Pak. J. Bot., 48(1): 21-28, 2016.

HALOPHYTIC COMPANION PLANTS IMPROVE GROWTH AND PHYSIOLOGICAL PARAMETERS OF TOMATO PLANTS GROWN UNDER SALINITY

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Abstract

Salinity becomes a major concern when soil salt concentration becomes excessive in growth medium. Halophytes are capable of accumulating high concentrations of NaCl in their tissues, thus using halophytic plants in crop rotations or even in mixed cropping systems may be a promising management practices to mitigate salt stress related yield loses. Salinity induced yield losses and related physiological parameters on tomato plants (*Lycopersicon esculentum* Mill. cv. SC2121) grown with or without halophytic companion plants (*Salsola soda* L. and *Portulaca oleracea* L.) were investigated in pot experiment. Treatments consist of four soil type (collected from Harran plain-Turkey) with similar physical properties but varying in salinity level: electrical conductivity (EC): 0.9, 4.2, 7.2, and 14.1 dS m^{-1}. The reduction in plant total dry weight was 24, 19, and 48% in soils with slight (4.2 dS m^{-1}), moderate (7.2 dSm^{-1}) and high (14.1 dS m^{-1}) salinity as compared to non-saline soil (0.9 dS m^{-1}), respectively. Leaf content of proline, malondialdehyde (MDA), catalase (CAT) and peroxidase (POX) enzyme activity increased with increasing level of salinity. In tomato plants grown in consociation with *Salsola soda*, salinity induced DM decrease was only 6, 12 and 28% in soils with slight, moderate and high salinity as compared to non-saline soil, respectively. However, when *Portulaca oleracea* used as companion plant, no significant change in biomass or fruit yield was observed. This study showed that mixed planting with *Salsola soda* in high saline soils may be an effective phyto-remediation technique that may secure yield formation and quality of tomato.

Key words: Phytoremediation, Tomato, Salt stress, Halophytes, Companion plants.

Introduction

Due to a change in global precipitation patterns, recent climate models predict that, incidences and duration of drought periods will increase in many regions of the world negatively affecting the productivity of field crops (Campbell, 2015). On the other hand, the world's food production will need to increase by up to 70% by 2050 as the world's population will reach 9.1 billion, 34 percent higher than today (Anon., 2012). Achieving this goal will be a big challenge due to the decreased availability of arable land, resulting from urbanization and land degradation. In this context, scientists have to develop new management practices for increasing productivity of per unit land specifically in developing countries.

Salinity is one of the major abiotic environmental stresses affecting about 6-7% of the world's total land area and agricultural productivity specifically in arid and semi-arid regions of the world (Grewal, 2010; Qadir *et al*., 2006). Salinity has an effect on almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. Salinization is the process of increasing concentration of total dissolved salts in soil solution either due to natural processes or anthropogenic actions (excess irrigation and fertilization) (Ghassemi *et al*., 1995). In saline soils, high concentrations of sodium (Na) and chlorine (Cl) ions within the plant root zone retards the growth of plants by either decreasing the water potential of root media or causing toxicity of Na and Cl in various plant organs (Panta *et al*., 2014).

Crop and forage species used in modern agriculture are generally grown in fertile soils and these plants are salt sensitive (glycophytes). Increase in soil salt content negatively influences multiple plant physiological processes, e.g. photosynthesis, respiration, transpiration, membrane properties, nutrient balance, enzymatic activity, and metabolic activities, cellular homeostasis, and hormone regulation (Kaya *et al*., 2013; Geilfus *et al*., 2015). Thus, depending on the severity of salt stress, germination rate, plant growth and importantly agronomic yield of agricultural products can significantly be reduced. For most species, a 10% yield decrease reported when EC of a soil solution increases over the 4-8 dS m^{-1} range (Ventura *et al*., 2014). The grain yield of maize (*Zea mays* L.) decreased by 21% for each unit increment in EC in the irrigation water (Blanco *et al*., 2008; Tufail *et al*., 2013; Fahad *et al*., 2012).

Salinity is a continuous process and its remediation is cost and labor-intensive. Remediation and proper utilization of saline soils including agronomic practices (e.g. use of salt tolerant varieties, and phytoremediation) may secure crop yield (Kaya *et al*., 2015; Hasanuzzaman *et al*., 2014). Number of biochemical, physical, or molecular approaches have been developed for reclaiming saline or sodic soils (Singh *et al*., 2012; Diacono *et al*., 2015). These methods are largely based on the elimination of toxic ions such as Na and Cl from soils (Flowers *et al*., 2014) or aim to improve crop productivity under stress conditions; and can be defined as physical reclamation (deep ploughing), chemical reclamation, plant control, water based approaches, etc. (Qadir *et al*., 2007; Karakas, 2013). However, due to the complexity of the salt tolerance mechanism, commercial success of most of these methods have been found to be limited, especially in semi-arid and arid areas (Flowers, 2004; Colla *et al*., 2006; Ashraf, 2009).

