SHORT REPORT

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Arbuscular mycorrhizal symbiosis improves growth and root nutrient status of citrus subjected to salt stress

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ABSTRACT: The application of microorganisms such as arbuscular mycorrhizal fungi to enhance salt resistance is quite well-known, but the interaction of mycorrhiza and salinity to growth, relative water content, and nutrient concentrations of salt-sensitive citrus (*Citrus tangerine*) seedlings has been less studied. The non-colonized seedlings and seedlings colonized by *Glomus mosseae* or *Paraglomus occultum* were exposed to salt stress by irrigation with 100 mM NaCl solutions. Salt stress significantly depressed *G. mosseae*-colonization but not *P. occultum*-colonization. Mycorrhizal association could markedly increase both plant performance (leaf number, leaf area, shoot, and root dry weights) and leaf relative water content of citrus seedlings exposed to salt stress. Root Na^+ concentrations were lower in mycorrhizal than in non-mycorrhizal seedlings under given salinity conditions. Mycorrhizal inoculation was found to promote root concentrations of K^+, Ca^{2+}, and Mg^{2+} at all salinity levels, although the differences for Mg^{2+} was not significant at the 100 mM NaCl level. The K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ ratios were higher in mycorrhizal than in non-mycorrhizal citrus roots subjected to salt stress. It seems that mycorrhizal inoculation possesses the potential to enhance salt tolerance of citrus.

KEYWORDS: *Citrus tangerine*, *Glomus mosseae*, ionic balance, *Paraglomus occultum*, salinity

INTRODUCTION

Salt stress is one of the major abiotic stresses in reducing plant growth and productivity worldwide[1]. About 20% of irrigated agricultural land and 2% of dryland agriculture are affected by salinity[2]. In sustainable agriculture, solutions to salinity problem should include both plant breeding for salt tolerance and the application of biological factors such as arbuscular mycorrhizal (AM) fungi (AMF)[3].

AMF are associated with the roots of over 90% of terrestrial plant species[4]. The symbiosis contributes to improve water use and nutrient uptake, especially for elements with low soil mobility, such as P and Zn, and it increases plant tolerance to various biotic and abiotic factors[5]. Most investigations have shown that AM symbiosis may enhance plant growth and vigour under salt stress conditions through a more efficient nutrient uptake, particularly of P, and through the production of osmoregulators[3,6–8].

Citrus is classified as salt sensitive[9]. The deleterious effects of salt stress lead to a reduction in fruit yield and quality[10]. Heretofore, only a study conducted by Graham and Syvertsen[11] found that the AM fungus *Glomus intraradices* did not influence the tolerance of citrus seedlings to salinity but enhanced Cl uptake.

The aim of this study is to evaluate the effects of AMF inoculation on growth and root nutrient status of citrus seedlings, in order to ascertain the eventual usefulness of such a technique for sustainable or organic orchards.

MATERIALS AND METHODS

The experiment was conducted in a plastic greenhouse without heating equipment at Jingzhou City, Hubei between March and July 2008. The experiment had a randomized complete factorial design with two factors: three mycorrhizal treatments (*Glomus mosseae*, *Paraglomus occultum*, and non-inoculated) and two salt levels (0 mM and 100 mM NaCl). Three replications per treatment were performed to give a total of 18 pots (three seedlings per pot).

The seeds of citrus (*Citrus tangerine* Hort. ex Tanaka) were surface-sterilized with 70% alcohol for 5 min and rinsed four times with distilled water. The seeds were sown into plastic pots (16 cm in depth and 20 cm in mouth diameter) filled with 3.4 kg of autoclaved (121 °C, 0.11 MPa, 2 h) soil mixture on 4 March 2008. The potted substrate was inoculated with *Glomus mosseae* and *Paraglomus occultum* before sowing by placing 15 g of inocula (soil, spores,

hyphae and infected roots) 5 cm below the surface of the soil. These inocula were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. Non-inoculated pots were supplied with 15 g sterilized substrate as control.

All of the seedlings were subjected to salinity stress after 85 days of AMF inoculation. The salinity was induced by adding 300 ml of 100 mM NaCl solutions (EC 9.7 dS/m), and the control (0 mM NaCl) seedlings were irrigated with 300 ml of distilled water (EC 0.1 dS/m). The soil was salinized stepwise to avoid osmotic shock using 25 mM NaCl per day.

