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Effects of mycorrhizal fungi inoculation on green pepper yield and mineral uptake under irrigation with saline water

Abstract

High salinity in soil or irrigation water has detrimental effects on plant nutrition and reduces crop growth and yield. In this study, the effects of pre–inoculation of green pepper (*Capsicum annuum* L., cv. Zingaro) with arbuscular mycorrhizal (AM) fungi on mineral uptake, growth and fruit yield under irrigation with saline water were investigated. Pepper seedlings were transplanted into nonsterile soil plots under polyethylene covered plastic house conditions and irrigated with saline water of three levels of ECw: nonsaline (0.5; NSW); medium (2.4; SW1) and high (4.8dSm^{-1}; SW2)] salinity levels. At pre–flowering stage (8–weeks after transplanting), AM inoculated plants had greater shoot and root dry matter and plant height than nonAM plants regardless of salinity level. Shoot concentrations and contents of P and K were higher and Na concentration and content were lower in AM compared with nonAM plants at pre–flowering stage. At harvest, fruit fresh yield, fruit weight, and fruit number per plant were higher in AM than nonAM plants. The enhancement in fruit fresh yields due to AM fungi was 38, 42 and 26% under NSW, SW1 and SW2 treatments, respectively. Results indicate that pre–inoculation of green pepper transplants with AM fungi improved nutrient uptake and fruit yield especially under moderate rather than severe salinity levels.

Keywords: mycorrhiza, *capsicum annuum*, fruit yield, salt stress, mineral

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Introduction

Salinity in soil or irrigation water are major environmental constraints to crop productivity and are increasing steadily in many parts of the world, especially in arid and semi–arid areas (e.g., Mediterranean region). Due to low precipitation in arid and semiarid regions, in addition to overexploitation of available water resources (e.g., ground water), low quality water (e.g., saline water) has been utilize for irrigation crops grown in plastic houses as well as those planted in open fields. High salinity of the irrigation water has detrimental effects on soil fertility and reduces crop growth and yield.[1–3] One of the strategies that have been used to counteract salinity stress involves growing crops that are tolerant to saline conditions.[4,5] However, alleviation of salinity problem using salt tolerant crops is consider expensive and often represents only a temporary solution.[6,7] Therefore, incorporating biological factors that enable plants to tolerate salt stress such as mycorrhizal fungi inoculum would be helpful in improving crop production under saline conditions.[6,8] Arbuscular mycorrhizal (AM) fungi are beneficial plant symbionts that form mutualistic relationships with roots of most crop plants. This association allows plants to explore larger volumes of soil to absorb more water and nutrients uptake (especially immobile nutrients as P, Zn, and Cu) which result in enhancement of plant growth and productivity.[1,9] This can be attained by increasing the surface area of soil explored via fungal hyphae, that extend into soil past zone of nutrient depletion.[9] Many recent studies have indicated that AM fungi could enhance the ability of plants to cope with salt stress by improving mineral nutrient absorption, maintaining ion balance, protecting enzyme activities and increasing water use efficiency.[1,10–13] AM fungi also enhance soil aggregation and water holding, both by extra radical hyphae in soil and exuding glomalin (glue like) which enhances soil structure.[14]

The most promising areas for practical use of AM fungi are during nursery seedlings production (e.g., horticultural crops), due to benefits that can be realize stronger growth of seedlings in nursery and improved performance after planting in the field.[6,15] Green sweet pepper (*Capsicum annuum* L.) is one of the economic important crops produced all over the world and its seedlings are produce in nurseries. Green pepper is considered sensitive to moderately sensitive to salinity stress.[16,17] However, AM fungi use in green pepper crop production is still less exploited compared to other crops of economic importance especially under field conditions.[18] Therefore, the symbiotic interactions between AM fungi and host plants under saline conditions need to be studied in order to optimize beneficial effects of AM fungi in enhancing crop growth and productivity. The objective of this study was to determine the effects of pre–inoculation of seedlings with arbuscular mycorrhizal (AM) fungi on mineral uptake, growth and yield of green pepper when irrigated with different levels of water salinity.

