Mycorrhizal infection ameliorates chlorophyll content and nutrient uptake of lettuce exposed to saline irrigation

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ABSTRACT

Salinity is one of the most serious environmental problems influencing crop growth. Today, the use of microorganisms as biofertilizers in agriculture is quite diffused, and good results have been obtained in terms of induction of resistance to biotic and abiotic stresses in crops. The effects of inoculation with a mixture of the mycorrhizal fungi *Glomus mosseae*, *G. intraradices* and *G. coronatum* have been investigated on lettuce (*Lactuca sativa* L.) cultivated at three different levels of salinity of the irrigation water (0, 1.5 and 3 g NaCl/l) and collected during three subsequent samplings. Dry mass production was significantly enhanced in the inoculated plants collected at the first sampling, and the effect was even more evident at the highest salinity; however, it was not observed at the latest samplings. The chlorophyll content and total foliar area were mostly enhanced by colonization with the mycorrhizal fungi. Moreover, mycorrhization significantly reduced Na and Cl plant uptake, and stimulated the absorption of K and P. The experiment suggests that mycorrhization can be a suitable way to induce salt-stress resistance in horticultural crops, and that it can show its best effects at medium-high salinity levels of the irrigation water.

Keywords: lettuce; salt stress; mycorrhization

During the last years, under the stimulus of a new environmental sensitivity in agriculture and the need for a sustainable management of agroecosystems, the role of microorganisms has become increasingly prominent in the conservation of fertility of soils (Lugtenberg et al. 2002, Welbaum et al. 2004). Several studies focused on the effects of soil microorganisms as biofertilizers, useful to surrogate chemical fertilization (Vessey 2003), to confer the resistance to pathogens for plants (Shanmugam et al. 2002) or for bioremediation of contaminated soils (Sorlini 1996).

Remarkable results were recorded in the case of the coincident application of organisms with different attitudes (Sarawgi et al. 1999, Wu et al. 2005). One of the most efficient combinations providing better growth and resistance to abiotic stresses is represented by *Rhizobium* sp. and mycorrhizal fungi (Azcón et al. 1991, Herrera et al. 1993, Baker et al. 1995, Ahmed 1996), due to a higher level of N-fixation by the *Rhizobium* stimulated by a better availability of phosphate to the mycorrhized plant (Becker et al. 1991).

Referring specifically to the mycorrhizal fungi, there are several examples of success in using them to provide resistance to abiotic stresses such as drought (Reid and Bowen 1979, Augè 2001), lack of nutrients (Sylvia et al. 1993, Subramanian and Charest 1999), high and low temperatures (Zak et al. 1998) and heavy-metals (Schutzendubel and Polle 2002).

The problem of progressive salinization of telluric waters in many areas of the world made the researchers focus on the possible use of mycorrhizal fungi to provide resistance to saline stress in crops; in many cases positive results were obtained (Rosendahl and Rosendahl 1991, Al-Karaki and Hammad 2001, Cantrell and Lindermann 2001, Yano-Melo et al. 2003). The induction of resistance is realized in two ways: first, via a limitation of the absorption of Na and Cl ions from the circulating solution, lowering their intake to levels tolerable for the plant, which is performed by the fungus (Al-Karaki and Hammad 2001, Mohammad et al. 2003); second, through a general amelioration of the nutritional status by means of a better bal-

ance of the intake of more and less available ions (Graham 1986). Other authors show how mycorrhizal fungi can improve the resistance to salinity by influencing the hormonal equilibrium of the plant (Danneberg et al. 1992), enhancing water uptake (Ruiz-Lozano and Azcón 1995), stimulating photosynthetic activity (Augè and Stodola 1990) and proline accumulation in the cytoplasm (Azcón et al. 1996).

The aim of this study was to investigate the effects of the infection with a mixture of arbuscular mycorrhizal fungi on the tolerance to saline stress of *Lactuca sativa* L., in order to put in evidence the eventual usefulness of such a technique for purposes of sustainable or organic horticulture.

MATERIAL AND METHODS

Location, plant material and growth conditions

The experiment was conducted in an iron-glass greenhouse with metal benches. The greenhouse was not heated, and natural light was not supplemented with lamps.

