Comparative Study of Substrate-Based and Commercial Formulations of Arbuscular Mycorrhizal Fungi in Romaine Lettuce Subjected to Salt Stress

Idoia Garmendia and Víctor Javier Mangas

Department of Environment and Earth Sciences, University of Alicante, P.O. Box 99, E- 03080 Alicante, Spain. Tel. 34 965903400 ext. 2419; Fax: 34 965909862;

Address correspondence to I. Garmendia; E-mail: idoia.garmendia@ua.es

ABSTRACT

To compare the effect of substrate-based and commercial arbuscular mycorrhizal fungi (AMF) in salt stress tolerance of Romaine lettuce a bifactorial analysis was carried out. Under non-saline conditions, only plants inoculated with formulation 1 stimulated shoot weight but not related with greater root AMF colonization. Phosphorus and potassium concentrations in leaves were improved by mycorrhizal association. Irrigation with 100 mM sodium chloride (NaCl) did not affect leaf relative water content and we observed no osmotic adjustment in leaves from non-mycorrhizal plants. However, root dry biomass and its starch content decreased, while leaf starch and root soluble sugar concentrations were enhanced. Lettuce inoculated with formulation 2 and substrate-based *Glomus intraradices* showed the highest root colonization percentages. Nevertheless, none of the mycorrhizal treatments induced a significant improvement on growth

of lettuce subjected to salt stress. Romaine lettuce seems to be a moderately tolerant variety to salinity and therefore, the contribution of AMF was minimized.

Keywords: *Glomus*, *Lactuca sativa*, salinity, horticulture, commercial mycorrhiza

INTRODUCTION

Lettuce is considered relatively sensitive to salinity showing a reduction of growth and yield quality (Kohler et al., 2009; Martínez et al., 1996), although it depends on the variety. Romaine lettuce, one of the most commonly used salad vegetable, seems to be one of the less sensitive varieties (Nasri et al., 2011). However, dry weight, height and color of Romaine lettuce is significantly changed by long-term irrigation with moderately high sodium chloride (NaCl) concentration (Kim et al., 2008). Salinization of agricultural soils and irrigation water is one of the major environmental problems for crop yield. Under saline conditions plants suffer osmotic stress, by limiting root water absorption, and ionic stress, resulting from high concentration of toxic ions within plant cells.

Arbuscular mycorrhizal fungi (AMF) can contribute to the salinity resistance of host plants by improving nutritional status, particularly of phosphorus (P) and nitrogen (N) (Jeffries et al., 2003; Ojala et al., 1983), enhancing osmotic adjustment (Augé, 2001; Azcón et al., 1996), increasing water use efficiency and uptake (Augé, 2001; Ruiz-Lozano and Azcón, 1995), stimulating photosynthetic activity (Augé and Stodola, 1990) and reducing oxidative damage (Augé, 2001). Other biological strategies to facilitate plant growth under salinity stress are the

use of plant growth-promoting bacteria (PGPR) as *Pseudomonas mendocina* (Kohler et al., 2009) or *Azospirillum brasilense* (Barassi et al., 2006). These bioprotectors can play a significant role in soilless greenhouse lettuce culture with limited good quality water resources. For example, *Azospirillum*-inoculated lettuce seeds had better germination and vegetative growth than non-inoculated controls after being exposed to NaCl (Barassi et al., 2006), and mycorrhizal symbiosis enhanced plant growth and leaf relative water content (Jahromi et al., 2008) and significantly reduced sodium (Na) and chloride (Cl) uptake of lettuce subjected to salt stress (Zuccarini, 2007).

Taken into account such considerations and the well known fact that the use of biological tools are useful for purposes of more sustainable horticulture, our objective was to compare the effect of substrate-based and commercial AMF inocula ameliorating the negative effect of saline conditions in soilless greenhouse Romaine lettuce.

