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The effects of *Arbuscular mycorrhiza* on the growth and physiological characteristics of grafted cucumber under salinity stress

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ABSTRACT - This study aimed to study the mutual effects of grafting and *Arbuscular mycorrhiza* on cucumber. The experimental design was a factorial experiment based on a randomized block design. Accordingly, treatments were mycorrhiza inoculation including non-inoculation mycorrhiza (AM1) as the control, mycorrhiza with 2400 spores per pot (AM2); and grafting, in which cucumbers *(Cucumis sativus* var. super daminos) were grafted on *Lagenaria siceraria* (R2) and non-grafted cucumbers (R1), and the salinity concentration included the control (C), without adding NaCl, medium salinity equal to 30 mM NaCl and high salinity equal to 60 mM NaCl with three replications. The results showed that growth parameters such as the number of male and female flowers, time of appearance of the first male and female flower, the node number of the first female flower, stem length, the number of nodes and leaves, shoot and root fresh weights, were decreased with salinity stress. Grafting also affected the time of appearance of the first male flower and in the node, the number of the first female flower appearance at high and moderate NaCl concentrations. The appearance of the first female flower was postponed under the high concentration of NaCl. Photosynthetic reduction detected in cucumbers subjected to salinity was associated with a decrease in their chlorophyll and mesophyll conductance. In addition to reducing the photosynthesis rate, chlorophyll fluorescence was also affected by moderate and high salinity stress. Generally, grafting and mycorrhiza application decreased the harmful effect of salinity, especially in moderate salinity.

INTRODUCTION

Environmental parameters such as temperature, humidity, aeration, rainfall and intensive nutrient fertilization lead to the nutrient imbalance in soils which can limits field cultivation (Ye et al., 2006). Besides this, extra salinity, acidification and nutrient fertilizer affect crop yield and quality. Microorganisms improve plant growth, nutrient absorption, stress tolerance and crop yield under suboptimal environmental conditions such as salinity, drought and low fertility stresses (Ye et al., 2006). Endomycorrhizal fungi (*Vesicular-Arbuscular mycorrhiza*, VAM) enhance plants' salinity resistance (Hajiboland et al., 2010). *A. mycorrhiza* (AM) could be reduced the adverse effect of water stress (Rooney et al., 2009). Soil and crop productivity is highly related to soil microbial communities, including mycorrhizal fungi (Rooney et al., 2009). Vesicles and arbuscules are fungal structures in root cortex that are generated with AM application (Quilambo, 2000). Mycorrhizal fungi can significantly increase nutrient uptake, particularly phosphorous uptake and transportation, within plant roots (Quilambo, 2000).

Salinity is one of the main abiotic stresses which can limits global agricultural productivity (Munns and Tester, 2008). Soil salinity happens due to the low rainfall, fertilizers with high salinity index, irrigation with saline water, unprincipled agricultural management, and the use of extra fertilizer. Sodium chloride (NaCl) is the most abundant salt in saline environments (Tester and Davenport, 2003). Furthermore, in arid and semiarid regions soil salinity has become a serious problem (Ravindran et al., 2007).

It has been reported that vegetative growth, yield, shoot, and root fresh and dry weight in tomatoes could be decreased by salinity stress. The harmful effect of salinity stress was due to the combination of osmotic and specific ion effects of Cl^- and Na^+ (Hajiboland et al., 2010). The high concentrations of toxic Na^+ and Cl^- ions in saline soils are absorbed by roots and transported to shoots and leaves through the xylem. So, accumulation of the extra Na^+ and Cl^- ions can prevent other ions' absorption. This ionic imbalance causes

oxidative stress by reactive oxygen species (ROS) production, which can inhibit several physiological and biochemical processes in plants and change the metabolism of carbohydrates, amino acids, and fatty acids (Verma and Mishra, 2005). It has been shown that grafting onto a salt-tolerant rootstock is an effective and environmentally friendly method for decreasing yield losses under salinity stress (Yang et al., 2012).

It is well known that the uptake of mineral nutrients by roots and the efficiency of photosynthesis of leaves are "source" forces for the plant's growth. Higher concentrations of Na^+, Cl^- and SO_4^{2-}ions in saline soils accumulate in plant cells and inhibit nutrient (e.g., nitrogen, phosphorus, and potassium) uptake (Zhang et al., 2011) and photosynthesis (Ait-El-Mokhtar et al., 2020). Salinity was found to inhibit specific enzymes involved in the synthesis of photosynthetic pigments, resulting in decreasing in chlorophyll content (Porcel et al., 2015). Salt stress can also cause significantly disturbed chlorophyll fluorescence (Sheng et al., 2015). The change of rapid chlorophyll fluorescence can reflect the change of photosynthetic electron transfer and also the function and stability of photosystem II (PSII) (Sheng et al., 2015) and it is widely regarded as an important parameter for diagnosing the operation of the photosynthetic apparatus in plants and analyzing the mechanism of plant response to stress (Harbinson, 2013).

Nowadays, grafting is used as an efficient method to increase abiotic stress resistance (i.e. salinity by decreasing ionic stress, drought, and low temperature) (Zrig et al., 2011). There are various studies on the effect of grafting under salinity stress on plant growth, ion accumulation, leaf Na^+ and Cl^- concentrations, yield, fruit quality, leaf pigments, root hydraulic conductance, etc. on watermelon (Colla et al., 2006a) and melon (Colla et al., 2006b).The goal of the present study was to investigate the effect of using AMs on grafted cucumbers to alleviate salinity stress on the cucumbers' growth and physiological characteristics.

MATERIALS AND METHODS

The experimental design was a factorial experiment based on a randomized block design. Treatments were mycorrhiza inoculation including non-inoculation mycorrhiza (AM1) as the control and mycorrhiza with 2400 spores per pot (AM2); and grafting, in which cucumbers *(Cucumis sativus* var. super daminos) were grafted on *Lagenaria siceraria* (R2) and non-grafted cucumbers (R1), and the salinity concentration included the control (C), without adding NaCl, medium salinity equal to 30 mM NaCl and high salinity equal to 60 mM NaCl with three replications.

