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Full Length Research Paper

The effect of endo-mycorrhiza (VAM) treatment on growth of tomato seedling grown under saline conditions

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This study was carried out in order to determine the effects of various study doses of mycorrhiza treatment on morphological characteristics and color quality of tomato seedlings (*Lycopersicon esculentum* L.) which are grown under 100 nM salinity stress. Two different types of tomatoes "Aspendos F_1" and "Donna F_1" were used in this study. Mycorrhiza treatment was performed by using ROOTS-novozymes endo-mycorrhiza fungus (VAM) to obtain 10, 50 and 100 mycorrhiza plants^{-1}. VAM treatment prevented the decrease in the length of the plants caused particularly by high level soil salinity, both in Aspendos and Donna species. In both species, the height of the plants which are grown under salt+M50 and salt+M100 conditions became bigger than the control plants and the plants grown under salt conditions. Furthermore, high mycorrhiza treatment also increased the weight of fresh stem and root but had no particular affect on dry stem and root. While chlorophyll a, b and total chlorophyll quantities of the mycorrhiza treatment with M50 and M100 doses grown under salt conditions were found to be high in comparison to control and salt treatment, carotenoid level was found to be low. Conclusively, mycorrhiza treatment to tomato seedlings which are grown under salt conditions was caused to have seedlings grown by preventing negative effects of salt, and provided a high quality growth and which kept the green color of the seedlings.

Key words: Color, mycorrhiza, *Lycopersicon esculentum* L., seedling length, root length, weight.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is considered a major vegetable crop in many parts of the world and mostly grown under irrigation, both in protected and open field conditions (Al-Karaki, 2006). Also, tomato is one of the most important vegetable species in Turkey with respect to its planting area, production and trade. As a matter of fact, Turkey is in the third rank, with respect to its production capacity with 9.945.043 MT in the world, after China and United States (FAO, 2007). Tomato also has a particular importance as being a raw material of agricultural industry besides its consumption as a fresh vegetable.

Tomato is a major vegetable plant, and it is moderately sensitive to salinity. It is necessary to conduct the extensive research on growth conditions under moderate salinity to produce vegetative growth. Tomato was selected a model crop for this study due to its commercial importance as a horticultural cash crop in many areas. Salinization of soil is a serious problem and is increasing steadily in many parts of the world, particularly in arid and semi-arid areas (Abdel-Latef, 2010). Saline soğuls occupy 7% of the earth's land surface (Ruiz-Lozano et al., 2001). At present, out of 1.5 billion ha of cultivated land around the world, about 77 million ha (5%) is affected by excess salt content (Sheng et al., 2008).

The effect of salinity concentration on plant growth has been salt studies in different tomato cultivars. Salinity adversely affected the vegetative growth of tomato, and it reduced plant length and dry weight (Adler and Wilcor, 1987). It had also been reported to reduce the fresh and dry weight of shoots and roots of tomato plants (Shannon et al., 1987; He e t al., 2007). Increased salinity over

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Table 1. Chemical composition of roots-novozymes (Endo-mycorrhiza (VAM).

Active ingredients	Amount	Inert ingredients	Amount (%)
Mycorrhiza	27.55 %	Humic acids	28.70
Glomus intraradices	25 viable organisms/gram	Cold water kelp extracts	18.00
Glomus mosseae	24	Ascorbic acid	12.00
Glomus aggregatum	24	Amino acid	6.00
Glomus clarum	1	Myo-Inositol	2.50
Glomus monosporus	1	Surfactant	2.50
Glomus deserticola	1	Thiamine(vitamin B1)	1.75
Glomus brasilianum	1	Alpha-tocopherol(vitamin E)	1.00
Glomus etunicatum	1		
Gigaspora margarita	1		
Total mycorrhiza	79 nos/gram		

4000 µg g^{-1} led to reduction in dry weight, leaf area, plant stem, and roots of tomato (Omar et al., 1982). The reduction of dry weights due to increased salinity may be as a result of combination of osmotic and specific ion effects of Cl and Na (Hajiboland et al., 2010). The leaf and stem dry weights of tomato were also reduced significantly in plants irrigated with saline nutrient solution in contrast with control parents (Satti and Al-Yahyai, 1995).

