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Arbuscular mycorrhizal symbiosis modifies the effects of a nitric oxide donor (sodium nitroprusside;SNP) and a nitric oxide synthesis inhibitor (Nω-nitro-L-arginine methyl ester;L-NAME) on lettuce plants under well watered and drought conditions

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Abstract Arbuscular mycorrhizal (AM) symbiosis is known to help the host plant to overcome environmental stresses as drought by a combination of multiple mechanisms including enhancing of root water uptake capacity. On the other hand, Nitric oxide (NO) is involved in regulating the response of plants to environmental stresses and colonization process of AM fungi. The objective of this research was to study how AM and non-AM lettuce plants responded to a NO donor (sodium nitroprusside; SNP) or to a NO synthesis inhibitor (Nω-nitro-L-arginine methyl ester hydrochloride; L-NAME) under well watered and drought conditions. Most remarkable results were that L-NAME increased the percentage of AM colonized roots under both water regimes and AM plants modified the shoot:root ratio by both chemicals under well watered conditions. Also, the deleterious effects of SNP treatment were partially prevented by AM symbiosis. Moreover, NO could be involved in the diminution of leaf water content under drought conditions, and SNP treatment seems to favor apoplastic water path inside roots. Therefore, different outcomes of relative water content, stomatal conductance and root hydraulic conductivity observed between AM and non-AM plants could be mediated by NO.

Keywords Arbuscular mycorrhizal (AM) symbiosis · Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) · Relative water content (RWC) · Root hydraulic conductivity (L) · Sodium nitroprusside (SNP) · Stomatal conductance

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1 Introduction

Arbuscular mycorrhizal (AM) symbiosis is the result of a biological interaction between AM fungi (AMF) and roots of most of the higher plants. This symbiosis provides an ecological niche to AMF in which they complete their life cycle and improves nutrient and water uptake capacity of the host plants (Gutjahr and Parniske 2013). Moreover, it is known that AM plants are more tolerant to a variety of stresses (Abbaspour et al. 2011; Porcel et al. 2012) due to a regulation of stomatal conductance (Ruiz-Lozano and Aroca 2010), an accumulation of specific solutes that decrease their osmotic potential, an improvement in root water transport properties and an increase in the activity of several antioxidant enzymes to overcome stressful conditions (Ruiz-Lozano 2003; Porcel et al. 2012).

Root hydraulic conductivity (L) is a parameter which estimates root water transport capacity. Water flow inside the roots can circulate through the apoplastic spaces (apoplastic path), or via the cell-to-cell path, through plasmodesmata or crossing the cell membranes through aquaporins (water channel protein) (Steudle 1997). These pathways act simultaneously, although a pathway can predominate over the others depending on environmental conditions. Thus, when transpiration rate is low, cell-to-cell pathway is predominant (Steudle 2000; Javot and Maurel 2002). Conversely, AM plants show an increase of water movement through apoplastic path (Bárzana et al. 2012), decreasing the expression and abundance of several aquaporins (Bárzana et al. 2014).

Nitric oxide (NO) is involved in many physiological processes, such as regulation of stomatal movement (Garcia-Mata and Lamattina 2001; Bright et al. 2006), regulation of photosynthesis (Takahashi and Yamasaki 2002), mitochondrial functionality (Zottini et al. 2002),

gravitropism and floral development (Hu et al. 2005), regulation of plant metabolism and senescence (Leshem et al. 1998; Guo and Crawford 2005), and induction of cell death (Pedroso and Durzan 2000).

Moreover, NO is known to regulate the multiple plant responses against several biotic and abiotic stresses and to alleviate some consequences caused by oxidative stresses (Beligni and Lamattina 1999; Garcia-Mata and Lamattina 2001; Crawford and Guo 2005). Focusing on drought, several studies have demonstrated that drought conditions induce an increase of endogenous NO (Gould et al. 2003; Arasimowicz-Jelonek et al. 2009a; Kolbert et al. 2010; Xiong et al. 2012). Fan and Liu (2012) observed that treatment with the NO donor sodium nitroprusside (SNP) enhanced endogenous NO concentration. Application of an inhibitor of the enzyme nitric oxide synthase (NOS) Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) decreased it (Tossi et al. 2009). Under drought conditions, exogenous NO applications resulted in an improvement of plant growth as well as maintenance of higher RWC, chlorophyll content and photosynthetic activity, and a reduction of lypooxigenase activity, stomatal conductance, and membrane ion leakage (Garcia-Mata and Lamattina 2001; Tian and Lei 2006; Lei et al. 2007; Arasimowicz-Jelonek et al. 2009b, a; Xiong et al. 2012). In addition, Xiong et al. (2012) proposed that SNP reduced the transpiration rate under well watered conditions, as drought stress does. Liu et al. (2010) observed that SNP reduced oxidative damage under chilling conditions, whereas plants treated with L-NAME- and 2-(4-carboxyphenyl)-4,4,5,5,-tetramethyllimidazoline-1-oxyl-3-oxide (cPTIO, a NO scavenger) showed lower activity of antioxidant enzymes.

