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Comparison of salinity effects on grafted and non-grafted eggplants in terms of ion accumulation, MDA content and antioxidative enzyme activities

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Abstract: Grafting onto resistant/tolerant rootstocks is known to alleviate the negative effects of abiotic stress factors like salinity by enhancing their enzymatic antioxidant defense system and having more efficient nutrient uptake. This study was carried out under greenhouse conditions, different rootstock/scion eggplant combinations were grown under two salinity treatments 1.8-2 dS/m (control) and 6-7 dS/m (stress) with seven eggplant genotypes as rootstocks (commercial and Turkish genotypes). Two genotypes were used as the scion. Leaf MDA and ions (Na^+, Cl^-, K^+ and Ca^{++}) content, antioxidant enzymes activity were evaluated as indicators for plant tolerance level. It was found that the rootstock-grafted plants were more efficient in preventing Na^+ ions to be transferred to the plants upper parts and had higher SOD, CAT, and APX activity levels compared to the self- and non-grafted plants which resulted in better tolerance and growth in these plants.

1. Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop

worldwide, its production reaches about 48.5 million ton while in Turkey eggplant production reaches 800-900 thousand tons (TUIK, 2015). One of the most stress factors that affect eggplant production is salinity. Salinity is a major environmental factor limiting plant growth and productivity, especially in the arid and semiarid regions (Parida and Das, 2005). Conflicting literature exists on eggplant tolerance to soil salinity and this difference could be related to the varieties or cultivars used and to the different environmental conditions in those studies (Ünlükara *et al*., 2010).

Overcoming salt stress problems would have a positive impact on agriculture production. Attempts have been made to improve salt tolerance of crops by traditional breeding programs but with limited success due to the complexity of the trait (Flowers, 2004). Even the use of genetic transformation of plants to raise their tolerance, despite its success in some cases (Rus *et al.,* 2001); the complexity of the trait and lack of public acceptance, limiting its wide spread and use (Munns*,* 2002). One way of avoiding or reducing losses in production caused by salinity would be to use the tolerant rootstocks. In relation to salt tolerance, many studies have been conducted to determine the response of grafted plants to salinity. According to these studies, the improvement of salt tolerance by grafting is related to the capability of rootstocks to reduce toxicity of Na^+ and/or Cl^- through exclusion and/or reduction of absorption of Cl^- by the roots, and the replacement or substitution of total K^+ by total Na^+ in the foliage (Estañ *et al*., 2005; Martinez-Rodriguez *et al.,* 2008). It is supposed that useful rootstocks should be able to reduce the uptake and transport of saline ions to the shoot, which will slow or prevent the accumulation of the toxic salt ions in the leaves (Usanmaz and Abak, 2018). Salt stress causes a range of adverse effects in plants, mainly ionic disorders, osmotic stress and nutritional imbalance. A common feature of these effects is the overproduction of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Ashraf and Foolad, 2007). Salt stress causes stomatal closure, which reduces the CO_2/O_2 ratio inside leaf tissues and inhibits CO_2 fixation (Hernández *et al.,* 2000). Plants antioxidant enzymes such (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S- transferase, GST) work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (Scandalios, 1997; Dixit *et al*., 2001; Shalata *et al*., 2001).

Superoxide dismutase (SOD) reacts with the superoxide radical at almost diffusion-limited rates to produce H_2O_2 (Scandalios, 1993). H_2O_2 is scavenged by peroxidases, especially ascorbate peroxidase (APX), and catalase (CAT).

In the present study, we exposed non-grafted, self-grafted and rootstock-grafted eggplants to conditions of salt stresses to investigate whether grafted plants could improve tolerance to salinity by alleviating the expression of antioxidant enzymes.

Using local genotypes in breeding programs is of vital importance to find new rootstocks with the ability to alleviate the effects of salinity and reduce its effect on plant growth and productivity.

