Protective effects of arbuscular mycorrhizae on laurustinus plants irrigated with treated wastewater under field conditions

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RESUMEN (Calibri 10p BOLD)

The combined effect of arbuscular mycorrhizal fungi (AMF) and two kinds of reclaimed water was evaluated on the physiological behavior, leaf nutrition and aesthetic value of laurustinus plants. AMF satisfactorily colonized the laurustinus roots, enhanced the structure of soil, increased beneficial nutrients and decreased toxic ions in leaves, stimulated flowering and enhanced the water status of the plants irrigated with reclaimed water. AMF not only ameliorated the negative effects of reclaimed water with high salinity, but also had a positive effect in well watered plants.

Palabras clave: salinity; *Glomus iranicum*; soil properties; ornamental plants**.**

1. Introduction

Currently, the overexploitation of available water resources and other environmental factors are leading to an increase in soil salinization [1]. Particularly in the Mediterranean area, protected horticultural and ornamental crops have to cope with the increasing salinization of irrigation water and soil, because of the intensive use of scarce water resources [2]. This suggests an urgent need to explore new alternative water resources such as reclaimed water to satisfy the crop water requirements. Reclaimed water usually contains a great concentration of nutrients that produce a direct benefit for crops. However, depending upon its source and treatment, reclaimed water may have high salt content, heavy metals or pathogenic organisms. In general, as salinity increases in the treated wastewater used for irrigation, the probability of crop problems increases.

Many researchers have shown that arbuscular mycorrhizal fungi (AMF) have a positive influence on cultivation systems [3]. They not only improve plant growth through increased nutrient uptake, but they also have 'non –nutritional' effects in stabilizing soil aggregates, in preventing erosion, and in alleviating the negative effects induced by salinity. AMF are able to develop several mechanisms to enhance the salt tolerance of host plants such as increased nutrient acquisition, maintenance of the K/Na ratio, biochemical changes, physiological changes and molecular or structural changes [4].

Although salinity may reduce the colonization capacity and the growth of fungal hyphae [5] some authors have demonstrated that mycorrhizal inoculation can alleviate the negative effects produced by saline irrigation in ornamental and wild plants [2, 6].

In this study, we evaluated the combined effect of arbuscular fungi inoculation and two kinds of reclaimed water with different electrical conductivity on the ion acquisition, water status, gas exchange and aesthetic value of *Viburnum tinus* plants, as well as the effect of reclaimed water on mycorrhizal root colonization and soil properties.

2. Materials and Methods

The experiment was performed on a one-year-old laurustinus (*Viburnum tinus* L.) (n=80) at the experimental farm of CEBAS- CSIC in Santomera (Murcia, Spain), using a planting pattern of approximately 1 m x 0.80 m.

On April 2012, laurustinus plants were collected from a nursery and were transplanted into the experimental plot. The soil was amended initially with 2 g L^{-1} of Osmocote Plus and every 3-4 months, a Hoagland solution (standard nutrient solution) were supplied through the drip irrigation system. The plants were irrigated twice a day and the volumetric water content (θ_v) of the soil profile was measured by time domain reflectrometry (TDR).

On 5 April 2013, the first saline period started with two irrigation treatments which consisted of a Control (EC < 0.9 dS m^{-1}) and a reclaimed wastewater, RW (EC: 4 dS m^{-1}) from a sewage treatment plant located in Campotejar (Murcia, Spain). Plants were irrigated so that the stem water potential of the Control plants did not exceed -0.8 MPa and depending on the season, climatic conditions and plant development. For each irrigation treatment, twenty plants were inoculated with *Glomus iranicum* var. *tenuihypharum* sp. *Nova*. Therefore there were four treatments in total: Control and RW treatments with and without mycorrhizal inoculation.

On 20 June 2013, the second saline period started with Control water and with a reclaimed wastewater, RW (EC: 6 dS m^{-1}) from another sewage treatment plant located in Mazarrón (Murcia, Spain). The second saline period ended on 18 December 2013.

2.1 <u>Root colonization and enzymatic mycorrhizal activity</u>

At the beginning and at the end of the experiment, the percentage of mycorrhizal root colonization was estimated in three roots per treatment following the gridline intersect method [7].