The seedlings were harvested 60 days after the salinity stress. Leaf area per plant, leaf number per plant, and shoot and root dry weights were measured. Relative water content (RWC) of the fifth fully expanded leaf from the shoot apex was evaluated by the method of Wu and Xia[12]. Oven-dried plant matter was comminuted and sieved through a 0.5 mm sieve. About 0.1 g of sieved material was used to extract inorganic ions. The cations K^+, Na^+, Mg^{2+}, and Ca^{2+} concentrations were determined directly using an Atomic Absorption Spectrometer (AI 1200, Aurora Instruments).

One portion of root fragments from every seedling was separated before drying in the oven for analysis of mycorrhizal colonization. Mycorrhizal colonization was assessed after clearing the 1-cm root fragments in 10% KOH at 90 °C and staining with 0.05% trypan blue in lacto phenol according to the method of Phillips and Hayman[13]. AM colonization was quantified according to the formula of Wu et al[14].

Data were subjected to a two-way ANOVA (with mycorrhizal and salinity treatments) using the SAS statistical software program. Fisher's protected least significant difference ($p < 0.05$) was used to perform multiple comparison of means. Percentage values were arcsine transformed before statistical analysis.

RESULTS AND DISCUSSION

There was no mycorrhizal colonization recorded in the non-inoculated seedlings. The seedlings inoculated with AMF had root colonization of 41–68% (Table 1). The highest colonization was in salt-free soils infected by *G. mosseae*, and the lowest colonization occurred in *G. mosseae*-colonized seedlings subjected to salt stress. Salinity significantly depressed *G. mosseae*-colonization but not *P. occultum*-colonization, implying that fungal species provide a different capacity to respond to salinity. This is in agreement with several studies on maize[15] and zucchini[16].

Salinity has been shown to decrease growth characteristics of citrus seedlings in saline soils (Table 1). The seedlings inoculated with AMF had higher leaf number, leaf area, shoot and root dry weights than non-AMF plants regardless of salinity levels, meaning that mycorrhizal seedlings under saline conditions grow better than non-mycorrhizal seedlings. Similar results were also observed for maize[15], zucchini[16], guayule[17], and *Strophostyles helvola*[18]. No significant differences in growth performance were recorded between *G. mosseae*-colonized and *P. occultum*-colonized seedlings. The mechanism by which arbuscular mycorrhiza mitigate growth reduction caused by salinity remains unresolved. Al-Karaki[19] concluded that enhanced growth of mycorrhizal plants in saline conditions is related partly to mycorrhizal-mediated enhancement of mineral nutrient uptake in host plants, especially P nutrition.

RWC was not significantly affected by salinity (Table 1). However, mycorrhizal inoculation markedly increased RWC irrespective of saline levels, compared to non-inoculated treatment. This may have been the result of improved water relation by AMF hyphae[20].

Root Na^+ concentrations increased with soil salinity in both mycorrhizal and non-mycorrhizal seedlings (Table 1), because NaCl was used to develop a salinity gradient. Root Na^+ concentrations were lower in mycorrhizal than in non-mycorrhizal seedlings under given salinity conditions, resulting from dilution effects due to growth enhancement by AMF colonization[16,19]. Root concentrations of K^+, Ca^{2+}, and Mg^{2+}, were higher for mycorrhizal than for non-mycorrhizal seedlings at all salinity levels, although the differences for Mg^{2+} was not significant at 100 mM NaCl level. It seems that improved plant nutrition by AM symbiosis allows cells to regulate and separate flowing ions more effectively[21]. These results suggest that AM colonization improves nutrient uptake by availability or transport of mycorrhizal hyphae, thus enhancing salt tolerance of mycorrhizal plants. Rabie[6] also reported similar responses in *G. clarum*-colonized mung bean plants irrigated with various dilutions of seawater.

The nutrient imbalance due to salt stress is well known, and results from the effects of salinity on nutrient availability, competitive uptake, transport, or partitioning within the plants[22]. K^+ is a competitor of Na^+ under saline conditions[23]. Maintenance of a high cytosolic K^+/Na^+ ratio is a key feature of plant salt tolerance[24,25]. The present study here confirmed that mycorrhizal roots kept a higher K^+/Na^+ ratio, implying that K^+ competed for the site of Na^+ on the cell membrane (Table 1). A similar increase in K^+/Na^+ ratio has also been reported previously[21]. The

Table 1 Effects of salinity and mycorrhizal inoculation (MI) on mycorrhizal colonization (MC), growth performance, leaf relative water content, root cation concentrations, and ionic balance of *Citrus tangerine* seedlings.