In the present study, two eggplant genotypes were grafted onto seven rootstocks to compare the ability of the different rootstock genotypes in increasing eggplant tolerance as it is related to the ability of the rootstock to 1) control the transport of Na^+ and Cl^-, 2) to maintain better K^+ and Ca^{++} uptake, 3) to increase the enzymatic defense mechanism scavenging the ROS induced by oxidative stress resulting in less leaf malodialdehyde (MDA) content. Another goal was to evaluate the potential of the Turkish genotypes Burdur and Mardin as rootstocks under salinity conditions in comparison to the commercial genotypes.

2. Materials and Methods

The field part of the experiment was carried out between August-November 2014, in a 300 m^2 plastic house belongs to the private sector (Genta General Agricultural Products Marketing Co.) in Antalya-Turkey while laboratory works and analysis were carried out in Ankara University Faculty of Agriculture, Departments of Horticulture and Agronomy laboratories.

Plant material

Two eggplant (*Solanum melongena* L.) genotypes, Naomi F_1 cv., a commercial cultivar, and Artvin, a salt sensitive breeding line, were used as scions. And for rootstocks five commercial genotypes were used,

AGR703 (*Solanum aethiopicum*), Köksal F_1, Yula F_1 and Vista (*S. incanum* x *S. melongena* hybrids), and Hawk (*S. torvum*), these genotypes are the most common used rootstocks in grafting eggplants due to their tolerance. In addition two local salt-tolerant genotypes Burdur and Mardin (*S. melongena* L.) were used as rootstocks, their tolerance to salinity were confirmed in previous studies screening for Turkish tolerant genotypes (Yaşar, 2003).

Grafting and salt treatment

Eggplant seeds were sown in germination trays filled with 2:1 peat:perlite. After sowing trays were kept under controlled conditions of temperature (25°C) and humidity (80%). When the seedlings reached 2-4 true leaves stage, grafting was carried out. The tube-grafting was used in this study because it is the most widely used grafting method in *Solanaceae* family (Rivard *et al.,* 2009). Then grafted seedlings were kept under controlled conditions of humidity (90%) for four days, then seedlings were transferred to the greenhouse under shading before planting for acclimatization. Ten days after grafting seedlings were placed in the plastic house and were ready for transplantation in 8 L plastic pots filled with 3:1 perlite:vermiculite.

In one of our earlier studies we examined these genotypes tolerance under salinity conditions at seedling stage in a hydroponic experiment (Talhouni, 2016), in this study we wanted to assess these same genotypes at flowering and fruit set stage. When plants reached the flowering and fruit set stage salinity treatment began. 6-7 dSm^{-1} water EC was used as the stress treatment by solving NaCl into the nutrition solution; according to the volume of barrels used in fertigation about 8 kg of iodine-free sodium chloride were required , while for the control the EC level was kept at 1.8-2 dSm^{-1} (no NaCl was added).

Leaf ion concentrations

After 60 days from salinity stress application, samples were taken from the control and the salinity treated plants for the different analysis. For the leaf-Na^+, Cl^-, K^+ and Ca^{++} concentration measurements, leaves were dried at 65°C for 48 hours, grounded, dissolved in 1% (v/v) HCl. For the analysis of Na^+, K^+ and Ca^{++} contents, atomic absorption spectrophotometer (Varian Spectra AA 220 FS) was used (Kuşvuran, 2012). While for Cl^-, titration procedure was followed as described by Taleisnik *et al.* (1997) using Buchler - Cotlove chloridometer.

Enzyme extractions and assays

Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at -80°C for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium phosphate buffer, pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 *g* for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C.

APX activity was determined by measuring the consumption of ascorbate by following absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme required to consume 1 µmole ascorbate min^{-1} (Cakmak and Marschner, 1992).

SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT.

Catalase (CAT) activity was measured as the decline in absorbance at 240 nm due to the decomposition decline of extinction of H_2O_2. The reaction was started by adding H_2O_2.

Lipid peroxide content

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction. Frozen samples were homogenized in a pre-chilled mortar with two volumes of ice-cold 0.1% (w/v) tricloroacetic acid (TCA) and centrifuged for 15 min at 15000 x *g*. Assay mixture containing 1 ml aliquot of the supernatant and 2 ml of 0.5% (w/v) thiobarbituric acid in 20% (w/v) tricloroacetic acid (TCA) was heated to 95°C for 30 min and then rapidly cooled in an ice-bath. After centrifugation (10000 x *g* for 10 min at 4°C), the supernatant absorbance (532 nm) was read and values corresponding to non-specific absorption (600 nm) were subtracted. The MDA content was calculated according to the molar extinction coefficient of MDA (155 mM^{-1} cm^{-1}).

