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Effect of Mycorrhizal Fungi on Morpho-physiologic and Nutritive Characteristics of Flying Dragon under Salinity Stress

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ABSTRACT: Citrus is one of the most important subtropical fruit trees that categorized under the salt sensitive horticultural crops. Arbascular mycorrhizal fungi (AM), by using a symbiotic relationship with plant root caused more accessibility of mineral nutrition in plants. In present study, two AM fungi (*Glomus mosseae* and *Paraglomus occultum*) and three level of salinity (50, 100 and 150 mM sodium chloride) in Flying Dragon seedling were evaluated. According to the results, with increasing of salinity levels mycorrhizal colonization percentage, relative water content, leaf number, leaf area, stem diameter, dry weight of root and shoot had decreased. Among the treatments, seedling in which inoculated with AM in compared with non-inoculated treatments had shown significantly difference. Mycorrhizal fungi had significantly shown lower Na^+ concentration and higher K^+, Ca^{2+} concentration, ratio of K^+/Na^+ and Ca^{2+}/Na^+ ratio in root seedlings. Also, between two used AM fungi in some measured parameters significant difference were found, that indicated different genus of AM shown different responses to salinity. Furthermore, inoculation treatments had recorded less salinity stress conditions. Finally, suggested that AM symbiosis with using leaf osmotic adjustment and ionic balance could mediated adverse effects of salinity.

Keywords: Arbascular mycorrhizal, citrus and salinity.

I. INTRODUCTION

Most areas in the world in which were under salinity, are on the rise. According to the reports, about 50 percent of world land have shown salinity. About 30 percent of Iran irrigated areas have been faced with salinity [7]. Citrus is one of the most important fruit crops in Iran. Citrus is highly sensitive to water and soil salinity. About 13 percent decrease of citrus yield per each 1 dS m^{-1} increase in salinity above 1.4 dS m^{-1} [20]. Rootstocks have a fundamental role in the development of citrus industry. All commercially-cultivated citrus cultivars are grafted onto rootstocks. Rootstocks have a large impact on scion growth habit and tolerance to abiotic stress. The effect of rootstocks on fruit production and quality has been extensively mentioned in literature [15, 17]. Flying dragon trifoliate orange used as a dwarf rootstock, in which has tolerance traits toward citrus tristeza virus, resistance traits toward phytophthora root rot and citrus.

Arbuscular mycorrhizal (AM) fungi are probably distributed in most soils and approximately 90% of higher plant species examined interact with AM fungi [5, 23]. AM are formed predominantly by the branching haustorial structures called arbascules, found in the cortical plant root cells and hyphal coils, as well as intercellular hyphal networks and external hyphae that extend into the soil. AM fungi are one of the most important soil microorganism that by expanding plant root interface with soil environment fascinated plant nutrients [19]. Symbiotic relationship leads to mutual exchange of inorganic nutrients such as zinc, phosphorus and carbon between fungus and plant. Also, it can increase plant tolerance to environmental stresses such as drought conditions and temperature stress [1, 26].

In common with most cultivars of trifoliate orange [13]. The fungi population is a key factor for successful plant growth. AM growth hyphae increased root level, water absorption efficiency and nutrient distribution specially phosphorus and zinc [22]. The researchers reported more biomass and less proline content in citrange "carrizo" inoculated with *Glomus intraradices* in compare with non-inoculated treatment under different salinity levels [4].

Two symbiosis AM (*Glomus mosseae* and *Paraglomus occultum*) through growth improving, photosynthetic rate and root structure could reduced adverse effects of salinity under 100 mM sodium chloride concentration [26]. However, AM fungi (*Glomus intraradices*) did not shown a positive impact on 30 and 60 mM salinity in *Citrus sinensis* seedlings [10]. Not only inoculated AM fungi (*Glomus* and *Gigaspora* species) have not shown any significant effect on nutrition elements content (Phosphorus, potassium, magnesium, chloride and sodium), but also other factors such as chlorophyll, compatible solutes in citrange "Troyer" under 100 and 150 mM salinity [15]. These dissociable results seem to be difficult to explain mycorrhizal function under salt stress condition.