Arbuscular mycorhizal fungi (AMF) widely occur in saline soils (Aliasgharzadeh et al., 2001). Salinity negatively affects the formation and function of mycorrhiazl symbiosis (Juniper and Abbott, 1993). The root colonization by AMF involves a series of morpho-physiological and biochemical events that are regulated as well as by environmental factors. The improving salt tolerance of arbuscular-mycorhizall-inoculated (AM) plants, are still unclear, although, the improved nutrition uptake may be one of the reasons (Poss et al., 1985).

In recent years, some studies have indicated that AMF can enhance the plant growth and uptake of nutrients, decrease yield losses of tomato under saline conditions and improved salt tolerance of tomato (Li et al., 1991 a, b; Ruiz et al., 1996; Al-Karaki, 2000; Al-Karaki et al., 2001; Huang et al., 2010; Abdel Latef and Chaoxing, 2011). This study was conducted to determine if inoculation of seedling with endo mycorrhiza (VAM) fungi alleviates salt effect on growth of tomato seedling when irrigated with saline water.

MATERIALS AND METHODS

Plant material

The experiments were conducted in greenhouse at the Vocational School of Ahi Evran University. The seeds of tomato (*L. esculentim* cv. Donna and cv. Aspendos) were obtained from the Beta Seed Inc. The seeds were planted into polyethylene trays. The trays were sterilized by immersing in 5% sodium hypochloride, and filled with peatmoss. The trays were kept in a greenhouse at an average temperature at 23±2°C and 65±5% relative humidity. Trays were irrigated with water without nutrients until cotyledone leaves emerged, after that, it was irrigated with water containing maxifol (with microelements) at a rate of 1501 g $100L^{-1}$ when the first leaves emerged. Unhealthy seedlings were excluded from the study. Tomato cultivars used in this study were moderately sensitive to salinity.

Endo-mycorrhiza fungus (VAM) treatment

For each treatment, ten seedlings having same length were labeled and mycorrhiza treatment was performed. The VAM inoculums (*Glomus* spp.) consisted of spores named endo roots mycorrhiza ınoculant (Novozymes Biologicals, Inc. 5400 Corporate Circle Salem, VA 24153) was used for mycorrhiza treatments (Table 1). For ten of mycorrhiza spores, 0.12658 g $plant^{-1}$ (M10), for fifty spores, 0.6329 g $plant^{-1}$ (M50) and for hundred of spores, 1.2658 g $plant^{-1}$ (M100) preparation were scaled and these were dissolved in 5 ml pure water. The prepared solution was injected to the plant by preventing drainage to its root. Only one treatment was performed in practice since mycorrhiza survives active in the soil for two years.

Salinity stress conditions

Salinity stress treatment commenced four days after AMF treatment. 100 mM salt concentration which was determined to be the limit value to create stress on the plant (because of tomato moderate tolerant to salt), was preferred. Plants to which no salinity stress was applied, were normally watered three times a day and the plants to which salinity stress treated plants were watered with water having 100 mM salt concentration during same watering intervals.

Morphological characteristics

Ten plants from each treatment were randomly selected after three weeks VAM inoculation. Then, plants were separated into leaves, shoots and roots, weighed to determine the fresh weight and dried at, 65°C for 48 h for the dry weight determination. Plant and root dry weight determined was used as a sensitive scale (at Shimadzu AY220- 0.0001g sensitivity).

Color substance analysis

Color analysis was performed on the leaves taken from the plants.

Table 2. Morphological characteristics of "*Aspendos*" species seedlings.