The model species was *Lactuca sativa* L., cv. Meraviglia d'Inverno, chosen for the rapidity of its biological cycle and for its ability to undergo infection of mycorrhizal fungi, as shown in literature (Ruiz-Lozano et al. 1996).

A preliminary infection trial was run to test the vitality of the inoculum: the seeds (unpelleted to avoid the risk of eventual interferences of biocide products with the mycorrhizal inoculum) were put to germinate in alveolate containers filled with peat. 0, 10, 20 and 30% in volume of crude inoculum was added and subsequently the roots were analyzed to estimate the rate of infection (Table 1). The roots were stained following the protocol of Phillips and Hayman (1970), and the rate of infection was evaluated by the gridline intersect method (Giovannetti and Mosse 1980). The inoculum used was a mixture of *Glomus mosseae*, *G. coronatum* and *G. intraradices*, provided by the CCS (Aosta, Italy).

Table 1. Root infection rate of inoculated and uninoculated plants for the preliminary test

	Level of salinity (g NaCl/l of irrigation water)		
	0	1.5	3
Without inoculum	0	0	0
With inoculum	45.2	34.3	29.7

Once the vitality of the inoculum was demonstrated, the seeds were placed in alveolate containers filled with growth substrate for germination, with or without the inoculum (10% in volume), and irrigated through floating in plastic tanks filled with constantly oxygenated tap water (EC = 0.84 dS/m).

One month after sowing, plantlets were transplanted into plastic pots filled with peat, and drip-irrigated with water at different levels of salinity according to the respective treatments. One month after transplant, fertirrigation was started, adding 1 g/l of soluble fertilizer 20/20/20 N/P/K Idrosystem® to the irrigation water, and this irrigation regime continued until the end of the trial. The fertilizer, specific for fertirrigation, was chosen because of its balanced supply of main elements and the low content of chlorine.

Experimental scheme

Six treatments were performed, derived from the factorial combination of three levels of salinity in the irrigation water and presence or absence of mycorrhizal colonization. The three levels of salinity were 0.84, 3.2 and 5.8 dS/m, corresponding to 0, 1.5 and 3 g NaCl per litre of irrigation water, respectively. Each combination of treatments was applied in triplicate to a set of six plants, and the pots were re-randomized daily in order to avoid a possible lack of homogeneity of light in the greenhouse that might alter plant responses to the treatments.

Measurements

Three samplings were done, at intervals of ten days, starting from the first day of fertirrigation. For each sampling, six plants were removed for every combination of the treatments, so that the third sampling coincided with the end of the trial. Total foliar area was measured on the plants collected at the third sampling with the leaf area meter (ΔT Area Meter MK2), Delta T-Devices, Burwell, UK. The chlorophyll content was measured at the beginning of the experiment (7 days after transplant) on all plants, and at the end (third sampling, before harvesting) on the remaining plants, with the portable chlorophyll meter (SPAD-502, Minolta).

Fresh and dry weights of shoots and roots of the plants were measured at each sampling; plant material was oven-dried at 75°C during three days for the dry weights, and successively the mineral content was analyzed. Content of Na, Ca, K (nitroperchloric digestion and flame photometry), Cl (the Zall method – Zall et al. 1956) and P (the Morgan method – Morgan 1941) was measured. One portion of about 1 g of roots from every plant was separated before drying in the oven for analysis of infection rate; the final dry weight was then proportionally adjusted, taking into account this subtraction. EC and pH of the irrigation water and of the substrate at the end of the experiment were measured, too.

Statistical analysis

Data were subjected to the analysis of variance using the SAS statistical software program; two-way ANOVA with interaction was used to determine significant differences for every parameter measured, among the various treatments grouped based on salinity level and presence/absence of colonization (values indicated in the tables). The significant values (indicated in the text) were therefore established performing multiple comparisons of means with the Tukey's test (SAS Institute 1990).

RESULTS

Growth

In all the treatments, an increase of both root and shoot dry biomass was observed from the first to the last sampling, with maximum values in the non-saline treatments (Table 2). At the third sampling, in particular, the production of non-saline non-inoculated plants was higher than that of medium-saline inoculated plants. Mycorrhizal inoculation positively affected root dry biomass of plants collected at the first sampling for all the salinity treatments (P = 0.018), and enhanced shoot dry biomass of plants under high saline stress (P = 0.035). It is noteworthy how the differences in dry biomass production between control and inoculated plants, and between plants irrigated with saline or non-saline water, became less pronounced passing from the first to the third sampling, the latter performed after three weeks of fertirrigation.

