

High salt induced oxidative damage and antioxidant response in tomato grafted on tobacco

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One of the major limitations on agricultural development in many countries is the high salinity of the groundwater used in irrigation. Grafted plants may exhibit phenotypic variations from scion and rootstock plants in terms of abiotic stress tolerance, and be a method for improvement of tolerance in agricultural practices. The aim of the present study was to investigate response of *Solanum lycopersicum* L. ('Elazığ') grafted on *Nicotiana tabacum* L. ('Samsun') and *Nicotiana rustica* L. ('Hasankeyf'), namely "Tomacco" plant (patent nr TR-2008-05391-B), to 10-d high NaCl irrigation. Physical development, chlorophyll a and b, total chlorophyll, total carotenoid, and anthocyanin levels were evaluated. Proline, lipid peroxidation, and electrolyte leakage levels were assayed in roots and leaves together with ascorbate peroxidase (APX) and catalase (CAT) activities. Considering alterations in chlorophyll contents, proline, malondialdehyde (MDA), and conductivity levels, and antioxidant enzyme activity levels scion and self-grafted plants seem to be more affected by salt treatments than tobacco and rootstock grafted plants. Tobacco roots seem to have better adaptive responses against salt stress in comparison to tomato as supported by changes in proline, APX, and CAT levels. Self-grafting experiments further supported grafting tomato onto tobacco rootstocks enhanced salt tolerance and adaptive response of scions and these changes seem to be dependent on rootstock rather than graft-induced changes. In conclusion, we demonstrated that previously defined graft unions of tomato on tobacco, which have increased fruit yield, had also enhanced tolerance to high salt stress and a promising technique for the cultivation of more salt tolerant varieties.

Key words: Grafting, *Solanum lycopersicum*, NaCl stress, *Nicotiana rustica*, *Nicotiana tabacum*.

INTRODUCTION

High salt concentration is a world wide problem causing a decrease in crop yields. One of the major limitations on agricultural development in many countries is the high salinity of the groundwater used in irrigation. In addition, extensive areas of irrigated land are becoming increasingly degraded by salinization and water logging resulting from over-irrigation and other forms of poor agricultural management (Ghassemi et al., 1995). Salinity affects approximately 20% of the world's cultivated land and nearly half of all irrigated land (Zhu, 2001).

Salinity inhibition of plant growth is the result of low osmotic potential of soil (water stress), nutritional imbalance, specific ion effect (salt stress) or a combination of these factors (Parvaiz and Satyawati, 2008). Plant species have developed different mechanisms to cope with these effects. The reduction of cellular osmotic potential by solute accumulation is one of the primary responses of plants to prevent the deleterious effects of salinity on osmotic adjustment. This can be achieved through the

accumulation of inorganic ions (Na⁺, Cl⁻, and K⁺) and/or compatible organic solutes (soluble carbohydrates, amino acids, proline, betaines, etc.) (Hasegawa et al., 2000). Environmental stress causes oxidative damage in nucleic acid, protein, and lipid molecules of plant tissues by disturbing the balance between the production of reactive oxygen species (ROS) and the quenching activity of the antioxidants (Spsychalla and Desborough, 1990). Various antioxidant molecules and enzymes contribute to the antioxidant defense mechanism of plants. Carotenoids, ascorbate, glutathione, and tocopherols are the primary antioxidant components. Some enzymes of the antioxidant defense of plants include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), peroxidases, and the enzymes of the ascorbate-glutathione cycle, ascorbate peroxidase (APX; EC 1.11.1.11), and glutathione reductase (GR; EC 1.15.1.1) (Aghaei et al., 2009).

Grafting has been widely used in agriculture, forestry and horticulture since the end of the 1920's. The process is used primarily for the purpose of changing varieties or cultivars, optimizing cross-pollination and pollination, benefiting from interstocks, perpetuating clones, producing certain plant forms, repairing damaged plants, increasing the growth rate of seedlings for the improvement of crop and vegetable yield and quality in agriculture, increasing resistance to biotic factors such as *Fusarium* species (Boughalleb et al., 2008) and abiotic stress tolerance of scion such as chilling (Zhou et al., 2007), and decreasing

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heavy metal toxicity (Rouphael et al., 2008). Grafted plants also exhibit phenotypic variations from scion and rootstock plants in terms of salinity tolerance, and grafting onto salt-tolerant rootstocks capable of inducing salt tolerance in the grafted shoots has been an effective method for improvement of salt tolerance in agricultural practices (Rivero et al., 2003). In the case of tomato plants, it was demonstrated that tomato seedlings grafted onto tomato rootstocks had lower NaCl accumulation in the xylem and leaves than ungrafted ones (Fernandez-Garcia et al., 2002), increased fruit yield under saline conditions (Martinez-Rodriguez et al., 2008), and improved photosynthesis performance (He et al., 2009). Similarly, grafting of tobacco onto tobacco rootstocks has been shown to induce salt resistance in tobacco with lower foliar concentrations of Na⁺ and Cl⁻, lipid peroxidation, and higher proline and sucrose concentrations (Ruiz et al., 2006). We have previously defined "Tomacco" plant (Patent nr TR-2008-05391-B) by grafting of tomato onto tobacco rootstocks as a novel and promising technique applicable to different tomato varieties for improvement of tomato plant performance and yield (Yasinok et al., 2009). In the present study, we addressed the question of whether grafting of tomato onto tobacco rootstocks affect physical development, pigment amounts, proline levels, lipid peroxidation, electrolyte leakage, and antioxidant enzymes activities under severe saline irrigation.

MATERIALS AND METHODS

Growth conditions and grafting

Locally cultivated tomato (*Solanum lycopersicum* L. 'Elazığ') and tobacco variants 'Samsun' (*Nicotiana tabacum* L.; S) and 'Hasankeyf' (*Nicotiana rustica* L.; H) were grown from seeds in a plastic greenhouse (Institute of Transplantation and Gene Sciences, Baskent University, Kazan-Ankara, Turkey) at 20-25 °C and 45-55% RH. Tobacco and tomato seeds were sown into cell plants containing seedling substrate (Seedling Substrate, Klasmann-Deilmann GmbH, Geeste, Germany) and grown for 50 and 25 d, respectively. Plants were transferred to soil in individual pots containing an animal based soil fertilizer (soil:fertilizer; 2:1), and grown for another 20-25 d in defined greenhouse conditions. The seeds of the scion were sown 30 d earlier than seeds of rootstocks to ensure necessary stem diameters at the time of grafting. Grafting was performed in the plastic greenhouses at the Institute of Transplantation and Gene Sciences, during 21-24 April 2009. The grafting of tomato plants on tobacco rootstocks was performed when the tobacco plants had 6-7 and the tomato plants had 3-4 real leaves. For the assessment of the effect of grafting on salinity, tomato plants were self grafted. Tomato plants (E) were grafted on tomato (E/E) and tobacco rootstocks (S/E and H/E for 'Elazığ' grafted on 'Samsun' and 'Hasankeyf', respectively) using the cleft grafting method, as previously described (Ersayin-

Yasinok et al., 2008). In brief, stock was cut down the middle of the stem at right angles with a sterile razor blade; the base of the scion was cut into a thin, narrow wedge and inserted into the cut of the rootstock. The graft union was covered with parafilm to enhance healing. Grafted plants were kept for 10 d under controlled conditions (22-25 °C and 85-90% RH).