Treatments	Plant length (cm)	Fresh stem weight (g)	Dry stem weight (g)	Root length (cm)	Fresh root Weight (g)	Dry root weight (g)
Control	5.94 ± 0.29 b*	0.81 ± 0.09**	0.13 ±0.02	15.45 ± 1.99	0.43 ± 0.05 d	0.04 ± 0.00 c
M10	7.50 ± 1.40 b	1.33 ± 0.39	0.19 ±0.06	16.67 ± 0.20	0.63 ± 0.12 cd	0.06 ± 0.01 bc
M50	7.70 ± 1.36 b	2.14 ± 0.56	0.28 ±0.08	17.11 ± 0.24	1.14 ± 0.16 b	0.08 ± 0.02 ab
M100	10.20 ± 0.10 a	2.22 ± 0.41	0.33 ±0.06	19.38 ± 0.21	1.65 ± 0.21 a	0.11 ± 0.02 a
Salt control	6.10 ± 0.55 b	1.61 ± 0.34	0.19 ±0.04	13.78 ± 1.53	0.59 ± 0.13 cd	0.04 ± 0.01 c
Salt+M10	6.10 ± 0.26 b	1.89 ± 0.06	0.22 ±0.02	15.59 ± 0.51	0.66 ± 0.03 cd	0.04 ± 0.00 c
Salt+M50	6.53 ± 0.93 b	2.15 ± 0.32	0.25 ±0.05	15.94 ± 2.03	0.81 ±0.12 bcd	0.06 ± 0.01 bc
Salt+M100	6.77 ± 0.34 b	2.32 ± 0.03	0.27 ±0.02	17.57 ± 1.15	0.90 ± 0.01 bc	0.07 ± 0.00 bc
LSD	2.433				0.3655	0.03106

*Letters show vertical comparisons among treatments at P ≤ 0.05 error level. **Nonsignificant at P ≤ 0.05 error level.

Fresh tissue (0.2 g) was sampled, fully expanded leaf were homogenized in 80% acetone and read using a UV/visible spectrophotometer at 470, 663, 652 and 645 nm. Total chlorophyll, chlorophyll a, b and carotenoid amount were determined according to Yürekli and Porgali (2006).

Chlorophyll a= 11.75 x (A663) – 2.35 x (A645)
Chlorophyll b = 18.61 x (A645) – 3.96 x (A663)
Carotenoid = 1000x (A470) – 2.27 Chlorophyll a – 81.4 Chlorophyll b /227

Statistical analysis

Statistical analysis of the data was done using analysis of variance (ANOVA) and Duncan's Multiple Range Test was used to evaluate treatment significance at P ≤ 0.05.

RESULTS AND DISCUSSION

Morphological characteristics

Aspendos species

The effects of the treatment on plant length, fresh root weight and dry root weight for 'Aspendos' species seedlings were found to be significant at statistical level. Maximum plant length that was 100 M (10.20 cm) during the studies was observed in the plants to which endo mycorrhiza fungus (VAM) was treated and the difference between this treatment and other treatments was found to be statistically significant (Table 2). Fresh stem weight varied between 0.81 g (Control) and 2.32 g (salt+100 M), dry stem weight varied between 0.13 g (Control) and 0.33 g (100 M), root length varied between 13.78 cm (salt control) and 19.38 cm (100 M).

Meanwhile, the differences observed between the treatments were not found to be statistically significant (Table 2). During the studies, fresh root weight of the plants to which 100 M VAM was applied, increased significantly (1.65 g) and this was followed by 1.14 g increase as a result of 50 M treatment and 0.90 g increase as a result of salt +100 M VAM treatment on the plants respectively. Likewise, the highest dry root weight is determined as 0.11 g in the plants to which 100 M VAM applied. Lowest dry root weight was statistically determined to be 0.04 g and determined respectively for control plants, salt control plants and salt +10 M treated plants (Table 2).

Donna species

Differences between the treatments with respect to plant height of 'Donna' specie seedlings were not statistically significant. These parameter values varied between 3.90 cm (control) and 5.80 cm (100 M) (Table 3). Fresh stem weight was found to be 100 M (1.95 g) in the plant to which VAM was treated, as the highest value. This was followed by 1.80 g in salt+100 M treated seedlings, 1.55 g in salt + 50 M treated seedlings and 1.51 g in 50 M VAM treated seedlings respectfully. Lowest fresh stem weight is observed in control plants as 0.69 g. Meanwhile, the difference between control and 10 M (0.78 g) and salt+10M (0.85 g) treated plants were not found statistically significant. Highest dry stem weight in 'Donna' species seedlings is determined to be 0.28 g in 100 M treated seedlings. Lowest dry stem weight was observed as 0.1 g in salt + 10 M treated seedlings.