In the same way, NO is involved in establishment of symbiosis. Calcagno et al. (2012) found that *Medicago truncatula* plants treated with AM fungal exudates, showed an increase of NO accumulation in their roots. Zhang et al. (2013) described an increase of NO synthesis in clover plants inoculated with *Glomus mosseae*. Similarly, Li et al. (2013b) observed that soybean plants inoculated with *Glomus intraradices* (currently known as *Rhizophagus irregularis*) also increased NO content in roots.

The aim of the present study was to investigate how AM symbiosis modified the response of lettuce plants to SNP or L-NAME application under well watered and drought conditions in terms of growth, stress symptoms, and water relation parameters. For that purpose, SNP or L-NAME were exogenously added to lettuce plants (*Lactuca sativa L.*), previously inoculated or not with the AMF *Rhizophagus irregularis* and grown under well watered or drought conditions. Plant growth, root AMF colonization, chlorophyll content, percentage of yellow leaves, stomatal conductance, daily rate of root water consumption, water status, and L were determined.

2 Material and methods

2.1 Experimental design and growth conditions

Plants of *Lactuca sativa* L. were used in a combined factorial design with three factors: (1) a biotic factor: plants were inoculated or not with the AMF *Rhizophagus irregularis*, (2) a physiological factor: untreated plants, plants treated with 0.5 mM SNP and plants treated with 1 mM L-NAME, (3) an abiotic factor: plants were grown under well watered or drought conditions. There were 12 different treatments with 10 replicates, totaling 120 plants. Five replicates of each treatment were used for L measurement, and the other 5 replicates were frozen in liquid nitrogen immediately after harvest for later use in other molecular and biochemical determinations. All physiological measurements and collection of samples were carried out 3 h after sunrise in order to avoid diurnal fluctuations in plant processes. The experiment was repeated twice with similar results, and the data of one experiment have been shown.

The experiment lasted 13 weeks from seed germination to harvest and was conducted under greenhouse conditions with temperatures ranging from 20 to 25 °C, 16/8 h light/dark photoperiod, a relative humidity of 50–60% and a photosynthetic photon flux density of 800 μE m^{-2} s^{-1}, as measured with a light meter (LICOR; Lincoln, NE, USA model LI-188B). Before the beginning of drought treatment, all plants received 10 ml of 80% Hewitt's nutrient solution (Hewitt 1952). Chemical treatments were applied during the last month twice a week (5 ml of 1 mM SNP and 5 ml of 2 mM NAME, being the final concentration in the pots of 0.5 mM for SNP treated plants and 1 mM for NAME treated plants). In the last week, plants were subjected to two water regimes before harvesting. Half of the plants were subjected to moderate drought (70% of field capacity) while the other half was maintained at field capacity. Soil moisture was measured with a ML2 ThetaProbe (AT Delta-T Devices Ltd., Cambridge, UK).

2.1.1 Soil and biological materials

Loamy soil was collected from Granada (Spain), sieved (5 mm), diluted with quartz sand (0.2 mm) [1:1, soil:sand, *v*/v] and sterilized by steaming (100 °C for 1 h on three consecutive days). The original soil used in this experiment had a pH of 8.1, 1.8% organic matter and nutrient concentrations (g kg^{-1}) as follows: N, 2.5; P, 6.2; K, 132. The soil texture was made up of 35.8% sand, 43.6% silt and 20.5% clay.

Mycorrhizal inoculum was bulked in to an open-pot culture of *Trifolium repens* L. mixed with *Sorghum vulgare* Pers. X *Sorghum* × *drummondii* (Steud.) Millsp. & Chase plants and consisted of substrate (vermiculite:sepiolite, 1:1), spores, mycelia, and infected root fragments. Five grams of inoculum were applied to each pot (500 g) at the same time as the seeds. The AMF species was *Rhizophagus irregularis* isolated BEG

121. Uninoculated pots received the same amount of sterilized inoculum and 2 ml aliquots of the filtered AM inoculum to provide a general microbial population free of AM propagules.

2.2 Physiological parameters

2.2.1 Biomass production

At harvest, shoots and washed roots of 5 plants per treatment were separated. Then, samples were dried in an oven (75 °C) for 2 days to determine dry weights (DW).