MATERIAL AND METHODS

Biological Material, Growth Conditions, and Experimental Design

Seeds of Romaine lettuce (*Lactuca sativa* L. var. *longifolia* cv. 'Parris Island') were germinated on washed sand. When one month old, 125 seedlings were transplanted to 3 L plastic containers filled with a mixture of perlite-coconut fiber-sand (1.5:1.5:1 v/v/v). When transplanted, plants were divided into five groups (25 plants per treatment): (a) non-mycorrhizal plants (NM), plants inoculated with a commercial product containing granular sand and clay with spores of a mixture

of *Glomus intraradices* (Schenck and Smith) and *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe [commercial inoculum 1 (CI_1)], (c) plants inoculated with a commercial product containing *G. intraradices* on granular expanded clay [commercial inoculum 2 (CI_2)], (d) plants inoculated with bulk inoculum of *Glomus intraradices* (Gi), and (e) plants inoculated with bulk inoculum of *Glomus mosseae* (Gm). Table 1 lists the abundance of propagules in different inocula and the rates of application into the potting substrate. Commercial formulations were used at 5x the recommended dose because previous studies with commercial inocula had indicated that the rate recommended by the manufacturers sometimes is too low for mycorrhizas to form within a reasonable time (Tarbell and Koske, 2007). Bulk inocula were supplied by Plant Biology Department of Navarra University (Navarra, Spain). These inocula were substrate-based and include root fragments, spores and hyphae from 3 months culture of leek and alfalfa grown in a mixture of perlite-coconut fiber (1:1 v/v). Infectivity of bulk inocula was evaluated by Most Probable Number (MPN) assay (Schenck, 1982) with *Sorghum bicolor* as the host plant. The bioassay was performed with five replicates in 200 mL pots (perlite-coconut fiber, 1:1 v/v) in a greenhouse (25/20ºC day/night and natural daylight), watered with deionized water and grown for 4 weeks. All inoculants were added to the planting hole and mixed with the surrounding potting substrate ensuring that good contact was achieved with runner roots.

Plants were drip irrigated weekly with 100 mL Long Ashton Nutrient Solution (LANS) (Hewitt, 1966) at one-quarter phosphorus strength to contribute to the establishment of mycorrhizal symbiosis (Azcón-Aguilar and Barea, 1997). In addition, plants received water to prevent wilting. The experiment was carried out in a greenhouse at 25/15ºC day/night and plants

received natural daylight supplemented with irradiation from sodium lamps Son-T Plus (Philips Nederland B.V., Eindhoven) during a photoperiod of 16 h.

Salt stress was induced by NaCl (100 mM in irrigation water) 2 months after transplanting. To avoid an osmotic shock, the concentration of NaCl was increased gradually during the first week to reach the desired NaCl concentration and maintained for additional 3 weeks. At the end of the experiment, the electrical conductivity of the substrate from non-saline pots and the pots cultivated under salinity was about 0.45 and 2.44 mS cm^{-1}, respectively. Two plant harvests were performed: the day before imposing the salt stress (two months after AMF inoculation) and after 4 weeks of saline conditions.

Plant Growth parameters, Water Status and Estimation of AMF Colonization

In each harvest, total dry matter (DM) of the different plant organs was determined after drying at 80ºC for 2 days. Relative water content (RWC) was estimated by a modification of Weatherley's method (1950) on youngest fully mature leaves.

Root samples were cleared and stained (Phillips and Hayman, 1970) and the percentage of AMF root colonization was assessed by examining a minimum of 100 1 cm root segments for each treatment (Hayman et al., 1976).

Mineral Analyses

Samples (0.25 g dry weight) were dry-ashed and dissolved in HCl according to Duque (1971). Phosphorus, potassium, magnesium, calcium, manganese, zinc, iron and sodium were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, Waltham, MA, USA). Total nitrogen was quantified after combustion (950ºC) of leaf dry matter with pure oxygen by an elemental analyzer provided with a thermal conductivity detector (TruSpec CN, Leco, St. Joseph, MI, USA). Youngest full-mature leaves were used for mineral analysis.