The experiment was performed for 4 months in terms of temperature 27±2 °C and 30 to 60% relative humidity (RH) and 6000 lux lighting with lamp in the form of 14 hours of lighting and 10 hours of blackout in a greenhouse of Isfahan University of Technology, Isfahan, Iran. The rootstock was chosen from a previous research (Mohammadnia and Haghighi, 2021). Scion seeds had been cultivated 10 days before rootstock seeds in cocopeat:perlite (1:1 w/w). Scion plants and rootstocks were cut beneath and above the first true leaves, respectively. Hole-insertion grafting was used. Grafted plants were transferred to a recovery greenhouse with high RH. Plants were kept for 2 weeks in recovery conditions and gradually adapted to normal greenhouse conditions. Grafted plants were transferred to 5 kg pots containing soil that had received mycorrhiza inoculum of *Glomus mosseae* according to mycorrhizal treatments. *G. mosseae* was taken from Touran Biotech Company (Shahroud, Iran). The interaction of mycorrhiza and root was observed with a light microscopic thorough examination of all roots, as stained with Chlorazol Black E at the root surface. Irrigation was used when the plant needed it. Plants were conducted to wire above the greenhouse. No pesticides were used. After 30 days' growth, salinity stress was induced by the addition of three different concentrations of the NaCl solution to each respective treatment that was followed by proper irrigation. NaCl solution was added twice a week. To prevent osmotic shock, the salinity stress was performed in two steps; rinsing with ordinary water was performed before irrigation with saline water for the second step.

All plants of three replications were harvested 60 days post-applying each treatment. Samples were analyzed for morphological parameters such as shoot length, root length, fresh weight, dry weight, nodes umber, and leaves number (i.e. total biomass). Also, flowering parameters have been recorded.

Measured Parameters

Plant Growth

The male and female flower, node, and leaves numbers were counted during the experiment. Stem length was determined using a ruler. The time of flowers' emergence and their node emergence were recorded.

Determination of Fresh and Dry Weight of Shoots and Roots

At the end of the experiment, the shoots were separated from the roots and each part was weighed, then they were oven-dried at 55 °C to measure the dry weight of the shoots and roots.

Determination of Chlorophyll Index

Chlorophyll Index (SPAD value) was recorded from adult leaves of plant ends using a chlorophyll meter (SPAD-502 plus, Japan). For this purpose, from each plant, three readings were performed on three separate leaves (a total of 9 readings per replicate), and then the average value was recorded.

Photosynthesis Trait Assay

Gas exchange parameters including photosynthesis rate, transpiration, stomatal conductivity, and intercellular CO_2 of stomata were measured from three replications per treatment using a portable photosynthesis meter (Li-Cor Li-3000, USA) on a sunny day. Mesophyll conductance (mmol CO_2. m^{-2} s^{-1}) was calculated by

dividing the photosynthetic rate by the sub-stomatal CO_2 concentration (Ahmadi and Siosemardeh, 2005).

Determination of Chlorophyll Fluorescence

The maximum quantum efficiency of the photosystem-II was measured by the ratio of variation chlorophyll fluorescence to maximum chlorophyll fluorescence (Fv/Fm) using a chlorophyll fluorescence apparatus (model OS-30, Minolta Corp). Chlorophyll fluorescence was measured by a portable fluorescence monitoring system (RS232, Handy PEA, UK). A clip was placed on the leaf for 30 min for dark adaptation. The initial (F0), maximum (Fm), and maximum quantum efficiency of the photosystem-II (Fv/Fm) were reported according to Maxwell and Johnson (2000).

Total Phenolic Content

The Folin–Ciocalteu method was used for measuring the total phenolic content. The UV absorbance was measured at 725 nm wavelength using a spectrophotometer (UV 160AShimadzu Corp., Kyoto, Japan) and a gallic acid standard curve (mg.100 g^{-1} fresh weight) (Singleton and Rossi, 1965).

Proline Concentration

Proline concentration was determined by a fast, simple, and accurate method based on the reaction of proline with the acid ninhydrin (Bates et al., 1973). H_2SO_4, 3% (v/v), was used to homogenize the leaves at 4 °C. The solution was incubated and then centrifuged at 5000 *g* for 20 min. The supernatant was mixed with 2.5% ninhydrin, 60% H_3PO_4 (v/v) and one ml of CH_3 COOH 100% (v/v). The UV absorbance was measured at 518 nm wavelength by a spectrophotometer (UV 160A- Shimadzu Corp., Kyoto, Japan).

Electrolyte leakage (EL)

Electrolyte leakage (EL) was measured using an electrical conductivity meter with the method of Lutts et al., (1995). Leaf pieces in distilled water were shaken at 100 rpm using a shaker at room temperature for 24 h. Equation 1 was used to measure EL.

Equation 1. EL (%) = initial conductance (measurement) / final conductance (measurement) × 100.

The initial conductance was measured using a conductivity meter. The tubes were then autoclaved at 115 °C for 10 min and the final readings (measurements) were recorded.

Determination of Total Antioxidant Activity

The total antioxidant activity of leaves was evaluated with DPPH assay as described by Koleva et al. (2002). Three mg of the sample was dissolved in five mL methanol stock and 1.4 ml of this solution was blended with 0.6 mL of DPPH solution. After 30 min the UV absorbance of the solution was recorded at 515 nm wavelength using a spectrophotometer (Shimadzu UV160A-Japan).

Statistical Analysis

Data were analyzed using Statistics 8 software (Tallahassee FL, USA). The Means were separated with the least significant difference by LSD test at the 1% and 5% probability levels.

RESULTS

The time of the appearance of the first male and female flowers and the node number of the first female flower were affected significantly (P <0.05) by the grafting treatments (Table 1). The time of appearance of the first male and female flowers was significantly decreased in the R2 treatment (Table 1) and the node number of the first female flower was significantly increased in the R2 treatment.

The number and time of appearance of the first male and female flowers per plant were affected significantly (P <0.05) by the mycorrhiza inoculation treatments (Table 1). The number of male and female flowers per plant increased in the AM2 treatment (Table 2) also, the time of appearance of the first male and female flower decreased in the mycorrhiza inoculation treatments (Table 2).