2.2.2 Symbiotic development

The percentage of mycorrhizal root length 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) (Phillips and Hayman 1970). The extent of mycorrhizal colonization was calculated according to the gridline intersect method (Giovannetti and Mosse 1980) ($n = 7$).

2.2.3 Leaf relative water content (RWC)

Small leaf pieces from the last fully developed leaves of 5 plants per treatment were weighed (fresh weight (FW)) immediately after harvesting, then placed in a water saturated vial at 4 °C for 24 h and weighed (turgid weight (TW)). The samples were then dried in an oven at 75 °C for 48 h to obtain the dry weights (DW). Then RWC was calculated using the following equation: ((FW – DW)/ (TW-DW)) × 100.

2.2.4 Stomatal conductance (gs)

Stomatal conductance was measured with a porometer system (Porometer AP4; Delta-T Devices Ltd., Cambridge, UK) following the manufacturer's instructions. Stomatal conductance (gs) measurements in lettuce plants were taken in two green leaves fully developed in 7 plants from each treatment during the four days before the harvest. The measurements were taken 3 h after sunrise.

2.2.5 Daily water consumption

During the last week, daily water consumption of each plant was controlled. The amount of water per day to be supplied to each pot for maintaining the percentage of soil moisture constant was recorded by using ML2 ThetaProbe (AT Delta-T devices Ltd., Cambridge, UK) apparatus. ThetaProbe apparatus measures volumetric soil moisture content by responding to changes in the apparent dielectric constant of moist soil. The volumetric soil moisture is the ratio between the volume of water present and the total volume of the sample. This is a dimensionless parameter, expressed as percentage (%vol). Actual soil water percentage was daily measured in each pot and the amount of water needed to reach the desired percentage was calculated and applied to the corresponding pot 2 h before sunrise, that is, 1 h before measurements. The soil moisture percentage for well watered plants was fished at 20% and for droughted plants, at 14%.

2.2.6 Chlorophyll content

Five samples of each treatment (100 mg aprox.) were frozen in liquid nitrogen and crushed in a mortar. One point five mililiters of 100% methanol were added, transferred into 2 ml Eppendorf tubes and gently shaken for 30 min at RT, before centrifuging at 17,700 g for 5 min at 4 °C. The supernatant was recovered and the pellet was dried in the oven to calculate DW. Thereafter, 1:25 diluted supernatants were measured in a spectrophotometer at 652.4 and 665.2 nm. Two replicates per sample were performed. Total chlorophyll concentration was calculated according to Lichtenthaler (1987).

2.2.7 Percentage of yellow leaves

Leaves from five plants per treatment were separated into yellow and green portions and the dry weight was determined. The percentage of yellow leaves was calculated using the following formula: (YLDW/ (YLDW + GLDW)) × 100 where YLDW is the dry weight of yellow leaves and GLDW is the dry weight of green leaves (Marulanda et al. 2010).

2.2.8 Root hydraulic conductivity (L)

L measurements were determined in 4 plants of each treatment by the free exudation method, as previously described (Aroca 2006). Under these conditions, water circulates through roots following the osmotic gradient between the root bathing solution and the root xylem, and according to the Steudle's model (Steudle and Peterson 1998), water only flows through the cell-to-cell pathway.

The stems of lettuce plants were cut just below the first branches and the pots were immersed in aerated tap water, resembling hydroponic conditions. By this way, only intrinsic hydraulic properties were determined, independently of the amount of water in the soil. A pipette connected to a silicon tube was attached to the stem. The liquid exuded from the root in the first 15 min was discarded to avoid phloem contaminations. Plants were maintained under exuding conditions for 90 min, and the exudates were collected and weighed. Also, the root dry weight of each plant was determined after incubating them during 2 days at 75 °C. The osmolarity of the exuded sap was determined using a cryoscopic osmometer (Osmomat 030, Gonotec Gmbh, Berlin, Germany). L was calculated as: $L = Jv/\Delta\Psi s$, where Jv is the exuded sap flow

rate expressed in a root dry weight basis and ΔΨs is the osmotic potential gradient between the exuded sap and the solution.

2.2.9 Statistical analysis

Data were subjected to multifactorial ANOVA analysis with three factors (biotic, physiological and abiotic factors). Post-hoc comparisons with the tukey's honesty-significant difference (HSD) tests were used to investigate differences between groups (lowercase letters) and chemical treatments for each condition (capital letters).

3 Results

3.1 Mycorrhizal colonization

Uninoculated plants did not show any mycorrhizal colonization. On the other hand, no chemical treated plants (NT) inoculated with *R. irregularis* presented 18% of mycorrhizal root length colonized. Treatment with SNP had no effects on mycorrhizal root length colonization (Fig. 1). However, plants treated with L-NAME increased mycorrhizal root length colonization up to 36%. Drought treatment had no effect on mycorrhizal root length colonization (Fig. 1).