Biochemical Analysis

These analyses were performed on the youngest full-mature leaves harvested at midday, frozen in liquid nitrogen and stored at -20ºC in each harvest for later quantifications. Photosynthetic pigment content of leaves was determined according to Séstak et al. (1971). Samples (20 mg of fresh leaves) were immersed in 5 ml of 96% ethanol at 80ºC for 10 min to extract the pigments. The absorbance of extracts was spectrophotometrically measured and the equations reported by Lichtenthaler (1987) were used to calculate pigment concentrations.

Total soluble sugars (TSS), starch and proline in roots and leaves were quantified in potassium phosphate buffer (KPB) (50 mM, pH= 7.5) extracts of fresh tissue (0.1 g). These extracts were filtered through four layers of cheesecloth and centrifuged at 28710 g for 15 min at 4ºC. The pellet was used for starch determinations (Jarvis and Walker, 1993). The supernatant was collected and stored at 4ºC for TSS and proline determinations. Total soluble sugars were analyzed spectrophotometrically with the anthrone reagent (Yemm and Willis, 1954). Free

proline was estimated by spectrophotometric analysis at 515 nm of the ninhydrine reaction (Irigoyen et al., 1992).

Statistics

Plant DM, leaf RWC, mycorrhizal colonization, and mineral concentration in leaves the day when salt stress was imposed were analyzed with one-way analysis of variance (ANOVA). Data on parameters measured after salinity treatments were subjected to a two-factor ANOVA. The variance was related to the main treatments (AMF and salt stress) and to the interaction between them. Means ± standard deviation (SD) were calculated and, when the *F*-ratio was significant, least significance differences were evaluated by the Tukey-b test. When only two treatments were compared, means ±SD were calculated and their differences tested for significance by using Student's *t*-test. Significance levels were always set at 5%.

RESULTS

There were significant differences in growth parameters between lettuce plants two months after been inoculated with different mycorrhizal fungi (Table 2). Plants inoculated with commercial formulation 1 had the greatest shoot biomass and reduced its root dry matter, while the rest of AMF inocula only brought down the root biomass comparing with NM plants. Mycorrhizal colonization achieved the 42% in Gi lettuce and around 1% in the rest of mycorrhizal treatments. Non-mycorrhizal plants remained uncolonized.

Referring to photosynthetic pigments, chlorophyll leaf concentrations were higher in Gi, Gm and CI_2 plants, and foliar carotenoids were accumulated specifically in lettuce inoculated with both bulk inocula (Table 2). Lettuce plants showed good water status with 90% of relative water content. Mineral concentrations in leaves varied from non-mycorrhizal to different type of AMF inocula applied (Table 3). Commercial formulation 1 induced higher levels of P and magnesium (Mg), Gi plants improved potassium (K) and zinc (Zn) concentration, and lettuce plants inoculated with substrate-based *G. mosseae* had greater foliar iron (Fe) concentration two months after they had been inoculated.

Under non-saline conditions and three months after seedlings were transplanted and inoculated, shoot biomass of CI_1 plants was still higher than the rest of treatments and root dry matter was lower than in NM plants (Table 4). In fact, all mycorrhizal treatments maintained the reduction of its root biomass. At that moment, root colonization achieved 1, 34, 40, and 11% in CI_1, CI_2, Gi, and Gm plants respectively. Leaf concentrations of chlorophyll in CI_2 and Gi plants and carotenoids in CI_2, Gi, and Gm plants were enhanced comparing with NM and CI_1 plants. Salinity did not change the growth trend of lettuce plants and did not affect photosynthetic pigments' level, although NM and Gi plants subjected to 100 mM of NaCl showed a reduction of root biomass comparing with their respective controls under non-saline conditions. The salt stress imposed was not as severe to reduce the relative water content of leaves with the exception of CI_1 plants. However, as a result of the high significant interaction between the two factors studied, salinity induced higher mycorrhizal root colonization in lettuce inoculated with substrate-based *G. intraradices.*