Materials and methods

Mycorrhizal inoculation and production of seedlings

Green pepper (cv. Zingaro) seeds were sown in polystyrene trays with 20–cm^3 cells filled with a mixture of peat moss and perlite (2:1, V/V). Half of the trays received the AM fungi (*Glomus mosseae*) at rates 10ml/planting cell (contains 400±20 propagules/cell) and placed directly beneath the seeds in planting cells. The added inoculums consisted of AM fungi colonized root fragments, spores and hyphae mixed with soil. The inoculum was isolated initially from a wheat (*Triticum durum* desf.) field in northern Jordan and multiplied in pot cultures using chickpea (*Cicer aritinum* L.) as host plant. Control treatments received no AM (nonAM) inoculum. Seedlings in trays

were grown on a bench with mist irrigation in the greenhouse until plants reach appropriate size for transplanting (35 days old). One day prior to transplanting into soils, 10 representative seedlings were taken from trays from both AM and nonAM treated plants and subjected to a destructive measurement, to assess for mycorrhizal colonization of roots and shoot growth (seedling length, stem width and the number of leaves per plant).

Transplanting and cultural conditions

The experiment was conducted under polyethylene covered plastic house during the period March to July 2014 on a nonsterile silty clay (fine, mixed, thermic, Typic Xerochrept) soil at the experimental farm of Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan. Before planting, a representative composite soil samples were taken from experimental area at a depth of 25cm and analyzed for major soil properties and indigenous AM fungi spores. Soil properties before planting were 8% sand, 45% silt, and 47% clay; 1.1% organic matter; pH 8.0 (soil: water, 1:1); electrical conductivity (ECe) 1.3dS^{-1}, 0.25P ($NaHCO_3$–extractable), 22.7K, 6.1 Na, 0.2 Fe, 0.02 Zn, and 0.03 Cu (5mM DTPA extractable) in mmolkg^{-1} soil. The initial search for indigenous AM fungi spores (assayed by wet sieving) yielded <2sporesg^{-1} air–dried soil. The experimental area was prepared manually and divided into the experimental plots thereafter. Plot dimensions were 1.6mx3.0m with four pepper rows in each plot. Before transplanting, 100kg ha^{-1}of N (as urea), 30kg ha^{-1}of P (as superphosphate), and 60kg ha^{-1}of K (as potassium sulfate) were applied and incorporated below the soil surface. Green pepper seedlings from both AM and nonAM treated plants were transplanted into planting rows at a plant population 10m^{-2} (spacing at 25cm and 40 cm within and between rows, respectively). Plants were fertilized with 30 and 50kg ha^{-1} of N (as urea) 30 and 60days after transplanting. Mean daily temperature was between 20 and 33ºC. Weeds were controlled by hand as needed.

Irrigation/salinity treatments

To insure the establishment of the seedlings, plants were irrigated with tap water for 3 weeks (via drip irrigation system), before being subjected to three irrigation/salinity treatments until the end of harvest as needed:

i. Nonsaline water (NSW) – tap water (ECw=0.5 dSm^{-1}).

ii. Medium saline water (SW1)– irrigation with saline water (ECw=2.4dSm^{-1}).

iii. High saline water (SW2) – irrigation with saline water (EC_w=4.8dSm^{-1}). The resulted soil EC_e at harvest was 1.5, 3.6 and 7.1dS m^{-1} for NSW, SW1 and SW2 treatments, respectively. Saline water was brought from a saline well located in Mafraq governate (Jordan), and its properties before dilution were ECw (6.1dS m^{-1}); pH 9.1; TDS (3835ppm); SAR (5.2); 24 K, 15 Ca, 25.2 Mg, 24.5 Na, 50Cl, 10.3 HCO_3, and 10.3 SO_4 in meq L^{-1}. Tap water was used as control treatment has the following properties: EC_w (0.5dS m^{-1}); pH 8.0; TDS (410ppm); SAR (0.5); 1.2 K, 3.4 Ca, 3.0 Mg, 1.0 Na, 1.8 Cl, 5.0 HCO3, and 0.2 SO_4 in meq L^{-1}.