3.2 Plant growth

Shoot dry weight (SDW) was affected by all factors, although a significant interaction between chemical treatment (physiological factor) and water regime (abiotic factor) was found (Table 1). Application of both SNP and L-NAME diminished SDW under well water conditions, being higher for SNP treated plants (Fig. 2a). Under drought conditions, SDW only decreased in non-AM plants treated with SNP (Fig. 2a). The AM symbiosis had not a great effect in SDW, being only marked the effects on well watered SNP-treated plants (Fig. 2a).

Although the biotic factor did not provide a significant effect in root dry weight (RDW) per se, a significant interaction of the three factors regarding RDW was found (Table 1), being both the physiological (chemical treatment) and abiotic (water regime) factors those with a higher effect on RDW. Application of both SNP and L-NAME diminished root dry weight (RDW), but only under well watered conditions, being the effect of L-NAME treatment higher in AM plants (Fig. 2b). RDW decrease caused by drought was not affected by chemical treatments or AM symbiosis (Fig. 2b).

The shoot:root ratio was increased by L-NAME treatment in AM plants and decreased by SNP treatment in non-AM plants under well watered conditions (Fig. 2c). No further significant changes were observed.

3.3 Stomatal conductance (gs)

All factors affected stomatal conductance (gs), as well as the interaction between biotic and physiologic factors, and between abiotic and physiologic ones (Table 1). In fact, gs mainly decreased due to drought, and the presence of AM fungi showed no effect (Fig. 3a). SNP treatment decreased gs in all treatments, except in AM plants subjected to drought (Fig. 3a). Treatment with L-NAME did not cause any significant effect on gs, except in non-AM plants under well water conditions in which a significant increase was observed (Fig. 3a).

3.4 Percentage of yellow leaves and chlorophyll content

The percentage of yellow leaves is a measurement indicatives of stress symptoms caused by a given treatment such as drought (Aroca et al. 2003; Marulanda et al. 2010). The

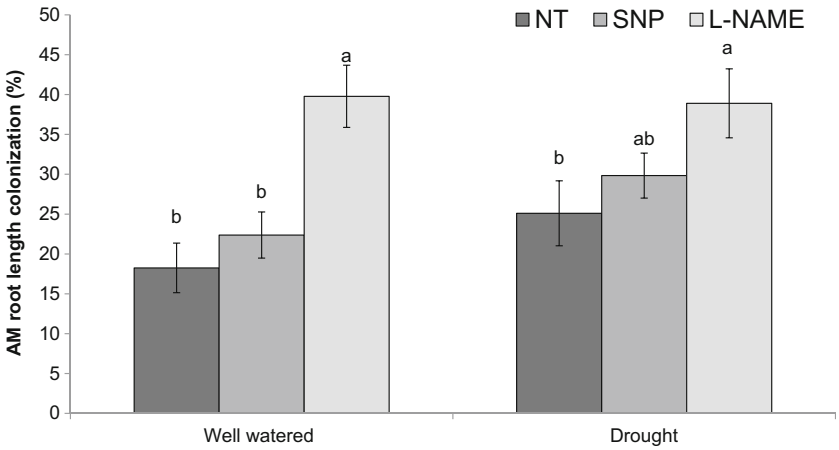

Fig. 1 Effect of water regime and SNP and L-NAME treatments on the percentage of mycorrizal root length colonization by *Rhizophagus irregularis* in lettuce roots. Seven plants of each treatment were analysed. Untreated plants (NT), plants treated with 0.5 mM SNP, and plants treated with 1 mM L-NAME. Well-watered plants (*dark bars*) and droughted plants (*grey bars*). Lowercase letters mean significant differences ($p < 0.05$) after TUKEY's HSD tests for all treatments. Bars represent mean ± SE ($n = 7$)

Table 1 Significance of sources of variation after three-way ANOVA analyses for following parameters: shoot dry weight (SDW), root dry weight (RDW), shoot:root ratio (S:R), stomatal conductance (*gs*), percentage of yellow leaves (YL), chlorophyll content (Chl), daily rate of root water consumption (WC), leaf relative water content (RWC) and root hydraulic conductivity (L). The sources of variation were AM symbiosis (B, biotic factor), water regime (A, abiotic factor) and chemical treatments (P, physiological factor), as well as their interactions

	B	A	P	BxA	BxP	AxP	BxAxP
SDW	*	***	***	ns	ns	**	ns
RDW	ns	***	**	ns	ns	***	*
S:R	ns	ns	ns	ns	ns	ns	ns
gs	*	***	***	ns	***	***	ns
YL	ns	***	***	ns	ns	**	ns
Chl	ns	***	***	**	ns	ns	***
WC	***	***	**	ns	ns	ns	**
RWC	ns	***	***	ns	ns	***	**
L	*	**	*	ns	ns	ns	ns

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant effect

changes observed were mainly due to abiotic and physiological factors (Table 1). SNP treatment only increased the percentage of yellow leaves under well watered in non-AM plants. In fact, AM plants were more resistant to SNP treatment in terms of percentage of yellow leaves (Table 1). Drought treatment increased the percentage of yellow leaves in all plants (Fig. 3b).

