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Response of Mycorrhiza-Inoculated Pepper and Amino Acids to Salt Treatment at Different Ratios

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ABSTRACT

Mycorrhiza has attracted interest as one of the microorganisms that increase a crop's salt stress tolerance. This study was conducted to determine the impacts of mycorrhiza inoculation and applying salt at different ratios on the yield of peppers and amino acid concentrations. The study was conducted in greenhouse conditions on loamy soils with four salt treatments, two mycorrhiza inoculations and a control in a complete randomized block design. The present study indicated that salt treatment alone was significantly correlated with crop stem and root amino acid concentrations, RWC% and leaf sizes, whereas applying mycorrhiza showed a positive relationship to stem height, stem and root wet weight, and root amino acids but led to a decloine in root serine and glutamine, and stem amino acid and glutamine. In conclusion, inoculating with mycorrhiza was observed to make a positive contribution to salt stress tolerance at different levels in almost all the parameters examined.

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KEYWORDS
Mycorrhiza; salt; pepper; amino acid

Introduction

Salt is an important abiotic stress factor which negatively influences crop growth and productivity, especially in arid and semiarid regions (Munns 2002). The soils impacted by salinity constitute about 7% of the total global land surface (Sheng et al. 2011). Excessive and uncontrolled irrigation, use of low-quality irrigation water, high groundwater and inadequate drainage conditions, climatic factors, natural salt rocks, and seawater are some of the reasons for salinity issues (Daşgan 2008; Maas and Grattan 1999; Munns and Tester 2008; Shannon 1984). Not only 954 million hectares of land worldwide but also 1.5 million hectares of annually irrigable land is affected by salinization. It is estimated that sustainable agricultural lands will be affected by an increase in salinity of 30% in 25 years and by 50% in the middle of 21st century (Asraf and Foolad 2007; Kusvuran 2010; Munnns 2005).

The operation of many metabolic activities or processes, and especially of photosynthetic activity, is adversely affected in crops exposed to salt stress, which influences crops by changing their structural, physiological and biochemical development, and molecular mechanisms. The negative impacts of changes caused by salt stress depend on the crop species, crop variety, ion variability (which causes salinity), ion concentration, and the duration of salt stress. In addition, the accumulation of some amino acids (alanine, arginine, glycine, serine, leucine and valine, amino acids, proline and nonprotein amino acids, and amides (such as glutamine and asparagines) has been reported in crops exposed to salt stress (Mansour 2000).

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Recently, the mycorrhiza mushroom, which is found in fossils that are millions of years old, has attracted interest as one of the microorganisms that increase crops' salt stress tolerance. Mycorrhiza, which is an important species of symbiotic life existing between crop roots and soil fungi, has a positive impact by increasing the crop's nutrient uptake, as well as resistance to biotic and abiotic stress conditions (Faber et al. 1991; George and Marschner 1996; Karagiannidis, Bletsos, and Stavropoulos 2001; Kothari, Marschner, and Römheld 1991; Marschner 1995; Ruiz-Lozana 2003; Slezack et al. 2000; Smith and Read 1997, 2008; Yano-Melo, Saggın, and Maıa 2003). It is well documented that mycorrhiza enhances crop tolerance against abiotic stress conditions such as high salinity, drought, and heavy metals (Forgy 2012; Ghazi, Hammad, and Rusan 2001; Kaya et al. 2009; Subramanian, Santhanakrishnan, and Balasubramanian 2006; Türkmen et al. 2005), and also improves nutrient uptake (Krikun et al. 1990; Poulton, Koıde, and Stephenson 2001; Tofino and Sanchez 1998; Waterer and Coltman 1989). In addition, Bowen (1980) stated that mycorrhiza could protect crops against stressors by retaining toxic elements. The activity of mycorrhiza has been determined to be different in many plant species and even in the same species (Krishhna et al. 1985; Sreennivasa and Rajashekhara 1989). Mycorrhiza was reported to not only enhance crop resistance against salinity stress but also crop growth by producing many hyphae, increasing mineral nutrition, eliminating nutritional imbalances, improving the condition of the crop water, and reducing the salt intake of the host plant (O'Keefe and Sylvia 1991; Smith and Read 1997; Weıssenhorn 2002).

Pepper (*Capsicum annuum* L.) is an important crop with agricultural preserve in Turkey and worldwide (Anonymous 2005). It is known that the pepper crop's roots normally form a symbiotic association with arbuscular mycorrhizal fungus (Martin and Stutz 2004; Sensoy 2007) and this provides many benefits (Davies et al. 2002; Garmendia, Goicoechea, and Aguireolea 2004; Salami 2002; Türkmen et al. 2005). Küçükyumuk, Gültekin, and Erdal (2014) evaluated the impacts of individual and combined vermicompost and mycorrhiza treatments on pepper growth and mineral nutrition, and found that mycorrhiza had positive impacts on crop wet and dry weight, and nutrient concentrations. It was reported by Russo (2006) that pepper fleas attained the highest dry matter weight and the crop attained its maximum height under the influence of mycorrhiza-grafting. Many studies reported that plants forming a symbiotic relationship with mycorrhiza were less affected by salt stress. Turkmen et al. (2008), studying the development and nutrient contents of pepper seedlings under salt stress, treated them with two different applications of mycorrhiza and reported that mycorrhiza increased stem and root length, stem diameter, and stem-rot wet and dry weight at both control and with 75 ppm salt stress. The present study aimed to the determine the impacts of mycorrhizal infections on pepper root and stem growth and the composition of the crops at different salt levels.