The effect of salinity in leaf K, Mg, manganese (Mn), Zn, iron (Fe), and sodium (Na) concentrations depended on the AMF inocula applied (Table 5). Under non-saline conditions, K was higher in plants treated with substrate-based inocula, Mg in CI_1 plants, Mn in CI_1 and Gi lettuce, Zn and Fe in all mycorrhizal treatments with the exception of Zn in CI_1 plants. Moreover, CI_1, CI_2 and Gi plants showed significantly higher P concentration in leaves than NM lettuce after 3 months of culture, despite all plants received phosphorus at one-quarter strength.. Salinity caused slight modifications in foliar nutrient concentrations. Only CI_1 plants showed higher P concentration in leaves than NM lettuce and K level was not enhanced due to mycorrhizal inoculation. However, salinity increased foliar Fe level in non-mycorrhizal plants, while plants inoculated with bulk inocula reduced it. Salt stress also decreased foliar Ca and Mg concentration in Gi and Gm plants, and enhanced leaf Mn in Gi, Gm, and CI_2 plants. As expected, all plants subjected to 100 mM NaCl treatment had higher Na concentration in leaves, with lower values in CI_1 plants.

Mineral analysis of commercial formulations was also assessed (Table 6). Commercial inoculum 1 had similar Ca, Fe, and Na concentrations as CI_2, although it showed significantly higher N, P, K, Mg, and Zn. In contrast, CI_2 showed greater Mn level.

Results concerning soluble solutes showed that CI_2 plants exhibited the highest leaf starch concentration after three months of culture under non-saline conditions (Figure 1, a1). However, all inoculated plants had lower root starch level than NM lettuce, especially CI_1 plants. Total soluble sugar concentration in leaves was similar in non-mycorrhizal and mycorrhizal plants (Figure 1, b1), although Gi plants showed lower root TSS level than NM plants. Under non-saline conditions, plants inoculated with commercial formulation 1 had the greatest leaf proline

concentration (Figure 1, c1), while root proline concentration was lower in plants treated with commercial formulations than with bulk inocula. Four weeks treatment with 100 mM NaCl altered carbohydrate and proline levels of lettuce leaves and roots (Figure 1, a2, b2, c2). Non-mycorrhizal, CI_1 and Gm plants increased foliar starch concentration comparing with their respective controls not subjected to salt stress, while all treatments with the exception of CI_1 plants reduced root starch level (Figure 1, a2). Total soluble sugars in leaves showed dissimilar behaviour depending on the mycorrhizal treatment (Figure 1, b2). Plants inoculated with CI_1 enhanced foliar TSS concentration due to growing with 100 mM NaCl and CI_2 plants reduced it. In roots, TSS level was maintained as under saline conditions in CI_1, CI_2 and Gi plants, but increased in NM and Gm plants. In reference to proline concentration, plants subjected to salt stress had similar root concentration in comparison with non-saline conditions (Figure 1, c2), although proline leaf concentration of CI_1 plants was enhanced.

DISCUSSION

Plants inoculated with substrate-based *G. intraradices* showed the highest and earliest root mycorrhizal colonization. According to Feldmann (1998), AMF isolates are not genetically homogeneous and thereby their function results in changes of mycorrhizal effectiveness. Formulations CI_1 and CI_2 contained *Glomus intraradices*, although only CI_2 plants showed good mycorrhizal establishment. Moreover, one of the commercial mycorrhizal product tested, CI_1, did not colonize lettuce roots. Studies with commercial formulations have indicated that the qualities of some inocula remain uncertain (Gaur et al., 1998; Tarbell and Koske, 2007). In fact,

the promises made about the product and the results seen by the end-users are often world's apart, showing that some mycorrhizal products available need greater regulation and control over the production and selling (Alten et al., 2002). According to Tarbell and Koske (2007), the failure of five of the eight commercial inocula to colonize roots of *Zea mays* when applied at the recommended rate by manufacturers, concerns about the quality and viability of some formulations.

Despite CI_1 lettuce plants did not establish symbiosis with AMF, they showed an enhanced shoot growth and leaf nutrient concentration that could be explained by the high levels of N, P, K, Mg, and Zn quantified after mineral analysis of the commercial product. Excessive P content of the formulation CI_1 could cause the inhibition of mycorrhizal establishment of lettuce roots. Alten et al. (2002) explained that the nutrient content of the mycorrhizal product can be of special importance if high doses of formulation must be used, thus in the processing of the inoculum especially the amount of P should be reduced.