Chlorophyll content was measured in the green parts of the leaves. SNP treatment increased chlorophyll content in AM plants under well watered conditions and in non-AM plants under drought conditions. L-NAME increased chlorophyll content exclusively in AM plants grown under drought conditions (Fig. 3c). Drought treatment only reduced chlorophyll content in non-AM plants (Fig. 3c).

3.5 Daily rate of root water consumption (WC)

Table 1 shows a significant interaction among the three factors regarding WC. Thus, the abiotic factor (drought) reduced WC in untreated plants, but AM symbiosis prevented this reduction in untreated and SNP treated plants (Fig. 4a). SNP decreased WC in plants grown under well watering conditions, although this effect was prevented by AM symbiosis. L-NAME only increased WC in AM plants under well watered conditions (Fig. 4a). Under drought conditions, AM plants had higher WC than non-AM plants, except in L-NAME treated ones (Fig. 4a).

3.6 Leaf relative water content (RWC)

AM symbiosis had no significant effect in RWC, so the statistically significant differences were mainly due to the abiotic and physiological factors (Table 1). Drought treatment significantly reduced RWC values in all treatments, except in non-AM plants treated with L-NAME. AM symbiosis reduced the

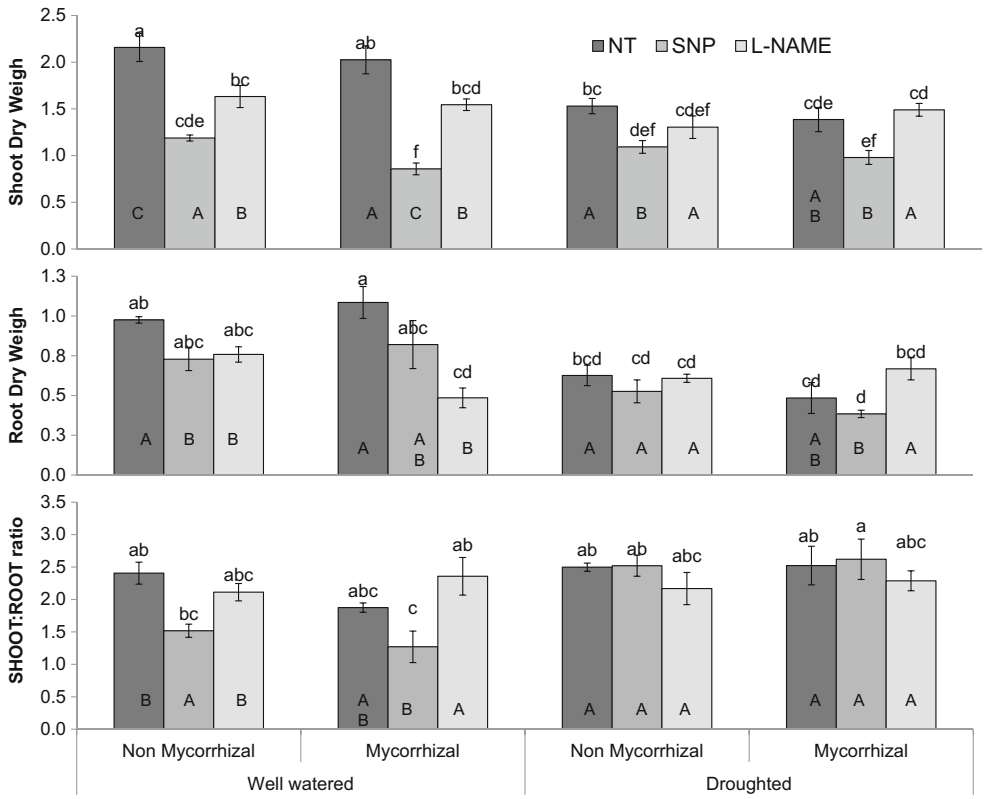

Fig. 2 **a** Dry weights of shoots and **b** roots, and **c** shoot:root ratio of lettuce plants untreated (NT), treated with 0.5 mM SNP or 1 mM L-NAME. Plants were either uninoculated (*dark bars*) or inoculated with *Rhizophagus irregularis* (*grey bars*) and cultivated under well-watered or drought conditions. Lowercase letters mean significant differences ($p < 0.05$) after TUKEY's HSD tests for all treatments, capital letters were used to show significant differences ($p < 0.05$) after TUKEY's HSD tests between the three chemical treatments for each growth conditions. Bars represent mean ± SE ($n = 5$)