Tomato is one of the most important vegetable crops grown in Mediterranean zone. Much of the tomato is grown in greenhouse conditions or soils close to the Mediterranean sea, where salinity problems already exist

because the well waters used for irrigation contain high amounts of soluble salts, mainly chlorides and sulphates. Phytoremediation techniques can be a promising management practices for enhancing crop salt tolerance (in our case tomato) in salt-impacted sites. Here, saline tolerant plants such as halophytes, bushes, and grasses have all been used as media to improve saline, sodic, or saline-sodic soils due to their high salt absorbance capacity (Rabhi *et al*., 2010). Halophytes absorb salt from soils and deposit it in their tissues, preventing crop plants from reaching toxic ions and do not pose a threat to crop plants in terms of ionic imbalance and physiological competetion (Zuccarini, 2008). In a study performed by Colla *et al*. (2006) the efficiency of *S. soda* as a companion plant with peppers was examined at 4.0 and 7.8 dS m^{-1}. The authors reported that the presence of *S. soda* decreased the EC value by 43% and increased total crop, marketable crop, and total biomass values by 26, 32, and 22%, respectively.

In this context, we set up a greenhouse study where the effects of salinity on tomato plants grown with or without halophytic companion plants (*Salsola soda* L. and *Portulaca oleracea* L.) was investigated. In addition to key agronomic parameters, we performed number of plant physiological analysis, e.g. leaf proline content, peroxidase enzyme activity, catalase enzyme activity, total protein content, lycopene, and vitamin C in fruit.

Materials and Methods

Soils with varying salinity level were collected from the Harran plain (36°52′39″N 39°02′02″E), Turkey. Detailed chemical and physical properties of the soils were shown in Table 1. Prior to the experiment, arable crops (wheat, maize, and barley) were grown on this soil. The treatments consist of four soil type: i) loam non-saline (LNS), ii) slide saline (LSS), iii) moderate saline (LMS), and iv) high saline (LHS). The upper 2 cm of soil were removed and the 10-15 cm soil section was collected for the experiment. Before use, soil samples were carefully air dried to allow sieving with a 4 mm mesh sieve. Tomato plants (cv. SC2121) were grown alone (TM) or in combination with *S. soda* (TMS) or *P. oleracea* (TMP) seedlings in 8 L pots containing 6 kg dry soil under controlled greenhouse condition.

Soils chosen for the experiment had following EC levels: LNS (EC=0.90 dS m^{-1}), LSS (EC=4.19 dS m^{-1}), LMS (EC=7.22 dS m^{-1}), and LHS (EC=14.05 dS m^{-1}). A trial was performed in a randomized block design with four replicates. Briefly, one week before transferring the tomato seedling into each pot, companion plant seeds were germinated at a rate of 30 companion halophytic plants per pot. Seven days after germination of halophytic plants in respective treatments, 45 days-old tomato seedlings grown in viols were transplanted individually into each pot at a rate of one tomato plants per pot. Pots were irrigated with an established drip irrigation system and soil water content was kept beetwen 31 and 45% water holding capacity (WHC) throughout the experiment. Plants were harvested 100 days after onset of treatments. Plants were separated into stems, leaves, and fruits. Fresh matter was analysed immediately after harvest. Dry matter (DM) of plant organs was determined after drying samples at 70° C to weight constancy.

Physiological and biochemical analysis: The membrane stability index (MSI) was determined based on the method of Premchandra *et al*. (1990). Briefly, at harvest 100 days after onset of treatments (DAO), leaf samples were cut into small pieces (c.a. 5 mm in length), and were placed in test tubes containing 10 mL of deionized distilled water. Tubes were then placed in a water bath at 40ºC for 30 min. The initial conductivity of the medium (C_1) was measured then samples were incubated at 100ºC for 10 min to expell electrolytes. Afterward, samples were cooled to 25ºC and a second conductivity measurement for the medium (C_2) was determined.

A chlorophyll determination was performed according to the method of Arnon (1949). For the analysis, a 0.5 g leaf sample was homogenized in 5 mL acetone:water (80:20% v/v) mixture and a reading with a UV spectrophotomer (UV-1700, Shimadzu) was obtained against an 80% acetone control for chlorophyll *a* at 663.5 nm and for chlorophyll *b* at 645 nm.