Statistical analysis

Randomized complete block design with three replicates was used. Each replicate included 108 pots (18 rootstock/scion combination * 2 salinity level * 3 plants of each combination) with one plant/pot. Data were subjected to Duncan's multiple range tests

using the SAS program (P≤0.01)(Version 6.12, SAS Institute Inc., Cary, USA).

3. Results

Na^+ concentrations

In general, the concentrations of Na^+ in the leaves increased significantly due to increased NaCl concentration (Table 1) with significant differences between grafting combinations, and a significant 'salinity x scion/rootstock combination' interaction at P≤0.01.

After 60 days of salinity stress application, the combinations that showed the least concentrations of Na^+ in their leaves were; (rootstock/scion) Köksal/Artvin, Vista/Naomi, Köksal/Naomi, AGR703/Naomi and AGR703/Artvin (9.75, 9.65, 9.92 10.02 and 10.46 µg/mg FW respectively) with increase rates of 3511, 1035, 4623, 1721, 3506% respectively (Fig. 1) which indicated that these rootstock genotypes could limit Na^+ to the leaves more successfully. No significant effects of grafting per se were noticed, no differences were observed between non-grafted and self-grafted combinations.

Cl^- concentration

As in leaf Na^+ concentration, leaf Cl^- concentration also increased significantly under salinity treatment in all combinations, with a significant differences and a significant 'salinity x scion/rootstock combination' interaction at P≤0.01 (Fig. 1). The highest Cl^- concentration was observed in Artvin, Naomi, (rootstock/scion) Artvin/Artvin, Naomi/Naomi, Mardin/Artvin, Mardin/Naomi (11.83, 10.81, 9.54, 9.06, 8.83 8.12 µg/mg FW) combinations, while the lowest was observed Köksal/Artvin, AGR703/Artvin, AGR703/Naomi and Burdur/Artvin (5.06, 5.06, 5.27, 5.49 µg/mg FW respectively) and there were no significant differences between non- and self-grafted combinations (Table 1).

K^+ concentrations

The amounts of K ion measured in leaf samples taken from plants treated with EC 6-7 dS / m NaCl gave lower values in some combinations than control plants (Table 1), the highest decrease in leaf K^+ content was observed in non-grafted Artvin (-9.82%).followed by (rootstock/scion) Artvin/Artvin with decrease rate of (-6.25%) (Fig. 1). The highest values were obtained from (rootstock/scion); Köksal/Artvin, Köksal/Naomi, Mardin/Naomi, AGR703/Artvin, Yula/Naomi (4.86, 4.64, 4.36, 4.30, 4.27 µg/mg FW, respectively) (Table 1). Among these combinations, Köksal/Artvin, AGR703/Artvin and Yula/Naomi had the highest K^+ ions increase rate (47.27%, 22.16%, 20.96%) (Fig. 1). Combinations that gave the lowest K^+ ion amount measurements were (rootstock/scion) Hawk/Artvin, Naomi, Burdur/Naomi, Naomi/Naomi,

Table 1 - Leaf ions concentration (µg/mg FW); in the different grafting combinations under control and salinity treatments