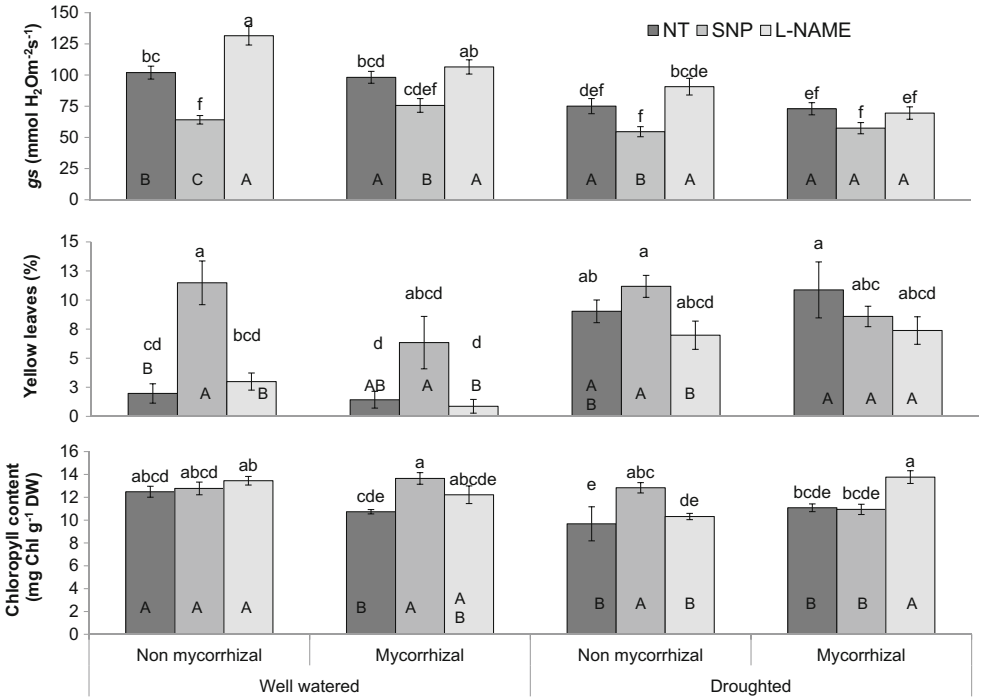

Fig. 3 **a** Stomatal conductance (*gs*) ($n = 56$), **b** chlorophyll content ($n = 5$), **c** percentage of yellow leaves ($n = 5$), of *L. sativa* plants. Plants were either uninoculated (*dark bars*) or inoculated with *Rhizophagus irregularis* (*grey bars*). Plants were untreated (NT), treated with 0.5 mM SNP or 1 mM L-NAME and cultivated under well-watered conditions or subjected to drought stress for one week. Bars represent mean ± SE. Lowercase letters mean significant differences ($p < 0.05$) after TUKEY's HSD tests for all treatments, capital letters were used to show significant differences ($p < 0.05$) after TUKEY's HSD tests between the three chemical treatments for each growth conditions

negative effect of drought on both untreated and L-NAME plants (Fig. 4b). The reduction of RWC in non-AM SNP treated plants was the only difference observed under well irrigation conditions (Fig. 4b).