Materials and methods

Experimental design

This study was conducted in a controlled greenhouse at Ahievran University, Kırşehir, Turkey from March to May 2016. The greenhouse mean temperature and relative humidity were 16°C at night and 24°C in the morning, and 74%, respectively, during the experiment. Seeds of *Capsicum annum* var. Cemele were germinated on 86-celled styrofoam trays filled with peat, then the homogenous and healthy seedlings were transplanted to pots of 30 × 20 x 20 cm after thirty days. The pots were filled with 1.5 kg air-dried soil and drainage-blocked pots (with plastics bags inside). The present experiment was established to evaluate the impacts of mycorrhiza treatments on pepper crop growth and nutrition when different rates of salt were applied. It was carried out in randomized block design and with four replications. The soil used in the experiment was transported from the Kirsehir Okse region and passed through a 2 mm diameter sieve. 74 days after the beginning of fruit setting, plants were harvested and the stem and root systems were sampled separately.

Table 1. Some physical and chemical properties of soils used in the present study.

Texture	Loam	K (mg kg^{-1})	600.0
pH (1:2.5)	7.72	Fe (mg kg^{-1})	6.46
EC (µs/cm)	0.209	Zn (mg kg^{-1})	4.05
CaCO3 (%)	14.6	Cu (mg kg^{-1})	1.71
N (%)	0.071	Mn (mg kg^{-1})	29.08
P (mg kg^{-1})	55.0		

Laboratory analysis and intial soil conditions

Soil texture and pH were determined using a Bouyoucos hydrometer (Bouyoucous 1951) and a 1:2.5 (soil: water) ratio with a glass electrode pH meter. Soil total salt was analyzed following Jakson (1958), and lime was determined with the Scheibler calcimeter according to Allison and Moodie (1965). Total nitrogen (N) was determined by the Kejdahl process (Bremner 1996), available potassium with 600 mg/kg according to Knudsen, Peterson, and Prat (1982), and available phosphorus by using Olsen et al. (1954). Some microelements such as Fe, Zn, Cu, and Mn, which are shown in Table 1, were analyzed according to the DTPA method (Lindsay and Norvell 1978). Initial soil analysis showed that the soils used in the present study were loamy, insufficient in nitrogen, had a high lime content, no salinity issues, and contained high levels of phosphosrus and exchangeable potassium. Moreover, the soil-available iron, zinc, manganese, and copper concentrations were above sufficient levels (Lindsay and Norvell 1978).

Crop properties and study treatments

The seedling used in this study, a local fillet pepper genotype of Cemele (*Capsicun annum* cv.), is tolerant to medium salinity. Seeds of *Capsicum annum* var. Cemele were germinated on 86-celled styrofoam trays filled with peat, then the homogenous and healthy seedlings were transplanted to pots of 30 × 20 x 20 cm after thirty days. The pots were filled with 1.5 kg air-dried soils and drainage-blocked pots (by plastics bags inside). Treatments included four applications of salt (S0 = 0 nM; S50 = 50 nM NaCl; S100 = 100 nM NaCl; and S150 = 150 mM NaCI) with two mycelial spore mycorrhizae (M0; 0 per crop, M100; 100 per crop) and also two with mycorrhiza and without mycorrhiza. In addition, endo-mycorrhiza fungus (VAM) obtained from the ROOTS-Novozymes company was applied to the root zone of the crops during the confusion. Crops were irrigated with pure water. After 74 days, plant sampling was conducted by cutting from the rootstock and washing the soil from the body of the plant. Furthermore, the crop height, diameter, leaf disc weight, root and stem wet weights and leaf width and length were measured. Harvested and washed crop root and stem samples were transferred to a deep-freeze unit until they were analyzed.

Statistical analysis

Statistical analysis was conducted to determine the impact of the treatments with regard to the complete randomized design and using the SPSS 20 V package. The influence of the salt and mycorrhiza applications was evaluated by analysis of variance according to the Duncan-LSD method and a 0.05 significance level (Düzgüneş et al. 1987).

Results and discussion

The plant's fruit formation stage was not studied because the amino acid accumulated in the plant's root system and its reaction on the plant's body in terms of the plant's tolerance plays a very important role in protecting from salt stress. The decrease in the mycorrhiza and mycorrhizae

Table 2. Stem wet weight (g/crop), height and diameter (cm), and root wet weights (g/crop) as impacted by Mikoriza and salt applications.

Applications		N	Stem			Root
			Wet weight	Height	Diameter	Wet weight
M_0	S_0	4	27.75 ± 2.91 b	39.75 ± 2.45 a	5.72 ± 0.22 b	20.18 ± 1.49 b
	S_{50}	4	19.93 ± 1.42 c	29.62 ± 1.95 c	5.09 ± 0.15 c	8.11 ± 0.35 c
	S_{100}	4	9.25 ± 0.81 de	21.67 ± 1.24 d	3.96 ± 0.29de	3.61 ± 1.16 de
	S_{150}	4	2.74 ± 0.37 f	17.22 ± 0.38 e	3.46 ± 0.26 e	1.56 ± 0.34 e
M_{100}	S_0	4	33.05 ± 1.51 a	42.52 ± 1.39 a	6.38 ± 0.20 a	24.64 ± 2.33 a
	S_{50}	4	21.40 ± 1.89 c	35.67 ± 0.54 b	5.58 ± 0.11bc	9.79 ± 0.56 c
	S_{100}	4	10.71 ± 0.34 d	23.50 ± 0.71 d	4.17 ± 0.27 d	4.92 ± 0.59 d
	S_{150}	4	5.85 ± 0.33 ef	18.25 ± 0.20 e	3.65 ± 0.19de	2.58 ± 0.23 de
	P value		0.000***	0.000***	0.000***	0.000***
	M_0	16	14.92B	27.07B	4.56B	8.37B
	M_{100}	16	17.75A	29.99A	4.95A	10.49A
	P value		0.000***	0.000***	0.000***	0.000***

***; significant at P < 0.001 levels.
The differences between mean values indicated by different letters are significant (P</0.05).

pepper stem wet weight (g crop^{-1}), height and diameter (cm), and root wet weights (g crop^{-1}) was found to be statistically significant (P < 0.01; Table 2) as a result of the increase in the ratios of salt used in the treatments.