Plant growth and fruit yield

At pre–flowering stage (8–weeks after transplanting), three plants were sampled randomly from each experimental plot for determination of shoot height, shoot and root dry matter, AM fungi roots colonization and mineral nutrients uptake. These samples were taken by a fork, fitted to excavate the soil volume under the area occupied by the plants. The rest plants in each plot were left to grow until end of season for fruit harvest. Sampled plants were washed by distilled water and separated into shoots and roots. Shoots were oven dried at 70°C for 48, weighed and saved for mineral analysis. Roots were rinsed free from soil and weighed and fresh subsamples (2 grams) were saved for assessment of AM fungi root colonization and the rest were oven dried and weighed. Total root dry weight was adjusted for weight of the subsample used for AM assessment. Green pepper fruits were harvested by hand many times during the growing season when they were reached a fully marketable size (fruit reached a shining green color). Total fruits harvested per plot were recorded to determine fresh fruit yield per unit area. Fruit number per plant and individual fresh fruit weight were also determined.

Determination of mycorrhizal colonization

Collected roots at pre–flowering stage were cut into 1cm fragments, thoroughly mixed, and representative fresh samples (1gram) were used for determination of root AM fungal colonization. Root samples were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol as described by Philips and Hayman,[19] and microscopically examined by estimation of root colonization by determining percentage of root segments containing hyphae, arbuscules and vesicles.[20]

Mineral analysis

Dried shoot samples were ground to pass 0.5mm sieve using a cyclone laboratory mill. The ground material was mixed thoroughly, and samples of 1.0g were ashed for five hours at 550°C in a muffle furnace, and then the ash was dissolved in 2N HCl for determination of the concentration of Na, K, and P. Sodium and K concentrations were analyzed by using flame photometer. Phosphorus was determined according to the yellow phosphorus–vanado–molybdate complex method by using spectrophotometer. Nitrogen concentration in shoots was determined by using the micro–Kjeldhl method. Mineral contents were calculated by multiplying of mineral concentration by corresponding dry weight of shoots.

Mycorrhizal enhancement effect

The overall enhancement effects of AM fungi inoculation on the green pepper fruit yield and shoot mineral contents (percentage change) of plants grown under nonsaline and saline conditions were calculated according to the following formula:[6]

i. Fruit yield (FY) change = $[(FY_{AM}–FY_{nonAM})/FY_{nonAM}]x100$

ii. Nutrient content (NC) change = $[(NC_{AM}–NC_{nonAM})/NC_{nonAM}]x100$

Experimental design and statistical analysis

The experiment consisted of a randomized complete block design with two factors:

i. AM fungi treatments (with and without AM fungi inoculation).

ii. Three water salinity levels (NSW, SWI and SW2).

iii. Each treatment was replicated 4 times. Data were statistically analyzed using analysis of variance in the MSTATC PROGRAM (Michigan State Univ, East Lansing, MI, USA). Probabilities of significance were used to indicate significance among treatments and interactions and LSDs ($P \leq 0.05$) were used to compare means.

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Results and discussion

Seedling quality before transplanting

The pre–inoculated seedlings with AM fungi were larger than non AM seedlings at transplanting; however, there were no significant differences in shoot growth (seedling length, stem width and the number of leaves per plant) between AM and non AM plants (Table 1). Mean AM fungi colonization of roots of AM pre–inoculated seedlings was 13.2%, while no AM fungi colonization was observed in the roots of non AM seedlings (Table 1). The primary purpose of nursery inoculation is not to promote plant growth at this stage of production, but to establish AM fungi on plant roots so that mycorrhizae will be efficiently transformed to the field.[6,15] The minimum level of colonization necessary for successful transfer of mycorrhizal plants to the field is ~10% which reported to spread rapidly to new roots after transplanting.[21] Results of this study indicated that the level of colonization with AM fungi before planting might be considered adequate for successful establishment of mycorrhizal plants after transplanting.