The proline content was determined according to the method of Bates *et al*. (1973). Acid-ninhydrin was used as a reagent. The reagent was made by dissolving (warming and agitating) 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid (the reagent remains stable for 24 hours at 4ºC). After weighing, leaves were frozen in liquid nitrogen and crushed immediately with a mortar and homogenized in 10 mL of a 3% (w/v) aqueous sulphosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper, then 2 mL of filtrate was mixed in a test tube with 2 mL of acid ninhydrin and boiled at 100°C for one hour. The reaction was terminated in an ice bath. The reaction mixture was extracted with 5 mL of toluene. Tubes were thoroughly shaken for 15-20 seconds and left for 20 min in order to achieve separation of the two layers. The chromophore containing toluene was removed and allowed to warm to room temperature. Absorbance was measured in a spectrophotometry (UV-1700-Shimadzu) at 515 nm against a toluene blank.

Peroxidase enzyme activity (POX, E.C.1.11.1.7) was determined by monitoring the increase in absorbance due to tetraguaiocol formation at 470 nm according to the method of Cvikrova *et al*. (1994). For the analysis, 0.5 g of plant material was homogenized in 5 mL of a 50 mM Na-phosphate buffer solution then 100 µL of extract was added to 3 mL of the reaction mixture (13 mM guaiacol, 5 mM H_2O_2, and 50 mM Na-phosphate, pH 6.5). The reaction was initiated with a H_2O_2 addition and was measured at 25°C at 470 nm using a UV spectrophotometer (UV-1700, Shimadzu) at two minute intervals until the 4th minute. One unit of POX activity is defined as a change of 0.1 absorbance unit per minute at 470 nm. Activity is expressed as enzyme units per gram of fresh weight (FW), U g^{-1} FW.

Catalase enzyme activity was determined by monitoring the decomposition of H_2O_2 according to the method of Milosevic and Slusarenko (1996). For the analysis, fifty µL of plant extract (as obtained above) was added to a 2.95 mL (10 mM H_2O_2, 50 mM potasium-phosphate buffer solution (pH 7.0), and 4 mM Na_2EDTA) reaction mixture and measured for 30 seconds at 240 nm and 25°C with a UV spectrometer (UV-1700, Shimadzu). One CAT activity unit (U) is defined as a change of 0.1 absorbance unit per minute. Activity is expressed as enzyme units per gram fresh weight, U g^{-1} FW.

The protein content of samples was determined according to the Coomassie Brilliant Blue G250 method using Bovine Serum Albumin as a standard, measured at the 595 nm colorimetric wavelength (Bradford, 1976). For the measurement of lipid peroxidation (MDA), the method designed by Sairam and Saxena (2000) was employed. For the analysis, leaf tissues (0.5 g) were homogenized with 5 ml of a 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 10,000 g for five minutes. To one milliliter of the supernatant, 4 mL of 20% v/v TCA containing 0.5% v/v thiobarbituric acid (TBA) was added. The solution was heated at 95°C for 30 min then quickly cooled on ice. The mixture was centrifuged again at 10,000 g for 5 min and the absorbance of the clean supernatant was measured at 532 and 600 nm.

$$\text{MDA}\,(\text{nmol g}^{-1}) = \frac{\text{Extract volume (ml)} \times \left[(A_{532} - A_{600})/(155\,\text{mM}^{-1}\,\text{cm}^{-1})\right]}{\text{sample amount (g)}} \times 10^3$$

A determination of the lycopene content was obtained according to the method of Barrett and Anthon (2001). One gram of tomato fruit was extracted with 10 mL of an ethanol:hexane solution (4:3). The mixture was centrifuged at 10,000 g for 10 min at room temperature then 100 µL of supernatant (0.01g) was added to 7 mL of an ethanol: hexane solution (4:3) mixture and vortexed. Following one hour incubation at room temperature, 1 mL of H_2O was added to the tubes and vortexed. The tubes were then kept in the dark in order for different phases to form. The top phase was obtained and measured at 503 nm against a hexane blank with a UV spectrophotometer (UV-1700, Shimadzu).

$$\text{Lycopene}\ (\mu\text{g g}^{-1}) = \frac{A_{503} \times 2.7}{172 \times (0.1\,\text{g ml}^{-1})} \times 537$$

The vitamin C content of fruits was determined following the methods of Oz (2002). Five grams of tomato fruit was homogenized in 25 mL of oxalic acid. The mixture was then centrifuged at 10,000 g for 10 min at room temperature. One milliliter of this mixture was added to 7 mL of a 1% oxalic acid solution and 8 mL of dye reagent. The dye reagent was prepared by dissolving 84 mg of $NaHCO_3$ in 80 mL of boiling distilled H_2O containing 100 mg of 2,6-dichloro phenol indophenol (2,6-DCPIP). The mixture was filtered and cooled, and diluted to 100 mL with d H_2O. Then, 25 mL of this solution was obtained, diluted to 500 mL with d H_2O, vortexed, and kept at 4°C until use. The mixture was then vortexed and measured at 518 nm against the oxalic acid and dye mixture.