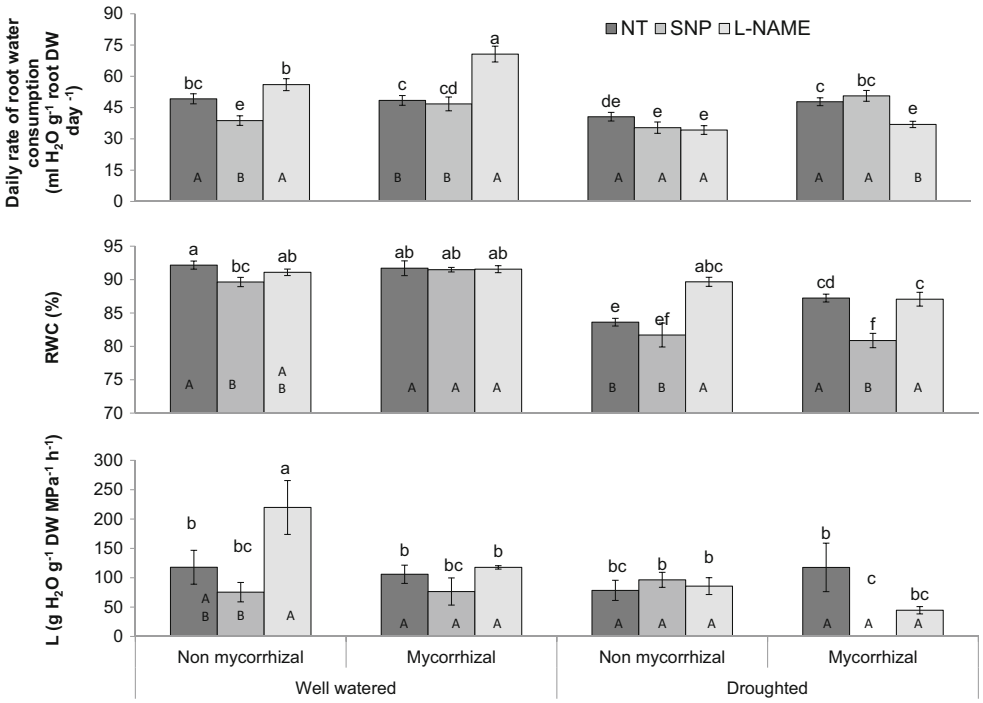

Fig. 4 **a** Daily rate of root water consumption ($n = 50$), **b** leaf relative water content (RWC) ($n = 5$), **c** root hydraulic conductivity (*L*) measured by free exudation method ($n = 5$) of *L. sativa* plants. Plants were either uninoculated (*dark bars*) or inoculated with *Rhizophagus irregularis* (*grey bars*). Lettuce plants treated with 0.5 mM SNP, plants treated with 1 mM L-NAME and control untreated plants (NT). The plants were cultivated under well-watered conditions or subjected to drought stress for one week. The *L* value of SNP-treated plants and inoculated could not be detected (n.d) under drought condition. Bars represent mean ± SE. Lowercase letters mean significant differences ($p < 0.05$) after TUKEY's HSD tests for all treatments, capital letters were used to show significant differences ($p < 0.05$) after TUKEY's HSD tests between the three chemical treatments for each growth conditions

3.7 Root hydraulic conductivity (L)

Overall, plants of the different treatments showed similar L values, except by non-AM plants treated with L-NAME under well watered conditions, which had the highest L value, and AM plants treated with SNP under drought conditions for which L was negligible (Fig. 4c).

4 Discussion

SNP and L-NAME have been largely used as chemicals to study the implication of NO as a molecular signal dealing with abiotic stresses (Fan and Liu 2012; Zhang et al. 2014; Liu et al. 2015). However, no data about the effects of these compounds are available in AM plants. Here the effects of SNP and L-NAME in AM and non-AM plants under well watered and drought conditions were investigated. Since both chemicals could have potential side effects on plant physiology besides NO contents regulation, the interpretation of our results should be taken with caution.

The most outstanding result was that L-NAME treatment increased the percentage of root length colonized by the AM fungus *R. irregularis*. Previously, it has been reported that AM symbiosis increased NO levels in the host roots (Calcagno et al. 2012; Zhang et al. 2013). This capacity of plants to regulate NO synthesis depends on the type of fungi, since the increase in response to AM fungi seems to be lower than that in response to a pathogenic fungi infection (Espinosa et al. 2014). Our results suggest that L-NAME may be controlling NO concentration by inhibiting NOS activity, and this may cause an increase of AM root colonization.

Although both SNP and L-NAME decreased SDW under well watered conditions, the diminution was higher in SNP treated plants. These results may indicate that certain amount of NO is needed to grow properly, but changes in the optimal level of NO could cause a nitrosative stress (Valderrama et al. 2007). Similar dual effect of NO was observed before by Clark et al. (2010), regarding root growth regulation by extracellular nucleotides. Curiously, the negative effects of SNP on plant growth were more severe in aerial parts than in roots, even when SNP was applied to the soil. This could be caused by an indirect effect of soil SNP on shoots, or by the translocation of SNP to aerial parts. Remarkably, SDW of AM plants was more affected by SNP treatment than that of non-AM plants under well watered conditions, maybe because AM roots have a higher water transport capacity than non-AM plants (Marulanda et al. 2003). In fact, AM plants consumed more water than non-AM plants under well watered conditions when exposed to SNP. Aerial parts of lettuce AM plants responded to SNP treatment with higher reduction of SDW, but with less damage symptoms because an increased of chlorophyll content and lower percentage of yellow leaves.

Similarly, L-NAME treatment under well watered conditions decreased RDW to a higher extent in AM plants than in non-AM plants. These plants also showed an increase of the percentage of colonized root length, which could cause the increase of the shoot:root ratio as previously reported (Zhang et al. 2015).