Salt treatments and physical development

Grafted and non-grafted tomato and tobacco plants with 6-8 leaves were evaluated for salt treatments. For salt treatment groups, 0.2, 0.4, and 0.6 M NaCl (Merck, Darmstadt, Germany) solutions were added to cups under the pots every 2 d for 10 d. Control groups were irrigated with distilled water in the same time intervals. At least two experimental setups, containing at least four plants and two samplings of leaf and root tissues, were used for the analysis of each plant.

After 10 d of irrigation, stem length and leaf number were assessed for the evaluation of physical development. To assess the increasing of length and leaf number prior to salt treatment plant length and leaf number were registered. At the end of the salt treatment, length and leaf number of plants were measured and alterations in length and leaf number were expressed as a percentage change according to initial measurements.

Pigment amounts

In order to determine the levels of chlorophyll a and b, total chlorophyll (a+b) and total carotenoid (xanthophylls and carotenes; x+c), small pieces of leaves (0.03-0.04 g) were put in pure acetone (Merck) and incubated at 4 °C for 4 d. Absorbance was measured at 470, 644.8, and 661.6 nm, and the concentrations of a and b, and x+c in leaves were calculated according to Lichtenthaler and Buschmann (2001). Anthocyanin content of leaves (0.03-0.04 g) and roots (0.05-0.06 g) was determined by crashing tissue samples in 1 mL of 79% (v/v) methyl alcohol (Merck) and 1% HCl (Merck) (v/v) solution. Samples were incubated at 4 °C for 4 d and the absorbance of the sample solution was measured at 530 and 657 nm ($\epsilon \approx 34\ 300\ \text{M}^{-1}\ \text{cm}^{-1}$) (Mancinelli, 1990; Giusti and Wrolstad, 2001).

Proline levels

Proline amount in leaves (0.8-1.0 g) and roots (0.3-0.5 g) was determined according to method described by Bates et al. (1973). In brief, samples were homogenized with mortar and pestle in liquid nitrogen, and the homogenates were suspended in sulfosalicylic acid (Merck). Samples were centrifuged at 14 000 rpm for 10 min, at 4 °C and 0.1 mL supernatant was transferred into a solution of 0.2 mL acid ninhydrin (Merck), 0.2 mL acetic acid (Merck) and 0.1 mL sulfosalicylic acid. After a 96 °C, 1 h incubation, 1 mL toluene (Merck) was added and samples were centrifuged at 14 000 rpm for 5 min. Upper phase was transferred into quartz cuvettes and absorbance was recorded at 520 nm

against toluene. Proline amount was calculated by using proline (Sigma-Aldrich, St. Louis, Missouri, USA) standard curve, and results are expressed as mg proline in g FW.

Lipid peroxidation

Lipid peroxidation was assayed by determining malondialdehyde (MDA) amount, a product of lipid peroxidation (Ohkawa et al., 1979). In brief, leaf and root samples were homogenized with mortar and pestle in liquid nitrogen, and the homogenates were suspended in trichloroacetic acid (TCA; Merck). Samples were centrifuged at 12 000 rpm for 15 min and supernatant was mixed with 1:1 volume of thiobarbituric acid (TBA; Sigma-Aldrich) in TCA as blank. Following 25 min incubation at 96 °C, samples were cooled to room temperature and centrifuged at 10 000 rpm for 5 min. Absorbance of the supernatant was recorded at 532 and 600 nm against TBA in TCA. After subtracting the non-specific absorbance at 600 nm, MDA concentration was calculated by its extinction coefficient of 155 mM⁻¹ cm⁻¹.

Electrolyte leakage (membrane permeability) test

Electrolyte leakage was determined by measurement of electrolytes that leaked from leaves and roots according to the method described by Nanjo et al. (1999). For the measurement of the conductance of leaves and roots, 4-5 leaves per plant and total root tissue, respectively, were immersed into 0.4 M mannitol (Merck) solution and incubated for 3 h at room temperature. Electrical conductance (C1) was measured by EC Meter (ECO 401, Adwa, Romania). After boiling the sample for 15 min, electrical conductance (C2) was measured again for the determination of total ion concentration by complete membrane disintegration. The percentage of ion leakage was calculated according to the formula (Nanjo et al., 1999): Electrolyte leakage (%) = (C1/C2) × 100.

Enzyme activities

For determination of APX activity (Wang et al., 1991), samples (leaves and roots) were homogenized with mortar and pestle in liquid nitrogen, and the homogenates were suspended in Tris-HCl (50 mM, pH 7.2) buffer containing 2% poly(vinylpolypyrrolidone) (Sigma), 2 mM ascorbate (Sigma), and 1 mM EDTA (Applichem, St. Louis, Missouri, USA). After centrifugation at 12 000 rpm for 20 min, at 4 °C, total protein amount in supernatants were determined according to the Bradford method (Bradford, 1976); 100 µg total protein was added to the assay solution (50 mM pH 6.6 potassium phosphate buffer with 2.5 mM ascorbate), and the reaction was initiated with the addition of H₂O₂. Decrease in the absorbance of ascorbate was recorded at 290 nm for 3 min against assay solution ($\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$).

For determination of CAT activity (Chance and Mahly, 1995), samples were homogenized with mortar and pestle in liquid nitrogen, and the homogenates were suspended

in suspension buffer (50 mM Tris-HCl, pH 7.8). After centrifugation at 12 000 rpm for 20 min, at 4 °C, total protein amount in supernatants was determined according to the Bradford method; 100 µg total protein was added to a potassium phosphate buffer (50 mM, pH 7) and a reaction was initiated with the addition of H₂O₂. Decrease in the absorbance H₂O₂ was recorded at 240 nm for 3 min against assay solution ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$).

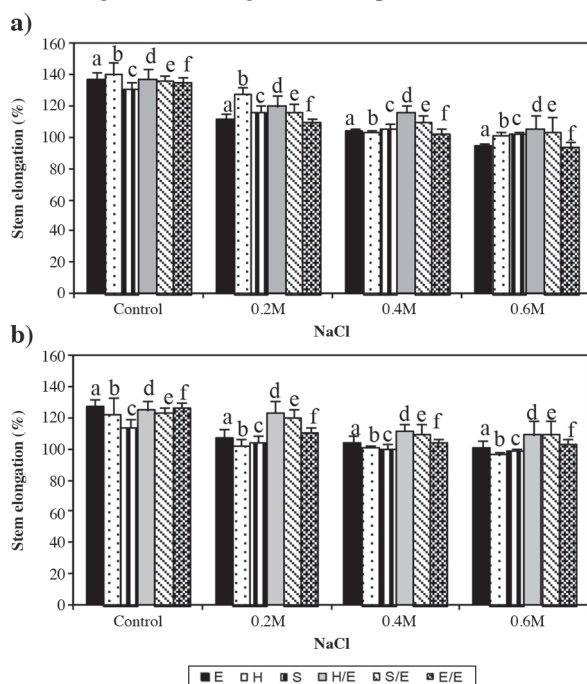
Statistical analysis

Statistical analyses were performed using SPSS 11.5 software (IBM Corporation, Armonk, New York, USA). All data are expressed as mean ± standard error of the means (SEM). Mean difference between the control and the salt treated groups of two cultivars were statistically evaluated using one-way ANOVA at 0.05 level and post hoc Tukey analyses were carried out to find groups whose mean differences were significant.