Mycorrhizal colonization

Assessment of AM colonization of roots at pre–flowering stage (8 weeks after transplanting) showed that both AM and non AM plants were colonized by AM fungi, even though the AM plants had much higher root AM fungi colonization (30.5 to 55.2%) than non AM plants (8 to 15%) (Table 2). The AM fungi root colonization in non AM plants might came from native mycorrhizae in soil of experimental field. The AM fungi root colonization in green pepper was reduced by salinity stress regardless of AM fungi inoculation status (Table 2). These findings are in agreement with other researchers working on different vegetable crops, who reported that salinity not only affects the host plant growth but also the AM fungi colonization.[2,6–8] Salinity can reduce AM colonization capacity, spore germination and inhibiting growth of hyphae of the fungus.[12,22,23] These reports have indicated that the negative effects of salinity on the AM fungus probably due to the direct effect of present salts on the fungi.[22,24]

Plant growth

At pre–flowering stage (8weeks after transplanting), salinity stress significantly reduced pepper plant height and shoot and root dry matter yields compared with the nonsaline treatment regardless of AM status (Table 2). However, AM colonization improved significantly shoots and root dry matter in the medium salt–stressed (SW1) and nonsaline (NSW) plants, but it did not significantly affect them in the high salt–stressed (SW2) plants (Table 2). Plant height was significantly higher in AM than nonAM plants regardless of salinity level (Table 2). The beneficial effects of mycorrhizal fungi on plant growth under saline conditions have been demonstrated in various plant species, by Al–Karaki[4] and Balliu et al.,[25] in tomato, Zuccarine[26] in lettuce, Pereira et al.,[18] Kaya et al.[7] and Cekic et al.[8] in pepper plants.

Nutrient uptake

At pre–flowering stage (8weeks after transplanting), applying saline water in irrigation decreased N, P and K, concentrations and contents regardless of AM status (Tables 3) (Table 4). However, AM inoculum application enhanced shoot P and K concentrations regardless of salinity level, although the differences for K concentrations were only significant under medium saline conditions. No significant differences between AM and non AM were noted for shoot N concentrations (Table 3). Shoot contents of N, P and K were generally higher for AM than nonAM plants regardless of salinity treatment (Table 4). However, shoot P contents are significantly higher in AM than nonAM plants grown under both saline and nonsaline conditions, while shoot N and K contents were significantly higher in AM than non AM plants only under nonsaline and medium (SW1) saline conditions (Table 4). The higher mineral nutrient uptake in AM compared to nonAM plants (higher contents of N, P and K) under saline conditions likely occurred because of improvement of soil exploration by mycorrhizal extraradical hyphae that extend beyond root depletion zone which resulted in reducing antagonistic effects of salinity on nutrients uptake.[25,27] Enhanced uptake of N, P and K by AM plants has been reported by many researchers for different vegetable crops grown under saline conditions.[25,27–29] Concentrations and contents of Na were significantly lower in the shoots of AM than nonAM pepper plants grown under both saline but not nonsaline conditions (Tables 3) (Table 4). The decrease in shoot Na contents in AM plants may partially be explained by a dilution effect due to an increase in dry matter accumulation of AM plants.[6] Evelin et al.[30] and Cantrell et al.,[10] reported that mycorrhizal root colonization appears to have a role in alleviating salt stress by lowering Na absorption by the root and translocation to shoot tissues.

Fruit yield

Fruit fresh yields and fruit number was significantly reduced by increasing salinity level compared to the nonsaline treatment (Table 5). However, fruit yields and fruit number of AM plants were higher than that of nonAM plants, although these differences were significant only under nonsaline and medium saline conditions (Table 5). Mean fruit weight was generally higher in AM than nonAM plants regardless of salinity level, but the differences for this parameter was only significant under nonsaline conditions (Table 5).

The beneficial effects of mycorrhizal fungi on fruit yield and components in green pepper under saline conditions have been demonstrated.[7,8,18] Many studies have indicated that AM fungi contribute to plant growth via enhancement of mineral nutrient uptake particularly that of P and N and hence improve salt tolerance in different vegetable crops grown under saline conditions.[2,12,25–29] Mycorrhizal inoculation has been also reported to reduce the negative effects of Na by maintaining vacuolar membrane integrity, which prevents this ion from interfering in growth metabolic pathways.[31] In the present study, mycorrhizal inoculation increased P and K uptake (concentrations and contents) and reduced Na concentrations and contents, thereby alleviating the adverse effects of salt stress on pepper plants (Tables 3) (Table 4).