The interactive effects of AM fungi on citrus plants have been reported several times, but th In this paper, we analyzed the impact of two mycorrhizal fungi under salinity stress. Our objectives were to determined how AM symbiosis can alleviate adverse effect of salinity and which of our mycorrhizal fungi show better results.

II. MATERIAL AND METHODS

Plant culture, mycorrhizal inoculation and treatments: Seed of Flying dragon were sterilized by immersion in 70% alcohol for 4 min, rinsed 5 times with distilled water and germinated in jiffy pots at 27ºC. Twenty-day-old seedlings were transferred to plastic pots containing autoclaved growing media (0.11 MPa, 121ºC, 2h) of soil, vermiculite and sphagnom (5:2:1). The soil was collected from the botanical garden of Ferdowsi University of Mashhad, Iran. The experimental pots were placed in greenhouse under natural light. The average day/night temperature was 27/19º C and relative humidity was 60-75%.

Two different mycorrhizal fungi called *Glomus mosseae* and *Paraglomus occultum* were provided by the Seed and Plant Improvement Institue, Karaj, Iran. 25 g of fungi per pot were used while non-AM fungi treatments received the same weight of growth media. The soil used in this study was collected from top layer of soil in Mashhad city, Khorasan Razavi Province, Iran. The soil (containing 34 mg/kg available nitrogen, 10 mg/kg phosphorus, 195 mg/kg available potassium, pH=7.8 and organic matter 22 g/kg) relative humidity from June to August 2014 .The experiment was conducted in a greenhouse under a temperature of 23-30, 12 h day/night and 70-75%. Seedlings were kept 90 days for more growth and adaptation and then exposed to different level of salinity. For avoiding an osmotic shock NaCl gradually added to each treatment. The experimental design

conducted in a completely randomized block design as a factorial form. First factor was four levels of salinity (0, 50, 100 and 150 mM NaCl) and the second factor was two different genotypes of mycorrhizal fungi. Six replicates of each treatment were applied. Control treatments were irrigated with distilled water.

Parameter measurements: After two months the experiment was terminated. Shoot, leaves and roots were separated. Shoot and root dry weight were measured after drying in oven at 72 ºC for two days. Leaf area index (LEI) was estimated by LAI-2200 Plant Canopy Analyzer (CID Bio-Science, USA). Relative water content (RWC) was measured by Wu and Xia [24]. The sucrose and glucose were determined by Wu method. Concentration of proline was measured by the method of Bates *et al* [2].

AM colonization was estimated in according with Melgar *et al* [14] with using light microscopy. The percentage of AM colonization was calculated according to following equation: Percentage of AM colonization = (Root length infected/Root length observed) × 100. Na^+, K^+ and Ca^+ concentrations in leaves were measured using Ca^+ was performed by atomic absorption spectrometer (AI1200, Aurora instrument limited, Canada).

Statistical Analysis: The data were analyzed by two factor ANOVA using JMP 7 software. Least significant difference (LSD, $\alpha<0.05$) was used in order to compare the significant difference between treatments.

RESULTS AND DISCUSSION

Salinity significantly decreased mycorrhizal colonization. Our results showed that mycorrhizal colonization of inoculated seedlings varied from 41.05 to 66.86% (Table 1). We did not recorded any mycorrhizal colonization in non-inoculated treatments. The highest colonization occurred in control treatment in which inoculated with *Paraglomus occultum*, and the lowest colonization was in 150 mM treatment of NaCl that inoculated with *Glomus mosseae* (Table 1). Salinity cause more remarkable decrease on colonization of *Glomus mosseae* than, *Paraglomus occultum.* This indicated that each different mycorrhizal species had different potential during salinity.

These data correlated with some researchers [7, 30] results. In compared with non-salinity treatments, salinity reduced leaf number, relative water content (RWE), stem diameter, leaf area index (LEI), root and shoot dry weight, regardless of the inoculation with AM fungi (Table 1). In most growth parameters, significant difference didn't reported between two AM fungi. The highest leaf number was found in control treatment including *G. mosseae* (14.72), and the lowest leaf number were in non- mycorrhizal in 150 mM of sodium chloride (8.55). By increasing salinity level, more leaf felled, however, plant inoculated with AM fungi had significantly more leaf. In control and 50 mM treatments, leaf RWE had shown significance difference between inoculated and non-inoculated seedlings.

