 weeks), where only less than 10% root colonization was achieved. Moreover, this positive effect was more evident under the most extreme conditions (80 mM), where *G. intraradices*-colonized plants grew 7 times more than non-mycorrhizal plants. Growth promotion was maintained until 7 weeks, with a 3 (40 mM) and 4-fold (80 mM) increase. Thus, AM symbiosis alleviates salt stress and allows lettuce plants to grow better under these unfavourable conditions and, interestingly, this beneficial effect begins since very early stages of the association. In the present work, a greater increase in AM-induced growth promotion than in the previous study was observed (Jahromi et al., 2008). It may be that applying salt stress from the beginning of the growing period, as occurs in nature, affects plant growth more drastically and that, under these sub-optimal conditions, the effect of the mycorrhiza is enhanced. Indeed, the mycorrhizal dependency of lettuce plants increased with salinity levels (Table 2).

AM-colonized plants exhibited a better performance of photo system II, as well as an enhanced stomatal conductance rate both under non-saline and under saline conditions. These two positive effects may also have accounted for the enhanced plant growth of AM-colonized plants, most probably by enhancing CO_2 fixation under salt stress. In this sense, several studies have shown a correlation between tolerance to abiotic stresses and maintenance of efficiency of photo system II, which also sustained plant productivity (Loggini et al., 1999; Redondo-Gómez et al., 2007; 2010; Ruiz-Sánchez et al., 2010). The higher values of photosynthetic efficiency in mycorrhizal plants indicate that the photosynthetic apparatus of these plants is less damaged by the salt stress imposed

(Germ et al., 2005; Redondo-Gómez et al., 2010). Although the oxidative status of plants was not measured in this study, the higher performance of photo system II and the enhanced stomatal conductance in mycorrhizal plants could have contributed to decreased photo-respiration, leading to a lower production of reactive oxygen species (ROS) in these plants (Cadenas, 1989; Ruiz-Sánchez et al., 2010), thus contributing to an enhanced salinity tolerance and growth.

ABA plays an important role in plant responses to abiotic stresses including salinity. As expected, we found out that ABA levels in non-colonized roots and AM roots increased as a consequence of salinity. Curiously, for non-AM plants this increase was only observed at 40 mM NaCl, while at 80 mM NaCl the ABA levels decreased and were similar to those of untreated plants. An induction in ABA was also detected in mycorrhizal plants subjected to salt stress, although to a lesser extent than in non-colonized plants. In any case, salt application enhanced the accumulation of ABA in AM plants until reaching a similar level than non-inoculated plants at 80 mM NaCl (Fig. 4). The decrease of ABA in non-AM plants at 80 mM NaCl, while AM plants increased steadily their ABA content is difficult to be understood. However, ABA is a signalling hormone which is finely regulated in response to the environmental conditions and the physiological plant status. For instance, in a previous study the ABA levels of AM lettuce plants decreased when exposed to 50 mM NaCl as compared to non-salt treated plants (Jahromi et al., 2008). Also, previous studies showed that mycorrhizal and non-mycorrhizal lettuce plants exhibit contrasting responses to ABA during drought stress and recovery (Aroca et al., 2008). Thus, results on ABA are mostly in agreement with previous studies in lettuce (Jahromi et al., 2008) and, when taken together with data on biomass production, efficiency of photosystem II and stomatal conductance, suggest that arbuscular mycorrhizal plants are less stressed than non-colonized plants under soil saline conditions. Interestingly, this reduced stress damage could explain the increased growth observed in mycorrhizal plants (Fig. 2). The ABA pattern coincided with that observed for the ABA biosynthesis gene *LsNCED2*, indicating a *de novo* production of this phytohormone in response to salinity. Moreover, since NCEDs catalyse the rate-limiting step in ABA biosynthesis (Taylor et al., 2005) and the expression of the other *LsNCED* genes did not change, our results could indicate that LsNCED2 is the enzyme responsible for ABA production in roots exposed to salt stress. Under non-saline conditions, a lower ABA content was detected in mycorrhizal roots compared to non-colonized plants. Similar results were obtained previously in roots of lettuce plants under non-stressful conditions or after recovery from drought (Aroca et al., 2008). Previous studies showed that AM symbiosis can alter the levels of ABA in the host plant and that, under drought stress, the levels of ABA are lower in AM-colonized than in non-colonized plants (Duan et al., 1996; Estrada Luna and Davies, 2003; Aroca et al., 2008), as we found here under non-saline conditions or under the lower salt level (40 mM NaCl). These results, together with those of other physiological parameters, support that AM symbiosis improves plant fitness.