Grafting combination	Na^+		K^+		Ca^{++}		Cl^-	
	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
Köksal/Artvin	0.27±0.00 a	9.75±0.25 a	3.30±0.15 ab	4.86±0.33 b	0.50±0.03 f	0.48±0.03 e	0.06±0.02 a	5.06±0.64 a
AGR703/Artvin	0.29±0.03 a	10.46±0.53 a-c	3.52±0.34 a-c	4.30±0.26 ab	0.49±0.02 f	0.41±0.04 de	0.06±0.02 a	5.06±0.90 a
Vista/Artvin	0.51±0.03 ab	12.14±0.86 b-e	3.89±0.11 a-e	3.93±0.53 a	0.46±0.02 b-f	0.41±0.03 de	0.12±0.04 a-d	6.30±1.10 a-c
Yula/Artvin	0.24±0.02 a	11.08±0.58 a-e	3.74±0.18 a-e	3.89±0.12 a	0.43±0.01 a-c	0.35±0.03 b-d	0.19±0.02 c-f	6.63±0.75 a-d
Burdur/Artvin	0.49±0.01 b	10.91±0.53 a-d	3.57±0.12 a-c	3.97±0.24 a	0.49±0.02 ef	0.39±0.05 c-e	0.09±0.03 ab	5.49±0.13 ab
Mardin/Artvin	0.61±0.09 b-d	12.36±0.29 c-e	3.70±0.23 a-d	4.22±0.09 ab	0.44±0.01 a-d	0.35±0.06 b-d	0.14±0.03 a-e	8.83±1.08 c-f
Hawk/Artvin	0.26±0.05 a	10.89±0.18 a-d	4.05±0.28 c-e	3.84±0.31 a	0.46±0.02 c-f	0.41±0.03 de	0.11±0.02 a-c	6.45±1.50 a-d
Artvin/Artvin	0.83±0.08 e-g	12.36±0.70 c-e	4.32±0.05 de	4.05±0.15 ab	0.41±0.01 a	0.35±0.02 b-d	0.19±0.02 c-f	9.54±1.21 e-g
Artvin	0.88±0.04 fg	12.77±0.46 de	4.38±0.28 e	3.95±0.13 a	0.40±0.01 a	0.29±0.02 ab	0.22±0.02 ef	11.83±0.62 g
Köksal/Naomi	0.21±0.02 a	9.92±0.37 ab	4.11±0.22 c-e	4.64±0.23 ab	0.49±0.02 d-f	0.31±0.01 a-c	0.09±0.04 ab	6.06±0.38 ab
AGR703/Naomi	0.55±0.12 ab	10.02±1.26 ab	3.87±0.06 a-e	3.96±0.28 a	0.48±0.01 d-f	0.36±0.02 b-d	0.10±0.04 a-c	5.27±0.55 a
Vista/Naomi	0.85±0.05 fg	9.65±0.71 a	3.67±0.15 a-d	4.15±0.08 ab	0.44±0.02 a-e	0.36±0.02 b-d	0.15±0.03 b-f	6.13±0.19 ab
Yula/Naomi	0.73±0.04 d-f	10.93±0.97 a-d	3.53±0.10 a-c	4.27±0.29 ab	0.42±0.01 a-c	0.25±0.02 a	0.18±0.05 b-f	7.51±0.54 a-e
Burdur/Naomi	0.49±0.05 b	11.14±1.16 a-e	3.50±0.36 a-c	3.88±0.23 a	0.48±0.01 d-f	0.40±0.01 c-e	0.13±0.01 a-d	6.37±1.01 a-c
Mardin/Naomi	0.68±0.04 c-e	12.07±0.13 b-e	3.95±0.32 b-e	4.36±0.24 ab	0.43±0.01 a-c	0.34±0.03 a-d	0.14±0.03 a-e	8.12±0.63 b-e
Hawk/Naomi	0.80±0.10 e-g	11.14±1.20 a-e	3.80±0.11 a-e	4.09±0.27 ab	0.41±0.01 ab	0.35±0.01 b-d	0.20±0.02 d-f	7.29±0.24 a-e
Naomi/Naomi	0.94±0.04 g	12.34±0.84 c-e	3.33±0.20 ab	3.96±0.36 a	0.40±0.01 a	0.34±0.01 a-d	0.22±0.02 ef	9.06±1.06 d-f
Naomi	0.52±0.06 ab	13.30±0.73 e	3.27±0.29 a	3.85±0.30 a	0.40±0.02 a	0.28±0.03 ab	0.23±0.03 f	10.81±1.41 fg
CV (%)	*43.07*	*9.78*	*8.81*	*6.83*	*8.01*	*15.22*	*38.38*	*27.17*
Treatment	**		**		**		**	
Combination	**		**		**		**	
Combination x treatment	**		**		**		**	

AGR703/Naomi, Artvin (3.84, 3.85, 3.88, 3.96, 3.96, 3.96 85 µg/mg FW). No significant differences were obtained for self-grafted combinations.