Mycorrhizal enhancement effect

The overall effects of AM fungi inoculation on the pepper yield and mineral contents (percentage–wise) of plants grown under nonsaline and saline conditions are summarized in Table 6. The enhancement in fruit fresh yields due to AM fungi was 38, 42 and 26% under nonsaline (NSW), medium (SW1) and high (SW2) saline water conditions, respectively. Under conditions of irrigation with medium saline water (WS1), the enhancement due to AM fungi inoculation in the shoot contents of N, P and K was significantly higher than those irrigated with nonsaline or high saline water treatments (Table 6). The enhancement values due to AM fungi inoculation for N, P, and K contents were 46, 66 and 60% for pepper grown under medium saline water conditions, respectively. However, no significant differences

in enhancement effects due to AM fungi inoculation between NSW and SW2 for these elements except for N contents were noted (Table 6). Pre–inoculation with AM fungi induced a regulatory effect on the translocation and hence content of Na in pepper shoots compared to nonAM plants under both nonsaline and saline conditions, but the effects were greater under saline conditions, when shoot Na contents reduced due to AM fungi inoculation by 5, 12 and 10% under NSW, SW1 and SW2 conditions, respectively (Table 6). These results demonstrate the favorable relationship between pepper and AM fungi, and shows that when roots were associated with AM fungi, the detrimental effect of the salinity stress decreased significantly, although salinity reduced mycorrhizal colonization. The beneficial effects due to AM fungi inoculation on growth and mineral nutrition were greatest at medium salinity level. Ronco et al.,[32] reported that AM fungi inoculation has ecological importance of AM association for plant survival and growth under salinity stress.

Although salinity reduced AM growth to a varying degree depending on salinity conditions, AM symbiosis can frequently increase plant tolerance to salinity stress.[6,33,34] A strategy for management of salinity through improvement of growth and nutrient uptake would be to adopt cultural practices that encourage root mycorrhization with appropriate AM prior to transplanting of horticultural crop in the field soil; especially the salinity level under field is not an adjustable variable. The finding that AM pepper plants irrigated with saline water had greater fruit fresh yield, fruit weight, and shoot DM than nonAM plants supports the hypothesis that pre–inoculated AM plants grow better than nonAM plants under saline conditions. Inoculation of transplants prior to salt exposure might help bypassing the potential inhibitory effects that salt could have on AM fungal spore germination. Such inhibitory effects of salinity on rate of mycorrhizal colonization have been reported.[12,22,23] The procedure used in this study of pre–inoculating transplant seedlings with AM fungi can be of practical importance in the cultivation of many horticultural crops grown under saline conditions.

Table 1 Root AM colonization, seedling length, stem diameter and number of leaves of AM and nonAM green pepper seedlings before transplanting[1]

AM treatment	AM colonization (%)	Seedling length (cm)	Stem diameter (mm)	Number of leaves/seedling
AM inoculated	13.2 a	11.3 a	5.1 a	6.2 a
Non inoculated	0.0 b	10.8 a	4.9 a	5.8 a

SD, Means in each column followed by same letter are not significantly different (P≤0.05) according to L.S.D.AM, mycorrhizal inoculated; NonAM, no inoculation with mycorrhiza.

Table 2 Root AM colonization, plant height, shoot and root dry matter (DM) yields after 8 weeks of seedlings transplanting of AM and nonAM pepper plants grown under different water / salinity regimes

Water/salinity regime	AM Fungi Status	Root AM colonization (%)	Plant height cm plant^{-1}	Shoot DM g plant^{-1}	Root DM g plant^{-1}
NSW	NonAM	15.0 d	35.5 bc	12.5 b	4.2 b
	AM	55.2 a	46.1 a	16.4 a	5.5 a
SW1	NonAM	10.0 de	33.3 cd	9.5 c	3.3 c
	AM	42.3 b	39.3 b	12.2 b	4.0 b
SW2	NonAM	8.0 e	30.5 d	7.2 d	2.4 c
	AM	30.5 c	34.9 c	9.0 cd	3.0 c

SD, Means in each column followed by same letter are not significantly different (P≤0.05) according to L.S.D.AM, mycorrhizal inoculated; NonAM, no inoculation with mycorrhiza.