Mineral content of tomato plants: The mineral content of the leaves and roots of tomato plants were determined according to the method of Chapman & Pratt (1961) with slight modifications. Samples burned at 500°C were homogenized in 5 mL 2N HCl. The homogenate obtained after filtration was analyzed by Inductively Coupled Plasma (ICP, Perkin Elmer) for the quantification of Na, K, Ca, and Mg ions. Chloride determinations for plant samples were obtained by the Mohr method using K_2CrO_7 as indicator in the titration of Cl ions with a $AgNO_3$ standard solution (Johnson & Ulrich, 1959; Kacar & Inal, 2008).

Statistical analysis: Data were subjected to an analysis of variance (ANOVA) at a significance level of 0.05 using Duncan's Multiple Range Test with the SPSS software program (Version 11.0). Data are presented as mean value ± the standard error.

Results

Tomato yield affected by salinity and companion plants: When tomato mono culture treatment (TM treatment), total DM formation and fruit yield in varied significantly among soil types (Table 2). Growth depression of tomato plants in high compared to low saline soils occurred rapidly. Here, total DM was 47, 19, and 24% lower in LHS, LMS, and LSS than in LNS treatment, respectively. Moreover, salinity induced fruit yield reduction was more pronounced than those in total DM. The decrease in fruit yield was 98, 71 and 16% in LHS, LMS, and LSS treatments when compared to LNS treatment, respectively. Leaf chlorophyll content in LHS treatment was more than 50% lower as compared to LNS treatment, which indicates degradation of chlorophyll molecules by excess formation of reactive oxygen species. Accordingly, CAT activity that detoxifies H_2O_2 increased also almost 6 fold in high saline TM treatment than in control plants (Table 4).

Presence of companion plants did not affect total DM or fruit yield in LNS treatment (non-saline) but promoted both DM and fruit yield under salinity. The fruit yields of tomato in TM treatments were 845.2, 295.7, and 20.3 g plant^{-1} in LSS, LMS, and LHS treatment, respectively. However, in TMS plants (when tomato plants grown with *S. soda*), fruit yields were 962.2, 564.7, and 229.7 g plant^{-1} in LSS, LMS, and LHS treatments, respectively. The data clearly showed that the effect of *S. soda* on fruit yield formation when planted with tomato was more pronounced specifically under high salinity. However, when *P. oleracea* was grown as companion plants, the fruit yield was almost similar to the plants in TM treatment.

Tomato leaf and root ion concentrations affected by salinity and companion plants: With increasing salinity levels, in soils Na and Cl content of the leaves increased significantly (Table 3). Here leaf Cl content increase only about two fold however leaf Na content increased about 10 fold when comparing TM plants grown under high and no-salinity. The latter indicates more resistance against the Cl uptake than Na. In high saline soils, leaf Na and Cl content was 46 and 28% lower in TMS treatment compared to TM. On the other hand, leaf Na and Cl content was about 28 and 11% in TMP treatment compared to TM plants. Overall treatments, root Na and Cl content remained almost constant or increased only slightly with the increase in salinity levels. The latter indicates that both companion plants reduced the Na and Cl uptake of tomato plants from the soils, while the effect was clearly greater in *S. soda* treatments.

The concentration of beneficial ions such as K, Ca, and Mg decreased with increase in salinity level in TM treatment (Table 3). However, the decrease was more pronounced for K and Ca. In TMS and TMP treatments, the decrease in K content due to salinity was still significant however plant Ca content was almost close to the non-saline control treatments. In line with the Na and Cl data, the beneficial impact of *S. soda* on beneficial ion uptake of tomato plant was found to be greater than that of *P. oleracea.*

Table 1. Physical and chemical characteristics of the soils (non-saline loam (LNS), slight saline loam (LSS), moderate saline loam (LMS), high saline loam (LHS) used in the experiment.

Salinity level	EC	pH	Na^+	Total C	Sand	Clay	Loam
	dS m^{-1}		(Meq L^{-1})	(%)	(%)	(%)	(%)
LNS	0.90	7.73	1.15	1.26	16	31	52
LSS	4.19	7.82	8.81	1.20	21	28	51
LMS	7.22	8.19	17.19	1.07	18	34	48
LHS	14.05	8.20	46.84	0.83	20	33	48

Table 2. Physiological growth parameters for tomato plants grown in saline soils either alone or in combination with companion plants.