<u>2.2 Soil and leaf analyses</u>

At the end of the first saline period and at the end of the experiment, the mineral content of three soil samples and the mineral content of four leaves per treatment were determined by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). Clhoride concentration was analysed by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.). The total-N (NT), total-C (CT) and organic- C (C_{org}) concentrations of the soil were also measured with an elemental analyser Flash EA 1112 Series- Leco Truspec. The OM content was determined by multiplying C_{org} by 1.72. Available-P was also analysed colorimetrically as molybdovanadophosphoric acid [8].

At the end of the first saline period the easily extractable glomalin (EEG), were determined in three soil samples per treatment by the Bradford method with bovine serum albumin as standard [9].

2.3. <u>Growth parameters, water status and gas exchange.</u>

At the end of the experiment, all the plants were visually evaluated as follows: (1) PIC, percentage of plants in ideal condition; (2) PAC, percentage of plants in acceptable condition; (3) PDB, percentage of plants with dry branches; and (4) DP, percentage of dry plants. The number of flowers per plant was counted at the end of the second saline period.

Throughout the experiment, stem water potential (Ψ_{stem}) was estimated on seven leaves per treatment, previously covered with aluminium foil and measured at noon with a Scholander pressure chamber. Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined on seven leaves per treatment at noon, using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA).

3. Results and discussion

The Inoculated plants for Control and RW treatments presented higher percentage of root colonization than non-inoculated plants. Nevertheless, for non- inoculated plants, these percentages presented relatively high levels, probably due to a native AMF proliferation, percentage that increased at the end of the second saline period (Table 1). On the other hand, only AMF colonization in inoculated RW plants decreased at the end of the second saline period respect to the end of the first saline period. Therefore, Na and Cl content present in the soil of the plants irrigated with RW could affect negatively the mycorrhizal colonization [4].

As regards inoculated plants, our results confirm that *Glomus iranicum* var. *tenuihypharum* sp. symbiosis was well established on laurustinus roots regardless of the type of water used.

In addition, the high content of the EEG observed in inoculated soils (Table 2) could be related to the higher content of C_{org} and OM, and the tendency of N_t to increase in inoculated soils respect to non-inoculated soils (Table 2) [10] creating stable aggregates.

Table 1 Percentage of initial (IC) and final (FC) root colonization

Mycorrhizal Parameters	Treatments				
	C	**C M**	**RW**	**RW M**	

IC (%)	59±1 b	84±5 a	62± 2 b	81±2 a	***
FC (%)	85±1 c	91± 3 a	69± 1 d	76± 1 b	***

*, **, ***, and ns indicate the level of significance at P≤0.05, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test.

Table 2. Effects of the irrigation water and AMF on the chemical properties and the easily extractable glomalin in soil (EEG) at the end of the first saline period

Mycorrhizal Parameters	Water			AMF			
	C	RW		+	-		
Available- P	15.89	11.31	**	13.34	13.61	ns	ns
NT (g/100g)	0.15	0.13	ns	0.15	0.13	ns	ns
CT (g/100g)	7.15	7.71	ns	7.62	7.24	ns	ns
C_{org} (g/100g)	1.36	1.36	ns	1.42	1.31	*	ns
OM (%)	2.34	2.34	ns	2.43	2.25	*	ns
C/N	9.49	10.28	ns	9.81	9.96	ns	ns
EEG (mg g^{-1} soil)	38.26	22.43	***	38.14	22.55	***	***

*, **, ***, and ns indicate the level of significance at P≤0.05, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test.

Leaf K content was decreased by AMF. However, the fungal activity enhanced the phosphorus as well as magnesium nutrition of plants and alleviated the adverse effect of salinity supressing toxic ions such as Na (table 3) [11].

Table 3. Effects of irrigation water and AMF on the mineral content at the end of the experiment

Leaf analyses (mg kg^{-1} DW)	Water			AMF			
	C	RW		+	-		
Cl	4475	12200	***	7850	8825	*	ns
Na	202	1771	***	743	1230	***	***
Ca	12584	14995	***	13600	13979	ns	**
Mg	4651	4152	***	4571	4233	***	***
P	1746	1458	***	1702	1502	***	ns
K	12020	15203	***	13028	14195	***	ns
B	197	202	*	202	205	*	***

*, **, ***, and ns indicate the level of significance at P≤0.05, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test.