RESULTS

Physiological changes under salt stress

Salt treatment caused a reduction in stem elongation and leafing (Figure 1) after a 10-d irrigation period; 0.2, 0.4, and 0.6 M salt treated non-grafted tomato (E) and self-grafted (E/E) plants had significantly decreased stem length and leafing when compared with untreated



Letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis.

E: Tomato 'Elaziğ', H: rootstock 'Hasankeyf', S: rootstock 'Samsun', H/E: 'Elaziğ' grafted on 'Hasankeyf', S/E: 'Elaziğ' grafted on 'Samsun', E/E: self-grafted 'Elaziğ'.

Figure 1. Effect of 10-d NaCl treatment on stem elongation (a) and leafing (b) in tomato grafted on tobacco.

controls. On the other hand, stem elongation of tobacco and S/E plants significantly decreased in 0.4 and 0.6 M salt treatments whereas the reduction was significant only for the 0.6 M treatment group of H/E plants (Figure 1a). Similarly, there was a significant decrease in leafing of non-grafted and self-grafted tomato plants of all salt treatment groups (Figure 1b). Reductions in leafing were significant in 0.4 and 0.6 M treatment groups of tobacco plants, where leafing decreased only in 0.6 M treatment groups of grafted plants. Considering stem elongation and leafing of control plants, reduction in these parameters was less in grafted plants when compared with tomato and self-grafted plants in all treatment groups.

Effect of salt treatment on pigment amount

Table 1 demonstrates chlorophyll a and b, carotenoid and anthocyanin contents of the control group. Table 2 summarizes the effects of salt treatment on the chlorophyll and carotenoid levels of plants. Chlorophyll a content in leaves of 0.4 and 0.6 M salt treated ‘Elaziğ’ and self-grafted ‘Elaziğ’ plants significantly decreased when compared

with the control group whereas there were significant reductions in leaf chlorophyll a contents of 0.6 M salt treated tobacco and grafted plants. There was a reduction in chlorophyll b content of 0.4 and 0.6 M salt treated ‘Elaziğ’ plants and all salt treated groups of self-grafted plants. As in chlorophyll a, chlorophyll b reductions were significant for 0.6 M salt treated tobacco and grafted plants. Except for the H/E plants, a decrease in total chlorophyll levels in tobacco and grafted plants due to 0.6 M salt treatment was observed. All salt treated self-grafted plants, and 0.4 and 0.6 M salt treated non-grafted plants had considerably lower total chlorophyll amounts when compared with control plants. Chlorophyll a to b ratio significantly increased in leaves of salt treated self-grafted plants. Similarly, the ratio increased in 0.4 and 0.6 M salt treated tomato plants and 0.6 M treatment groups of S and S/E plants. Levels of carotenoid significantly decreased in leaves of 0.6 M salt treated ‘Hasankeyf’ plants, whereas total chlorophyll to carotenoid ratio significantly decreased in all self-grafted salt treated plants, and 0.6 M salt treated plants except for the H group.

Table 1. Pigment contents (chlorophyll a and b, total chlorophyll (a+b), a/b ratio, total carotenoid (x+c), and total chlorophyll to carotenoid ratios (a+b/x+c), and anthocyanin) of control plants.

Tissue	Plants													
	E		H		S		H/E		S/E		E/E			
	L	R	L	R	L	R	L	R	L	R	L	R		
a, mg g ⁻¹ FW	0.80±0.07	-	0.28±0.01	-	0.57±0.01	-	0.88±0.14	-	1.04±0.09	-	1.03±0.02	-		
b, mg g ⁻¹ FW	0.46±0.09	-	0.14±0.01	-	0.37±0.02	-	0.58±0.22	-	0.75±0.04	-	0.66±0.15	-		
a + b, µg g ⁻¹ FW a/b	1.14±0.17	-	0.41±0.01	-	0.96±0.02	-	1.45±0.33	-	1.78±0.04	-	1.82±0.09	-		
(Ratio)	2.05 ± 1.18	-	1.95±0.08	-	1.43±0.01	-	1.87±0.46	-	1.40±0.20	-	1.41±0.18	-		
x+c, mg g ⁻¹ FW (a+b)/(x+c)	0.18±0.01	-	0.10±0.00	-	0.13±0.01	-	0.20±0.05	-	0.21±0.05	-	0.19±0.02	-		
(Ratio)	6.58±0.99	-	4.30±0.01	-	8.33±0.40	-	8.10±3.03	-	9.01±1.82	-	10.80±1.80	-		
Anthocyanin, µmol g ⁻¹ FW	0.30±0.01	0.17±0.02	0.05±0.01	0.28±0.03	0.08±0.01	0.22±0.02	0.67±0.10	0.24±0.01	1.18±0.02	0.44±0.09	0.35±0.08	0.15±0.01		

Results are presented as values ± SE.

E: Tomato ‘Elaziğ’, H: rootstock ‘Hasankeyf’, S: rootstock ‘Samsun’, H/E: ‘Elaziğ’ grafted on ‘Hasankeyf’, S/E: ‘Elaziğ’ grafted on ‘Samsun’, E/E: self-grafted ‘Elaziğ’, L: leaf, R: root.

Table 2. Effect of NaCl treatment on leaf chlorophyll a and b, total chlorophyll (a+b), a/b ratio, total carotenoid (x+c), and total chlorophyll to carotenoid ratios (a+b/x+c) relative to control groups in tomato grafted on tobacco.