Ca^{++} concentrations

EC 6-7 dSm^{-1} NaCl treatment led to a decrease in leaf Ca^{++} concentration in all combinations (Fig. 1) with significant differences between treatments and combination (P≤0.01) and with a significant 'salinity x scion/rootstock combination' interaction. The highest leaf Ca^{++} concentrations were obtained in (rootstock/scion) AGR703/Artvin, Köksal/Artvin, Burdur/Naomi, Hawk/Artvin and Vista/Artvin (0.48, 0.42, 0.41, 0.41 µg/mg FW, respectively). No significant effects were observed in self-grafted combinations.

Antioxidant enzyme activities

Salt treatments increased superoxide dismutase (SOD) activities in all of the plants (Table 2). However,

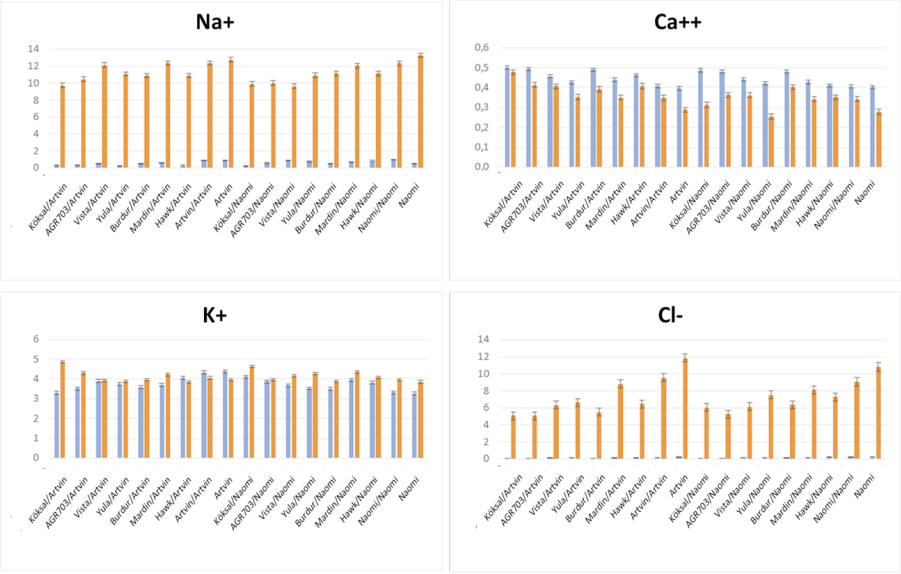

Fig. 1 - Leaf ions concentration (µg/mg FW); in the different grafting combinations under control (blue) and salinity treatments (red).

Table 2 - SOD, CAT, and APX enzymes activities (µmol/g FW) in the different grafting combinations under control and salinity treatments