The decline of stomatal conductance caused by SNP treatment had been previously observed (Fan and Liu 2012). AM symbiosis diminished such reduction. On the other hand, L-NAME increased stomatal conductance only in non-AM plants. It is possible that AM mycelia avoided the translocation of L-NAME to shoots. AM could accumulate L-NAME in the roots as described for other substances (Wu et al. 2009) or even metabolize it, since L-NAME is an arginine-related substance. In this sense, it has been found that arginine can be degraded to ornithine by AM fungi (Jin 2009).

The decrease of RWC by SNP treatment in non-AM plants under well watered conditions could be caused by a concomitant descent of daily rate of root water consumption (WC). By contrast, WC of AM plants did not decrease by SNP treatment.

L-NAME treatment increased WC in AM plants under well watered conditions, which could be caused by above described increase in root length colonization. However, the opposite result was observed under drought conditions. Thus, the well described increase of WC under drought conditions by AM symbiosis (Marulanda et al. 2003; Khalvati et al. 2005) (Fig. 4a) could be reduced by L-NAME treatment. As expected, RWC was lower in non-AM than in AM plants under drought conditions, as described elsewhere (Wu and Xia 2006; Li et al. 2015). However, SNP treatment in AM plants caused a descent of RWC values down to the values of non-AM plants. Conversely, L-NAME treatment raised RWC values of non-AM plants up to those of AM plants. Therefore, NO could be one of the causes of RWC decrease by drought in non-AM plants.

Although usually SNP treatment increased RWC of stressed plants (Garcia-Mata and Lamattina 2001; Dinler et al. 2014), Li et al. (2013a) also found a reduction of RWC in SNP-treated maize plants susceptible to chilling stress. Liu et al. (2013) found an increase of NO contents in leaves of rice plants colonized by AM fungi independently of the N content and growth temperature. To our knowledge, there are no more other reports regarding NO contents in leaves of AM plants. Our results suggest that AM symbiosis could diminish NO accumulation in leaves under drought conditions and then partially avoid the diminution of RWC, since L-NAME increased RWC of non-AM plants up to the values of AM ones. In fact, Hao et al. (2008) suggested that NO production under drought stress is mediated by NOS-like enzymes, the type of enzymes inhibited by L-NAME.

Our root hydraulic conductivity (L) values were in the same range than those previously reported in lettuce plants (Aroca et al. 2008), although here no differences between AM and non-AM plants were found. This could be caused by a different percentage of AM colonized root length (being lower in the present study), or maybe the treatment was not stressful enough. In fact, drought treatment only caused a descent of L in L-NAME non-AM plants and in SNP-treated AM plants, indicating that the applied drought treatment was mild. L is Most commonly reduced by drought stress to avoid loss of water from roots to soil as the water potential of the soil decreased (Aroca et al. 2012). L-NAME treatment only increased L in non-AM plants under well watered conditions, and SNP treatment only decreased L in AM plants under drought conditions. So the response of L to L-NAME and SNP was different between AM and non-AM plants, and also depends on the soil water regimen. Di Pietro et al. (2013) found a reduction in L values of Arabidopsis plants treated with a NO donor diethylamine NONOate, but here the descent of L by SNP was only observed in AM plants. These data indicate a clear effect of NO over AM symbiosis (Calcagno et al. 2012; Zhang et al. 2013) caused a different response of L. Therefore, the different L behavior previously observed between AM and non-AM plants (Aroca et al. 2007; El-Mesbahi et al. 2012) could be partly due to changes in the endogenous balance of NO levels.

SNP treatment in AM plants under drought conditions was able to reduce L almost to zero. Since we measured L under atmospheric pressure, only water moving through the cell-to-cell pathway was determined (Steudle and Peterson 1998). However, these plants had similar gs and higher WC than non-AM plants. Hence, we suggest that SNP treatment may be able to increase the proportion of water circulating by the apoplastic path. Since it was demonstrated that AM symbiosis enhances the water circulating by the apoplastic path (Bárzana et al. 2012), it is possible that SNP further stimulates it.

In summary, AM and non-AM plants responded differently to SNP and L-NAME treatments. It seems that NO could be inhibiting the spread of mycelia growth within the roots. Moreover, AM plants were more sensitive to both chemicals in terms of growth, although those changes were reflected in different shoot:root ratio values. Also, NO could be involved in the reduction of both RWC and stomatal conductance caused by drought as well as in the increasing RWC in AM plants. In addition, the different behavior of L in AM and in non-AM plants could be caused by different NO levels and by the enhancement of apoplastic path in response to SNP treatment in AM plants. However, more studies are needed to elucidate the NO involvement in regulating water relations under drought conditions in AM plants. Studies analyzing the NO contents in AM plants under drought conditions are also required to confirm our results using different chemicals.

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