NaCl		Leaf chlorophyll and carotenoid change (relative to control; % ± SE)					
		a	b	a+b	a/b	x+c	(a+b)/(x+c)
0.2 M	E	92.52 ± 11.41	79.92 ± 11.02	82.36 ± 8.88	120.97 ± 11.53	117.71 ± 6.14	82.49 ± 2.51
	H	98.10 ± 5.68	100.73 ± 2.98	99.82 ± 4.83	100.88 ± 0.77	101.02 ± 10.45	95.81 ± 12.13
	S	99.46 ± 0.96	97.36 ± 2.70	96.94 ± 0.04	109.30 ± 4.90	95.70 ± 6.00	94.40 ± 5.96
	H/E	84.56 ± 8.84	67.53 ± 11.59	71.99 ± 8.03	122.07 ± 9.31	115.06 ± 9.25	71.76 ± 7.23
	S/E	84.80 ± 10.44	69.37 ± 0.99	80.35 ± 16.51	128.31 ± 25.29	116.42 ± 8.20	68.45 ± 8.74
	E/E	77.28 ± 5.67	49.27 ± 6.32g	61.86 ± 5.50k	179.64 ± 10.12p	118.10 ± 4.03	42.49 ± 2.84u
0.4 M	E	70.29 ± 6.63a	72.93 ± 13.73h	68.30 ± 7.091	137.74 ± 15.92q	118.69 ± 9.38	59.03 ± 3.33v
	H	98.82 ± 5.06	101.36 ± 9.95	98.95 ± 1.36	101.27 ± 12.81	84.83 ± 3.57	90.60 ± 11.53
	S	98.19 ± 3.57	83.95 ± 6.16	78.98 ± 2.00	124.59 ± 6.40	97.83 ± 2.88	68.75 ± 3.33
	H/E	88.91 ± 11.85	65.87 ± 9.91i	71.06 ± 7.25	123.92 ± 12.22	112.00 ± 8.57	71.80 ± 7.14
	S/E	84.93 ± 3.13	70.45 ± 0.70	83.45 ± 3.03	133.49 ± 4.76	114.54 ± 10.19	69.51 ± 3.14
	E/E	57.70 ± 1.96b	34.20 ± 1.83g	45.24 ± 1.74k	187.65 ± 4.89p	117.68 ± 5.30	46.70 ± 1.98u
0.6 M	E	54.09 ± 13.43a	35.14 ± 4.23h	45.15 ± 6.011	134.15 ± 15.25q	119.01 ± 19.71	44.17 ± 6.92v
	H	44.54 ± 8.53c	53.47 ± 7.06e	47.91 ± 8.11m	102.56 ± 3.00	62.53 ± 14.98t	81.15 ± 15.92
	S	67.18 ± 8.11d	48.90 ± 6.97f	58.56 ± 7.46n	146.48 ± 4.72r	94.51 ± 6.26	57.12 ± 5.34z
	H/E	82.79 ± 19.19e	43.02 ± 8.60i	71.51 ± 18.10	130.82 ± 11.49	112.64 ± 17.59	55.61 ± 9.17w
	S/E	56.09 ± 5.37f	22.58 ± 2.72j	36.47 ± 0.49o	192.75 ± 14.98s	113.44 ± 12.14	45.06 ± 1.17y
	E/E	59.35 ± 4.53b	33.46 ± 4.21g	45.91 ± 4.00k	200.43 ± 14.30p	115.23 ± 4.15	38.41 ± 1.38u

Different letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis.

E: Tomato ‘Elaziğ’, H: rootstock ‘Hasankeyf’, S: rootstock ‘Samsun’, H/E: ‘Elaziğ’ grafted on ‘Hasankeyf’, S/E: ‘Elaziğ’ grafted on ‘Samsun’, E/E: self-grafted ‘Elaziğ’.

Anthocyanin levels in leaves and roots of 'Elazığ' and self-grafted 'Elazığ' plants did not change (Table 3); however, 0.4 and 0.6 M salt treatment caused an increase in anthocyanin levels of 'Hasankeyf' and 'Samsun' leaves, and 0.6 M salt treatment caused an increase in grafted tomato plants. The 0.4 and 0.6 M salt treated 'Samsun' and tomato plants grafted on 'Samsun' rootstocks, and 0.6 M salt treated 'Hasankeyf' and tomato plants grafted on 'Hasankeyf' rootstocks had elevated levels of anthocyanin in roots (Table 3).

Effect of salt treatment on proline amount

Proline levels in leaves and roots of control plants are presented in Table 4. There was a considerable increase in leaf proline levels of 'Elazığ' and self-grafted plants in all salt treatment groups (Figure 2). Increases in proline levels of grafted plants were relatively lower when compared with E and E/E groups. However, proline amount in

Table 3. Effect of NaCl treatment on leaf and root anthocyanin levels relative to control groups in tomato grafted on tobacco.

NaCl		Anthocyanin change (Relative to control; % ± SEM)	
		Leaf	Root
0.2 M	E	96.82 ± 4.49	151.86 ± 57.40
	H	224.10 ± 59.27	105.03 ± 2.34
	S	169.03 ± 14.43	129.36 ± 12.07
	H/E	139.55 ± 20.77	102.56 ± 4.60
	S/E	152.80 ± 9.65	124.90 ± 9.97
	E/E	100.15 ± 10.75	101.85 ± 12.20
0.4 M	E	106.76 ± 17.75	156.72 ± 24.06
	H	279.14 ± 30.95a	116.05 ± 12.78
	S	278.10 ± 67.36b	167.17 ± 7.07e
	H/E	175.53 ± 19.85	128.19 ± 22.56
	S/E	155.19 ± 10.95	178.28 ± 2.39f
	E/E	127.66 ± 11.33	123.50 ± 22.91
0.6 M	E	125.80 ± 4.33	149.41 ± 20.08
	H	364.33 ± 40.14a	148.02 ± 9.80g
	S	376.35 ± 44.88b	177.35 ± 9.96e
	H/E	183.48 ± 21.24c	167.30 ± 0.33h
	S/E	178.14 ± 7.02d	182.51 ± 17.75f
	E/E	142.17 ± 11.14	128.31 ± 12.31

Different letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis.

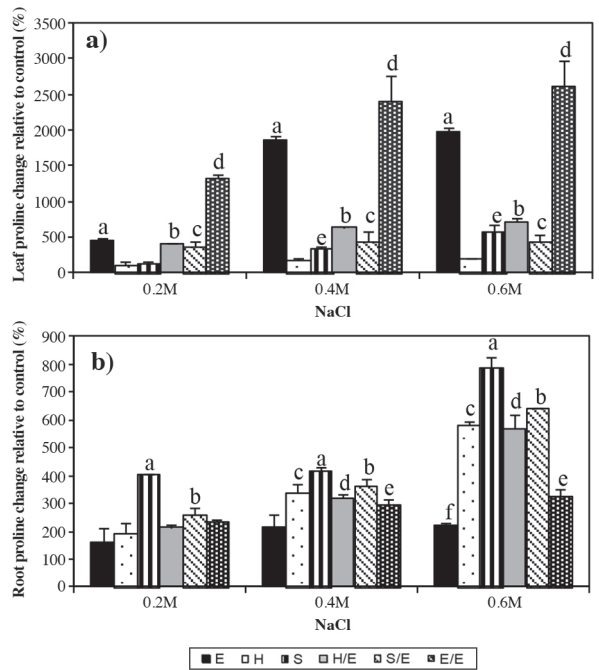
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Table 4. Proline and malondialdehyde (MDA) amounts, membrane permeability, ascorbate peroxidase (APX) and catalase (CAT) activities of control plants in tomato grafted on tobacco.

Tissue	Plants											
	E		H		S		H/E		S/E		E/E	
	L	R	L	R	L	R	L	R	L	R	L	R
Proline, mg g ⁻¹ FW	0.03±0.00	0.10±0.03	0.07±0.00	0.09±0.00	0.02±0.02	0.06±0.01	0.12±0.01	0.06±0.01	0.15±0.04	0.06±0.01	0.04±0.01	0.14±0.02
MDA, nmol g ⁻¹ FW	5.80±0.28	1.76±0.21	6.47±0.82	1.86±0.13	4.81±0.85	3.73±0.32	9.41±1.41	2.17±0.31	9.57±1.56	1.51±0.67	7.90±1.09	2.98±0.34
Membrane permeability, %												
APX, μmol ASC min ⁻¹ mg ⁻¹ protein	7.14±0.59	44.66±8.75	14.59±4.11	16.19±3.79	11.92±4.56	25.42±5.50	8.03±0.72	40.15±2.65	9.66±1.23	39.04±4.65	9.87±1.30	28.92±3.99
CAT, nmol H ₂ O ₂ min ⁻¹ mg ⁻¹ protein	0.73±0.36	2.64±0.78	2.55±0.07	16.25±1.12	6.82±1.61	6.31±1.12	1.70±0.45	17.27±3.27	2.05±0.49	9.92±0.39	0.39±0.05	2.27±0.43
	4.36±1.75	115.08±49.23	18.81±1.60	763.63±90.52	38.33±0.97	467.10±84.33	5.20±1.66	329.43±78.32	13.22±5.83	246.97±59.42	193.78±25.20	39.68±5.95

Results are presented as values ± SE.