Mycorrhizal symbiosis can increase shoot and root dry weight of lettuce (Jahromi et al., 2008; Ruiz-Lozano and Azcón, 2000) or maintain as in non-mycorrhizal controls (Kohler et al., 2009). Under non-saline conditions, lettuce plants respond to mycorrhizal inoculation maintaining shoot biomass but reducing the root dry matter without a negative effect in nutrient concentration in leaves. In fact, after three months of culture, K, Mg, Zn, Fe, and P levels in leaves increased in some inoculated treatments. Moreover, mycorrhizal symbiosis enhanced chlorophyll and carotenoid concentration of lettuce leaves in accordance with previous work of Zuccarini (2007). Higher levels of photosynthetic pigments in mycorrhizal lettuce can be related to a greater nutritional status of plants. However, plants inoculated with CI_1 did not show this

increase in foliar photosynthetic pigment concentration, probably due to a dilution effect caused by its higher shoot biomass. According to Balsam et al. (2011), arbuscular mycorrhizal fungi can enhance nutritional quality and potentially beneficial compounds for human diet (as photosynthetic pigments) in lettuce plants consumed as salads.

Lactuca sativa responses to salt stress have been highly variable according to the cultivar (Shannon et al., 1983). Romaine lettuce is considered a less sensitive lettuce variety (Nasri et al., 2011) although other authors describe as sensitive (Mahmoudi et al., 2010). In our case, four weeks irrigation with 100 mM NaCl, achieving to an electrical conductivity of the substrate of 2.44 mS cm^{-1}, did not affect shoot growth, leaf RWC or foliar photosynthetic pigment concentration in non-mycorrhizal plants, although root biomass was reduced. In addition, the resulting saline condition was enough to reduce root starch concentration with a concomitant increase in leaves. According to Schellenbaum et al. (1998), salinity can induce a preferential partitioning of carbohydrates to the roots, although root starch storage could decrease as a consequence of a decline in photosynthesis due to salinity. On the other hand, salinity enhanced mycorrhizal root colonization by bulk *G. intraradices.* Kohler et al. (2009) described that the level of colonization in roots of mycorrhizal lettuce plants decreased significantly with increasing NaCl concentration, while Cantrell and Linderman (2001) did not observe significant differences in AMF root colonization as salt concentration increased. In any case, this fact did not enhanced salt tolerance of Gi plants.

Plants inoculated with formulation 1 and subjected to saline conditions showed a reduction in leaf RWC despite an enhanced foliar proline and TSS concentration. To avoid the osmotic stress caused by salinity, plants may accumulate inorganic ions like K^+ and low-

molecular-weight solutes as proline to maintain the internal osmotic potential (Hasegawa et al., 2000), and mycorrhizal symbiosis can improve salt tolerance by improving this osmorregulation (Augé, 2001; Azcón et al., 1996). Despite CI_1 plants increased leaf osmolite concentration, this was not high enough to counterbalance the osmotic stress. In contrast, these plants had less leaf Na concentration than the rest of treatments, probably due to a dilution effect caused by its greater shoot biomass.

Mycorrhization in relation to salt stress did not enhanced K acquisition but induced higher foliar Mn concentration in Gi and Gm plants. Manganese uptake is competitive with other cations as Mg, K, Ca and Na (Jones, 2003), and therefore, can be related to some extent to the ability of Na cation exclusion preventing Na leaf accumulation and its osmotic injury. On the other hand, the capacity of mycorrhizal fungi to improve some nutrient availability as P,was not maintained under saline conditions, with the exception of CI_1 plants. Higher leaf P concentration observed in CI_1 plants may be related to the nutrient content of the formulation applied.