The time of appearance of the first male and female flowers was affected significantly (P <0.05) by the salinity treatments (Table 1). The time of appearance of the first female flower increased significantly with 60 mM NaCl salinity compared with 30 mM NaCl salinity (Table 2), whereas the time of appearance of the first male flower decreased significantly with 60 mM NaCl salinity compared with the control treatment (Table 2).

The stem length, number of nodes per plant, number of leaves per plant, shoot and root fresh weight, and shoot dry weight were affected significantly (P <0.05) by mycorrhiza and salinity treatments (Table 3). The application of mycorrhiza significantly enhanced the amount of all growth parameters including stem length, number of nodes per plant, number of leaves per plant, shoot and root fresh weight, and shoot dry weight, as shown in Table 4. In high salinity treatment, growth parameters such as stem length (23%), number of nodes per plant (16.61%), number of leaves per plant (1.99%), shoot (58.46%) and root (84%) fresh weight, and shoot dry weight (51.90%) were decreased as compared to the control treatment (Table 4).

Transpiration, Photosynthesis and mesophyll conductance were affected significantly (P <0.05) by the treatments (Table 5). The use of grafting significantly decreased the amount of transpiration (4.79%) and mesophyll conductance (20%) (Table 6). The highest amount of photosynthesis, transpiration, and mesophyll conductance was found in mycorrhiza application treatment (Table 6). Photosynthesis decreased significantly in salinity treatment. The lowest amount of photosynthesis, transpiration and chlorophyll fluorescence were found in S3 treatment (Table 6).

Table 1. Analysis of variance of the effect of grafting, mycorrhiza, and salinity on growth parameters of cucumber

Treatments	df	Number of male flowers per plant	Number of female flowers per plant	Time of appearance of the first male flower	Time of appearance of the first female flower	The node number of the first female flower
Rootstock	1	21.78^{ns}	1708.44^{ns}	134.891*	49.858*	10.1977*
Mycorrhiza	1	3249*	5184*	928.900*	158.838*	0.0044^{ns}
Salinity	2	293.58^{ns}	496.75^{ns}	2.673*	17.642*	0.2803^{ns}
Rootstock× Mycorrhiza	1	747.11^{ns}	1444^{ns}	30.686^{ns}	2.003^{ns}	0.0044^{ns}
Rootstock× Salinity	2	1384.03^{ns}	127.53^{ns}	1.590^{ns}	8.466^{ns}	6.8197^{ns}
Mycorrhiza× Salinity	2	74.08^{ns}	401.08^{ns}	3.196^{ns}	15.038^{ns}	1.4470^{ns}
Rootstock× Mycorrhiza× Salinity	2	344.19**	204.08**	1.418**	11.056**	2.9678**
Error		741.46	558.83	0.982	6.550	1.1301
CV		9.54	14.73	2.70	6.43	6.50

ns: no significant, ** significant at 1% and * significant at 5% probability, respectively.

Table 2. The main effect of grafting, mycorrhiza and salinity on growth parameters of cucumber

Treatments	Number of male flowers per plant	Number of female flowers per plant	Time of appearance of the first male flower (day after grafting)	Time of appearance of the first female flower (day after grafting)	The node number of the first female flower
Rootstock					
R1	27.72±0.11a	23.22±0.2a	38.70±0.12a	41.01±0.14a	3.467±0.11b
R2	29.27±0.10a	9.44±0.12a	34.66±0.13b	38.61±0.15b	4.55±0.17a
Mycorrhiza					
AM1	19.00±0.14b	4.33±0.12b	41.98±0.18a	41.96±0.16a	4.02±0.12a
AM2	38.00±0.13a	28.33±0.17a	31.38±0.19b	37.66±0.14b	4.00±0.14a
Salinity					
S1	4.65E^{-03} ±0.001a	12.25±0.11a	37.12±0.15a	39.69±0.14ab	4.11±0.16a
S2	6.34E^{-03} ±0.001a	23.75±0.12a	36.75±0.16ab	38.66±0.15b	3.83±0.12a
S3	8.62E^{-03} ±0.001a	13.00±0.14a	36.16±0.14b	41.08±0.17a	4.08±0.18a

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at $P < 5\%$.

Table 3. Analysis of variance effects of grafting, mycorrhiza, and salinity on stem length, number of nodes and leaves, shoot and root fresh weight, and shoot dry weight of cucumber

	df	Stem length	Number of nodes per plant	Number of leaves per plant	Shoot fresh weight	Shoot dry weight	Root fresh weight
Rootstock	1	308.5^{ns}	8.380^{ns}	8.517^{ns}	1495.11^{ns}	51.415^{ns}	4.69^{ns}
Mycorrhiza	1	28179.9*	852.514*	344.336*	8855.08*	364.308*	1757.97*
Salinity	2	741.6*	22.972*	16.799*	970.07*	146.880*	100.45*
Rootstock× Mycorrhiza	1	10.9^{ns}	0.001^{ns}	28.949^{ns}	411.27^{ns}	66.931^{ns}	3.19^{ns}
Rootstock× Salinity	2	475.6^{ns}	33.435^{ns}	49.298	680.01^{ns}	50.820^{ns}	196.37^{ns}
Mycorrhiza× Salinity	2	992.8^{ns}	14.945^{ns}	23.812^{ns}	545.35^{ns}	116.228^{ns}	183.00^{ns}
Rootstock× Mycorrhiza× Salinity	2	125.2**	1.258**	2.474**	224.68**	83.770**	183.84**
Error		385	46.190	34.360	72.61	38.189	85.41
CV		3.96	5.96	8.19	11.57	14.40	10.57

ns: no significant, ** significant at 1% and * significant at 5% probability, respectively.