With increasing of salt levels stem diameter and leaf area index (LEI) remarkably decreased, but colonized seedling especially in low salt concentration partly displayed better condition. In LEI and stem diameter, according to the results of mycorrhizal inoculation, even in high salinity (150 mM) had shown significance difference (Table 1). Analysis of dry biomass showed that all root and shoot dry weight decreased with the increasing of sodium chloride (Table 1). In compared with un-inoculated treatments, under salinity stress, both AM fungi treatment recorded significantly higher LEI and dry biomass.

Table 1: Effect of salinity and arbascular micorrhizal inoculation on colonization rate and some growth parameters of Flying dragon seedling (*Glomus mosseae* = Gm, *Paraglomus occultum* = Po, No micorrhiza = Nmi).

Salt concentration (mM)	Micorrhiza type	Colonization (%)	Leaf number	Relative water content (%)	Stem diameter (cm)	Leaf area (cm^2)	Root dry matter (g)	Shoot dry matter (g)
	Gm	63.91 ^{a}	14.72^{a}	70.95 ^{a}	0.20 ^{a}	48.42 ^{a}	0.19 ^{a}	0.36 ^{a}
0	Po	66.86 ^{a}	13.45^{ab}	71.45 ^{a}	0.22 ^{a}	47.25 ^{a}	0.18 ^{a}	0.35 ^{a}
	Nmi	0	12.52 ^{b}	65.32^{ab}	0.21^{a}	39.63 ^{b}	0.18 ^{a}	0.33^{b}
50	Gm	62.50 ^{a}	14.34 ^{a}	68.63 ^{a}	0.20 ^{a}	45.22 ^{a}	0.17^{ab}	0.32 ^{b}
	Po	63.26^{a}	13.25^{ab}	70.55 ^{a}	0.21 ^{a}	45.38 ^{a}	0.15 ^{b}	0.33 ^{a}
	Nmi	0	11.22^{c}	61.12 ^{b}	0.18^{bc}	38.11 ^{b}	0.14 ^{b}	0.26^{bc}
100	Gm	44.51 ^{b}	12.30 ^{b}	67.11^{ab}	0.19^{ab}	38.55 ^{b}	0.12^{bc}	0.27^{ab}
	Po	61.75^{ab}	12.40 ^{b}	66.02^{ab}	0.19^{ab}	39.01^{ab}	0.12^{bc}	0.26^{bc}
	Nmi	0	10.33^{c}	56.43^{bc}	0.16 ^{d}	32.41 ^{c}	0.11 ^{bc}	0.17^{c}
	Gm	41.05 ^{b}	12.11 ^{b}	64.56^{ab}	0.17 ^{d}	33.04 ^{c}	0.10 ^{c}	0.24^{bc}
150	Po	55.83^{ab}	12.05 ^{b}	64.50^{ab}	0.15^{e}	30.65 ^{c}	0.11 ^{bc}	0.25^{bc}
	Nmi	0	8.55^{d}	52.25 ^{c}	0.14 ^{e}	25.73 ^{d}	0.06^{c}	0.16 ^{c}

Same letter within each column indicates no significant difference among treatments $\alpha<0.05$.

During increasing of salinity level, sucrose content in inoculated treatments decreased, while in un-inoculated ones it was reverse (Table 2). Only in 100 mM sodium chloride treatments observed different significant between two AM fungi. In addition, the inoculated AM fungi had higher concentration of glucose than un-inoculated treatments (Table 2). Proline concentration was increased 22% and 32% in *G. mosseae* and *P. occultum* respectively. Although proline content increased with salinity intensity, no significant difference were observed between two AM fungi.

Table 2: Effect of salinity and two arbascular micorrhizal on concentration of osmotic compounds in leaf text of Flying dragon seedling (*Glomus mosseae* = Gm, *Paraglomus occultum* = Po, Control = No micorrhiza).