Under certain stresses as nutrient deficiency, the host plant increases the production of strigolactones to promote AM fungal development and symbiosis establishment (Yoneyama et al., 2007; López-Ráez et al., 2008). As above mentioned, strigolactones are also germination stimulants of root parasitic plant seeds (Bouwmeester et al., 2007; López-Ráez et al., 2011b). Other secondary metabolites than strigolactones, although a lesser extent, have also been shown to have germination-stimulatory activity (Bouwmeester et al., 2003). However, it was shown that the main known strigolactones are present in the 50% acetone fraction (López-Ráez et al., 2008). Here, a bioassay based on seed germination of *P. ramosa* using the 50% acetone fractions from root extracts was used to indirectly quantify strigolactone production by lettuce plants. We show that another stress different than nutrient deficiency such as salinity negatively affects strigolactone production in non-mycorrhizal plants (Fig. 5). As far as we know, this is the first study describing the influence of a different stress than nutrient deficiency in the biosynthesis of strigolactones. Interestingly, the presence of *G. intraradices* in the medium produced the opposite effect on the production of strigolactones, with an increase of up to 5-fold at higher salt concentrations. This fact is opposite with the observed levels of mycorrhization, where a reduced colonization was detected in response to salinity. It was shown that salts induce changes in the length and in other morphological properties of the AM fungus, thus affecting their symbiotic capacity (Juniper and Abbott, 2006; Jahromi et al., 2008). In this scenario, it might be that the negative effect of NaCl on the developing AM fungus counters the positive effect of strigolactones, decreasing mycorrhizal colonization. Thus, there is a need for the plant to overcome the stress and it seems that somehow the plant is able to sense the presence of the fungus producing strigolactones as a 'cry for help' cue. On the other hand, the pattern of strigolactone production in the presence of *G. intraradices* and salt fitted with that of the ABA content, suggesting an interaction between both phytohormones. A regulatory role for ABA in strigolactone biosynthesis was previously proposed as tomato ABA-deficient mutants blocked at different steps in the ABA biosynthetic pathway and plants treated with specific inhibitors showed a reduced capacity of producing strigolactones (López-Ráez et al., 2010). Our results with lettuce seem to confirm this ABA-strigolactone interaction, although further research is required to ascertain how it occurs.

In the absence of salt stress, a clear reduction in strigolactone production was envisaged in mycorrhizal plants. Similarly, it was shown that AM fungi colonization in pea induced less germination in *Orobanche* and *Phelipanche* species than non-colonized plants, suggesting a reduction in strigolactones in those plants (Fernández-Aparicio et al., 2010). Moreover, we showed that AM symbiosis also lead to a reduction in the germination stimulatory activity of tomato exudates for seeds of *P. ramosa*, and analytically demonstrated that this reduction was caused by a reduction in the production of strigolactones (López-Ráez et al., 2011a). Therefore, it seems that the reduction in strigolactone production induced by AM symbiosis is a conserved phenomenon across the plant species. It has been suggested that this strigolactone reduction might be involved in the regulation of mycorrhization as a plant strategy to avoid excessive colonization, a phenomenon known as autoregulation (García-Garrido et al., 2009). However, the mechanism by which AM symbiosis reduces strigolactone production is so far unknown.

In conclusion, it is shown here that AM symbiosis alleviates the negative effects of salt stress in lettuce plants by altering the hormonal profiles and affecting plant physiology in the host plant, allowing plants to grow better under these unfavourable conditions. The results confirm the potential of arbuscular mycorrhizas in protecting host plants against unfavourable environmental conditions and pave the way for applying AM symbiosis in sustainable agriculture in arid and semiarid areas. In addition, an influence of strigolactone production by salinity is proposed, indicating that other stresses different than nutrient deficiency may affect the biosynthesis of this new class of plant hormone. Interestingly, an increase in strigolactone production was suggested under salt stress in mycorrhizal plants, likely to further stimulate AM fungal development and symbiosis, and thus overcome the stress. Finally, although further research is required to elucidate the intrinsic mechanisms by which strigolactone biosynthesis is regulated under salt stress and along the mycorrhization process, our results suggest a possible involvement of ABA on such production.

Acknowledgements

This work was supported by grants AGL2009-07691 and AGL2011-25403 from the National R&D Plan of the MINCIN, and project P11-CVI-7107 financed by Junta de Andalucía (Spain). We thank Dr. Maurizio Vurro and Dr. Angela Boari for supplying the seeds of *P. ramosa*.