Chlorophyll content

The chlorophyll content, similar for all the treatments at the beginning of the experiment, became significantly different by the end of the

Table 2. Effects of mycorrhization and salinity on growth, chlorophyll content and leaf area of plants of *Lactuca sativa* L. at the three different days of sampling, and on the electrical conductivity of the substrate at the end of the experiment; significance values of two-way ANOVA are given

Salinity	Infection	1st sampling			2nd sampling		3rd sampling				
		s dw (g)	r dw (g)	Chl 0 (°Spad)	s dw (g)	r dw (g)	s dw (g)	r dw (g)	Chl 1 (°Spad)	leaf area (cm^2)	EC extract (mS/cm)
0	–	5.619	0.884	14.11	7.961	2.019	13.014	2.622	22.17	4405.8	2.04
	+	5.469	1.168	14.00	7.700	1.951	10.254	3.322	23.89	3697.7	1.41
1.5	–	4.890	0.755	13.56	6.931	1.991	10.819	3.130	25.56	3407.0	3.85
	+	4.342	1.254	13.67	6.358	1.455	8.721	2.032	25.67	3136.9	3.83
3	–	3.802	0.781	13.78	7.111	1.774	10.226	2.584	31.17	2741.4	5.65
	+	4.221	1.109	14.78	6.041	1.655	8.677	2.323	28.89	2963.5	4.79
Analysis of variance											
Salinity		20.3***	35.6***	ns	9.5**	21.3***	7.1**	11.0**	6.5**	23.4***	50.2***
Infection		12.5**	29.6***	ns	11.6**	10.3**	6.5**	12.1**	3.5*	7.3**	6.7**

s dw – shoot dry weight; r dw – root dry weight; Chl 0 – chlorophyll content at the beginning of the experiment; Chl 1 – chlorophyll content at the end of the experiment; EC extract – electrical conductivity of the saturated extract of the substrate; ns – not significant; *P = 0.05; **P = 0.01; ***P = 0.001

trial (P = 0.034 between irrigation treatments; P = 0.047 between control and inoculum) (Table 2). The main discriminating factor was the irrigation adopted, showing a positive correlation between salinity and chlorophyll content; the highest value was for inoculated plants at 3 g/l NaCl, the lowest for control plants irrigated with non-saline water.

Foliar area

Leaf area tended to decrease with higher salinity; mycorrhized plants showed on average bigger leaf area than the control ones (Table 2).

Ion content

Na concentrations tended to rise in leaves and roots at high salinity (Table 3). Plants infected with mycorrhizal inoculum showed significantly lower levels of Na in all organs; this effect was the most evident at 3 g/l NaCl (P = 0.025 in shoots, P = 0.009 in roots). The response of plants to inoculation in terms of Cl content was less pronounced; still, infected plants showed lower concentrations than the control ones. The allocation of Cl was preferentially at root level, while Na concentrations were higher in the shoots.

The levels of K, antagonist of Na, were reduced by saline treatments. The concurrent presence of mycorrhizal colonization allowed higher concentrations of K in the leaves of plants that underwent high saline stress; a slight reduction of K content was observed in roots of inoculated plants for all the irrigation treatments. Ca showed similar trends as K, with lower concentrations in tissues of infected plants, compared with the control ones. Both K and Ca showed preferential allocation in shoots; the shoot:root ratio of their concentrations was reduced by the mycorrhizal inoculum. Phosphorus concentrations were not strongly affected by salinity level; however, mycorrhization significantly enhanced its content in shoots (P = 0.008).

EC and pH of the substrate

The electrical conductivity of the substrate at the end of the trial resulted proportional to the level of salinity adopted, and was higher where plants had not been infected with the inoculum (Table 2). pH of the substrate did not show significant differences between the different treatments.