Salt concentration (mM)	Micorrhiza type	Sucrose (mg/g)	Glucose (mg/g)	Proline (mg/g)
	Gm	8.35^{bc}	38.65^{a}	0.27^{ab}
0	Po	8.77^{ab}	42.11 ^{a}	0.25^{b}
	control	7.21^{cd}	37.32 ^{a}	0.19 ^{c}
50	*Gm*	8.30^{bc}	37.22 ^{a}	0.2^{ab}
	Po	8.46^{bc}	42.10 ^{a}	0.26^{ab}
	control	7.28 ^{cd}	29.67 ^{b}	0.19 ^{c}
100	*Gm*	7.69 ^{c}	39.76 ^{a}	0.29 ^{ab}
	Po	6.42^{d}	36.66 ^{a}	0.31 ^{a}
	control	9.87 ^{b}	23.65^{c}	0.19 ^{c}
	Gm	7.03 ^{cd}	35.50^{a}	0.33^{a}
150	Po	6.14 ^{d}	37.10 ^{a}	0.33 ^{a}
	control	12.55 ^{a}	19.87 ^{c}	0.20^{c}

Same letter within each column indicates no significant difference among treatments $\alpha<0.05$.

In high salinity treatments (100 and 150 mM), proline content have been shown significantly difference in compare with no AM fungi inoculation (Table 2). According to the table above, just in control treatment (o mM), between two AM fungi observed significant difference. Compared to the control treatment, Na^+ but not K^+ concentrations were markedly increased under 100 mM sodium chloride (Table 3). With comparing two different AM fungi, no significant difference were found in K^+, Na^+, Ca^+, K^+/ Na^+ and Ca^+/ Na^+. Under the non salinity and salinity conditions, AM symbiosis notably decreased the Na^+ concentration in compared with non-inoculated treatments. Salinity significantly decreased the ratio of K^+/ Na^+ and Ca^+/ Na^+ (Table 3). Leaves of AM seedlings have shown higher K^+/Na^+ and Ca^+/Na^+ ratios than the non-AM inoculated seedlings at three salinity levels.

Table 3: Influence of salinity and different arbascular fungi on some ionic concentration of Flying dragon seedling.

Salt concentration (mM)	Micorrhiza type	Potassium mg/g)	Sodium (mg/g)	Calcium (mg/g)	K^+/Na^+	Ca^{2+}/Na^+
	Gm	18.31[a]	4.86[d]	7.85[ab]	3.76[a]	1.61[a]
0	Po	17.79[a]	5.02[d]	7.56[ab]	3.54[a]	1.50[a]
	control	16.12[b]	5.35[d]	6.53[c]	3.01[ab]	1.22[b]
50	*Gm*	18.11[a]	6.78[cd]	7.83[ab]	2.67[b]	1.15[b]
	Po	17.05[a]	7.34[bc]	7.51[ab]	2.32[b]	1.02[bc]
	control	14.42[c]	7.86[c]	6.56[c]	1.83[c]	0.83[c]
100	*Gm*	15.78[b]	9.22[c]	8.15[a]	1.71[c]	0.88[c]
	Po	15.81[b]	9.14[c]	8.07[a]	1.72[c]	0.88[c]
	control	14.21[c]	10.93[b]	7.13[bc]	1.30[c]	0.65[d]
.	*Gm*	13.68[c]	11.56[b]	8.22[a]	1.18[cd]	0.71[d]
150	Po	13.75[c]	12.45[b]	8.19[a]	1.10[cd]	0.65[d]
	control	11.61[d]	14.77[a]	7.78[ab]	0.78[d]	0.52[e]

Colonization by AM fungi was proved in Flying Dragon seedlings, and no colonization was observed in control (un-inoculated seedlings). A reduction in mycorrhizal fungi colonization in citrus plants grown under saline condition was expected since it has been shown that AM fungi may be influenced by salinity during spore germination. This is consistent with other results [16, 22]. According to the results colonization rate of two AM fungi displayed different results, suggesting that *P. occultum* contained a better colonization than *G. mosseae*. Significant increasing in growth parameters, namely, height, diameter, root length, and leaf area was more evident for the seedlings inoculated with *G. fasciculatum* and *G. mosseae.* Salinity reduces the water potential of the roots, causing reduction in growth rate, along with a suite of metabolic changes similar to those caused by water stress [21]. From the present results it can be deduced that the reduction in plant growth due to increased salinity can be attributed to the osmotic effects of salts. Osmotic stress is a problem stemming from salt stress, and the resulting decrease in chemical activity causes cells to lose turgor [15, 17].