Table 4. The main effects of grafting, mycorrhiza, and salinity on stem length, number of nodes and leaves, shoot and root fresh weight, and shoot dry weight of cucumber

Treatments	Stem length (cm)	Number of nodes per plant	Number of leaves per plant	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)
Rootstock						
R1	58.28±0.11a	14.36±0.12a	10.59±0.15a	29.83±0.12a	2.971±0.16a	8.38±0.19a
R2	64.52±0.13a	15.39±0.14a	9.55±0.17a	16.66±0.11a	5.41±0.18a	9.12±0.17a
mycorrhiza						
AM1	31.67±0.13b	9.70±0.13b	6.78±0.11b	7.21±0.1b	0.94±0.12b	1.61±0.1b
AM2	91.13±0.12a	20.04±0.14a	13.36±0.10a	39.28±0.1a	7.44±0.1a	15.89±0.1a
Salinity						
S1	70.69±0.10a	16.55±0.16a	11.18±0.17a	31.37±0.14a	8.19±0.14a	11.29±0.15a
S2	59.12±0.13b	14.26±0.15b	10.35±0.12b	25.33±0.1b	3.08±0.10ab	9.55±0.17b
S3	54.39±0.11b	13.80±0.11c	8.69±0.15c	13.03±0.16c	1.31±0.1b	5.43±0.12c

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%.

Table 5. Analysis of variance of the effect of grafting, mycorrhiza and salinity on photosynthesis parameters of cucumber

	Photosynthesis	Chlorophyll	Transpiration	Mesophyll conductance	Chlorophyll fluorescence
Rootstock	83.2656 ^{ns}	142.802 ^{ns}	0.76563 *	0.00143 *	0.00444 ^{ns}
Mycorrhiza	26.7765 *	13.963 ^{ns}	2.57474 *	0.06302 *	0.00109 ^{ns}
Salinity	173.418 *	15.176 *	2.77750 *	0.00236 *	0.00018 *
Rootstock× Mycorrhiza	193.906 ^{ns}	77.910 ^{ns}	3.44102 ^{ns}	0.00590 ^{ns}	0.00037 ^{ns}
Rootstock× Salinity	80.7799 ^{ns}	15.509 ^{ns}	5.40130 ^{ns}	0.00164 ^{ns}	0.00103 ^{ns}
Mycorrhiza× Salinity	3.84286 ^{ns}	3.252 ^{ns}	1.72990 ^{ns}	0.04516 ^{ns}	0.00098 ^{ns}
Rootstock× Mycorrhiza× Salinity	71.9821 **	16.658 **	6.56890 **	0.00214 **	0.00004 **
Error	0.00007741	12.476	0.0007741	0.0007741	0.00058
CV	0.01	22.66	1.01	1.83	5.44

ns: no significant, ** significant at 1% and * significant at 5% % probability, respectively.

Table 6. The main effect of grafting, mycorrhiza and salinity on photosynthesis parameters of cucumber.

Treatments	Photosynthesis (μ mol m^{-2} s^{-1})	Chlorophyll (Spad value)	Transpiration (mm mol^{-2} s^{-1})	Mesophyll conductance (m mol m^{-2} s^{-1})	Chlorophyll fluorescence
Rootstock					
R1	10±0.10a	13.60±0.10a	6.26±0.1a	0.05±0.001a	0.6±0.1a
R2	10±0.10a	17.58±0.13a	5.96±0.10b	0.04±0.002b	0.6±0.1a
Mycorrhiza					
AM1	18±0.12b	16.21±0.12a	5.84±0.1b	0.04±0.001b	0.5±0.1a
AM2	20±0.11a	14.96±0.1a	6.38±0.12a	0.05±0.003a	0.4±0.2a
Salinity					
S1	18±0.14a	16.52±0.12a	6.40±0.14a	0.06±0.001a	0.8±0.1a
S2	16±0.16b	15.91±0.11b	6.38±0.13b	0.03±0.002c	0.6±0.2b
S3	13±0.17c	14.34±0.11b	5.56±0.11c	0.04±0.001b	0.4±0.1c

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%.

Phenol, proline, antioxidant, and electrolyte leakage were affected significantly (P <0.05) by the salinity treatment (Table 7). Antioxidant (25.38%) and electrolyte leakage (10%) were increased significantly in mycorrhiza inoculation (AM2) treatment compared with AM1 (non-inoculation mycorrhiza) treatment. Meanwhile, phenol, proline, antioxidant, and electrolyte leakage were raised with increasing salinity treatment (Table 8). The lowest values of phenol (0.50 ppm), proline (5.84 ppm), and antioxidant (26.61%) were found in the control treatment and the lowest value of electrolyte leakage (40.76%) was observed in the S2 treatment (Table 8).

Table 7. Analysis of variance of the effect of grafting, mycorrhiza and salinity on phenol, proline, antioxidant, and electrolyte leakage of cucumber

Treatments	Phenol	Proline	Antioxidant	Electrolyte Leakage
Rootstock	0.000565 ^{ns}	0.00367 ^{ns}	237.324 ^{ns}	812.806 ^{ns}
Mycorrhiza	0.0001326 ^{ns}	0.008779 ^{ns}	359.639*	135.872*
Salinity	0.0002676*	0.004163*	69.123*	150.842*
Rootstock× Mycorrhiza	0.001863 ^{ns}	0.003642 ^{ns}	5.555 ^{ns}	3.420 ^{ns}
Rootstock× Salinity	0.008744 ^{ns}	0.004180 ^{ns}	287.697 ^{ns}	0.407 ^{ns}
Mycorrhiza× Salinity	0.000195 ^{ns}	0.005957 ^{ns}	16.064 ^{ns}	730.302 ^{ns}
Rootstock× Mycorrhiza× Salinity	0.000215**	0.0001202**	213.026**	203.255**
Error	0.000337	0.002879	133.115	46.777
CV	0.30	8.09	9.25	15.86

ns: no significant, ** significant at 1% and * significant at 5% % probability, respectively.