Table 3 Shoot concentrations of N, P, K, and Na after 8 weeks of seedling transplanting of AM and nonAM green pepper plant grown under different water/ salinity regimes.

Water/salinity regime	am fungi status	N (mg/g)	P(mg/g)	K(mg/g)	Na(mg/g)
NSW	NonAM	22.6a	2.8 b	18.7ab	3.2d
	AM	23.3 a	3.2 a	19.7 a	2.3d
SW1	NonAM	17.1b	2.4 c	12.5 c	13.1b
	AM	19.3 b	3.1ab	16.2 b	9.0 c
SW2	NonAM	12.7 c	2.1 d	10.8 c	15.4 a
	AM	14.3c	2.5c	12.3 c	11.2 b

SD, Means in each column followed by same letter are not significantly different (P≤0.05) according to L.S.D.AM, mycorrhizal inoculated; NonAM, no inoculation with mycorrhiza.

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Table 4 Shoot contents of N, P, K, and Na after 8 weeks of seedlings transplanting of AM and nonAM green pepper plants grown under different water/salinity regimes

Water/salinity regime	AM fungi status	N (mg/plant^{-1})	P(mg/plant^{-1})	K(mg/plant^{-1})	Na(mg/plant^{-1})
NSW	NonAM	283 b	35.0 b	234 b	40 d
	AM	382 a	52.5 a	323 a	38 d
SW1	NonAM	162 d	22.8 c	124 c	124 a
	AM	236 c	37.8 b	198 b	109 bc
SW2	NonAM	91 e	15.1 d	78 d	111 b
	AM	129 de	22.5 c	111 cd	101 c

SD, Means in each column followed by same letter are not significantly different (P≤0.05) according to L.S.D. AM, mycorrhizal inoculated; NonAM, no inoculation with mycorrhiza.

Table 5 Fruit fresh yield, fruit number, and fruit weight of AM and nonAM green pepper plants grown under different water/salinity regimes

Water/salinity regime	AM fungi inoculation	Fruit yield (Kg m^{-2})	Fruit number m^{-2}	Fruit weight (g)
NSW	NonAM	8.2 b	62 b	132bc
	AM	11.3 a	80 a	141 a
SW1	NonAM	6.2c	49 c	126 b
	AM	8.8 b	67 b	131 b
SW2	NonAM	3.8 d	33 d	115 c
	AM	4.8 d	40 d	121bc

SD, Means in each column followed by same letter are not significantly different (P≤0.05) according to L.S.D. AM, mycorrhizal inoculated; NonAM, no inoculation with mycorrhiza.

Table 6 Percentage change in fruit yield and shoot nutrient contents due to AM and nonAM of green pepper grown under nonsaline (NSW) and saline (SW1 and SW2) water conditions

Water/Salinity Regime	Fruit Yield (%)	Shoot Nutrient Content (%)			
		N	P	K	Na
NSW	38b	35b	50b	38 b	-5 c
SW1	42a	46a	66a	60 a	-12 a
SW2	26 c	42a	49b	42 b	- 9 b

SD, Means in each column followed by same letter are not significantly different (P≤0.05) according to L.S.D. AM, mycorrhizal inoculated; NonAM, no inoculation with mycorrhiza.

Conclusion

It is apparent that pre–inoculation of green pepper transplants with AM fungi have positive enhancement effects in reducing the effects of salt stress through enhancing plant growth, fruit yield and nutrient uptake under relatively medium salinity levels. These results might indicate that sensitive to moderately sensitive crops to salt stress (e.g., green pepper) can benefit from mycorrhizal inoculation under ecosystem that relatively affected by medium salt levels. In view of these results, it is possible to recommend that mycorrhizal inoculation can attain reasonable growth and fruit yield of sweet pepper under moderate saline conditions.

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Conflict of interest

There is no any conflict of interest exists.

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