Salinity level	Plant combination	Average FW (g)	Average DM (g)	Average fruit weight (g)	MSI (%)
LNS	TM	114.7 ± 3.9a	14.2 ± 0.4a	1004.4 ± 48.8a	32.6 ± 1.0a
	TMS	120.2 ± 8.2a	13.4 ± 0.8a	1020.1 ± 42.2a	32.4 ± 0.6a
	TMP	106.7 ± 6.6a	11.9 ± 0.7a	952.6 ± 21.6a	33.4 ± 5.8a
LSS	TM	98.1 ± 2.4b	10.8 ± 1.2a	845.2 ± 24.1a	48.2 ± 0.4a
	TMS	115.1 ± 4.5a	12.6 ± 2.2a	962.8 ± 18.3a	32.0 ± 2.0b
	TMP	100.0 ± 4.1b	10.9 ± 1.1a	896.7 ± 35.3a	34.6 ± 2.5b
LMS	TM	76.5 ± 3.2b	11.5 ± 1.3a	295.7 ± 33.2b	49.9 ± 1.4a
	TMS	110.7 ± 4.8a	12.0 ± .0.8a	564.7 ± 21.0a	34.3 ± 1.7b
	TMP	81.7 ± 3.1ab	8.9 ± 0.5a	462.9 ± 38.9a	35.6 ± 1.1b
LHS	TM	46.5 ± 1.47b	7.5 ± 0.77b	20.3 ± 5.77b	52.4 ± 2.13a
	TMS	72.5 ± 2.52a	9.7 ± 0.12a	229.7 ± 17.85a	34.6 ± 1.88b
	TMP	44.7 ± 2.66b	7.6 ± 0.08b	23.6 ± 3.45b	37.5 ± 1.79b

The $p \leq 0.05$ significance level for various plant combinations in each salinity level was indicated with different letters using Duncan's Multiple Range Test. The means are expressed (±) standard error. A tomato grown alone is expressed as (TM), the Tomato + *S. soda* companionship as (TMS), and the Tomato + *P. oleracea* companionship as (TMP)

Antioxidant levels affected by salinity and companion plants: With increasing salinity level soils tested in the present experiment, leaf chlorophyll concentration in TM treatments decreased significantly. In high saline TM treatments, chlorophyll concentration was almost 2/3 fold lower than in non-saline control TM plants. In moderate or high saline soils, leaf chlorophyll content of tomato plants was significantly higher in TMS and TMP treatment than in TM treatment ($p \leq 0.05$; Table 4). In line with the biomass data, chlorophyll content in TMS (0.5 mg g^{-1} leaf) was significantly higher than in TMP (0.3 mg g^{-1} leaf) treatment in high saline soils.

Expectedly, leaf proline, POX, and CAT levels also increased significantly (about 3 fold) in TM treatment when salinity level of soil increased. On the other hand, the latter increased only slightly in TMS treatment (not significant in many cases) when comparing salinity levels. Here, leaf proline concentrations in high saline soils were 15.8, 7.9, and 10.1 µmol g^{-1} leaf DM in TM, TMS and TMP treatment respectively. Whereas, proline levels of tomato leaves in non-saline soils were about 6.0 µmol g^{-1} leaf DM in all treatments. The latter clearly showed that when *S. soda* was used as companion plant in saline soils, tomato leaf chlorophyll content remains high whereas leaf proline content remains low. The MDA content (as a result of lipid peroxidation) was about 2 fold higher in tomato plants planted in LHS as compared to LNS soils in TM treatment. However, in LMS and LHS soils, tomato plants grown with companion plants had much lower MDA content (significant at $p \leq 0.05$). Furthermore, MDA levels were significantly lower TMS treatment than in TMP under high salinity situation.

Fruit quality affected by salinity and companion plants: Quality parameters of tomato fruit (e.g. EC, pH, vitamin C, and the lycopene content) were measured at harvest. Expectedly, salt stress increased EC values of tomato fruit juice in respective treatments. Here, EC values in TM was 5.5, 5.9, 8.3, 17.8 dS m^{-1} in LNS, LSS, LMS, and LHS treatments, respectively. When companion plants were co-cultivated with tomato plants (TMS and TMP), the EC values of fruits grown under moderate and high salinity conditions were remarkably lower than TM fruits ($p \leq 0.05$; Table 5). In line with that fruit lycopene content especially in TMS treatments were found to be significantly higher than fruits in TM treatment under salinity. Vitamin C content of the fruits was slightly higher under salinity, however the difference was not significant. Only under conditions of high salinity in LHS treatment, vitamin C content was slightly lower in TMS and TMP than in TM fruits.

Table 3. The leaf and root ion content for tomato plants grown in saline soils either alone or in combination with companion plants.