However, the difference between the treatments with respect to dry stem weight was not found to be statistically significant (Table 3). No significant difference was observed between the values of various treatments with respect to root height. The root height values of the seedlings varied between 1.93 cm (control) and 19.39 cm (100 M) (Table 3). Generally, arbuscular mycorrhiza fungus treatment caused increase in height of the seedlings for both species grown under with and without salt conditions; however, when the difference between control treatments did not show significance for Donna species, statistically significant differences were observed between 100 M treatment and other treatments in

Table 3. Morphological characteristics of '*Donna'* species seedlings.

Treatment	Plant length (cm)	Fresh stem weight (g)	Dry stem weight (g)	Root length (cm)	Fresh root Weight (g)	Dry root weight (g)
Control	3.90 ± 0.55**	0.69 ± 0.12 c*	0.12 ± 0.03 cd	11.93 ± 0.77**	0.51 ± 0.06 bc	0.05 ± 0.01 b
M10	4.17 ± 0.34	0.78 ± 0.08 c	0.13 ± 0.03 cd	14.16 ± 2.37	0.57 ± 0.06 bc	0.05 ± 0.01 b
M50	5.10 ± 0.35	1.51 ± 0.13 ab	0.25 ± 0.02 a	14.44 ± 1.04	1.02 ± 0.06 a	0.08 ± 0.01 a
M100	5.80 ± 0.94	1.95 ± 0.19 a	0.28 ± 0.02 a	19.39 ± 3.63	1.25 ± 0.04 a	0.10 ± 0.00 a
Salt control	4.83 ± 0.58	1.10 ± 0.24 bc	0.16 ± 0.03 bcd	12.18 ± 0.40	0.45 ± 0.15 c	0.03 ± 0.01 bc
Salt+M10	4.07 ± 0.09	0.85 ± 0.08 c	0.10 ± 0.01 d	12.75 ± 1.03	0.40 ± 0.06 c	0.02 ± 0.00 c
Salt+M50	4.37 ± 0.20	1.55 ± 0.19 ab	0.18 ± 0.02 bc	13.09 ± 1.02	0.75 ± 0.12 b	0.04 ± 0.01 bc
Salt+M100	4.93 ± 0.38	1.80 ± 0.18 a	0.22 ± 0.02 ab	14.29 ± 1.37	0.69 ± 0.10 bc	0.04 ± 0.00 bc
LSD		0.4945	0.06418		0.2685	0.02106

*Letters show vertical comparisons among treatments at $P \leq 0.05$ error level. **Non-significant at $P \leq 0.05$ error level.

Aspendos seedlings. This obtained result is compliant to the results taken for pepper (İkiz et al., 2009), onion (Goussous and Mohammed, 2009) and vegetables (Matsubara et al., 1994) which showed that, AMF inoculation resulted in higher growth in comparison to control treatment. It is stated that biological growth of natural arbuscular mycorrhiza fungus increased the quality of seedlings in seedbeds (Bagyaraj et al., 1989).

In our study, VAM treatment increased the quality of the seedlings due to the increase in plant height and fresh stem weight of the plants (Table 3). When salinity stress is compared with control dry weight of the stem, root and leaf and leaf blade are decreased due to direct increase in ion toxicity or due to indirect effect of osmotic imbalance between soil/plant caused by salt ions (Abdel, 2010). In our study, where dry stem weights were higher than control and salt control treatment, in VAM treated plants grown under salinity stress dry root weights were found lower or same with control and salt control treatment; however, particular significance is not found between the treatments ($P \leq 0.05$). Fresh stem weight significantly increased in plants to which a high level of VAM (50 and 100 M) treatment was applied, in comparison to low level of VAM (10 M) treatment applied to both plants, with and without salinity stress in Donna species; but this increase was not statistically significant in 'Aspendos' species (Tables 2 and 3). These increased results may be in correlation with the increase in nutrient intake. Wang et al. (2008) found out that, the mycorrhiza inoculation at the initial stage of plant growth caused increase in plant growth at seedbed and caused increase in their performance after their planting in the field. Fresh stem weight became higher in both species in our study in comparison to control but this increase was not statistically significant in Aspendos species but it was significant in Donna species. Similar results were obtained in the height of the stems. Therefore, AMF treatment at initial growth stage of the tomato seedlings may cause increase in vegetative growth. Bryla and Koide (1998) stated that, high level of growth parameters in 31 days tomato seedlings, to which VAM was applied, is the result of its treatment during initial growth of tomatoes. Similarly, Javeid and Riaz (2008) determined that increase in vegetative growth of gladiola species is the result of VAM treatment and inoculation during initial growth stage.