Eggplant	SOD		CAT		APX		MDA	
	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
Köksal/Artvin	217.03±8.00 a	635.46±27.84 gh	139.90±13.74 ab	593.91±28.44 h	2042.77±12.67 h	5387.96±560.98 e	5.12±0.29 a-d	10.44±0.54 a
AGR703/Artvin	207.06±6.28 a	645.64±38.29 h	129.82±10.00 ab	576.91±27.73 gh	2020.91±97.38 h	4820.66±545.40 de	5.02±0.13 ad	10.23±1.24 a
Vista/Artvin	215.92±11.75 a	464.88±44.39 c-f	113.03±11.99 a	484.17±25.96 c-f	1758.36±28.48 e-h	3829.29±568.57 a-d	4.86±0.27 ab	12.83±0.26 ab
Yula/Artvin	193.54±6.29 a	414.49±26.02 b-d	103.86±6.46 a	409.13±1.88 bc	1765.71±141.18 e-h	3307.96±550.14 a-c	5.12±0.26 a-d	10.14±0.61 a
Burdur/Artvin	208.97±10.32 a	508.88±55.72 d-f	131.44±4.26 ab	548.67±19.02 e-h	1882.51±52.64 f-h	3839.81±57.28 a-d	5.19±0.10 a-d	11.16±0.44 a
Mardin/Artvin	195.91±10.91 a	470.99±16.48 c-f	138.76±14.25 ab	434.69±6.55 b-d	1395.16±235.41 b-d	3105.27±93.76 ab	5.49±0.25 a-e	11.50±0.21 a
Hawk/Artvin	203.44±10.29 a	436.05±50.00 b-e	118.68±15.42 a	464.12±25.38 c-e	1926.37±199.83 gh	4303.30±32.77 b-e	4.80±0.31 a	11.95±0.98 ab
Artvin/Artvin	186.29±10.04 a	341.33±58.60 ab	123.87±6.38 ab	350.16±52.43 ab	1134.59±102.54 ab	2731.28±37.49 a	6.32±0.13 fg	12.54±0.15 ab
Artvin	189.95±12.89 a	295.15±16.97 a	113.49±7.29 a	374.84±27.07 b	903.43±37.94 a	2614.61±37.06 a	6.34±0.33 g	14.81±1.23 b
Köksal/Naomi	190.92±25.52 a	553.92±32.30 f-h	116.33±14.37 a	553.17±30.84 f-h	1968.59±112.34 g-h	4427.68±48.03 c-e	4.95±0.18 a-c	10.93±1.20 a
AGR703/Naomi	213.81±4.16 a	491.39±39.05 d-f	165.69±17.37 b	503.21±14.17 d-g	1924.53±144.55 gh	4326.69±614.68 b-e	4.87±0.23 ab	10.54±1.04 a
Vista/Naomi	194.09±17.87 a	457.84±29.92 c-f	124.91±15.83 ab	405.95±29.79 bc	1547.82±154.87 c-f	3072.32±48.96 ab	5.59±0.25 b-f	11.18±0.16 a
Yula/Naomi	200.41±10.91 a	427.66±11.33 b-e	99.34±6.30 a	401.18±19.92 bc	1499.31±168.98 c-e	3663.61±635.13 a-d	5.26±0.28 a-d	11.06±0.93 a
Burdur/Naomi	196.48±16.49 a	534.86±35.10 e-g	117.08±28.01 a	503.48±27.99 d-g	1813.06±32.67 e-h	3789.02±90.92 a-d	5.09±0.39 a-d	10.36±0.85 a
Mardin/Naomi	191.44±20.27 a	378.05±10.08 a-c	104.70±15.90 a	367.67±14.99 ab	1608.48±112.47 d-g	3448.81±529.02 a-c	5.71±0.19 d-g	10.86±0.74 a
Hawk/Naomi	193.35±9.53 a	421.65±43.89 b-d	140.49±24.51 ab	360.07±7.01 ab	1695.49±80.45 d-h	4471.40±613.60 c-e	4.80±0.12 a	10.89±0.92 a
Naomi/Naomi	201.11±19.08 a	272.74±22.75 a	125.68±6.97 ab	366.67±43.39 ab	1205.04±58.49 a-c	2693.56±51.33 a	6.05±0.16 e-g	14.66±2.05 b
Naomi	199.68±10.99 a	271.57±36.99 a	123.98±8.26 ab	289.02±39.88 a	1033.83±54.71 ab	2782.04±593.00 a	5.66±0.18 c-g	14.91±1.65 b
CV (%)	*4.65*	*24.53*	*12.87*	*19.94*	*21.9*	*21.7*	*9.37*	*13.58*
Treatment (T)	**		**		**		**	
Combination (C)	**		**		**		**	
C x T	**		**		**		**	

in the rootstock-grafted plants, SOD activity increased faster and with higher rates than in the non- and self-grafted plants. Köksal/Artvin and AGR703/Artvin had the highest SOD activity level (645.64 and 635.46 Umol/min/mg FW respectively). Followed by Köksal/Naomi, Burdur/Naomi, and Burdur/Artvin combinations (553.92, 534.8, and 508.88 Umol/min/mg FW respectively). The highest increase rate in SOD activity was obtained for AGR703/Artvin combination (211.8%) while Naomi/Naomi and Naomi combinations had the lowest increase with rates of 35.62 and 36% respectively (Fig. 2). Naomi and self-grafted plants had the lowest values of the enzyme activity with no significant differences between non- and self-grafted combinations.