Salinity	Plant combination	Na g kg^{-1}		K g kg^{-1}		Ca g kg^{-1}		Mg g kg^{-1}		Cl g kg^{-1}	
		Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
LNS	TM	1.9 ± 0.1a	4.7 ± 0.2a	20.3 ± 0.9a	7.9 ± 0.1a	34.3 ± 1.6a	14.3 ± 1.3a	6.5 ± 0.4a	4.7 ± 0.2a	19.5 ± 1.5a	17.0 ± 1.0a
	TMS	1.7 ± 0.1a	4.3 ± 0.7a	20.8 ± 0.8a	8.4 ± 0.2a	36.5 ± 1.4a	15.6 ± 0.1a	6.3 ± 0.3a	4.5 ± 0.1a	18.0 ± 1.0a	15.0 ± 1.0a
	TMP	1.6 ± 0.1a	4.4 ± 0.5a	19.5 ± 1.1a	7.9 ± 0.1a	35.7 ± 3.1a	16.9 ± 0.1a	6.5 ± 0.2a	4.8 ± 0.0a	20.0 ± 1.0a	14.0 ± 1.0a
LSS	TM	9.9 ± 0.1a	10.5 ± 2.0a	15.6 ± 0.8a	7.0 ± 0.2a	32.2 ± 4.3a	17.2 ± 0.1a	5.9 ± 0.1a	4.8 ± 0.3a	34.0 ± 2.0a	20.0 ± 1.0a
	TMS	5.9 ± 0.1b	7.4 ± 0.3b	17.9 ± 0.5a	7.1 ± 0.1a	33.9 ± 7.6a	13.9 ± 0.1a	5.7 ± 0.1a	5.4 ± 0.1a	26.5 ± 1.5a	15.0 ± 0.0a
	TMP	6.6 ± 0.1b	6.9 ± 0.0b	15.6 ± 0.9a	7.3 ± 0.2a	33.0 ± 3.2a	16.6 ± 0.1a	6.0 ± 0.4a	5.2 ± 0.2a	31.0 ± 1.0a	17.0 ± 2.0a
LMS	TM	16.4 ± 1.1a	15.2 ± 0.3a	12.6 ± 0.8b	3.2 ± 0.2b	26.5 ± 0.9b	9.4 ± 0.1a	6.2 ± 0.7b	5.7 ± 0.3a	39.0 ± 1.0a	27.0 ± 2.0a
	TMS	9.1 ± 0.9b	10.0 ± 0.1b	16.7 ± 0.3a	4.1 ± 0.3a	32.6 ± 1.1a	13.3 ± 0.1a	7.0 ± 0.2a	6.4 ± 0.4b	27.5 ± 2.5b	20.0 ± 0.0b
	TMP	11.8 ± 0.5b	8.5 ± 0.9b	15.4 ± 0.4a	3.5 ± 0.0a	32.8 ± 0.9a	13.0 ± 0.1a	7.4 ± 0.8a	6.1 ± 0.0b	32.5 ± 2.5a	126.0 ± 1.0b
LHS	TM	22.2 ± 1.1a	19.0 ± 1.1a	9.6 ± 0.2b	2.3 ± 0.0b	22.9 ± 0.4b	7.79 ± 0.1a	6.2 ± 0.2b	5.5 ± 0.0a	44.5 ± 0.5a	135.0 ± 1.0a
	TMS	11.9 ± 0.3b	11.4 ± 1.6b	12.4 ± 0.3a	3.3 ± 1.2a	32.6 ± 1.3a	10.4 ± 0.1a	7.2 ± 0.2ab	6.1 ± 0.2a	32.0 ± 1.0b	28.0 ± 1.0b
	TMP	15.9 ± 0.6b	15.7 ± 0.8b	11.7 ± 0.9a	3.2 ± 0.0b	29.9 ± 1.3a	9.6 ± 0.1a	7.4 ± 0.3a	5.8 ± 0.0a	39.5 ± 0.5a	30.0 ± 1.0a

The p≤0.05 significance level for various plant combinations in each salinity level was indicated with different letters using Duncan's Multiple Range Test. The means are expressed (±) standard error. A tomato grown alone is expressed as (TM), the Tomato + *S. soda* companionship as (TMS), and the Tomato + *P. oleracea* companionship as (TMP)

Discussion

The effect of salt stress on plant development has been investigated by many researchers and it has been reported that the biochemical and physiological development of crop plants affected negatively, e.g. low photosynthesis efficiency and decreases in chlorophyll content (Mehta *et al*., 2010; Poór *et al*., 2011; Jamil & Rha, 2013); mineral deficiencies, and such as, K and Ca ions (Sohail *et al*., 2009; Ning *et al*., 2015; Bhuiyan *et al*., 2015), increases in membrane permeability (Mansour, 2013), the degradation of cell wall structures (Le Gall *et al*., 2015; Al-Harbi *et a*l., 2015). In the present study, increase in salinity levels decreased both total DM and fruit yield significantly. For most species, a 10% yield decrease reported when EC of a soil solution increases over the 4-8 dS m^{-1} range (Ventura *et al*., 2014). However, yield of maize (*Zea mays* L.) decreased by 21% for each unit increment in EC in the irrigation water (Blanco *et al*., 2008). In the present study, the latter was about 10-15% for each unit increment in EC in the irrigation water. Under salt stress, plants forced to produce more antioxidant enzymes (CAT, POX, APX, GR, etc.) to protect against the destructive effects caused by free oxygen radicals. Expectedly, in the present study, tomato plants produced more proline, and the activity of MDA, CAT and POX was much higher under salt stress (Table 3).