Table 8. The main effect of grafting, mycorrhiza and salinity on phenol, proline, antioxidant, and electrolyte leakage of cucumber

Treatments	Phenol (ppm)	Proline (ppm)	Antioxidant (%)	Electrolyte Leakage (%)
Rootstock				
R1	0.61±0.01a	5.46±0.091a	26.71±0.021a	38.38±0.041a
R2	0.61±0.01a	7.61±0.012a	32.07±0.031a	47.88±0.013a
Mycorrhiza				
AM1	0.61±0.01a	4.88±0.081a	26.08±0.011b	41.07±0.015b
AM2	0.61±0.01a	8.19±0.013a	32.70±0.012a	45.19±0.0124a
Salinity				
S1	0.50±0.01b	5.84±0.092b	26.61±0.031b	41.42±0.013ab
S2	0.61±0.01a	6.06±0.014a	31.46±0.041a	40.76±0.015b
S3	0.61±0.01a	6.71±0.0111a	30.09±0.0122a	47.20±0.014a

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%.

The time of appearance of the first male and female flowers and the number of male and female flowers per plant and the node number of the first female flower were affected significantly (P <0.01) by the rootstock× mycorrhiza× salinity interaction treatments (Table 1). The numbers of male and female flowers per plant were highest in R1×AM2×S2 treatment (Table 9). The lowest number of these two traits was found in R1×AM1×S3 treatment, however, they were not statistically different from the others (Table 9). The highest time appearance of the first male and female flowers was found in R1×AM1×S2 treatment (Table 9). The lowest amount of these traits was observed in R2×AM2×S2 treatment. The cucumber grafted onto *L.siceraria* showed the highest node number of the first female flower (R2×AM1×S1 treatment) compared with non-grafted plants. The lowest node number of the first female flower was observed in R1×AM2×S1 treatment (Table 9).

The stem length, number of nodes and leaves, shoot and root fresh weight and shoot dry weight were affected significantly (P <0.01) by the rootstock× mycorrhiza× salinity interaction treatments (Table 3). The comparison table of the mean interactions of salinity and mycorrhiza also showed that with increasing salinity levels, the stem length, number of nodes and leaves, shoot and root fresh weight and shoot dry weight were decreased (Table 10). The lowest amounts of these parameters were found in the R1×AM1×S3 treatment, although no significant difference was observed with those of some other treatments (Table 10). In the R1×AM1×S3 treatment, stem length (47.64%), number of nodes per plant (38.29%), number of leaves per plant (52.2%), shoot fresh weight (86.19%), shoot dry weight (78.35%), root fresh weight (76.11%) were decreased as compared to the R1×AM1×S1 (control) treatment (Table 10). Mycorrhiza application slightly reduced the negative effects of salinity treatment; however, with increasing the salinity level, all growth parameters were decreased, although they were not significantly different (Table 10). The highest number of nodes and shoot fresh weight were found in the R1×AM2×S1 treatment. Grafted cucumbers showed a better response to all growth parameters under salinity conditions. However, these reactions were not statistically significant in all treatments (Table 10). With the application of grafting and mycorrhiza, the highest amount of stem length was observed in the absence of salinity (R2×AM2×S1 treatment). However, a decrease in all growth parameters was observed in mycorrhiza-treated grafted cucumbers under saline conditions (Table 10).

Photosynthesis, chlorophyll, transpiration, mesophyll conductance, and chlorophyll fluorescence were affected significantly (P <0.01) by the rootstock× mycorrhiza× salinity interaction treatments (Table 5).

The rate of photosynthesis was decreased in all treatments using salinity (Table 11). Although the

application of mycorrhiza in saline conditions improved the photosynthesis rate, however, this increase was not significant. Also, in mycorrhiza inoculation and graft combination treatment, the photosynthesis rate decreased in the high saline conditions compared with lower salinity and control (Table 11). The highest photosynthesis rate was found in the R2×AM2×S1 treatment (Table 11).

Chlorophyll SPAD was decreased in all treatments in the salinity conditions and only it was increased only when mycorrhiza inoculation and graft were combined (R2×AM2×S3). However, this increase was not significant (Table 11).

Transpiration was observed in the highest value (7.59±0.1 $m^{-2}s^{-1}$) in R1×AM1×S3 (Table 11). This trait was significantly increased in mycorrhiza inoculation and graft combination treatments in both salinity and non- salinity conditions compared with corresponding non-mycorrhiza inoculation and graft combination treatments in both salinity and non- salinity conditions. Transpiration was recorded the lowest value (5.33±0.1 $m^{-2}s^{-1}$) in R2×AM1×S3 treatment in this study (Table 11).

Mesophyll conductance was increased in mycorrhiza inoculation and graft combination treatments under moderate salinity (R1×AM2×S2) and decreased in high salinity (R2×AM2×S3) conditions (Table 11). Non-grafted cucumber (R1) under mycorrhiza inoculation condition (AM2) significantly increased mesophyll conductance at S2 and S3 salinity levels, as compared to the control (AM1); this increase was greater in S2 treatment than that of S3 treatment. Transpiration did not show the specific changes although it is increased in R1×AM1×S3 (Table 11).

Chlorophyll fluorescence was changed significantly in treatments and showed a significant decrease in the R1×AM2×S2 and R1×AM2×S3 treatments compared with other treatments (Table 11).

Proline, antioxidant and electrolyte leakage were affected significantly (P <0.01) by the rootstock× mycorrhiza× salinity interaction treatments (Table 7). There was a significant decrease in proline content with mycorrhiza inoculation in R1×AM2×S1 treatment (Table 12).

Antioxidants increased by increasing the salinity level in AM1×R1×S3 compared to AM1×R1×S2 (Table 12). In other words, it can be concluded that in this study, antioxidants increased at moderate and high levels of salinity in non-grafted and no mycorrhiza inoculated cucumbers (R1×AM1×S2 and R1×AM1×S3), and this increase was 52.18% and 58.86% more than that of the value of control treatment (i.e. R1×AM1×S1), respectively (Table 12).

The lowest percent of electrolyte leakage was observed in R1×AM2×S1 and R1×AM2×S2 treatments in them the grafting was not done. Electrolyte leakage increased (to 55.13±0.11) by grafting only in R2×AM2×S3 treatment (Table 12).