E: Tomato 'Elazığ', H: rootstock 'Hasankeyf', S: rootstock 'Samsun', H/E: 'Elazığ' grafted on 'Hasankeyf', S/E: 'Elazığ' grafted on 'Samsun', E/E: self-grafted 'Elazığ', L: leaf, R: root.



Letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis.

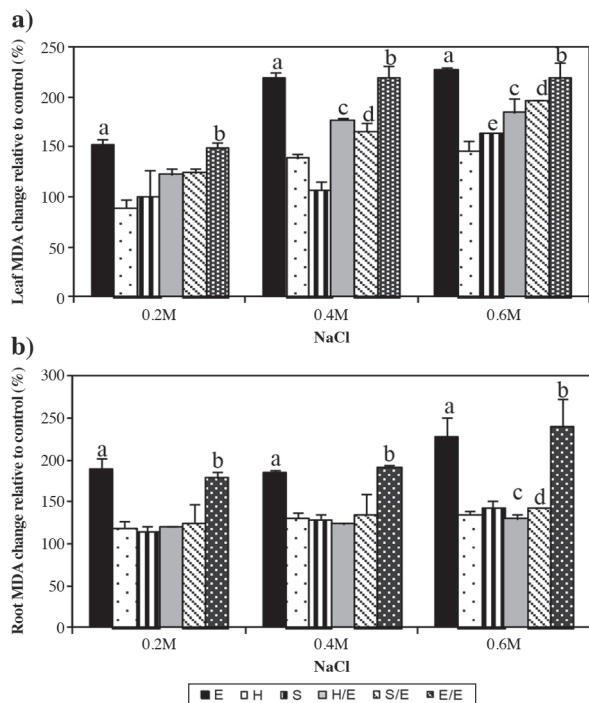
E: Tomato 'Elazığ', H: rootstock 'Hasankeyf', S: rootstock 'Samsun', H/E: 'Elazığ' grafted on 'Hasankeyf', S/E: 'Elazığ' grafted on 'Samsun', E/E: self-grafted 'Elazığ'.

Figure 2. Effect of NaCl treatment on proline levels of leaves (a) and roots (b) relative to the control groups in tomato grafted on tobacco.

'Samsun' leaves increased significantly due to 0.4 and 0.6 M salt treatment whereas there was no significant change in the leaves of 'Hasankeyf' plants.

Effect of salt treatment on MDA levels

The MDA levels of leaves and roots of the control groups are summarized in Table 4. Lipid peroxidation increased significantly in leaves of salt treated 'Elazığ' and self-grafted 'Elazığ' plants (Figure 3a). Although, 0.4 and 0.6 M salt treatment caused a significant increase in MDA levels in the leaves of the grafted 'Elazığ'



Letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis. E: Tomato 'Elazığ', H: rootstock 'Hasankeyf', S: rootstock 'Samsun', H/E: 'Elazığ' grafted on 'Hasankeyf', S/E: 'Elazığ' grafted on 'Samsun', E/E: self-grafted 'Elazığ'.

Figure 3. Effect of NaCl treatment on MDA levels of leaves (a) and roots (b) relative to the control groups in tomato grafted on tobacco.

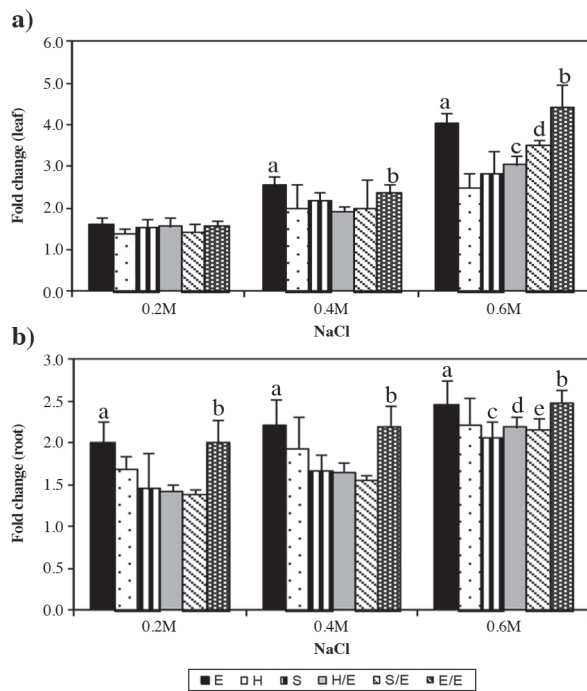
plants, percent change in leaf MDA levels of the grafted plants were lower than that in E and E/E plants, which indicates higher oxidative damage in the leaves of E and E/E plants. Concordantly, lipid peroxidation was higher in roots of salt treated E and E/E plants when compared to tobacco and tobacco rootstock grafted tomato plants (Figure 3a).

Effect of salt treatment on leaf and root membrane permeability

The permeability of leaf and root membranes of the control groups are summarized in Table 4. In concordance with changes in lipid peroxidation levels, results of the electrolyte leakage test demonstrated that salt treated E and E/E plants had considerably higher increases in leaf and root membrane permeability when compared with that of H/E and S/E plants (Figure 4). Leaf membrane permeability of tobacco cultivars did not change due to salt treatment, whereas the levels increased in root membranes after 0.6 M salt treatment.

Effect of salt treatment on enzyme activities

Table 4 demonstrates the APX and CAT activities of the control groups. APX activity significantly increased in leaves of all salt treated 'Elazığ' plants and 0.4 and 0.6 M salt treated self-grafted 'Elazığ' plants (Table 5). On



Letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis. E: Tomato 'Elazığ', H: rootstock 'Hasankeyf', S: rootstock 'Samsun', H/E: 'Elazığ' grafted on 'Hasankeyf', S/E: 'Elazığ' grafted on 'Samsun', E/E: self-grafted 'Elazığ'.

Figure 4. The effect of NaCl treatment on membrane permeability of leaves (a) and roots (b) relative to the control groups in tomato grafted on tobacco.

the other hand, 0.4 and 0.6 M salt treated S and 0.6 M salt treated S/E groups had elevated APX activities in leaves. Similarly, all salt treatment groups of S and S/E plants had increased root APX activity, whereas the increments were significant in the roots of 0.4 and 0.6 M salt treatment groups of E and H plants.

CAT activity increased in leaves of salt treated E plants and 0.4 and 0.6 M salt treated H/E plants (Table 5). Conversely, there was a significant decline in CAT activity in leaves of S plants due to salt treatment. Similarly, 0.6 M salt treated E and self-grafted plants had significantly increased root CAT activity, whereas H and H/E plants had decreased CAT activity in roots.

DISCUSSION

Salinity decreases the crop quality and yield by imposing toxic ion effect and water stress, a condition of the reduced ability of a plant to extract water from its external environment and transport it through tissues. At the physiological level, salt stress adversely affects various growth and development parameters of plants. At the cellular level, salt stress enhances ROS production, which in turn results in alterations in the antioxidant defense of plants. The extent of these physiological and biochemical effects determines the salt tolerance/sensitivity of the plants.