Color substances

***Aspendos* species:** In this study, chlorophyll a content values in 'Aspendos' species varied between 0.91 and 2.15 mg g^{-1} FW (Table 4). Lowest chlorophyll content determined in salt control treated seedlings and highest chlorophyll a values are obtained from seedlings to which were 50 and 100 M treated (Table 4). Chlorophyll b content determined in salt control and salt+10 M (0.42 and 0.48 mg g^{-1}FW, respectively) treated seedlings. Meanwhile, mycorrhiza treatment under conditions without salt stress caused increased chlorophyll b content; highest chloropyhll b values were obtained in 100 M (0.90 mg g^{-1} FW) treatment and it was followed in 50 M treatment by the value of 0.86 mg g^{-1} FW. Furthermore, 100 M treatment made to plants under salinity stress caused higher chloropyhl b content (0.76 mg g^{-1} FW) than control plants (Table 4).

Total chlorophyll content was found higher in seedlings, which were treated with 50 M (2.96 mg g^{-1} FW) and 100 M (3.05 mg g^{-1} FW) in comparison to other seedlings. Total chloropyhl content in plants treated with Salt + 50 M and salt + 100 M (2.52 and 2.67 mg g^{-1} FW respectfully) were higher in comparison to the values obtained in control (2.17 mg g^{-1} FW) and salt + 10 M treated (2.27 mg g^{-1} FW) plants and the difference between these treatments were statistically found significant (Table 4). Likewise the total chlorophyll content, mycorrhiza treatments also had positive affects on carotenoid contents of the plants. Carotenoid content was high in the plants which were treated with 100 M (211.15 mg g^{-1} FW) and 50 M (208.73 mg g^{-1} FW) lowest carotenoid content

Table 4. Chlorophyll a, b and total chlorophyll and carotenoid contents of '*Aspendos*' species seedlings.

Treatment	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Control	1.56 ± 0.03 d*	0.61 ± 0.05 d	2.17 ± 0.07 c	162.76 ± 6.00 b
M10	1.68 ± 0.07 cd	0.59 ± 0.02 d	2.27 ± 0.10 c	164.10 ± 6.92 b
M50	2.10 ± 0.03 a	0.86 ± 0.03 ab	2.96 ± 0.07 a	208.73 ± 3.23 a
M100	2.15 ± 0.02 a	0.90 ± 0.03 a	3.05 ± 0.05 a	211.15 ± 3.17 a
Salt control	0.91 ± 0.10 f	0.48 ± 0.05 e	1.39 ± 0.08 e	85.90 ± 10.20 d
Salt+M10	1.31 ± 0.03 e	0.42 ± 0.02 e	1.73 ± 0.04 d	119.39 ± 2.50 c
Salt+M50	1.82 ± 0.07 bc	0.70 ± 0.05 cd	2.52 ± 0.09 b	168.10 ± 7.62 b
Salt+M100	1.91 ± 0.00 b	0.76 ± 0.02 bc	2.67 ± 0.02 b	178.01 ± 0.85 b
LSD	0.1667	0.1079	0.2113	17.56

*Letters show vertical comparisons among treatments at P ≤ 0.05 error level.

Table 5. Chlorophyll a, b and total chlorophyll and carotenoid contents of '*Donna'* species seedlings.

Treatment	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Control	1.57 ± 0.03 c	0.56 ± 0.01 c	2.13 ± 0.04 c	159.66 ± 3.34 c
M10	1.92 ± 0.17 b	0.82 ± 0.01 b	2.74 ± 0.18 b	180.00 ± 20.80 bc
M50	2.10 ± 0.01 ab	0.82 ± 0.01 b	2.93 ± 0.03 b	205.06 ± 1.90 ab
M100	2.25 ± 0.01 a	1.07 ± 0.05 a	3.33 ± 0.07 a	227.82 ± 3.98 a
Salt control	1.43 ± 0.04 c	0.52 ± 0.02 c	1.95 ± 0.06 c	153.56 ± 4.18 c
Salt+M10	1.01 ± 0.08 d	0.31 ± 0.03 d	1.32 ± 0.11 d	98.09 ± 7.89 d
Salt+M50	1.66 ± 0.04 c	0.60 ± 0.01 c	2.26 ± 0.04 c	155.04 ± 5.14 c
Salt+M100	1.66 ± 0.11 c	0.60 ± 0.06 c	2.27 ± 0.17 c	155.50 ± 10.90 c
LSD	0.2473	0.09822	0.3140	27.83