The highest stem and root wet weight (33.05 and 24.64 g crop^{-1}) were monitored under the M100S0 (100 mycorrhizal spores per crop and zero nm NaCl salt) treatment which refers to different Duncan groups, respectively. Another group contained the second highest stem and root wet weight, which were observed as 27.75 and 20.18 g crop^{-1} under M0S0 (zero mycorrhizal spores per crop and zero nm NaCl salt). In addition, crop stem and root wet weight, crop height, and crop diameter were significantly (P < 0.01) decreased by increasing the salt concentration. The impacts of mycorrhiza on the pepper crop stem and root wet weight, stem diameter, and stem height were also significant (P < 0.01). Applications on crops with (M100) and without (M0) mycorrhiza referred to different Duncan groups (Table 2). Moreover, the pepper stem wet weights, height and diameter, and root wet weights with mycorrhiza were greater than those without mycorrhiza but the same ratio of salt (P < 0.01; Table 2). Similarly, Kaya et al. (2009) reported that mycorrhiza-inoculated peppers attained higher stem and root weights than those not inoculated at 50 and 100 mM of salt stress. It is well documented in many studies that salt stress leads to a decline in crop leaf, stem and root weight (AliDinar, Ebert, and Ludders 1999; Chartzoulakis and Klapaki 2000; Hernandez et al. 1995; Naseer, Nisar, and Ashraf 2001; Yamato, Ikeda, and Iwase 2008). Applying mycorrhiza increased crop stem and root wet weight and crop height and diameter by reducing the adverse influences of salt in all parameters (Table 2). Turkmen et al. (2008) studied the impacts of two different mycorrhiza treatments on the development and nutrient concentrations of salt-stressed pepper seedlings and reported that mycorrhiza treatment increased stem and root heights, wet weight and dry weight as well as stem diameter under both control and at 75 ppm salt stress. Mycorrhiza-inoculated crops were reported to have higher leaf, stem, and root wet and dry weights in comparison to those without mycorrhiza in other studies using corn (Sönmez et al. 2012), banana (Yano-Melo, Saggin, and Maia 2003), tomato (Al-Karaki 2000), lettuce (Jahromi et al. 2008; Ruiz-Lozano and Azcón 2000), chickpea (Garg and Shikha 2010), and pepper (Kaya et al. 2009; Russo 2006). As shown Table 3, salt treatments affected leaf relative water content (%RWC), leaf length and width in a statistically significant manner (P < 0.001). Mycorrhiza inoculation also impacted %RWC (P < 0.05), leaf length and width (P < 0.001) (Table 3). In summary, a positive correlation was observed between the salt concentration and the %RWC and leaf sizes. However, these reductions were partially mitigated by mycorrhizal applications (Table 3).

Mycorrhiza inoculation protects the proportional water content of crop leaves especially under high salt concentrations. In other words, without mycorrhiza inoculation and in accordance with

Table 3. Impacts of mycorrhiza inoculations and salt applications on RWC% and leaf size (cm).

Applications		N	% RWC	Leaf Lenght	Leaf Width
	S_0	4	96.00 ± 1.80 a	10.78 ± 0.43 ab	4.91 ± 0.35 b
	S_{50}	4	91.27 ± 3.02 ab	10.19 ± 0.33 bc	4.86 ± 0.16 b
M_0	S_{100}	4	81.31 ± 7.27 b	7.97 ± 0.65 de	4.04 ± 0.47 cd
	S_{150}	4	60.01 ± 8.25 c	5.75 ± 0.78 f	3.01 ± 0.19 e
	S_0	4	91.14 ± 4.70 ab	11.80 ± 0.60 a	5.65 ± 0.27 a
	S_{50}	4	88.70 ± 2.63 ab	10.86 ± 0.53 ab	5.31 ± 0.21 ab
M_{100}	S_{100}	4	83.27 ± 4.38 b	9.12 ± 0.80 cd	4.59 ± 0.41 bc
	S_{150}	4	80.10 ± 2.53 b	6.95 ± 0.60 ef	3.65 ± 0.30 de
	P values		0.000***	0.000***	0.000***
M_0		16	82.15 ± 15.2B	8.68 ± 2.08 B	4.21 ± 0.86 B
M_{100}		16	85.81 ± 5.58A	9.69 ± 1.97 A	4.80 ± 0.83 A
	P values		0.043*	0.000***	0.000***

*, and ***; significant at P</0.055 and 0.001 levels, respectively.
The differences between mean values indicated by different letters are significant (P</0.05).

increases in the ratio of salt applied, the %RWC decreased and reached 60.01% under the highest ratio of salt (M0S150), whereas mycorrhiza-inoculated peppers attained 81.10% under M100S150 treatment. Due to the lower proportion of leaf relative water content, more necrotic stains were found in the mycorrhiza-inoculated crop leafs under a high salt concentration in comparison to those without the mycorrhiza inoculation. Cekic, Unyayar, and Ortas (2012) not only reported two different types of mycorrhiza (*Glomus mosseae* and *G. intraradices*) which increased the proportional water content of pepper under the impact of salt stress but also stated that crops inoculated with *G. intraradices* in particular had a higher proportional water content than those inoculated with *Glomus mosseae*. Similarly, Öncel and Keleş (2002) found that salt stress led to a significant decline in the proportional water content of wheat. Furthermore, Jahromi et al. (2008) testified that there were increased plant growth and leaf relative water content under the impact of mycorrhizal symbiosis.