Table 5. Effect of NaCl treatment on ascorbate peroxidase (APX) and catalase (CAT) activities of leaves and roots relative to control groups in tomato grafted on tobacco.

NaCl		Change in APX activity (Relative to control; % \pm SEM)		Change in CAT activity (Relative to control; % \pm SEM)	
		Leaf	Root	Leaf	Root
0.2 M	E	181.05 \pm 28.99b	109.57 \pm 6.45	161.15 \pm 5.38m	117.89 \pm 10.45
	H	118.46 \pm 13.99	103.06 \pm 4.29	73.59 \pm 2075	43.03 \pm 3.33
	S	167.84 \pm 7.48	152.68 \pm 31.34b	27.34 \pm 2.10k	81.34 \pm 21.26
	H/E	120.14 \pm 14.19	97.67 \pm 7.05	151.36 \pm 8.26	86.81 \pm 12.16
	S/E	166.51 \pm 25.45	157.06 \pm 11.39d	120.40 \pm 25.83	69.17 \pm 12.21
	E/E	141.13 \pm 26.52	96.56 \pm 34.17	95.21 \pm 13.49	128.07 \pm 12.62
0.4 M	E	176.44 \pm 11.26b	146.01 \pm 5.47c	171.83 \pm 30.69m	135.35 \pm 30.43
	H	146.95 \pm 25.32	133.42 \pm 1.31a	75.43 \pm 4.44	48.42 \pm 10.05
	S	211.95 \pm 10.52a	219.07 \pm 38.78b	21.71 \pm 2.11k	77.46 \pm 3.11
	H/E	125.45 \pm 10.11	122.36 \pm 33.30	168.34 \pm 19.82s	70.86 \pm 0.77
	S/E	154.27 \pm 21.79	184.34 \pm 8.52d	123.47 \pm 18.82	70.46 \pm 5.23
	E/E	264.97 \pm 46.75c	143.88 \pm 16.05	91.92 \pm 34.18	123.02 \pm 20.28
0.6 M	E	257.15 \pm 30.40b	171.54 \pm 6.19c	314.24 \pm 69.57m	200.42 \pm 13.78t
	H	226.60 \pm 30.44d	165.32 \pm 2.14a	71.06 \pm 12.80	39.82 \pm 0.10p
	S	231.02 \pm 19.60a	245.36 \pm 32.41b	26.22 \pm 4.86k	67.16 \pm 2.20
	H/E	132.03 \pm 29.90	160.99 \pm 1.43f	255.61 \pm 15.41s	39.70 \pm 5.42q
	S/E	191.58 \pm 20.55e	210.06 \pm 9.68d	132.63 \pm 27.98	73.02 \pm 14.74
	E/E	293.54 \pm 77.10c	134.40 \pm 19.54	92.97 \pm 15.99	153.76 \pm 5.81r

Different letters represent significant difference between control and treatment groups ($P < 0.05$) according to one-way ANOVA analysis.

E: Tomato 'Elazığ', H: rootstock 'Hasankeyf', S: rootstock 'Samsun', H/E: 'Elazığ' grafted on 'Hasankeyf', S/E: 'Elazığ' grafted on 'Samsun', E/E: self-grafted 'Elazığ'.

Grafting has been used in the production of many crop species of economical importance, including tomato, for the purpose of enhancing production. So far the technique has been well established for increasing the tolerance of relatively sensitive scions to soil pathogens. More recently, the use of the technique has been extended to increase tolerance against abiotic factors. For example, tomato production, which is one of the most widely grown crops in the world, is often concentrated in semi-arid regions and saline waters are frequently used for irrigation purposes (Martinez-Rodriguez et al., 2008). To date, few studies have demonstrated improvement of salt tolerance of crop cultivars by grafting onto relatively tolerant cultivars (Fernandez-Garcia et al., 2002; Martinez-Rodriguez et al., 2008; Zhu et al., 2008; He et al., 2009). Previously, we defined grafting of tomato cultivars onto different tobacco rootstocks as a novel and comparable technique to tomato-tomato grafting (Yasinok et al., 2009). In that study, a 30% increase in total fruit yields was obtained by grafting 'Elazığ' onto *N. tabacum* 'Samsun'. In the present study, the effects of grafting tomato onto two locally cultivated tobacco species in Turkey, namely 'Hasankeyf' and 'Samsun', were studied for their physiological and biochemical responses under increasingly higher NaCl stress. Plants were irrigated with 0.2, 0.4, and 0.6 M NaCl water for 10 d. Stem lengths and the leafing of the plants (Figure 1) demonstrated that salt treatments caused significant decline in growth of 'Elazığ' and self-grafted 'Elazığ' plants, whereas growth retardation became considerable only above 0.4 M for tobacco rootstock grafted tomato plants.

Analysis of the chlorophyll content of leaves demonstrated that high salt concentration caused a reduction in chlorophyll a, b, and total chlorophyll amounts (Table 2). Previously it was reported that salinity could affect chlorophyll concentration of leaves through

inhibition of the synthesis of chlorophyll or through an acceleration of its degradation (Reddy and Vora, 1986). In addition to total chlorophyll content, a significant decrease in photosynthetic quantum and in net CO₂ uptake were also observed in two sorghum varieties exposed to high salinity (> 0.2 M) (Netondo et al., 2004). As such, decreases in chlorophyll contents seem to be correlated to the extent of salt stress that is encountered by the leaves. Accordingly, chlorophyll content of tobacco and grafted tomato plants seems to be less effected than that of 'Elazığ' and self-grafted 'Elazığ' plants, considering the percentage decline in these parameters and that the significant declines were observed above 0.4 M in tobacco and grafted plants. Chl b is found exclusively in the pigment antenna system, whereas Chl a is present in the reaction centers of photosystems (PS) I and II and in the pigment antenna. The light-harvesting pigment protein LHC-I of the photosynthetic pigment system PS I has a higher a/b ratio than that of LHC-II of PS II. The level of LHC-II of PS II is variable and shows a light adaptation response (Lichtenthaler and Buschmann, 2001). Increase in the chl a/b ratio of 'Elazığ' and self-grafted plant leaves may be related to the enlargement of the PS I system. The weight ratio of chlorophylls a and b to total carotenoids is an indicator of the greenness of plants. Lower values for the ratio (a+b)/(x+c) are an indicator of senescence, stress, and damage to the plant and the photosynthetic apparatus, which is expressed by a faster breakdown of chlorophylls than carotenoids (Lichtenthaler and Buschmann, 2001). Reduction in the ratio of leaves of 'Elazığ' and self-grafted plants was more considerable when compared to tobacco and grafted plants (i.e. there was no significant change in the ratio for 0.2 and 0.4 M salt treated tobacco and grafted plants), which demonstrates that non-grafted and self-grafted plants were more severely affected by salt treatments. In addition, decrease in chlorophyll contents

and $(a+b)/(x+c)$ signifies that self-grafted plants seem to be less tolerant of the lethal effects of salt when compared with non-grafted 'Elazığ' plants. The difference may be related to graft induced unfavorable changes in self-grafted plants, which seem to be well compensated by high stem water contents of tobacco rootstocks (Yasinok et al., 2009).