Table 9. The effect of different levels of grafting, mycorrhiza and salinity on growth parameters of cucumber

Treatments			Number of male flowers per plant	Number of female flowers per plant	Time of appearance of the first male flower (day after grafting)	Time of appearance of the first female flower (day after grafting)	Node number of the first female flower
R1	AM1	S1	13.00±0.12b	6.33±0.11b	45.18±0.112a	43.10±0.12a	2.13±0.12e
R1	AM1	S2	18.33±0.11ab	5.00±0.12b	45.37±0.121a	43.66±0.11a	3.33±0.11cde
R1	AM1	S3	9.66±0.103b	2.66±0.113b	44.33±0.114a	42.00±0.13a	4.66±0.13abc
R1	AM2	S1	27.66±0.101ab	27.00±0.112ab	34.00±0.112d	37.33±0.12bcd	3.66±0.11b-e
R1	AM2	S2	63.66±0.238a	63.66±0.114a	31.66±0.115e	39.66±0.14abc	4.33±0.12a-d
R1	AM2	S3	34.00±0.145ab	34.00±0.113ab	31.66±0.116e	40.33±0.12ab	2.66±0.14de
R2	AM1	S1	18.00±0.114ab	3.33±0.114b	39.00±0.113bc	40.66±0.11b	5.66±0.15a
R2	AM1	S2	17.66±0.123ab	5.33±0.111b	40.00±0.121b	40.00±0.13abc	3.66±0.12b-e
R2	AM1	S3	37.33±0.131ab	3.35±0.121b	38.00±0.114c	40.33±0.15ab	4.33±0.13a-d
R2	AM2	S1	33.00±0.143ab	13.00±0.111b	30.33±0.121e	35.66±0.11cd	5.33±0.14ab
R2	AM2	S2	21.33±0.151ab	21.00±0.121b	30.00±0.131e	33.00±0.13d	3.66±0.12b-e
R2	AM2	S3	48.33±0.21ab	11.33±0.131b	30.66±0.121e	40.00±0.12abc	4.66±0.16abc

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%.

Table 10. The effects of different levels of grafting, mycorrhiza, and salinity on stem length, number of nodes and leaves, shoot and root fresh weight and shoot dry weight of cucumber

Treatments			Stem length (cm)	Number of nodes per plant	Number of leaves per plant	Shoot fresh weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)
R1	AM1	S1	40.17±0.12de	11.88±0.11abc	9.06±0.12ab	22.02±0.11bc	2.31±0.11b	1.80±0.12b
R1	AM1	S2	22.70±0.11e	8.33±0.12bc	5.66±0.11b	4.59±0.10c	0.52±0.12b	0.57±0.14b
R1	AM1	S3	21.03±0.13e	7.33±0.14c	4.33±0.10b	3.04±0.11c	0.50±0.13b	0.43±0.11b
R1	AM2	S1	107.67±0.15a	23.00±0.11a	17.33±0.11a	67.66±0.12a	7.03±0.14a	9.55±0.12a
R1	AM2	S2	92.42±0.14ab	20.38±0.11ab	18.06±0.10a	62.50±0.13ab	6.60±0.11b	7.2±0.13ab
R1	AM2	S3	65.69±0.11bcd	15.21±0.11abc	9.10±0.11ab	17.81±0.13bc	0.84±0.012b	4.84±0.12b
R2	AM1	S1	26.93±0.12e	9.33±0.13bc	6.33±0.10b	3.75±0.11c	0.53±0.012b	0.58±0.11b
R2	AM1	S2	30.70±0.11de	8.66±0.13bc	5.66±0.10b	4.41±0.12c	0.77±0.012b	0.83±0.12b
R2	AM1	S3	48.50±0.11cde	12.66±0.14abc	9.66±0.11ab	5.42±0.11c	0.99±0.011b	5.42±0.11b
R2	AM2	S1	108.00±0.13a	22.00±0.12a	12.00±0.12ab	32.05±0.12abc	4.4±0.10ab	7.3±0.12ab
R2	AM2	S2	92.33±0.12ab	19.66±0.12ab	12.00±0.12ab	31.17±0.12abc	3.87±0.12b	5.4±0.14ab
R2	AM2	S3	80.67±0.13abc	20.00±0.11ab	11.66±0.11ab	24.48±0.11abc	2.89±0.13b	4.00±0.11b

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%.

Table 11. The effects of different levels of grafting, mycorrhiza, and salinity on photosynthesis parameters of cucumber

Treatment			Photosynthesis ($\mu mol\ .\ m^{-2}\ s^{-1}$)	Chlorophyll (SPAD value)	Transpiration ($mmol\ .\ m^{-2}s^{-1}$)	Mesophyll conductance ($mmol\ .\ m^{-2}\ s^{-1}$)	Chlorophyll fluorescence ($Fv\ .\ Fm^{-1}$)
R1	AM1	S1	19.34±0.1a	16.81±0.1ab	5.70±0.1h	0.06±0.001b	0.06±0.001a
R1	AM1	S2	14.23±0.1c	16.08±0.1ab	5.61±0.1i	0.02±0.0013d	0.04±0.0011bc
R1	AM1	S3	13.45±0.1c	14.17±0.1ab	7.59±0.1a	0.02±0.0013d	0.02±0.001c
R1	AM2	S1	16.34±0.1b	14.42±0.1ab	6.87±0.1d	0.05±0.0012c	0.03±0.0012bc
R1	AM2	S2	17.36±0.1ab	11.92±0.1bc	6.43±0.1f	0.08±0.0014a	0.03±0.0012bc
R1	AM2	S3	17.09±0.1ab	8.16±0.1c	5.33±0.1j	0.06±0.001b	0.02±0.0011c
R2	AM1	S1	16.00±0.1b	18.13±0.1a	6.95±0.1c	0.06±0.001b	0.05±0.001abc
R2	AM1	S2	14.76±0.1c	17.20±0.1ab	5.70±0.1h	0.06±0.001b	0.06±0.001a
R2	AM1	S3	15.90±0.1b	14.85±0.1ab	3.52±0.1k	0.02±0.0013d	0.06±0.001ab
R2	AM2	S1	19.43±0.1a	17.62±0.1ab	7.32±0.1b	0.06±0.001b	0.06±0.001a
R2	AM2	S2	18.54±0.1a	17.50±0.1ab	6.52±0.1e	0.02±0.0013d	0.04±0.0011bc
R2	AM2	S3	17.45±0.1ab	20.15±0.1a	5.77±0.1g	0.009±0.0012e	0.06±0.001a

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%

Table 12. The effects of different levels of grafting, mycorrhiza, and salinity on phenol, proline, antioxidant, and electrolyte leakage of cucumber.