Inoculating with mycorrhiza and increasing the ratios of the salt used in the treatments were positively correlated with leaf length and width, which decreased linearly, but these reductions were lower under mycorrhiza inoculation than in those plants that had not been inoculated with mycorrhiza (Table 3).

The impacts of mycorrhiza and salt treatments on root amino acid concentrations are shown in Table 4; their impacts on stem amino acids are given in Table 5. Salt treatmetns significantly influenced crop root amino acid concentrations ($P < 0.001$), whereas mycorrhiza applications significantly affected serine ($P < 0.05$), glutamine ($P < 0.05$), arginine ($P < 0.05$), tyrosine ($P < 0.01$), hydroxy proline ($P < 0.05$), and proline ($P < 0.01$) contents in roots (Table 4). In general, increasing salt concentration linearly increased root aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, thio, tryptophan, phenylalanine, isoleucine, leucine and lysine contents but led to a decline in the concentration of cysteine (Table 4).

In addition to these observations, the root arginine, alanine, tyrosine, hydroxyproline, sarcosine and proline contents increased with an increase in salt ratio, declined at the S100 salt ratio and again increased at the S150 ratio. Root valine and methionine contents were linearly decreased by increasing salt ratios until the S100 salt ratio, but again increased under the S150 salt ratio. In general, stem aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, thio, methionine, tryptophan, phenylalanine, leucine, lysine, hydroxyproline, sarcosine and proline contents were increased linearly by increased salt concentration whereas arginine, alanine, tyrosine, cystine and the leucine contents were determined to be reduced under the S100 salt ratio and to further increase under the S150 ratio. A decline in serine and glutamine contents and increases in arginine, tyrosine, hydroxy proline and proline concentrations resulted when crop root amino acid concentrations were under the co-impacts of mycorrhiza and salt (Table 4). In addition, when root amino acid concentrations were under mycorrhiza inoculation, serine and

Table 4. Impacts of mycorrhiza and salt applications on root amino acid content (pmol/µl).

Applications		N	Aspartat	Glutamat	Asparagin	Serin
M_0	S_0	4	2385.82 ± 155.51^{bcd}	2125.39 ± 352.90^{bcd}	2954.83 ± 248.33^{bc}	5478.33 ± 439.33^{d}
	S_{50}	4	2422.72 ± 236.40^{bcd}	2583.99 ± 222.68^{abcd}	3298.68 ± 265.98^{abc}	6675.64 ± 498.99^{cd}
	S_{100}	4	2512.33 ± 569.26^{abcd}	2743.95 ± 789.96^{abc}	4244.78 ± 1530.03^{ab}	9394.82 ± 1891.26^{ab}
	S_{150}	4	3377.03 ± 276.50^{ab}	3788.61 ± 420.83^{a}	4467.51 ± 468.18^{ab}	10887.69 ± 1062.99^{a}
M_{100}	S_0	4	1546.51 ± 138.94^{d}	1432.28 ± 142.70^{d}	2178.10 ± 186.51^{c}	5849.37 ± 359.54^{cd}
	S_{50}	4	1730.32 ± 310.42^{cd}	1657.42 ± 233.64^{cd}	2827.82 ± 475.99^{bc}	5167.61 ± 535.47^{d}
	S_{100}	4	3480.85 ± 795.07^{a}	3565.45 ± 784.10^{a}	4802.34 ± 883.10^{a}	6634.25 ± 366.44^{cd}
	S_{150}	4	2683.33 ± 582.54^{abc}	3184.77 ± 815.50^{ab}	3935.59 ± 433.69^{ab}	7807.10 ± 978.68^{bc}
	P		0.000**	0.000**	0.000**	0.000**
M_0	Mean	16	2674.48 ± 523.32	2810.49 ± 768.25	3741.45 ± 981.81	8109.12 ± 2433.71A
M_{10}	Mean	16	2360.25 ± 928.73	2459.98 ± 1091.03	3435.97 ± 1153.62	6364.59 ± 1141.40B
	P		0.248	0.302	0.426	0.014**

Applications		N	Glutamin	Histidin	Glisin
M_0	S_0	4	2633.61 ± 363.54c	926.99 ± 134.18^{c}	769.71 ± 31.41^{b}
	S_{50}	4	2732.09 ± 314.25c	1060.32 ± 132.53^{c}	930.51 ± 190.05^{ab}
	S_{100}	4	4223.72 ± 364.28a	1641.46 ± 352.55^{ab}	1195.26 ± 60.57^{a}
	S_{150}	4	3733.06 ± 265.85ab	1716.06 ± 404.12^{a}	1163.65 ± 91.95^{a}
M_{100}	S_0	4	3070.58 ± 122.23bc	1300.40 ± 196.90^{abc}	1175.13 ± 27.93^{a}
	S_{50}	4	2706.62 ± 111.75c	988.58 ± 150.28^{c}	1043.18 ± 85.93^{ab}
	S_{100}	4	2777.13 ± 255.79c	1138.77 ± 138.84^{bc}	1117.81 ± 174.42^{a}
	S_{150}	4	2789.13 ± 371.90c	1176.58 ± 141.99^{bc}	1141.46 ± 253.98^{a}
	P		0.000**	0.000**	0.002**
M_0	Mean	16	3330.63 ± 753.55A	1336.21 ± 439.22	1014.79 ± 205.80
M_{100}	Mean	16	2835.87 ± 258.62B	1151.09 ± 183.65	1119.40 ± 152.08
	P		0.019*	0.130	0.112