Table 3. Ion content, expressed as percentage on the dry weight (dw) of leaves and roots of plants subject to the different combinations of treatments; significance values of two-way ANOVA are given

Salinity	Infection	Na		Cl		K		Ca		P	
		shoot (% dw)	root (% dw)	shoot (% dw)	root (% dw)	shoot (% dw)	root (% dw)	shoot (% dw)	root (% dw)	shoot (% dw)	root (% dw)
0	–	3.84	3.06	2.30	2.55	4.36	1.49	2.10	1.10	0.599	0.529
	+	3.91	2.94	1.23	1.30	3.94	1.33	2.00	1.06	0.656	0.573
1.5	–	4.32	4.60	3.35	3.60	3.29	1.77	2.20	1.00	0.539	0.519
	+	4.19	4.06	1.08	2.30	3.31	1.51	1.80	0.90	0.735	0.486
3	–	5.27	4.18	3.60	4.35	3.20	1.58	2.40	1.20	0.499	0.483
	+	4.97	3.20	1.03	2.80	4.17	1.01	1.84	1.10	0.831	0.434
Analysis of variance											
Salinity		14.4***	15.6***	12.3***	15.4***	12.4***	6.9**	8.4**	8.6**	9.5**	8.7**
Infection		19.3***	9.8***	11.0***	19.1***	7.3**	13.3***	8.3**	5.0*	14.1***	5.3*
Salinity × infection		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns – not significant; *P = 0.05; **P = 0.01; ***P = 0.001

DISCUSSION

The different levels of salinity of the irrigation water turned out to be generally a stronger discriminating factor than the presence or absence of inoculum; in fact, negative effects in terms of growth, production and physiological status of the plants induced by salinity were more remarkable than the benefits provided by mycorrhization. The differences in weight between infected and control plants and between plants at different levels of salinity became less prominent from the first to the last sample, which suggests that fertirrigation partially counterbalanced salinity, but at the same time made the effect of mycorrhization less relevant.

Mycorrhizal inoculum gives significant results only at high salinity levels, while at medium salinity the effect is not so remarkable. This is in accordance with other examples reported in literature, according to which mycorrhization gives the best results at high salinity; for example Mohammad et al. (2003) demonstrated how the inoculation of *G. intraradices* on *Hordeum vulgaris* started to be useful only when the conductivity of the saturated extract of the substrate was 16.6 dS/m.

It was shown that the chlorophyll content depends on the salinity level as well; on average, it was higher in inoculated plants, as already observed by other authors (Abdel and Mohamedin 2000, Dìaz Franco and Garza Cano 2006). Moreover, inoculated plants under salt stress reach levels of photosynthetic capacity (estimated by the chlorophyll content) even superior to those of non-stressed plants, showing that in this respect mycorrhization is capable to fully counterbalance salt stress.

The most remarkable effect of mycorrhization in relation to salt stress was observed in terms of reduction of Na and Cl uptake, which is a known strategy of resistance to salinity in plants naturally tolerant to salt (Greenway and Munns 1980). Furthermore, Na and Cl reduction allows a higher K absorption (Al-Karaki and Hammad 2001), and a generally more balanced mineral nutrition. The increase of P content in leaves of colonized plants supports the opinion of a more balanced nutrient uptake; it confirms the observations of Giri and Mukerji (2004), and provides evidence of the capacity of mycorrhizal fungi to solubilize elements otherwise scarcely available for the plant. The proportionally lower P content in roots of infected plants can be eventually explained by an induction of its translocation to the aerial parts performed by mycorrhizal fungi.

We can hence conclude that the inoculation with mycorrhizal fungi can represent a valid instrument for salt stress reduction in horticultural crops, but its positive effects start to be manifest only at high levels of salinity, while at low levels the choice of performing mycorrhizal infection can result in an anti-economical strategy. We therefore suggest that the optimal use of mycorrhizal fungi to oppose salinization of water and soils in horticulture should be intended in terms of a long-term soil remediation, by applying the fungi to subsequent crop cycles in order to create a better rhizospheric environment for the following crop and allow the adaptation to salinity of the mycorrhizal strains adopted. The adoption of an integrated approach, joining different strategies, can be a valid choice too, however, a preliminary evaluation of costs and benefits is necessary. This necessity is well represented by the positive effect of additional irrigation against salinity, resulting at the same time in a reduction of the importance of mycorrhization effect.

Acknowledgements

The author sincerely thanks to Dr. Marianne Tveit for her suggestions about the paper and statistical assistance, and Mr. Jan Kysela for help in revision.

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Received on February 23, 2007

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