Treatment			Phenol ($mgkg^{-1}$)	Proline ($mgkg^{-1}$)	Antioxidant (%)	Electrolyte Leakage (%)
R1	AM1	S1	0.61±0.01a	0.039±0.0011b	19.01±0.11c	42.79±0.12bcd
R1	AM1	S2	0.61±0.01a	0.062±0.0012ab	28.93±0.12b	44.90±0.11abc
R1	AM1	S3	0.61±0.01a	0.045±0.0011b	30.02±0.15a	52.99±0.15ab
R1	AM2	S1	0.61±0.01a	0.014±0.0013d	18.45±0.13c	30.38±0.14e
R1	AM2	S2	0.61±0.01a	0.058±0.0014b	20.09±0.12bc	26.85±0.13e
R1	AM2	S3	0.61±0.01a	0.019±0.0012c	20.10±0.14bc	32.33±0.16de
R2	AM1	S1	0.61±0.01a	0.047±0.0011b	24.11±0.13b	56.05±0.12a
R2	AM1	S2	0.61±0.01a	0.055±0.0013b	31.23±0.11a	48.36±0.14ab
R2	AM1	S3	0.61±0.01a	0.045±0.0014b	27.31±0.12b	45.98±0.17abc
R2	AM2	S1	0.61±0.01a	0.075±0.0011ab	18.56±0.13c	36.45±0.12cde
R2	AM2	S2	0.61±0.01a	0.085±0.0011a	21.01±0.12ab	45.30±0.13abc
R2	AM2	S3	0.61±0.01a	0.079±0.0011ab	19.9±0.11bc	55.13±0.11a

According to the LSD test, a column in each treatment and means followed by the same letter is not significantly different at P <5%

DISCUSSION

Growth decline is the common reaction of most plants to salinity conditions (Rouphael et al., 2008). Yetisir et al., (2006) and Jones et al., (1989) reported that salinity stress affected yield through the decreased number of leaves, nodes, female flowers, and stem length in watermelon, and cucumber. Similarly, Parthasarathi et al. (2021) reported that salinity stress reduced the growth parameter of potato and grafted plants. However, that reduction was not significant.

It seems, the inoculation of mycorrhiza under salinity stress could not provide conditions for the absorption of water and nutrients in cucumber.

Various mechanisms have been mentioned to discuss the effect of mycorrhiza on the vegetative growth of plants. The most important one was reported to be the effect of mycorrhiza on the absorption of nutrients such as nitrogen, phosphorus, and potassium in the soil (Abdelhafez and Abdel-Monsief, 2006).

Grafting can be used to mitigate salt stress, in vegetable production (Bie et al., 2017). Ortas and Ustuner, (2014) and Borde et al., (2011) explained that with mycorrhiza inoculation, flowering time occurred earlier in the plant. In the present study it has been shown that mycorrhiza inoculation was effective at the time of appearance of male flowers under salinity conditions in cucumber. However, Savvas et al., (2010) reported that the response of grafted cucumbers to the presence of salinity stress might be non-identical, depending on rootstock genotype. Some researchers have explained that at NaCl concentration, arbuscular mycorrhizal fungi do not have beneficial effects on plants and could not inhibit their salinity damage (Juniper and Abbott, 2006; Sheng et al., 2008).

Salinity stress decreases chlorophyll by reducing the amount of magnesium in the plant (Sheng et al., 2008). It has been reported that mitigation of vegetative growth in cucumbers under conditions of salinity stress has been related to decreasing photosynthesis and mesophyll conductance (Yang et al., 2012). In confirmation of this, the rate of photosynthesis was decreased by 30% with increasing salinity (Table 7) in the current study. On the basis of similar findings, Canakci and Karaboga,(2013) and Sun et al. (2015) indicated that chlorophyll reduction and mesophyll conductance were common consequences of salinity stress in cucumbers (*Cucurbita sativus* L.) Other researchers have shown that inoculation of mycorrhiza could improve plant chlorophyll because inoculation of mycorrhiza fungi increases magnesium uptake in the plant (Derbew et al., 2007). However, in the current study, the mycorrhiza application could not mitigate the unfavorable effects of salinity on SPAD value (Table 7). In agreement with this result, researchers have explained that salinity negatively affected mycorrhiza efficiency in photosynthesis and the water status of maize (Sheng et al., 2011). However, other researchers have found that photosynthesis rate and stomatal conductance are positively affected by mycorrhiza inoculation in comparison to control treatment (no mycorrhiza inoculation) under salt stress conditions (Wu et al., 2010). One of the most important roles of mycorrhiza has been shown to be increasing the promoting chlorophyll content (Gogoi and Singh, 2011). It has been also reported that inoculation of mycorrhizal fungi raises magnesium uptake in tomato plants (Elahi et al., 2010) and increases chlorophyll synthesis in bermudagrass (Wu et al., 2011). The same results were observed in this study.

Similar results have also been reported by Haghighi et al., (2017), who explained that mycorrhiza application could increase photosynthesis and mesophyll conductance under salt stress in cucumbers.

Some researchers have reported that pumpkin rootstock could improve the photosynthetic features of watermelon scion under environmental stress (Nawaz et al., 2017). Parthasarathi et al., (2021) explained that salinity stress in plants increased transpiration, which enhances sodium absorption from the growing environment. The same researchers have expressed that the rootstock directly affects plant transpiration in saline conditions (Parthasarathi et al., 2021).