Anthocyanins are natural, highly water soluble pigments found mainly in the vacuoles of many plant species. Anthocyanin biosynthesis may vary between the different developmental stages and may be induced by environmental factors such as visible and UVB radiation, cold temperatures and water stress. For example, anthocyanin accumulation has been shown to be induced by sucrose (Hara et al., 2004). The subsequent production and localization of anthocyanins in the root stem and leaf tissues may allow the plant to develop resistance to a number of environmental stresses (Chalker-Scott, 1999). The accumulation of water soluble solutes in plant tissues helps plants to maintain turgidity in high levels of external ion concentrations and uptake of water, which is the basis of osmoprotection in salt tolerant plants. Anthocyanin levels increased in leaves of tobacco and grafted plants in salt concentration above 0.4 M, whereas the levels remained unchanged in the leaves of 'Elazığ' and self-grafted 'Elazığ' plants (Table 3). Although its accumulation in roots was less when compared with that of leaf tissue, 0.4 M salt treatment and salt concentration above 0.2 M caused anthocyanin accumulation in roots of tobacco 'Samsun' and 'Samsun' rootstock grafted plants. As in previous studies, anthocyanin accumulation in leaves and roots of these groups seems to be an osmoprotective response against water stress exerted by high salt concentration.

Cytosolic proline helps to maintain osmotic adjustment as well as contributing to membrane stability and reducing the disruptive effect of NaCl on cell membrane as a free radical scavenger (Mansour, 1998). Proline amount increased in the leaves of 'Elazığ', grafted and self-grafted plants of all salt treatment groups (Figure 2A). Conversely, it increased in leaves of 'Samsun' plants above 0.2 M salt concentrations but remained unchanged in salt treated 'Hasankeyf' leaves. Increase in leaf proline amount was considerably higher in E and E/E compared with H/E, S/E, and S groups in all treatment groups. Interestingly, proline amounts in the roots (Figure 2b) increased in all salt treatment groups of S and S/E plants, in salt concentrations above 0.2 M for H and H/E, and to a lesser extent in E/E groups, due to an increase in proline levels in E plants in salt treatments of 0.6 M only. In fact, toxic ion effects are expected in root tissue more than in leaves due to the uptake of ions from roots. However, if the ions are transported to the leaves, the effects of salt become apparent. A low transportation rate of Na⁺ and Cl⁻ to the leaves and the ability to compartmentalize these ions in vacuoles to prevent their build-up in cytoplasm or cell

walls is one of the mechanisms of salt tolerance (Munns, 2002). In terms of proline accumulation, more ions might have been transported to the leaves of E, E/E, H/E, and S/E, which in turn may have caused proline accumulation in leaves (Li, 2009). So higher proline accumulations (especially above 0.2 M salt concentrations) might have been an initial response of the tobacco roots to high salinity, which in turn lowered the amount of Na⁺ and Cl⁻ transported to leaves, causing less damage to leaf tissue and lowering the osmoprotective response. This finding was also supported by increased levels of lipid peroxidation, which is an indication of oxidative damage and the adverse effects of the salt on membranes, due to the accumulation of toxic ions and ROS, in E and E/E leaves of all treatment groups. However, these levels were unchanged in leaves of H and 0.2 and 0.4 M salt treated S plants, as well as considerably lower lipid peroxidation levels in leaves of grafted plants (Figure 3A). In addition, lipid peroxidation increased significantly in the roots of E and E/E plants of all treatment groups of 'Samsun' (and to a lesser extent in 0.6 M salt treated S and S/E groups), whereas levels remained unchanged in the roots of H and H/E plants. In addition to lipid peroxidation, increased ion conductivity is an indication of the disruption of membrane integrity, which is the result of oxidative damage to the membrane structure. In accordance with the alterations in the MDA levels, conductivity in the roots of E and E/E considerably increased in all treatment groups (Figure 3b). However, leaves of the 0.2 M salt treated E and E/E plants seem to have preserved membrane integrity, despite increased lipid peroxidation (Figure 3a). Nevertheless, alterations in both leaf and root membrane conductivity values resulting from salt treatments in tobacco and grafted plants were considerably lower than that of non-grafted and self-grafted tomato plants.

Catalase and ascorbate peroxidase are among the enzymatic ROS scavenging mechanisms in plants. APX, GPX, and CAT subsequently detoxify hydrogen peroxide. APX requires ascorbate and GSH regeneration system. Since the extent of oxidative stress in a cell is determined by the amounts of superoxide, hydrogen peroxide, and hydroxyl radicals, the balance of SOD, APX, and CAT activities is important for the suppression of toxic ROS levels in a cell. Increase in APX levels was a result of the salt treatment on the roots of S and S/E, treatments above 0.2 M in the roots of E and H, and treatment at 0.6 M in H/E. On the other hand, APX levels increased in the leaves of salt treated E, in E/E and S in salt concentrations above 0.2 M, and in H and S/E in salt concentrations of 0.6 M. Conversely, CAT activity decreased significantly in roots of 0.6 M salt treated H and H/E, whereas it increased significantly in roots of 0.6 M salt treated E and E/E plants. Interestingly, an increase in CAT activity was observed in the leaves of salt treated E plants and 0.4 and 0.6 M salt treated H/E plants, in contrast to its decline in salt treated S plants. Apel and Hirt (2004) proposed

that changing the balance of scavenging enzymes would induce compensatory mechanisms, such as reducing CAT activity, and upregulating other scavenging enzymes such as APX and GPX. Likewise, in the present study APX activity increased while CAT activity decreased in leaves and roots of tobacco (Table 5). Firstly, APX has a higher affinity for H₂O₂ (μ M range) than CAT (mM range) (Mittler, 2002). Secondly, the ascorbate-glutathione cycle is found in all compartments of the cells whereas CAT is only present in peroxisomes (Mittler, 2002). In conclusion, APX plays a more crucial role in controlling the ROS level, although CAT is indispensable when high levels of ROS are produced (Willekens et al., 1997), which is also supported by the finding that APX activity was remarkably higher than CAT activity (i.e. units were expressed as μ mol ASC min⁻¹ mg⁻¹ protein and nmol H₂O₂ min⁻¹ mg⁻¹ protein, respectively; Table 4) in all groups studied. Accordingly, upregulation of both APX and CAT activities in tomato roots and of APX activity in tobacco roots, together with downregulation of CAT activity, demonstrate ROS levels that are more elevated in roots of tomato than in roots of tobacco. This, together with other parameters, indicates higher tolerance of tobacco to the lethal effects of NaCl. In terms of leaf tissue, grafted plants had elevated levels of CAT activity in contrast to decreased levels in the leaves of tobacco. Nonetheless, the alterations were still considerably lower when compared to levels of non-grafted and self-grafted tomatoes. Furthermore, results of the APX and CAT activities indicated that APX was the responsible enzyme for salt mediated ROS in tobacco cultivars, and both APX and CAT seemed to be important defense enzymes for tomato.