Due to their salinity tolerance and their ability to desalinate soils, in the past, halophytes have been used to improve crop yields (Grafienberg *et al*., 2003). Previous studies clearly showed that when the halophyte species *Salsola* spp., *Chenopodium* spp., and *Portulaca* spp. used in crop rotations in saline soils, crop yield may increase due to salt removal from soil via their harvested biomass (Grieve and Suarez, 1997; Agnihotri and Kumar, 2015). In recent years, these halophyte species have been used as companion plants in vegetable crops grown under saline-sodic conditions (Hasannuzman *et al*., 2014).

In the present experiment, when tomato plants were grown under saline conditions, significant decrease in chlorophyll content, biomass and fruit yield was measured. Use of both halophytic species as companion plants under excess salinity situations decreased plants susceptibility to salinity and enhanced total DM and fruit yield. On the other hand, when *S. soda* used as companion plants, tomato yielded much better than when *P. oleracea* was used. No remediation effects of *P. oleracea* under high saline conditions indicates that this halophyte most likely was not compatible for the given environmental conditions as compared to *S. soda*. Grafienberg *et al*. (2003) reported that *S. soda* plants resulted in no negative effects on tomato plants in a non-saline environment, while a higher density (15 g m^{-2}) of *P. oleracea* had negative effects on the yield of tomatoes. However in the present study, we did not observe any negative impact of *P. oleracea* on the yield of tomato plants under non-saline conditions.

Table 4. The biochemical response of tomato plants grown alone or in combination with companion plants under saline conditions.

Salinity level	Plant combination	Chlorophyll mg g^{-1}	Proline µmol g^{-1}	POX unit g^{-1}	CAT unit g^{-1}	MDA nmol g^{-1}
LNS	TM	0.6 ± 0.0a	6.2 ± 0.3a	5.7 ± 0.7a	0.4 ± 0.1a	4.3 ± 0.9a
	TMS	0.6 ± 0.1a	6.0 ± 0.1a	5.8 ± 0.2a	0.4 ± 0.2a	3.8 ± 0.2a
	TMP	0.6 ± 0.0a	5.9 ± 0.5a	6.1 ± 0.1a	0.4 ± 0.0a	4.7 ± 0.1a
LSS	TM	0.5 ± 0.0b	14.3 ± 0.6a	11.7 ± 1.5a	0.9 ± 0.1a	5.3 ± 0.5a
	TMS	0.6 ± 0.0a	6.2 ± 0.2b	5.3 ± 0.1b	0.5 ± 0.1b	5.1 ± 0.1a
	TMP	0.6 ± 0.0a	6.5 ± 0.5b	6.1 ± 0.9b	0.5 ± 0.0b	5.8 ± 0.2a
LMS	TM	0.3 ± 0.0b	15.3 ± 0.9a	11.0 ± 0.7a	1.2 ± 0.1a	6.6 ± 0.2a
	TMS	0.5 ± 0.0a	8.0 ± 0.3b	5.1 ± 0.9b	0.6 ± 0.1b	5.4 ± 0.2b
	TMP	0.4 ± 0.0a	9.5 ± 1.0b	7.3 ± 0.8b	0.6 ± 0.0b	5.9 ± 0.1b
LHS	TM	0.2 ± 0.0b	15.8 ± 2.0a	13.6 ± 1.4a	2.2 ± 0.3a	9.1 ± 0.1a
	TMS	0.5 ± 0.0a	7.9 ± 0.9b	6.6 ± 0.7b	0.6 ± 0.0b	5.6 ± 0.2c
	TMP	0.3 ± 0.0b	10.1 ± 1.1b	6.1 ± 0.9b	0.8 ± 0.1b	6.9 ± 0.4b

The p≤0.05 significance level for various plant combinations in each salinity level was indicated with different letters using Duncan's Multiple Range Test. The means are expressed (±) standard erors. A tomato grown alone is expressed as (TM), the Tomato + *S. soda* companionship as (TMS), and the Tomato + *P. oleracea* companionship as (TMP)

Table 5. The improvement of tomato fruit quality parameters with companion plants under various salinity levels.