*Letters show vertical comparisons among treatments at P ≤ 0.05 error level.

was observed as 119.39 mg g^{-1} FW in the seedlings which were treated with salt + 10 M. This case indicates that the positive affects of mycorrhiza treatment for the plants under salinity stress can only be observed after mycorrhiza level exceeds 50 numbers per plant. In the seedlings of this species, salinity stress caused negative affects on carotenoid content of the plant and the lowest carotenoid content was statistically recorded as 85.90 in this treatment (Table 4).

***Donna* species:** Although Chlorophyll a content in Donna species seedlings were determined to be 2.25 mg g^{-1} FW in the plants which were treated with 100 M, the recorded value (2.10 mg g^{-1} FW) for the plants treated with 50 M, was also included in the same statistical group with that value. Chlorophyll a quantity was determined to be 1.66 mg g^{-1} FW for the plants treated with Salt + 50 and 100 M and therefore, the difference between these and control treatment (1.57 mg g^{-1} FW) was not statistically significant. The lowest chlorophyll a content was recorded in the plants treated with salt + 10 M (Table 5). Chlorophyll b content was recorded to be high in the plants treated with 100 M (1.07 mg g^{-1} FW); the lowest chlorophyll b content was recorded in the plants treated with salt + 10 M (0.31 mg g^{-1} FW). Particularly, it was observed that, chlorophyll b content in the plants treated with salt + 50 and 100 M increased upto the chlorophyll b content level of control treatment. Similar results were obtained for the total chlorophyll parameters (Table 5). Carotenoid content of the plants were determined to be at highest level at 227.82 mg g^{-1} FW in samples treated with 100 M VAM.

Similarly, the lowest carotenoid content in other pigments was determined to be 98.09 mg g^{-1} FW in the plants to which salt + 10 M VAM treatment applied (Table 5). Chlorophyll values of the plants grown in salty soil significantly get reduced due to suppression of particular enzymes which are responsible for synthesis of photosynthetic pigments (Murkute et al., 2006). In our study, chlorophyll a (0.91 and 1.31 mg g^{-1} FW respectively), chlorophyll b (0.48 and 0.42 mg g^{-1} FW respectively) and total chlorophyll (1.39 and 1.73 mg g^{-1} FW respectively) quantities of the Aspendos species treated with salt control and salt + 10 M, were found to be significantly low in comparison to control and 50 and 100 M, VAM treatments ($P \leq 0.05$). Results obtained with Donna Species are similar. Reduction in intake of minerals required for Chlorophyll biosynthesis (for example Mg) reduces the chlorophyll content of the leaves (Sheng et al., 2008). In our study, chlorophyll content of the plants treated with salt control and salt + 10 M were found low.

In the studies performed by various researchers, chlorophyll content of the mycorrhizal plant leaves which were grown under high salt conditions, was found to be high (Colla et al., 2008; Kaya et al., 2009; Hajiboland et al., 2010). When there is mycorrhiza in the environment, the antagonistic affect of sodium on magnesium intake is being balanced and suppressed (Giri et al., 2003). In our studies, chlrorophyl a, b and total chlorophyll contents of the Aspendos species plants treated with salt + M 50 and M 100 were found to be significantly high in comparison to the samples of control and salt control treatments ($p \leq 0.05$).

Conclusion

VAM inoculating tomato seedlings grown under saline conditions did not promote significant seedling growth but positively affected growth and development resulting in higher fresh stem and root weight and chlorophyll content with beneficial for tomato growth and yield. Generally, the data obtained from cv. Aspendos is better than cv. Donna. So in saline soils, cv. Aspendos could be grown successfully after inoculation of VAM.

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