Applications		N	Tionin	Arginin	Alanin
	S_0	4	15804.62 ± 2221.98^{b}	18474.66 ± 870.78^{cd}	18085.31 ± 796.32^{c}
	S_{50}	4	16159.12 ± 881.90^{b}	23439.90 ± 4232.88^{abc}	26006.03 ± 3983.14^{ab}
	S_{100}	4	19396.06 ± 1437.24^{ab}	13715.59 ± 520.97^{d}	16306.95 ± 2291.52^{c}
	S_{150}	4	20908.17 ± 1506.22^{a}	22797.97 ± 2936.55^{abc}	22608.18 ± 3125.20^{abc}
	S_0	4	18872.45 ± 1930.25^{ab}	18394.00 ± 1042.17^{cd}	18840.93 ± 1811.95^{c}
	S_{50}	4	20530.91 ± 2227.45^{a}	21233.33 ± 873.39^{bc}	19627.61 ± 1031.69^{bc}
	S_{100}	4	18847.73 ± 915.67^{ab}	26235.54 ± 3139.40^{ab}	26186.04 ± 3529.41^{a}
	S_{150}	4	21164.38 ± 1199.48^{a}	27346.92 ± 3348.48^{a}	28995.58 ± 3726.22^{a}
	P		0.000**	0.000**	0.000**
	Mean	16	18066.99 ± 2639.90B	19607.03 ± 4663.10B	20751.62 ± 4663.57
	Mean	16	19853.87 ± 1816.58A	23302.45 ± 4336.60A	23412.55 ± 5088.67
	P		0.033*	0.027*	0.134

Applications	N	Tirosin	Sistin	Valin	Methionin
S_0	4	2219.49 ± 322.01^{de}	933.33 ± 120.69^{ab}	785.30 ± 142.03^{bc}	1238.40 ± 155.57^{ab}
S_{50}	4	2633.38 ± 126.49^{bcd}	670.92 ± 168.55^{bcd}	719.59 ± 167.55^{bcd}	919.87 ± 206.22^{bc}
S_{100}	4	1998.09 ± 94.71^{e}	575.37 ± 65.21^{cd}	485.69 ± 69.98^{cd}	898.07 ± 125.63^{bc}
S_{150}	4	2737.41 ± 271.34^{abcd}	928.95 ± 129.15^{ab}	981.06 ± 131.44^{ab}	1205.17 ± 165.98^{ab}
S_0	4	2466.76 ± 135.18^{cde}	421.73 ± 50.68^{d}	383.37 ± 23.57^{d}	597.92 ± 67.71^{c}
S_{50}	4	2809.25 ± 407.80^{abc}	448.83 ± 63.51^{d}	421.36 ± 49.22^{cd}	729.30 ± 102.88^{c}
S_{100}	4	3163.51 ± 171.73^{ab}	1170.87 ± 164.53^{a}	1254.27 ± 283.51^{a}	1538.02 ± 151.31^{a}
S_{150}	4	3260.53 ± 220.80^{a}	810.83 ± 213.05^{bc}	1014.09 ± 228.75^{ab}	1185.17 ± 188.90^{ab}
P		0.000**	0.000**	0.000**	0.000**
Mean	16	2397.10 ± 370.26B	777.15 ± 198.24	742.92 ± 217.89	1065.38 ± 219.81
Mean	16	2925.02 ± 396.64A	713.07 ± 339.86	768.28 ± 421.67	1012.61 ± 404.45
P		0.001***	0.520	0.832	0.650

Applications	N	Triptofon	Fenilalanin	İzolösin	Lösin
S_0	4	1497.38 ± 160.16^{e}	682.92 ± 72.80^{c}	254.19 ± 41.74^{d}	208.02 ± 10.45^{d}
S_{50}	4	1669.76 ± 231.81^{de}	699.44 ± 63.64^{c}	265.18 ± 44.01^{d}	232.47 ± 29.92^{cd}
S_{100}	4	2711.54 ± 194.60^{ab}	1554.12 ± 162.97^{a}	646.22 ± 176.25^{a}	483.76 ± 39.60^{a}
S_{150}	4	3028.04 ± 198.97^{a}	1121.62 ± 125.4^{b}	518.48 ± 144.79^{ab}	349.01 ± 32.83^{b}
S_0	4	2344.44 ± 406.74^{bc}	1196.11 ± 64.67^{b}	485.68 ± 85.19^{abc}	458.71 ± 39.75^{a}
S_{50}	4	1556.61 ± 243.47^{de}	812.94 ± 71.35^{c}	298.24 ± 61.43^{cd}	313.90 ± 41.24^{bc}
S_{100}	4	1773.22 ± 127.16^{cde}	741.51 ± 68.15^{c}	307.79 ± 26.80^{bcd}	296.19 ± 44.34^{bc}
S_{150}	4	2082.21 ± 307.21^{cd}	712.67 ± 75.30^{c}	302.64 ± 34.30^{bcd}	272.31 ± 33.70^{bcd}

(*Continued*)

Table 4. (Continued).