Catalase (CAT) activity increased under salt treatment in all combinations compared to the control plants (Table 2), (rootstock/scion) Köksal/Artvin and AGR703/Artvin had the highest CAT activity (593.91 and 576.91 µmol/min/mg FW respectively). Followed by Köksal/Naomi, Burdur/Artvin, AGR703/Naomi and Burdur/Naomi. On the other hand Artvin and Naomi (non-grafted) and the self-grafted plants had the lowest CAT activity. Burdur/Naomi had the highest increase rate in CAT activity (330%) while non-grafted Naomi had the lowest rate (133%) (Fig. 2). No significant differences were observed between non- and self-grafted combinations.

Under NaCl-salinity conditions, ascorbate peroxidase (APX) activity was increased in all plants (Fig. 2). However, AGR703/Artvin and Köksal/Artvin had the highest APX activity levels indicating their better tolerance level (4820.66 and 5387.96 µmol/min/mg FW respectively) as shown in Table 2, while the lowest APX enzyme activity was found in non-grafted plants Naomi and Artvin indicating their poor tolerance. No significant differences were obtained between non- and self-grafted combinations.

Malondialdehyde (MDA)

Salinity resulted in a significant increase on the MDA content compared to the controls due to oxidative stress induced peroxidation (Fig. 2). With regard to the MDA, significant differences were found among grafting combinations and a significant 'salinity x rootstock/scion combination' interaction at P≤0.01 (Table 2). According to the results Naomi, Naomi/Naomi and Artvin were found to be more sensitive with MDA content increase rate of 163.4, 142.3, 133.6% respectively (Fig. 2). No significant differences were observed between non- and self-grafted combinations.

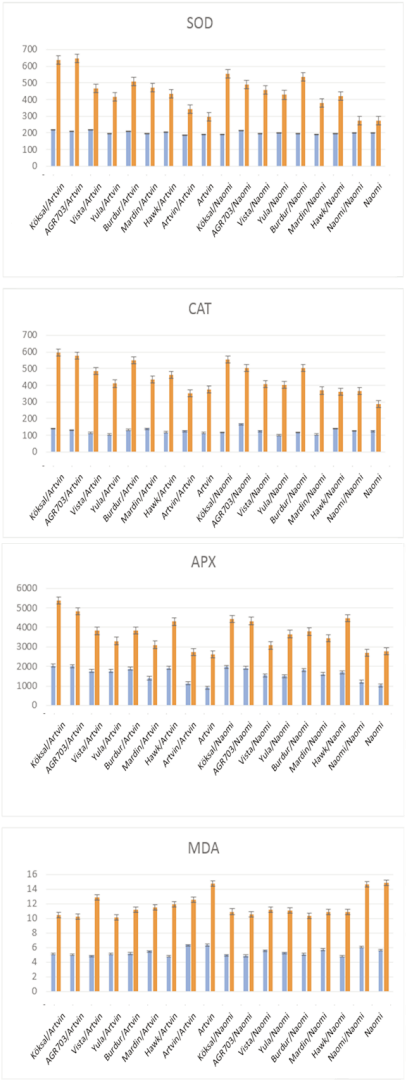

Fig. 2 - SOD, CAT and APX enzymes activities (µmol/g FW) in the different grafting combinations under control (blue) and salinity treatments (red).