CONCLUSIONS

One of the commercial formulations of AMF tested did not efficiently colonize lettuce roots. The positive effect on plant growth and nutrition attributed to this formulation was caused by the high mineral content included in the commercial product. In contrast, lettuce inoculated with bulk *G. intraradices* and commercial formulation 2 showed the highest root colonization rates with increased leaf P and photosynthetic pigment concentrations. A more balanced mineral nutrition together with the maintenance of the photosynthetic capacity (estimated by chlorophyll

concentration) in mycorrhizal plants could help to counterbalance salt stress. However, four weeks irrigation with 100 mM NaCl was not severe enough to cause noticeable damage to Romaine lettuce. Mycorrhizal inoculation will be more effective alleviating salt stress with more sensitive lettuce varieties and/or more negative saline conditions.

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Figure 1. Starch (a1, a2), total soluble sugars (b1, b2) and proline (c1, c2) concentrations in non-mycorrhizal (NM) and mycorrhizal lettuce plants inoculated with commercial inocula 1 (CI_1) or 2 (CI_2), and substrate-based *Glomus intraradices* (Gi) or *Glomus mosseae* (Gm) three months after transplanting and subjected to different salt concentrations. Means ± SD (n=7-9 plants) were compared with the Tukey-b test. Within each parameter histograms with the same letter do not differ significantly (P<0.05).

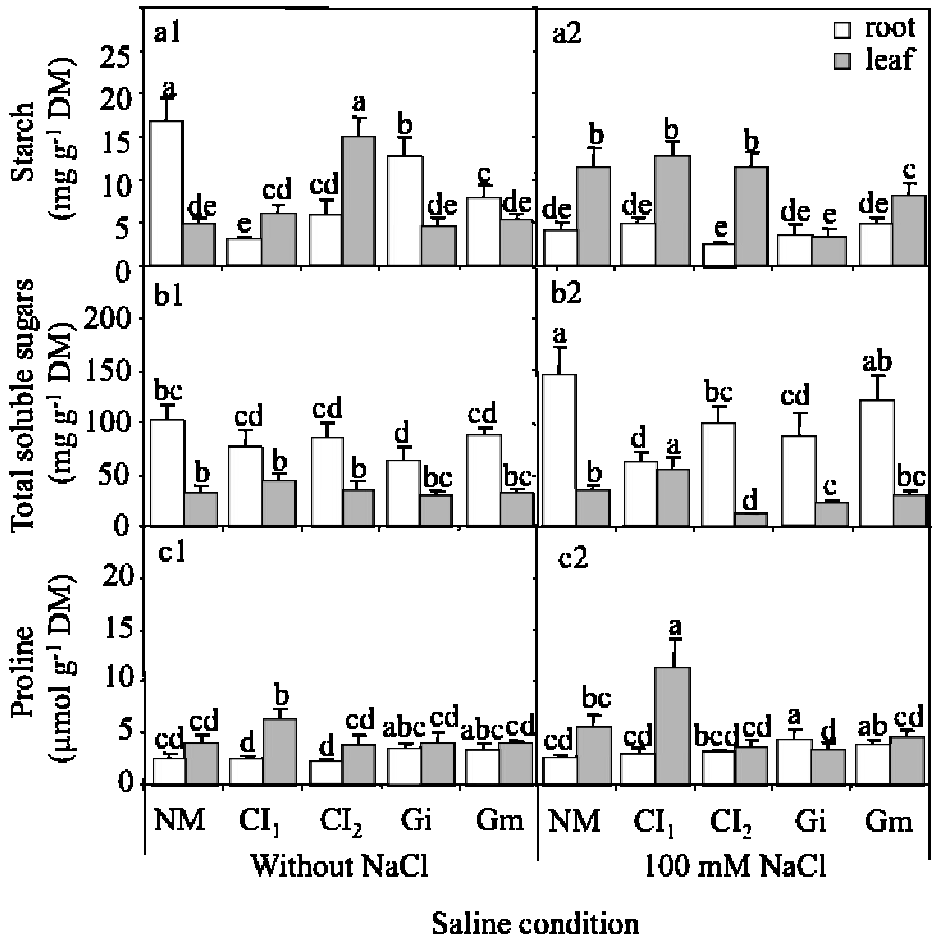

Table 1 Number of propagules in formulations of AMF and rates of inoculum applied.