References

Al-Karaki GN. Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza 2000;10:51–4.
Aliasgharzadeh N, Saleh Rastin N, Towfighi H, Alizadeh A. Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. Mycorrhiza 2001;11:119–22.
Aroca R, Vernieri P, Ruiz-Lozano JM. Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. J Exp Bot 2008;59:2029–41.
Bacaicoa E, Zamarreño AM, Leménager D, Baigorri R, García-Mina JM. Relationship between the hormonal balance and the regulation of iron deficiency stress responses in cucumber (*Cucumis sativus* L.) plants. J Am Soc Hort Sci 2009;134:589–601.
Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH. Secondary metabolite signalling in host-parasitic plant interactions. Curr Opin Plant Biol 2003;6:358–64.
Bouwmeester HJ, Roux C, Lopez-Raez JA, Bécard G. Rhizosphere communication of plants, parasitic plants and AM fungi. Trends Plant Sci 2007;12:224–30.
Cadenas E. Biochemistry of oxygen toxicity. Annu Rev Biochem 1989;58:79–110.
Christmann A, Moes D, Himmelbach A, Yang Y, Tang Y, Grill E. Integration of abscisic acid signalling into plant responses. Plant Biol 2006;8:314–25.
Cuartero J, Bolarín MC, Asíns MJ, Moreno V. Increasing salt tolerance in the tomato. J Exp Bot 2006;57:1045–58.
Duan X, Neuman DS, Reiber JM, Green CD, Saxton AM, Augé RM. Mycorrhizal influence on hydraulic and hormonal factors implicated in the control of stomatal conductance during drought. J Exp Bot 1996;47:1541–50.
Duncan DB. Multiple range and multiple F-tests. Biometrics 1955;11:1–42.
Estrada Luna AA, Davies Jr FT. Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micropropagated chile ancho pepper (*Capsicum annuum*) plantlets during acclimatization and post-acclimatization. J Plant Physiol 2003;160:1073–83.
Evelin H, Giri B, Kapoor R. Ultrastructural evidence for AMF mediated salt stress mitigation in *Trigonella foenum-graecum*. Mycorrhiza 2012, in press, http://dx.doi.org/10.1007/s00572-012-0449-8
Evelin H, Kapoor R, Giri B. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 2009;104:1263–80.
Fan L, Dalpé Y, Fang C, Dubé C, Khanizadeh S. Influence of arbuscular mycorrhizae on biomass and root morphology of selected strawberry cultivars under salt stress. Botany 2011;89:397–403.
Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 2002;12:185–90.
Fernández-Aparicio M, García-Garrido JM, Ocampo JA, Rubiales D. Colonisation of field pea roots by arbuscular mycorrhizal fungi reduces *Orobanche* and *Phelipanche* species seed germination. Weed Res 2010;50:262–8.
García-Garrido JM, Lendzemo V, Castellanos-Morales V, Steinkellner S, Vierheilig H. Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. Mycorrhiza 2009;19:449–59.
Germ M, Kreft I, Osvald J. Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.). Plant Physiol Biochem 2005;43:445–8.
Giovannetti M, Mosse B. Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 1980;84:489–500.
Giri B, Kapoor R, Mukerji KG. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol Fertility Soils 2003;38:170–5.
Gómez-Roldán V, Fermas S, Brewer PB, Puech-Pagés V, Dun EA, Pillot JP, et al. Strigolactone inhibition of shoot branching. Nature 2008;455:189–94.
Hause B, Mrosk C, Isayenkov S, Strack D. Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 2007;68:101–10.
Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. Microbial Ecol 2008;55:45–53.
Juniper S, Abbott L. Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 1993;4:45–58.
Juniper S, Abbott LK. Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. Mycorrhiza 2006;16:371–9.
Kapulnik Y, Delaux PM, Resnick N, Mayzlish-Gati E, Wininger S, Bhattacharya C, et al. Strigolactones affect lateral root formation and root-hair elongation in Arabidopsis. Planta 2011;233:209–16.
Kay R, Chan A, Daly M, McPherson J. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science 1987;236:1299–302.
Kohlen W, Charnikhova T, Lammers M, Pollina T, Tóth P, Haider I, Pozo MJ, de Maagd RA, Ruyter-Spira C, Bouwmeester HJ, López-Ráez JA. The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. New Phytol 2012;196:535–47.
Kumar A, Sharma S, Mishra S. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation, and mycorrhizal dependency of *Jatropha curcas* L. J Plant Growth Regul 2010;29:297–306.
Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta C_t$ method. Methods 2001;25:402–8.
Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol 1999;119:1091–9.
López-Ráez JA, Charnikhova T, Fernández I, Bouwmeester H, Pozo MJ. Arbuscular mycorhizal symbiosis decreases strigolactone production in tomato. J Plant Physiol 2011a;168:294–7.
López-Ráez JA, Charnikhova T, Gómez-Roldán V, Matusova R, Kohlen W, De Vos R, et al. Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytol 2008;178:863–74.
López-Ráez JA, Kohlen W, Charnikhova T, Mulder P, Undas AK, Sergeant MJ, et al. Does abscisic acid affect strigolactone biosynthesis? New Phytol 2010;187:343–54.
López-Ráez JA, Pozo MJ, García-Garrido JM. Strigolactones: a cry for help in the rhizosphere. Botany 2011b;89:513–22.
Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp are derived from the carotenoid pathway. Plant Physiol 2005;139:920–34.
Ohmiya A. Carotenoid cleavage dioxygenases and their apocarotenoid products in plants. Plant Biotechnol 2009;26:351–8.
Oxborough K, Baker NR. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 1997;54:135–42.
Parker C. Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Manag Sci 2009;65:453–9.
Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Brit Mycol Soc 1970;55:158–61.
Porcel R, Aroca R, Ruiz-Lozano JM. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron Sust Dev 2012;32:181–200.
Pozo MJ, Azcón-Aguilar C. Unravelling mycorrhiza-induced resistance. Curr Opin Plant Biol 2007;10:393–8.
Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moenne-Loccoz Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 2009;321:341–61.
Raghavendra AS, Gonugunta VK, Christmann A, Grill E. ABA perception and signalling. Trends Plant Sci 2010;15:395–401.
Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos EM, Luque T, et al. Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. Ann Bot 2007;100:555–63.
Redondo-Gómez S, Mateos-Naranjo E, Figueroa ME, Davy AJ. Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*. Plant Biol 2010;12:79–87.
Ruiz-Lozano JM, Porcel R, Azcon R, Aroca R. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants. New challenges in physiological and molecular studies. J Exp Bot 2012;63:4033–44.
Ruiz-Sánchez M, Aroca R, Muñoz Y, Polón R, Ruiz-Lozano JM. The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J Plant Physiol 2010;167:862–9.
Ruyter-Spira C, Kohlen W, Charnikhova T, van Zeijl A, van Bezouwen L, de Ruijter N, et al. Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? Plant Physiol 2011;155:721–34.
Sanan-Mishra N, Pham XH, Sopory SK, Tuteja N. Pea DNA helicase 45 overexpression in tobacco confers high salinity tolerance without affecting yield. Proc Natl Acad Sci USA 2005;102:509–14.
Sawada Y, Aoki M, Nakaminami K, Mitsuhashi W, Tatematsu K, Kushiro T, Koshiba T, Kamiya Y, Inoue Y, Nambara E, Toyomasu T. Phytochrome- and gibberellin-mediated regulation of abscisic acid metabolism during germination of photoblastic lettuce seeds. Plant Physiol 2008;146:1386–96.
Smith FA, Grace EJ, Smith SE. More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. New Phytol 2009;182:347–58.
Smith SE, Read DJ. Mycorrhizal symbiosis. London: Academic Press; 2008.
Stockinger H, Walker C, Schüssler A. '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytol 2009;183:1176–87.
Taylor IB, Sonneveld T, Bugg TDH, Thompson AJ. Regulation and manipulation of the biosynthesis of abscisic acid, including the supply of xanthophyll precursors. J Plant Growth Regul 2005;24:253–73.
Ünlükara A, Cemek B, Karaman S, Erşahin S. Response of lettuce (*Lactuca sativa* var. crispa) to salinity of irrigation water. N Z J Crop Hort Sci 2008;36:265–73.

Wilde P, Manal A, Stodden M, Sieverding E, Hildebrandt U, Bothe H. Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. Environ Microbiol 2009;11:1548–61.
Xie XN, Yoneyama K, Yoneyama K. The strigolactone story. Ann Rev Phytopathol 2010;48:93–117.
Yamato M, Ikeda S, Iwase K. Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions. Mycorrhiza 2008;18: 241–9.
Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y, et al. Nitrogen deficiency as well as phosphorous deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 2007;227: 125–32.