4. Discussion and Conclusions

As a result of high salinity level, Na^+ and Cl^- ions can be accumulated in toxic levels in plant tissues depends on the plant species. Even though these two ions are suitable for osmotic adjustment, excess concentrations will be toxic enough to prevent plant growth. In the study, combinations Vista/Artvin, Köksal/Artvin, Vista/Naomi, and Köksal/Naomi had less Na^+ accumulation in their tissues which indicates that these combinations were able to keep Na^+ ions away from their leaves. Concerning leaf Cl^-, Vista/Artvin combination had the least concentration. Grafted plants tend to hold Na^+ and Cl^- ions in their root tissues preventing them from being translocated to the shoots and leaves in high concen-

trations (Levitt, 1980; Estañ *et al*., 2005). Most vegetables like, cucumbers, melons, tomatoes and eggplant are injured by excess Na^+ ions (Tester and Davanport, 2003). In Giuffrida *et al.* (2009), increased NaCl level led to Na^+ concentration increase in tomato leaves and fruits. Kuşvuran *et al.* (2007), under salinity conditions Na^+ and Cl^- were accumulated in higher rates in the salinity-sensitive melon plants compared to the salinity-tolerant ones.

K^+ decrease rate was different between the different combinations. Akinci and Lösel (2012), different eggplant genotypes showed different tolerant level to salinity. Pala cv. showed better tolerance to salinity compared to Kemer and Aydın Siyahı cultivars with better K^+/Na^+ ratio. Yaşar *et al.* (2006), in tissue culture study on eggplant, there was an increase in Na^+ and Cl^- tissues concentrations with decrease in K^+ and Ca^{++} due to salinity. However there were significant differences between different genotypes, the salinity-tolerant MK and BB showed higher K^+ and Ca^{++} concentrations compared to the salinity sensitive GR and AH genotypes. Consequently MK and BB had higher K^+ and Ca^{++} uptake decreased under stress treatment (Savvas and Lenz, 2000). In a similar study on pepper, the same results were obtained (Aktaş *et al.,* 2002).

Combinations with the highest leaf Ca^{++} concentrations under salinity were Vista/Naomi, AGR703/Naomi and Burdur/Artvin. And maybe for this reason Burdur genotype can be considered a potential rootstock for increasing eggplant tolerance against salinity. The decrease in Ca^{++} uptake due to NaCl salinity was observed by many authors, and in contrary to K^+, the decrease was not due to the competition between Na^+ and K^+ at the absorption site on the root surface, it was always found because of the decline in the transpiration rate under stress conditions (Maggio *et al.,* 2007). In Gao *et al.* (2005), under stress conditions of low temperatures (5°C) grafted eggplants maintained higher leaf Ca^{++} concentrations compared to the non-grafted plants which gave the grafted plants higher tolerance under such stress conditions.

Plant adaptation to salinity may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and/or through the induction of antioxidant enzymes (SOD, CAT, GR, and APX, etc.) (Sevengör, 2010). In the present study, rootstock-grafted plants had higher activity of antioxidant enzymes under salinity conditions, which was translated to lower MDA content in their leaves which means these combinations, were less affected by the ROS-induced lipid peroxidation and they were more tolerant to salinity than the non- and self-grafted plants.

MDA content always found higher in salinity-sensitive plants compared to salinity-tolerant ones (Yaşar, 2003; Kuşvuran *et al*., 2015) and a significant relation between MDA content and antioxidant enzymes activity is first proven by Shalata and Tal (1998). Meloni *et al.* (2001) in cotton, Yaşar (2003) in eggplant, Doğan (2004) in tomato, and Sevengör (2010) in pumpkin, all found that MDA content was low in plants with high antioxidant enzymes activity under salinity stress conditions.

In this study, antioxidant enzymes activity showed a higher increase in rootstock-grafted plants compared to the non- and self-grafted plants, this increase was significantly different between grafting combinations. In another study where cucumber was grafted onto salinity-tolerant rootstock, H_2O_2 level was found to be low, whereas SOD, CAT, POD enzymes activity level were found higher. Öztekin and Tuzel (2011), CAT activity level differed according to the rootstock genotypes, but always was higher in the grafted plants compared to the non-grafted plants.

All results indicated that grafting per se had no significant role in alleviating negative effects of salinity as there were no significant differences between non- and self-grafted combinations in all parameters measured in this study.

In general, local genotypes (landraces) are adapted to prevailing environmental conditions like salinity. In this work, the local Turkish genotype Burdur showed a good potential to compete commercial genotypes. On the other hand, Mardin was way behind and did not show enough potential in this study.

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