Treatment	Number of propagules per litre of formulation*	Number of propagules per litre according to manufacturers	Recommended rate from manufacturers (mL per pot)	Formulation applied per pot (mL)
Commercial 1 (CI_1)	-	75,000	4-8	25
Commercial 2 (CI_2)	-	200,000	5	25
G. intraradices (Gi)	2,280	-	-	100
G. mosseae (Gm)	2,230	-	-	100

*based on data from a Most Probable Number (MPN) bioassay.

Table 2 Shoot and root dry matter (DM), mycorrhizal colonization, leaf relative water content (RWC) and photosynthetic pigment concentration in non-mycorrhizal (NM) and mycorrhizal lettuce plants inoculated with commercial inoculum 1 (CI_1) or 2 (CI_2) and substrate-based *Glomus intraradices* (Gi) or *Glomus mosseae* (Gm) two months after transplanting and before the salt stress was imposed.

Treatment	Shoot DM (g plant^{-1})	Root DM (g plant^{-1})	Mycorrhizal colonization (%)	RWC (%)	Chl a+b (mg g^{-1} DM)	Carotenoids (mg g^{-1} DM)
NM	2.8 b	1.4 a	-	91.5 a	11.8 c	1.8 b
CI_1	8.3 a	1.1 b	0.5 b	84.2 a	14.4 bc	2.4 ab
CI_2	1.3 b	0.6 c	0.9 b	94.9 a	16.9 ab	2.9 ab
Gi	1.6 b	0.5 c	42.0 a	92.0 a	19.4 a	3.4 a
Gm	1.7 b	0.5 c	1.3 b	94.4 a	20.4 a	3.5 a

Means (n=4 plants) were analysed with one-way ANOVA, and least significant differences were evaluated by the Tukey-b test. Within each column values followed by a common letter are not significantly different ($P < 0.05$).

Table 3 Foliar concentration of nutrients in non-mycorrhizal (NM) and mycorrhizal lettuce plants inoculated with commercial inoculum 1 (CI_1) or 2 (CI_2) and substrate-based *Glomus intraradices* (Gi) or *Glomus mosseae* (Gm) two months after transplanting and before the salt stress was imposed. Otherwise as for Table 2.

Treatment	N ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Na ($mg\ kg^{-1}$)
NM	22.6 a	1.0 b	36.9 b	7.8 a	3.1 b	70.11 ab	49.39 b	87.63 b	7544 a
CI_1	26.0 a	3.3 a	47.8 ab	9.0 a	4.4 a	108.68 a	45.83 b	101.02 b	5445 a
CI_2	24.0 a	1.2 b	44.0 ab	8.4 a	2.9 b	48.97 b	52.92 b	84.25 b	5909 a
Gi	28.7 a	1.4 b	56.2 a	9.4 a	3.8 ab	80.74 ab	92.32 a	123.79 ab	6756 a
Gm	25.8 a	1.3 b	45.1 ab	8.1 a	3.2 b	68.15 ab	49.23 b	156.97 a	5865 a

Table 4 Shoot and root dry matter (DM), mycorrhizal colonization, leaf relative water content (RWC) and photosynthetic pigment concentration in non-mycorrhizal (NM) and mycorrhizal lettuce plants inoculated with commercial inoculum 1 (CI_1) or 2 (CI_2) and substrate-based *Glomus intraradices* (Gi) or *Glomus mosseae* (Gm) three months after transplanting and subjected to different salt conditions.