Applications	N	Triptofon	Fenilalanin	İzolösin	Lösin
P		0.000**	0.000**	0.000**	0.000**
Mean	16	2226.68 ± 699.89	1014.53 ± 383.18	421.02 ± 202.73	318.32 ± 116.15
Mean	16	1939.12 ± 403.31	865.81 ± 210.07	348.59 ± 96.33	335.28 ± 83.22
P		0.165	0.184	0.207	0.638
Applications	**N**	**Lisin**	**Hidroksi Prolin**	**Sarkozin**	**Prolin**
S_0	4	32109.25 ± 3172.18^{b}	36949.33 ± 1741.56^{cd}	36170.62 ± 1592.65^{c}	4438.98 ± 644.01^{de}
S_{50}	4	32318.24 ± 1763.81^{b}	46879.80 ± 8465.77^{abc}	52012.07 ± 7966.29^{ab}	5266.76 ± 252.99^{bcd}
S_{100}	4	40792.13 ± 5782.14^{a}	27431.19 ± 1041.94^{d}	32613.90 ± 4583.05^{c}	3996.19 ± 189.42^{e}
S_{150}	4	41816.35 ± 3012.45^{a}	45595.94 ± 5873.10^{abc}	45216.36 ± 6250.41^{abc}	5474.82 ± 542.68^{abcd}
S_0	4	37744.90 ± 3860.51^{ab}	37288.01 ± 1629.28^{cd}	37681.87 ± 3623.90^{c}	4933.52 ± 270.36^{cde}
S_{50}	4	41061.82 ± 4454.91^{a}	42466.66 ± 1746.79^{bc}	39255.22 ± 2063.39^{bc}	5618.50 ± 815.60^{abc}
S_{100}	4	37695.47 ± 1831.35^{ab}	52471.09 ± 6278.80^{ab}	52372.08 ± 7058.83^{a}	6327.03 ± 343.46^{ab}
S_{150}	4	42328.76 ± 2398^{a}	54693.85 ± 6696.97^{a}	57991.16 ± 7452.45^{a}	6521.06 ± 441.60^{a}
P		0.001**	0.000**	0.000**	0.000**
Mean	16	36758.99 ± 5772.23	39214.07 ± 9326.21B	41503.25 ± 9327.13	4794.19 ± 740.53B
Mean	16	39707.74 ± 3633.15	46729.91 ± 8504.03A	46825.09 ± 10177.35	5850.03 ± 793.28A
P		0.094	0.024*	0.134	0.001***

glutamine contents significantly decreased ($P < 0.05$), whereas tionine ($P < 0.05$), arginine ($P < 0.05$), tirosine ($P < 0.001$), hydroxyproline ($P < 0.05$), and proline ($P < 0.001$) significantly increased (Table 4).

Salt treatments significantly influenced the crop stem amino acid concentrations ($P < 0.001$), whereas mycorrhiza applications impacted stem glutamine ($P < 0.01$), arginine ($P < 0.05$), tirosine ($P < 0.01$), and systine ($P < 0.05$) contents (Table 5). In general, salt concentration and root aspartate, glutamate, aspargin, serine, glutamine, histidine, glycine, thionine, arginine, valine, methionine, tryptophan, phenylalanine, leucine, lisin, hydroxyproline, sarcosine, and proline contents were linearly increased (Table 5). Similar to those in the roots, stem arginine, alanine, tirozine, systine, valine, and izolosine contents were increased by increases in salt concentration except under S100 treatment, when they declined. Concentrations of some crop amino acids (alanine, arginine, glisine, serine, losine, valine, amino acids, proline, and non-protein amino acids) and amides (such as glutamine and asparagine) were increased under salt stress. When the co-impacts of mycorrhiza and salt on crop stem amino acid contents were determined, all the above-mentioned amino acids (aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, thio, arginine, alanine, tyrosine, cystine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, hydroxy proline, sarcosine and proline) linearly increased (Table 5).

Mycorrhiza-inoculated stem amino acid and glutamine contents significantly ($P < 0.01$) declined, whereas tirozine ($P < 0.001$) and systine ($P < 0.05$) were increased (Table 5). Some of the crop amino acid (alanine, arginine, glisine, serine, losine, valine, amino acids, proline, non-protein amino acids, and amides (such as glutamine and asparagines) contents increased under salt stress (Mansour 2000). It was determined that crop proteins accumulated under salinity conditions could be used again (Singh et al. 1987) and would provide for nitrogen accumulation, which can play a role in osmotic regulation. The root proline concentration of salt-tolerant clover doubled, even though the proline concentration in salt-sensitive plants increased more slowly (Petrusa and Winicov 1997).

Mycorrhiza inoculation made a positive contribution to crop salt tolerance at different levels in almost all parameters observed. However, these positive impacts were observed to significantly decrease, especially under a high salt concentration. The reason for this is presumably the inhibition of the formation of hyphae due to the inhibition of the mycorrhizal spore germination phases by a high salt concentration.

Table 5. Impacts of mycorrhiza and salt applications on steam amino acid concentrations (pmol/µl).

Applications	N	Aspartat	Glutamat	Asparagin	Serin
S_0	4	6118,81 ± 127,43^{c}	5425,35 ± 583,77^{c}	8279,73 ± 1143,05^{d}	19516,90 ± 768,69^{cd}
S_{50}	4	7130,03 ± 1040,20^{bc}	7665,26 ± 946,11^{bc}	9876,66 ± 1206,03^{cd}	21595,89 ± 2872,23^{bcd}
S_{100}	4	7429,39 ± 776,97^{bc}	7831,26 ± 318,19^{bc}	11645,14 ± 836,63^{bc}	23855,72 ± 5737,64^{bc}
S_{150}	4	11662,66 ± 1662,2^{a}	13086,42 ± 2139,17^{a}	15405,54 ± 2228,97^{a}	36244,66 ± 1894,8^{a}
S_0	4	5413,36 ± 192,36^{c}	5159,08 ± 633,27^{c}	7766,42 ± 314,62^{d}	16842,07 ± 2049,56^{d}
S_{50}	4	5881,34 ± 199,45^{c}	5688,60 ± 771,68^{c}	9624,97 ± 483,08^{cd}	17193,25 ± 913,71^{cd}
S_{100}	4	8867,06 ± 1553,31^{b}	9081,96 ± 1483,05^{b}	12255,65 ± 1582,11^{bc}	23454,24 ± 2627,97^{bcd}
S_{150}	4	8899,53 ± 1564,66^{b}	10542,81 ± 2245,58^{ab}	13098,14 ± 915,65^{ab}	26842,24 ± 3629,09^{b}
P		0.000**	0.000**	0.000**	0.000**
Mean	16	8085,23 ± 2386,41	8502,08 ± 3101,30	11301,77 ± 3030,71	25303,30 ± 7358,44
Mean	16	7265,33 ± 1951,73	7618,12 ± 2665,61	10686,30 ± 2348,75	21082,95 ± 4919,11
P		0,296	0,394	0,526	0,066