NaCl	MI	MC	leaf RWC	Leaf	Leaf area	Dry weight (g)		Ionic concentration (mg/g)				Ionic balance		
(mM)		(%)	(%)	No.	(cm^2)	Shoot	Root	Na^+	K^+	Ca^{2+}	Mg^{2+}	K^+/Na^+	Ca^{2+}/Na^+	Mg^{2+}/Na^+
0	GM	68.26^{a}	69.93^{a}	13.1^{a}	49.5^{a}	0.31^{ab}	0.18^{a}	4.95^{d}	18.19^{a}	7.81^{ab}	3.13^{a}	3.69^{a}	1.58^{a}	0.64^{a}
	PO	65.91^{a}	72.33^{a}	12.3^{ab}	47.1^{a}	0.32^{a}	0.19^{a}	5.10^{d}	17.76^{a}	7.41^{ab}	3.22^{a}	3.49^{a}	1.45^{b}	0.63^{a}
	-	0	64.29^{bc}	11.4^{c}	39.3^{b}	0.25^{c}	0.15^{b}	5.95^{c}	16.28^{b}	6.49^{c}	2.90^{b}	2.74^{b}	1.09^{c}	0.49^{b}
100	GM	40.61^{b}	67.60^{ab}	11.4^{c}	34.1^{b}	0.26^{c}	0.12^{b}	9.13^{b}	15.66^{b}	8.13^{a}	2.31^{d}	1.72^{c}	0.89^{d}	0.25^{cd}
	PO	60.67^{a}	71.16^{a}	12.1^{bc}	38.3^{b}	0.26^{bc}	0.12^{b}	9.08^{b}	15.87^{b}	8.08^{a}	2.70^{c}	1.75^{c}	0.89^{d}	0.30^{c}
	-	0	59.85^{c}	10.2^{d}	26.8^{c}	0.19^{d}	0.09^{c}	10.88^{a}	14.48^{c}	7.17^{bc}	2.42^{d}	1.33^{d}	0.66^{e}	0.22^{d}

GM = *G. mosseae*, PO = *P. occultum*

Same letter within each column indicates no significant difference among treatments ($p < 0.05$).

competition of K^+ due to mycorrhization may induce the decrease of Na^+, thus enhancing salt tolerance of mycorrhizal plants. The Ca^{2+} cation is an important factor in the resistance of plants to salinity. In the present study, mycorrhizal citrus roots maintained a higher Ca^{2+}/Na^+ ratio under given salinity conditions (Table 1). An increased Ca^{2+}/Na^+ ratio under saline conditions may help to keep membrane integrity[26] and protect host plants against salt damage. It is well known that Mg^{2+} is a constituent of chlorophyll and an enzyme activator. A higher Mg^{2+}/Na^+ ratio was observed in mycorrhizal citrus roots exposed to salt stress (Table 1). This suggests that AMF reduce the antagonistic effect of Na. Giri and Mukerji[27] also reported that mycorrhizal colonization alleviated salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions, resulting from reduced Na^+ and improved Mg^{2+} uptake.

Although mycorrhizal association between AMF and their hosts is usually nonspecific, many studies have confirmed the existence of physiological or morphological differences within the species and even within geographic or ecotypic isolates of AMF[28]. It is widely accepted that an isolate from saline soil would have a higher capacity to promote plant growth under saline stress[29,30]. The present study observed that there were few differences between *G. mosseae*- and *P. occultum*-colonized seedlings under 100 mM NaCl conditions (Table 1), although *P. occultum* originated from a saline soil of Beijing and *G. mosseae* was from a non-saline soil of Sitsang. A similar result was observed by Tian et al[28] in cotton plants inoculated with two *G. mosseae* isolates from non-saline and saline soils. Ruiz-Lozano and Azcón[31] reported that a *Glomus* sp. isolated from saline soil protected lettuce plants from the detrimental effects of salt by the stimulation of root development, whereas the effects of *G. deserticola* originating from non-saline soil were based on improved plant nutrition. It seems that isolated AMF from saline and non-saline soils possess different physiological mechanisms to enhance salt tolerance of hosts.

CONCLUSIONS

The present investigation shows that inoculation with *G. mosseae* or *P. occultum* alleviates the detrimental effect of salinity on the growth of *C. tangerine* and improves nutrient acquisition of roots, demonstrating the potential of AMF colonization for the protection against salt damage of citrus plants.

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