Treatment	Shoot DM (g plant^{-1})	Root DM (g plant $^{-1}$)	Mycorrhizal colonization (%)	RWC (%)	Chl a+b (mg g^{-1} DM)	Carotenoids (mg g^{-1} DM)
Without NaCl						
NM	3.1 b	1.7 a	-	89.8 ab	13.6 bc	2.2 c
CI_1	8.5 a	1.2 bc	0.6 d	84.4 ab	15.0 bc	2.1 c
CI_2	2.0 b	0.8 d	33.9 b	92.8 a	18.2 a	3.0 a
Gi	2.9 b	1.3 b	39.6 b	82.8 ab	18.7 a	2.9 a
Gm	2.6 b	1.1 bcd	11.4 c	83.8 ab	16.7 ab	2.7 ab
100 mM NaCl						
NM	2.7 b	1.3 b	-	90.7 a	12.4 c	2.1 c

CI_1	8.9 a	1.3 b	0.4 d	79.3 b	16.8 ab	2.4 bc
CI_2	2.2 b	0.7 d	38.2 b	89.8 ab	16.5 ab	2.7 ab
Gi	3.0 b	0.9 cd	58.8 a	85.2 ab	16.1 ab	2.6 ab
Gm	2.5 b	0.8 d	8.1 c	86.2 ab	17.4 ab	2.9 a
Saline stress	*	**	***	ns	ns	ns
AMF	***	***	***	***	***	***
Interaction	*	ns	***	ns	*	ns

Data were analysed with two-way ANOVA with AMF and salt stress as the main effects. Means (n=7-9 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-b test. ns, *, **, and *** indicated respectively non-significant or significant at 5%, 1% and 0,1 % levels. Within each column values followed by a common letter are not significantly different ($P < 0.05$).

Table 5. Foliar concentration of nutrients in leaves in non-mycorrhizal (NM) and mycorrhizal lettuce plants inoculated with commercial inoculum 1 (CI_1) or 2 (CI_2) and substrate-based *Glomus intraradices* (Gi) or *Glomus mosseae* (Gm) three months after transplanting and subjected to different salt conditions. Otherwise as for Table 4.

Treatment	N ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Na ($mg\ kg^{-1}$)
Without NaCl									
NM	29.6 abc	1.6 c	56.8 bcd	16.3 a	5.7 b	89.1 d	64.1 ef	85.8 c	8430 c
CI_1	28.0 bc	3.8 a	52.4 cd	15.3 ab	6.8 a	133.9 bc	52.8 f	103.4 ab	3519 c
CI_2	33.3 a	2.4 b	60.0 abc	14.8 ab	5.2 bc	84.2 d	105.3 c	113.7 ab	4878 c
Gi	33.4 a	2.5 b	68.9 a	15.1 ab	5.8 ab	131.7 bc	157.8 a	114.0 ab	6478 c
Gm	31.1 ab	2.1 bc	68.3 a	15.1 ab	5.8 ab	118.5 cd	97.8 c	111.4 ab	5954 c
100 mM NaCl									
NM	27.7 bc	2.2 bc	57.2 bcd	14.1 ab	5.4 b	119.9 cd	84.5 de	120.2 a	24936 a
CI_1	27.1 c	3.9 a	62.7 ab	15.6 ab	6.8 a	161.8 b	64.5 ef	117.9 ab	17506 b
CI_2	30.1 abc	2.4 b	58.5 bcd	12.4 bc	4.4 c	139.5 bc	137.0 b	88.5 bc	26059 a
Gi	29.0 abc	2.2 bc	64.5 ab	11.4 c	4.3 c	212.5 a	172.4 a	86.1 c	25357 a

Gm	26.9 c	2.0 bc	50.1d	12.0 bc	4.4 c	170.3 b	84.6 de	88.2 bc	29896 a
Saline stress	***	ns	ns	***	***	***	***	ns	***
AMF	***	***	***	*	***	***	***	ns	**
Interaction	ns	ns	***	ns	**	*	**	***	*

Table 6. Concentration of nutrients in commercial formulations 1 (CI_1) and 2 (CI_2) of AMF.

Formulation	N ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Na ($mg\ kg^{-1}$)
CI_1	12.0 a	8.9 a	15.2 a	18.2 a	18.0 a	307.5 b	190.2 a	20302 a	1402 a
CI_2	1.8 b	0.2 b	1.5 b	16.8 a	2.6 b	1196.0 a	44.8 b	17130 a	1219 a

Means (n= 4) were compared with the Student's *t*-test within each column. Values followed by a common letter are nor significantly different ($P<0.05$).