In this research, inoculated seedling had shown higher concentrations of glucose and sucrose in compared with non-inoculated seedlings. Exogenous mycorrhizal fungi increased sucrose content in leaves under no salinity condition, while under stress, AM fungi dramatically decreased sucrose content. The reason of sucrose decreasing is obligate symbiosis, that caused AM fungi uptake plant root carbon. So, lower levels of sucrose in salt stress treatments and higher glucose content indicated that sucrose transforming to hexose support the symbiotic development [24, 26]. AM-inoculated Fly Dragon seedling had higher contents of proline in leaves than non-mycorrhizal plants grown in saline soil. The observed increase in proline in salt grown mycorrhizal plants is in a good conformity with the results of other researchers [11, 12]. This increment in proline could be due to the induction of proline biosynthesis enzymes and reduction of oxidation to glutamate.

According to this study and many previous papers, AM treatments exhibited low Na^+ and high K^+ and Ca^{2+} contents compared to non-inoculated plants. It seems that the role of AM fungi in alleviating salinity partially due to the prevention of Na^+ uptake. In onion, AM treatments had higher contents of K+ in shoots under salinity [18]. By maintaining a high ionic balance such as K^+/Na^+ ratio in cytoplasm and Na+ controlling, AM improved plant nutrition under salt stress. Many researchers have indicated that arbuscular mycorrhizal related to plant growth via enhancement of mineral nutrient uptake [21, 28]. Reduced the negative effects of sodium by maintaining vacuolar membrane integrity, and therefore preventing this ion from disturbing the metabolic pathways.

By saving membrane integrity, the compartmentalization will become better within vacuoles and ions. In the present investigation, stem diameter, shoot and root dry weights of AM seedlings were higher than non-inoculated seedlings under different salinity level, that confirms the results of some previous papers [6, 29]. Colonization of *G. versiforme* significantly increased the plant height, stem diameter, leaf numbers, and dry mass [8]. Grafting seedling trifoliate orange which were inoculated with *G. mosseae* significantly increased the plant height, stem diameter, leaf area, and shoot length of seedling [26, 27]. The results of them showed that Arbuscular mycorrhizal fungi inoculation could increase plant growth, such as plant height, stem diameter, leaf area, shoot dry weight, root dry weight.

Generally, salinity prevented plant growth through water deficit and adverse sodium and chloride effects [8]. Salinity has typically been evaluated by the biomass production [9]. Although salinity reduced biomass of the seedling, but AM seedlings have shown higher dry biomass as compared to non-inoculated seedlings, which indicate that AM improved seedling growth under salinity. In citrus, the effects of salinity on growth reduction is not only related to the osmotic causes but also were results of gradual accumulation of toxic levels of Cl^- and Na^+. In most plants, the sodium accumulation is mostly a greater obstacle than the accumulation of chloride. Citrus is relatively unique in this respect since Cl^- accumulation is greater problem in leaves. The decrease of K^+ content by salinity might be due to the direct effect of Na^+ replacing K^+. In trifoliate orange seedling, AM colonization significantly increased the concentration of P, K^+ and Ca^{2+} under ample water [6, 25]. In the present study K^+ and Ca^+ decreased with increasing salinity, that were in agreement with those found by many reports [14, 18, 28].

Under salt stress, plant growth and biomass suffered a setback. The reasons may be the non-availability of nutrients and the expenditure of energy to counteract the toxic effects of NaCl. However, mycorrhization was found to increase the compatibility of the host plant by enhancing its growth. Several researchers have reported that AM fungi-inoculated plants grew better than non-inoculated plants under salt stress [8, 24, 29]. Mycorrhizal fungi varied in their ability to improve citrus cultivar growth, that is because of AM species showed different responses to different nutrient uptake, particularly less mobile elements such as phosphorus, zinc and copper [3]. Mycorrhizal inoculation also increases plant resistance against stress conditions such as salt, drought, and temperature as well [11]. Conclusion based on various studies on effect of several mycorrhizal inoculums on the seedling growth, it was clear that AM fungi could be infected effectively, and their shoot and root growth, especially fibrous root growth, was significantly improved, compared with the control [27].

For alleviating the adverse effects of salinity in Flying Dragon rootstock, inoculating with mycorrhizal fungi is suggested. However, in respect of AM symbiosis, the fundamental pertinent mechanism pathways are still not completely clear.

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