The decrease in Ψ_{stem} of RW plants from week 14, as a result of the increase of EC in soil, and their high volumetric content (data not shown) in soil suggested that salts dissolved in reclaimed water caused difficulty for roots in absorbing water from the soil (Fig. 1). However, the mycorrhizal inoculation improved the stem water potential and improved slightly stomatal conductance of inoculated RW plants respect to the non-inoculated plants, with the beneficial effects of AMF becoming more visible at the end of the second saline period, when the level of stress increased (Fig. 1) [12].

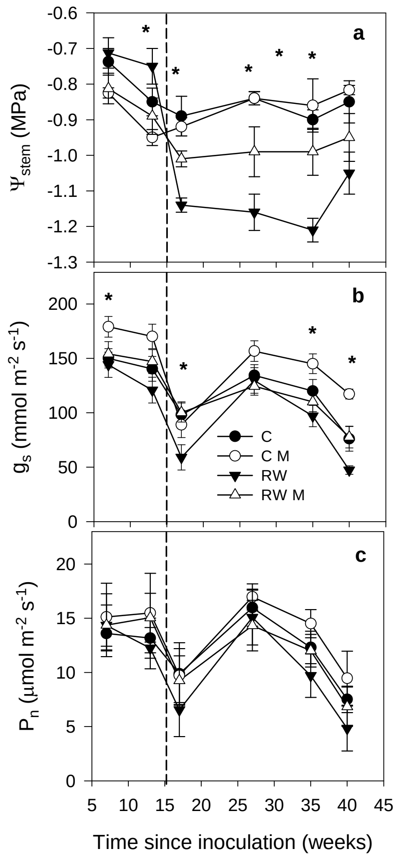

Figure 1*. Stem water potential (Ψ_{stem}) (a), stomatal conductance (g_s) (b) and photosynthesis rate (Pn) (c) of laurustinus plants during the experiment.*

Although there were no significant differences in the photosynthesis rate between treatments, at the end of the saline period, inoculated Control plants showed 100% of PIC, followed by non-inoculated Control plants, with 90% of PIC (Fig. 2). The lowest PIC values were found in non-inoculated RW plants, (35% of PIC and 5% of DP) probably due to salts reducing the chlorophyll content in leaves which resulted in leaf chlorosis. On the other hand, AMF produced 65% of PIC and 35% of PAC in RW plants. The number of flowers was also increased by the mycorrhizal inoculation, reaching in the inoculated Control plants the highest number of inflorescences per plant (Fig. 2). Similar results were found by Navarro et al. (2012) [2].

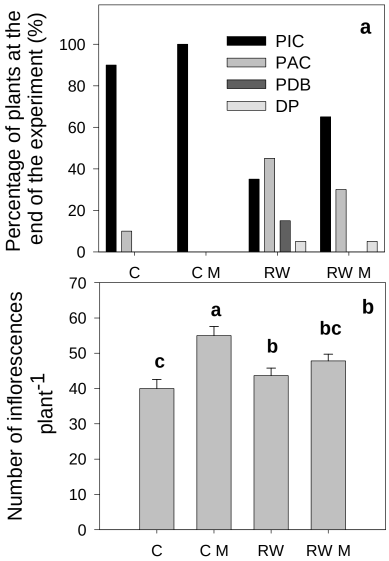

Figure 2. *Percentage of plants according to the visual characteristic of laurustinus plants (a) and number of inflorescences per plant (b)*

4. Conclusions

The salts dissolved in the RW induced a difficulty in taking water from the soil and osmotic stress which provoked a considerable percentage of plants with chlorotic leaves. In spite of the development of native AMF, the results suggested that the inoculation of *Glomus iranicum* var. *tenuihypharum* sp. *Nova* improved the aesthetic value of the laurustinus plants in both irrigation conditions, enhancing the structure of soil, increasing beneficial nutrients and decreasing toxic ions, stimulating flowering and enhancing the g_s rates. AMF not only ameliorated to a certain extent the negative effects of reclaimed water with high salinity, but also had a positive effect in well watered plants.

5. Acknowledgements

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6. References

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