Salinity level	Plant combination	EC dS m^{-1}	pH	Vitamin C mg 100 g^{-1}	Lycopene µg g^{-1}
LNS	TM	5.5 ± 0.3a	4.5 ± 0.0a	29 ± 0.5a	12.9 ± 1.8a
	TMS	5.0 ± 0.3a	4.4 ± 0.0a	33 ± 0.5a	13.5 ± 0.5a
	TMP	5.6 ± 0.2a	4.4 ± 0.1a	29 ± 1.5a	14.9 ± 1.5a
LSS	TM	5.9 ± 0.7a	4.3 ± 0.1a	32 ± 1.5a	7.3 ± 0.5a
	TMS	5.4 ± 0.2a	4.5 ± 0.0a	31 ± 1.5a	7.8 ± 0.5a
	TMP	5.7 ± 0.0a	4.3 ± 0.1a	28 ± 0.5a	8.6 ± 0.5a
LMS	TM	8.3 ± 0.2a	4.4 ± 0.1a	33 ± 2.5a	4.1 ± 0.2b
	TMS	6.9 ± 0.7b	4.3 ± 0.1a	30 ± 1.5a	5.8 ± 0.1a
	TMP	7.3 ± 0.3b	4.4 ± 0.1a	32 ± 1.0a	5.3 ± 0.3a
LHS	TM	17.8 ± 0.7a	4.2 ± 0.0a	41 ± 1.0a	0.5 ± 0.2b
	TMS	10.6 ± 0.9c	4.4 ± 0.3a	35 ± 2.0b	5.2 ± 1.8a
	TMP	13.7 ± 0.0b	4.3 ± 0.0a	37 ± 0.5b	0.6 ± 0.0b

The p≤0.05 significance level for various plant combinations in each salinity level was indicated with different letters using Duncan's Multiple Range Test. The means are expressed (±) standard erors. A tomato grown alone is expressed as (TM), the Tomato + *S. soda* companionship as (TMS), and the Tomato + *P. oleracea* companionship as (TMP)

Proline and MDA levels, accepted as common stress indices, were greatly reduced in tomato plants when they were grown with the companion plants under saline conditions. The latter can safely be attributed to the salt uptake companion plants that removed toxic ions such as Na and Cl from the rhizosphere of tomato plants. Our results contradict those of Zuccarini (2008) who reported that *P. oleracea* yielded better than *S. soda* in terms of ion uptake. Zuccarini (2008) also reported that *S. soda* caused excessive competition against *Solanum lycopersicon* due to its fast growth. However, the seed density of *S. soda* was almost eight times more than that of the *P. oleracea* used in this study. In the work presented here, the density of seeds for both species was equal in the pot culture. Therefore, in the given experimental conditions, it can safely conclude that use of both halophyte species did not cause any yield losses due to competition between halophyte species and tomato.

Due to the effect of companion plants, tomato plants took up much less toxic ions. The reduced uptake of toxic ions in TMS and TMP tomato plants due to companion plant activity reduced antioxidant enzyme activities significantly as compared to TM plants under high salinity situation. Similar findings were also reported for *S. soda* plants by Grafienberg *et al*. (2003) who suggested that the improvement of tomato plants by companion plants under saline conditions (1.3 and 6.5 dS m^{-1}) was achieved via the synthesis of substances used for fruit development instead of building up substances for mechanisms of stress tolerance. Reductions in stress metabolites and the uptake of toxic ions allowed tomato plants to use more energy to build up organic components such as lycopene and proteins instead of producing substances for defence mechanisms. In this study, salinity stress did not play an important role on the vitamin C content of tomato plants although salinity stress increased

slightly the fruit contents of vitamin C. Similar results were reported by Navarro *et al*. (2006) for squash fruits.

Conclusions

The present study clearly showed that the method provided here for soil and plant remediation using companion plants could be used for the improvement of saline soils and crop plant performance under excess salinity (as phyto-remediation technique) and is an alternative to other biochemical (hormones, amino acids, plant activators, and fertilizers), genetic (the transfer of stress-resistant genes to crop plants), physical (the improvement of plant growth media), and biological (the use of microorganisms) methods. Being low-cost and eco-friendly, the method would be beneficial in areas where soil stress is a big issue and could be applied for the short and long term improvement of crop plants.

The results indicate that the use of *S. soda* and *P. oleracea* as desalinating companion plants under slight and moderate salinity conditions are an attractive strategy for increasing tomato fruit production. In this study, we also found that the performance of *S. soda* under high salinity levels was much more remarkable for reducing the effect of salinity stress than *P. oleracea*.

Acknowledgments

This research is a part of the corresponding author's PhD thesis. Biochemical analyses were performed in the laboratory of Dr. Murat Dikilitas. Dr Dikilitas made substantial contributions to data interpretation, and to the drafting and revision of the manuscript. All authors read and approved the final manuscript.

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(Received for publication 25 March 2015)