The results of the present study, similar to those reported by several researchers on stress indices like proline and antioxidant (Evelin et al., 2012; Hajlaoui et al., 2010), did not comply with some others (Sheng et al., 2011; Borde et al., 2011). For example, Sheng et al., (2011) explained that the effect of mycorrhiza application and grafting combination on plant proline could be different depending on the plant species. A higher accumulation of antioxidants in grafted plants is one of the signs of tolerance in saline conditions (Goreta et al., 2008). The results of the present study confirmed the results of some other researchers which have shown that mycorrhiza-treated plants and grafted plants could accumulate more antioxidants and proline contents in mung beans (Evelin et al., 2012) and cucumbers (Haghighi et al., 2017), which in turn would protect plants against salinity stress.. It has been shown that the accumulation of proline and antioxidants in mycorrhiza inoculated and grafted plants could increase the osmotic potential and act as a response to salinity stress, as well as improve plant tolerance to salinity (Hajlaoui et al., 2010). Sun et al., (2015) have found that salinity could be correlated with oxidative stress in *Cucumis sativus*. In this case, cell membrane stability has been mentioned as a scale to differentiate salinity stress-tolerant plants from susceptible ones (Sun et al., 2015).

This study also confirmed other reports on the role of grafting in vegetable crops including cucumber grown under saline conditions (Colla et al., 2010). Yan et al., (2018) also reported that grafted watermelon onto summer squash had better membrane stability, as compared to non-grafted watermelon in salinity. In this study, although mycorrhiza inoculation and grafting treatment separately caused the reduction of ion leakage, however, it was shown that the application of both treatments together did not reduce ion leakage. Although salinity can affect negatively *A. mycorrhiza* fungi. It was shown that growth and performance of mycorrhizal plants improved under salt stress conditions (Porcel et al., 2015). These positive effects have been explained by improved host plant nutrition, higher K^+/Na^+ ratios in plant tissues, and a better osmotic adjustment by the accumulation of compatible solutes such as proline, glycine betaine, or soluble sugars (Porcel et al., 2015). Plants inoculated with *A. Mycorrhiza* also improved photosynthetic and water use efficiency

under salt stress (Porcel et al., 2015). It has also been reported that plants inoculated with *A. mycorrhiza* could enhance the activity of antioxidant enzymes to cope with the reactive oxygen species generated by salinity (Porcel et al., 2015).

CONCLUSIONS

In this study, it has been shown that the amounts of all growth parameters were decreased in salinity-stressed levels. Mycorrhiza inoculation was stimulated only at the time of the appearance of the first male flower at the moderate NaCl concentration. Differences in growth parameter values of grafted cucumbers were significant only in the time of appearance of the first male flower and in the node number of the first female flower at high and moderate NaCl concentrations compared to those of non-grafted cucumbers. The appearance of the first female flower was postponed under the high concentration of NaCl in R2×AM2×S3 treatment. The photosynthetic rates of non-grafted and grafted cucumber plants were sensitive to salinity stress.

Photosynthetic reduction in cucumbers subjected to salinity was associated with a decrease in their chlorophyll content and mesophyll conductance. Grafting cucumber onto *L. siceraria* rootstock decreased the photosynthetic rate, transpiration, and mesophyll conductance of cucumbers under salinity conditions. Proline content increased at a moderate level of salinity compared to the control in all treatments, and this increase was significant only in mycorrhiza application treatments alone. The results of the present study showed that electrolyte leakage increased in non-grafted plants under salinity stress including R1×AM1×S2 and R1×AM1×S3 treatments, although this increase was not statistically significant. The lowest antioxidant content was found in R1×AM2×S1 and R2×AM2×S1 treatments. Antioxidant increased in R2 treatments at AM1×S3.

In general, using grafting in cucumber seems to be more effective than mycorrhiza inoculation in decreasing stress indices under moderate salinity. Photosynthesis and antioxidant parameters and then growth parameters were more affected by salinity. Grafting was more effective than mycorrhiza in keeping stress indices in a suitable condition.

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تأثیر *Arbuscular mycorrhiza* بر خصوصیات فیزیولوژیکی و رشدی خیار پیوندی تحت تنش شوری

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چکیده – این مطالعه با هدف بررسی تأثیر متقابل پیوند و *Arbuscular mycorrhiza* بر روی خیار انجام شد. طرح آزمایشی، آزمایش فاکتوریل بر اساس بلوک‌های کاملا تصادفی بود. تیمارها مایه‌زنی مایکوریزا شامل بدون مایه‌زنی مایکوریزا ((AM1) به عنوان شاهد و میکوریزا با ۲۴۰۰ اسپور در هر گلدان (AM2)، و پیوند، که در آن خیار (*Cucumis sativus* var. super daminos) بر *Lagenaria siceraria* پیوند شد (R2) و خیارهای غیر پیوندی (R1) و غلظت شوری شامل شاهد (C)، بدون اضافه نمودن NaCl ، شوری متوسط برابر با غلظت ۳۰ میلی مولار NaCl و شوری زیاد برابر با غلظت ۶۰ میلی مولار NaCl با سه تکرار بود. نتایج نشان داد که پارامترهای رشد مانند تعداد گل‌های نر و ماده، زمان ظهور اولین گل نر و ماده ، تعداد گره اولین گل ماده ، طول ساقه ، تعداد گره و برگ ، وزن تر شاخساره و ریشه در تنش شوری کاهش یافت. پیوند فقط در زمان ظهور اولین گل نر و در تعداد گره اولین گل ماده در غلظت‌های بالا و متوسط NaCl اثر گذاشت. ظهور اولین گل ماده تحت غلظت بالای NaCl به تعویق افتاد. کاهش فتوسنتز تشخیص داده شده در خیارهای تحت شوری با کاهش کلروفیل و هدایت مزوفیل آن‌ها همراه بود. علاوه بر کاهش میزان فتوسنتز ، فلورسانس کلروفیل نیز تحت تأثیر تنش شوری متوسط و زیاد قرار گرفت .بطور کلی، اثر متقابل پیوند و میکوریزا به خیار کمک می‌کند تا اثر مضر شوری، به ویژه شوری متوسط را کاهش دهد.