Applications	N	Glutamin	Histidin	Glisin
S_0	4	9091,37 ± 1190,48^{cd}	3264,50 ± 285,04^{b}	2760,85 ± 160,07^{e}
S_{50}	4	9110,29 ± 688,06^{cd}	3537,22 ± 528,39^{b}	3153,70 ± 187,8^{cde}
S_{100}	4	10820,89 ± 271,57^{ab}	4182,80 ± 670,68^{b}	3223,86 ± 455,11^{bcde}
S_{150}	4	12453,16 ± 797,13^{a}	5692,59 ± 1124,42^{a}	3688,87 ± 203,95^{abc}
S_0	4	7908,99 ± 765,46^{d}	3321,96 ± 315,27^{b}	3020,45 ± 156,05^{de}
S_{50}	4	9031,31 ± 346,34^{cd}	3284,46 ± 334,27^{b}	3474,27 ± 145,91^{abcd}
S_{100}	4	9796,33 ± 520,66^{bc}	4237,86 ± 738,76^{b}	3752,73 ± 278,91^{ab}
S_{150}	4	9545,39 ± 814,02^{bcd}	4033,14 ± 371,09^{b}	3860,59 ± 278,35^{a}
P		0.000**	0.000**	0.000**
Mean	16	10368,93 ± 1605,45A	4169,28 ± 1165,59	3206,83 ± 421,61B
Mean	16	9070,51 ± 942,72B	3719,36 ± 607,80	3527,02 ± 390,66A
P		0,009**	0,181	0,034*

Applications	N	Tionin	Arginin	Alanin
S_0	4	7902,31 ± 1110,99^{c}	9237,33 ± 435,39^{cd}	9042,65 ± 398,16^{c}
S_{50}	4	8079,56 ± 440,95^{bc}	11719,95 ± 2116,44^{abc}	13003,01 ± 1991,57^{ab}
S_{100}	4	10198,03 ± 1445,53^{ab}	6857,79 ± 260,48^{d}	8153,47 ± 1145,76^{c}
S_{150}	4	10454,08 ± 753,11^{a}	11398,98 ± 1468,27^{abc}	11304,09 ± 1562,6^{abc}
S_0	4	9436,22 ± 965,12^{abc}	9197,00 ± 521,08^{cd}	9420,46 ± 905,97^{c}
S_{50}	4	10265,45 ± 1113,72^{a}	10616,66 ± 436,69^{bc}	9813,80 ± 515,84^{bc}
S_{100}	4	9423,86 ± 457,83^{abc}	13117,77 ± 1569,70^{ab}	13093,02 ± 1764,70^{a}
S_{150}	4	10582,19 ± 599,74^{a}	13673,46 ± 1674,24^{a}	14497,79 ± 1863,11^{a}
P		0.001**	0.000**	0.000**
Mean	16	9158,50 ± 1511,33	9803,52 ± 2331,55B	10375,81 ± 2331,78
Mean	16	9926,94 ± 908,29	11651,23 ± 2168,30A	11706,27 ± 2544,34
P		0,092	0,027*	0,134

Applications	N	Tirosin	Sistin	Valin	Methionin
S_0	4	1109,74 ± 161,01^{de}	2281,58 ± 193,39^{c}	786,21 ± 33,25^{d}	1913,98 ± 66,04^{c}
S_{50}	4	1316,69 ± 63,24^{bcd}	3963,43 ± 380,11^{b}	950,90 ± 79,41^{cd}	2361,37 ± 514,87^{bc}
S_{100}	4	999,04 ± 47,35^{e}	2053,22 ± 429,97^{c}	924,19 ± 63,72^{cd}	2883,02 ± 394,48^{ab}
S_{150}	4	1368,70 ± 135,67^{abcd}	3799,44 ± 411,82^{b}	1218,05 ± 74,16^{a}	3481,93 ± 678,45^{a}
S_0	4	1233,38 ± 67,59^{cde}	2631,06 ± 300,88^{c}	890,23 ± 94,15^{cd}	2277,49 ± 123,32^{bc}
S_{50}	4	1404,62 ± 203,90^{abc}	2869,19 ± 178,66^{c}	994,29 ± 41,42^{bc}	2141,98 ± 98,52^{bc}
S_{100}	4	1581,75 ± 85,86^{ab}	4544,96 ± 503,27^{ab}	1148,22 ± 81,04^{ab}	2539,74 ± 387,78^{bc}
S_{150}	4	1630,26 ± 110,40^{a}	5082,00 ± 456,85^{a}	1208,87 ± 97,67^{a}	2695,77 ± 467,70^{abc}
P		0.000**	0.000**	0.000**	0.000**
Mean	16	1198,55 ± 185,13B	3024,42 ± 949,34B	969,84 ± 171,66	2660,08 ± 736,67
Mean	16	1462,51 ± 198,32A	3781,81 ± 1139,40A	1060,41 ± 148,86	2413,75 ± 359,02
P		0,001***	0,050*	0,121	0,239