CONCLUSIONS

In this study, physiological and biochemical responses of tomato 'Elazığ' grafted on tobacco (*Nicotiana tabacum* L. 'Samsun' and *Nicotiana rustica* L. 'Hasankeyf') rootstocks to increasing NaCl concentrations were analyzed in both root and leaf tissues. Considering alterations in chlorophyll contents, proline, malondialdehyde, and conductivity levels, and antioxidant enzyme activity levels, leaves and roots of tomato and self-grafted tomato plants seems to be more affected by salt treatments than tobacco and rootstock grafted tomato plants. Tobacco roots seem to have better adaptive responses against salt stress in comparison to tomato as supported by changes in proline and ascorbate peroxidase and catalase levels. Grafting tomato onto tobacco rootstocks enhanced salt tolerance and adaptive response of scions; these changes seem to be dependent on the rootstock impacts rather than graft induced changes, as supported by self-grafting experiments. In conclusion, we demonstrated that previously defined graft unions of tomato on tobacco, which have increased fruit yield, also showed enhanced tolerance to high salt stress and appears to be a promising

technique for the cultivation of more salt tolerant varieties by conventional breeding practices, such as grafting.

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LITERATURE CITED

- Aghaei, K., A.A. Ehsanpour, and S. Komatsu. 2009. Potato responds to salt stress by increased activity of antioxidant enzymes. *Journal of Integrative Plant Biology* 51:1095-1103.
- Apel, K., and H. Hirt. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Reviews in Plant Biology* 55:373-399.
- Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* 39:205-207.
- Boughalleb, N., M. Mhamdi, B. El Assadi, Z. El Bourgi, N. Tarchoun, and M.S Romdhani. 2008. Resistance evaluation of grafted watermelon (*Citrullus lanatus* L.) against fusarium wilt and fusarium crown and root rot. *Asian Journal of Plant Pathology* 2:24-29.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248-254.
- Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology* 70(1):1-9.
- Chance, B., and A.C. Mahly. 1995. Assay of catalases and peroxidases. *Methods in Enzymology* 2:764-817.
- Ersayın-Yaşınok, A., F.I. Şahin, F. Eyidoğan, M. Kuru, and M. Haberal. 2008. Changes in nicotine levels of nicotine in fruits and leaves of the tobacco-grafted tomatoes. *Dialysis, Transplantation and Burns* 19(2):61-68.
- Fernandez-Garcia, N., V. Martinez, A. Cerdá, and M. Carvajal. 2002. Water and nutrient uptake of grafted tomato plants grown under saline conditions. *Journal of Plant Physiology* 159:899-905.
- Ghassemi, F., A.J. Jakeman, and H.A. Nix. 1995. *Salinisation of land and water resources: Human causes, extent, management and case studies*. UNSW Press, Sydney, Australia, and CAB International, Wallingford, UK.
- Giusti, M.M., and R.E. Wrolstad. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. p. F1.2.1-F1.2.13. In R.E. Wrolstad (ed.) *Current protocols in food analytical chemistry*. John Wiley and Sons, New York, USA.
- Hara, M., K. Oki, K. Hoshino, and T. Kuboi. 2004. Effects of sucrose on anthocyanin production in hypocotyl of two radish (*Raphanus sativus*) varieties. *Plant Biotechnology* 21:401-405.
- Hasegawa, P.M., R.A. Bressan, J.K. Zhu, and H.J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. *Annual Reviews in Plant Physiology and Plant Molecular Biology* 51:463-499.
- He, Y., Z. Zhu, J. Yang, X. Ni, and B. Zhu. 2009. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environmental and Experimental Botany* 66:270-278.
- Li, Y. 2009. Physiological responses of tomato seedlings (*Lycopersicon esculentum*) to salt stress. *Modern Applied Science* 3(3):171-176.
- Lichtenthaler, K.H., and C. Buschmann. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. p. F4.3.1-F4.3.8. In R.E. Wrolstad (ed.) *Current protocols in food analytical chemistry*. John Wiley and Sons, New York, USA.

- Mancinelli, A.L. 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. *Plant Physiology* 92:1191-1195.
- Mansour, M.M.F. 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiology and Biochemistry* 36:767-772.
- Martinez-Rodriguez, M.M., M.T. Estañ, E. Moyano, J.O. Garcia-Abellan, F.B. Flores, J.F. Camposa, et al. 2008. The effectiveness of grafting to improve salt tolerance in tomato when an 'excluder' genotype is used as scion. *Environmental and Experimental Botany* 63:392-401.
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7:405-410.
- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell and Environment* 25:239-250.
- Nanjo, T., M. Kobayashi, Y. Yoshihara, Y. Kakubari, K. Yamaguchi-Shinozaki, and K. Shinozaki. 1999. Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Letters* 461(3):205-210.
- Netondo, G.W., J.C. Onyangoa, and E. Beck. 2004. Sorghum and salinity II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science* 44:806-811.
- Ohkawa, H., N. Ohishi, and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95:351-358.
- Parvaiz, A., and S. Satyawati. 2008. Salt stress and phytochemical responses of plants-a review. *Plant Soil and Environment* 54:89-99.
- Reddy, M.P., and A.B. Vora. 1986. Changes in pigment composition, Hill reaction activity and saccharides metabolism in Bajra (*Pennisetum typhoides* S & H) leaves under NaCl salinity. *Photosynthetica* 20:50-55.
- Rivero, R.M., J.M. Ruiz, and L. Romero. 2003. Role of grafting in horticultural plants under stress conditions. *Food, Agriculture and Environment* 1:70-74.
- Rouphael, Y., M. Cardarelli, E. Rea, and G. Coll. 2008. Grafting of cucumber as a means to minimize copper toxicity. *Environmental and Experimental Botany* 63(1-3):49-58.
- Ruiz, J.M., J.J. Ríos, M.A. Rosales, R.M. Rivero, and L. Romero. 2006. Grafting between tobacco plants to enhance salinity tolerance. *Journal of Plant Physiology* 163:1229-1237.
- Spychalla, J.P., and S.L. Desborough. 1990. Superoxide dismutase, catalase and alpha-tocopherol constant of stored potato tubers. *Plant Physiology* 94:1214-1218.
- Wang, S.Y., H. Jiao, and M. Faust. 1991. Changes in ascorbate, glutathione and related enzyme activities during thiodiazuran-induced bud break of apple. *Plant Physiology* 82:231-236.
- Willekens, H., S. Chamnongpol, M. Davey, M. Schravdner, C. Langebartels, M. Van Montagu, et al. 1997. Catalase is a sink for H₂O₂ and is indispensable for stress in C3 plants. *EMBO Journal* 16:4806-4816.
- Yasinok, A.E., F.I. Sahin, F. Eyidogan, M. Kuru, and M. Haberal. 2009. Grafting tomato plant on tobacco plant and its effect on tomato plant yield and nicotine content. *Journal of Science of Food and Agriculture* 89:1122-1128.
- Zhou, Y., L. Huang, Y. Zhang, K. Shi, J. Yu, and S. Nogués. 2007. Chill-induced decrease in capacity of RuBP carboxylation and associated H₂O₂ accumulation in cucumber leaves are alleviated by grafting onto figleaf gourd. *Annals of Botany* 100:839-848.
- Zhu, J.K. 2001. Plant salt tolerance. *Trends in Plant Science* 6(2):66-71.
- Zhu, J., Z. Bie, Y. Huang, and X. Han. 2008. Effect of grafting on the growth and ion concentrations of cucumber seedlings under NaCl stress. *Soil Science and Plant Nutrition* 54:895-902.