Applications	N	Triptofon	Fenilalanin	İzolösin	Lösin
S_0	4	1799,19 ± 85,94c	3400,36 ± 480,82^{b}	2134,70 ± 168,22^{bc}	3155,32 ± 285,36^{d}
S_{50}	4	3253,63 ± 398,79a	3910,47 ± 210,29^{ab}	2398,67 ± 140,25^{ab}	5045,89 ± 353,01^{ab}
S_{100}	4	2139,47 ± 60,67bc	4322,84 ± 462,21^{a}	1892,13 ± 120,65^{c}	4255,13 ± 519,79^{bc}
S_{150}	4	3533,72 ± 503,47a	4375,57 ± 307,65^{a}	2546,34 ± 202,46^{a}	5599,87 ± 600,86^{a}
S_0	4	2145,33 ± 84,52bc	3989,45 ± 270,11^{ab}	2072,10 ± 107,68^{bc}	4024,76 ± 382,17^{cd}
S_{50}	4	2164,34 ± 157,95bc	4171,28 ± 263,34^{a}	2293,22 ± 159,54^{ab}	4053,10 ± 360,62^{bcd}
S_{100}	4	3402,53 ± 351,03a	3943,83 ± 150,04^{ab}	2492,92 ± 125,32^{a}	4896,44 ± 373,22^{abc}
S_{150}	4	2446,09 ± 118,34b	4484,25 ± 255,66^{a}	2535,00 ± 158,91^{a}	5610,12 ± 448,40^{a}

(*Continued*)

Table 5. (Continued).

Applications	N	Triptofon	Fenilalanin	İzolösin	Lösin
P		0.000**	0.002**	0.000**	0.000**
Mean	16	2681,51 ± 807,23	4002,32 ± 529,31	2242,96 ± 296,08	4514,06 ± 1033,18
Mean	16	2539,58 ± 560,16	4147,21 ± 306,84	2348,31 ± 227,22	4646,11 ± 764,56
P		0,568	0,351	0,268	0,684
Applications	N	Lisin	Hidroksi Prolin	Sarkozin	Prolin
S_0	4	3476,93 ± 206,08^{c}	2008,90 ± 119,76^{b}	5921,29 ± 450,14^{b}	141,80 ± 32,76^{b}
S_{50}	4	5203,05 ± 338,09^{a}	5183,56 ± 1307,92^{a}	6723,57 ± 588,74^{ab}	406,06 ± 61,36^{a}
S_{100}	4	3933,68 ± 225,83^{c}	2464,93 ± 596,59^{b}	6305,95 ± 246,69^{ab}	365,46 ± 74,57^{a}
S_{150}	4	5644,32 ± 474,09^{a}	5532,27 ± 1123,70^{a}	7298,22 ± 793,95^{a}	358,04 ± 58,72^{a}
S_0	4	4018,35 ± 280,79^{c}	2494,50 ± 300,78^{b}	6246,82 ± 162,26^{ab}	151,10 ± 3,19^{b}
S_{50}	4	4259,54 ± 121,72^{bc}	2765,41 ± 266,72^{b}	6949,36 ± 347,19^{ab}	188,38 ± 18,21^{b}
S_{100}	4	5311,85 ± 354,79^{a}	5205,68 ± 1392,01^{a}	7034,98 ± 468,75^{ab}	423,81 ± 57,99^{a}
S_{150}	4	5000,18 ± 552,22^{ab}	5772,22 ± 655,38^{a}	7328,52 ± 539,23^{a}	440,30 ± 80,64^{a}
P		0.000**	0.000**	0.002**	0.000**
Mean	16	4564,50 ± 963,18	3797,42 ± 1820,79	6562,26 ± 725,63	317,85 ± 118,35
Mean	16	4647,48 ± 633,61	4059,45 ± 1654,70	6889,92 ± 547,37	300,90 ± 143,57
P		0,775	0,673	0,160	0,718

Conclusion

Mycorrhiza has attracted interest as one of the microorganisms that increases crops' tolerance to salt stress. This study was conducted to determine the impacts of mycorrhiza inoculation and different ratios of salt on peppers and amino acid concentrations. The study was conducted in greenhouse conditions on loamy soils with four salt treatments, two mycorrhiza inoculations, and a control in a complete randomized block design. The present study indicated that applying salt alone was significantly correlated with the crop stem and root amino acid concentrations, RWC% and leaf sizes, whereas applying mycorrhiza showed positive relationships to stem height, stem and root wet weight, and root amino acids but led to a decline in root serine and glutamine contents, and stem amino acid and glutamine contents. In conclusion, mycorrhiza inoculation was observed to make a positive contribution to crop salt tolerance at different levels in almost all the parameters observed.

The present study was conducted to determine the response of mycorrhiza-inoculated pepper and amino acids under different rates of salt stress. Salinity stress conditions had a negative effect on some properties such as plant stem and root growth, leaf size, relative water content (RWC), plant length, width, amino acid content and this effect increased more when salinity was applied. To become tolerant of stress conditions, pepper plants have tried to adapt to adverse conditions through changes in amino acid. Applying salt was associated with the crop stem and root amino acid concentrations, RWC and leaf sizes and plant height, diameter, stem wet weight, root wet weight, and the amino acid content of stem and root significantly decreased whereas inoculating plants with mycorrhiza alleviated plants' stress and increased RWC, and the amino acid content of stem and root.

These results suggest that stress acclimation may involve differences in the partitioning of photosynthetic characteristics, water content and osmotic solutes in stressed plants. Since salinity is a common feature of arid and semiarid environments, plants have developed mechanisms to tolerate salinity as well as a lack of water using different fungi and microbes. Our results strongly support the contention that mycorrhiza inoculation made a positive contribution to crop salt tolerance at different levels in almost all the parameters observed.

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