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Physiological, biochemical and mineral dimensions of green bean genotypes depending on Zn priming and salinity

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ABSTRACT

The effects of salinity and zinc (Zn) priming on the physical and mineral composition of green bean genotypes were investigated on two green bean genotypes ('Şeker Fasulye' and 'Local Genotype') by soaking seeds in 0.05% Zn ($ZnSO_4.7H_2O$) solution and by exposing to salt stress by applying 50, 100 and 150 mM NaCl after first true leaf emergence. Plants not exposed to salt stress were treated as control. The effects of Zn priming on the salt tolerance of genotypes, fresh and dry weight of plant leaf relative water content (RWC), loss of turgidity (LOT), Na, K, Ca and Zn concentrations in the leaves, stem and root portions of plants were evaluated. The NaCl concentrations led to significant variations in the examined parameters. The highest concentration of salt (150 mM) caused fading in leaves and led to inhibition of growth and development. Salt application generally reduced the fresh and dry weights of plants of both genotypes where Zn priming showed an amendatory effect. Leaf RWC decreased with salt applications while LOT increased but Zn priming had no amendatory effect on these parameters. 'Şeker Fasulye' genotype was found to be relatively more salt tolerant than 'Local Genotype' on the basis of the investigated parameters. Zinc priming decreased the Na and Ca concentrations in plant organs; however, a decrease in K concentration was observed due to increase in NaCl.

Key words: Green bean, Leaf relative water content, Loss of turgidity, Salt stress, Zinc priming.

INTRODUCTION

Any increase in water usage can lead to salinity problems in soils if salt exists in the soil and/or irrigation water is salty, salinity decreases nutrition intake by plants and reduces the growth of a plant.

Plants wither under certain high salt concentrations in soil even if there is enough water in the soil due to osmotic reasons since water intake of plants decreases when salt concentration increases. In addition, salinity causes sodium (Na^+) and chlorine (Cl^-) ions to increase which results in ionic imbalance (K^+ and Na^+) in the protoplasm (Tavakkoli *et al*. 2010). In order to understand these mechanisms, researchers have focused on ion accumulations and transfers, especially Na^+, K^+ and Ca^{+2} that appear in plant organelles under salt stress.

Salinity is a common problem in arid and sub-arid regions but Zn deficiency also limits the plant productivity in such areas (Cakmak, 2000). Zinc plays a significant role in plant nutrition due to its regulatory functions in many enzyme systems like production of carbohydrate, nucleic acid and chlorophyll synthesis and in metabolism of the auxin. An appropriate amount of Zn is effective in decreasing Na concentration in plants since in case of Zn deficiency in cells, cell membranes become hyper-permeable or certain compounds exude from the roots (Cakmak, 2000). When Zn deficiency occurs together with salinity and aridness, the yield of a plant decreases. Zinc deficiency slows down the development and growth of seedlings, particularly in the early growth stages (Impa *et al*., 2013).

Green bean plant is sensitive to salt stress (Assimakopoulou *et al.*, 2015) and when salinity and Zn are lacking, different bean genotypes have significant differences in their grain yield and vegetative growth (Hacisalihoglu *et al.*, 2004; Dasgan and Koc, 2009). These differences occurring among the same species indicate that there is a relationship between the salt stress tolerance of genotypes (Assimakopoulou *et al.*, 2015) and Zn adaptation mechanisms (Hacisalihoglu *et al*., 2004). This study focuses on reaction of green bean genotypes to different salinity levels and Zn priming.

MATERIALS AND METHODS

Plant materials and seed priming: Seeds of two green bean genotypes 'Şeker Fasulye' and 'Local Genotype' were soaked in 0.05% solution of $ZnSO_4.7H_2O$ and incubated for 6 hours at 25°C (Harris *et al*. 2008). After soaking, the seeds were washed with deionized water. Control seeds were non-primed.

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Growth conditions and salt treatment: The seeds were sown in a seed-plot (31.5 x 51.5 cm) filled with a mixture of peat, perlite and vermiculite (2:1:1) and treatments were precisely imposed in a randomized block design having three replicates. Each replicate consisted of 10 plants. Plants were grown in a plant growth chamber (DAIHAN WGC-1000, South Korea), at 26°C/18°C (day/night) temperature having about 70% relative humidity and a light density of 450 molμm^{-2}s^{-1} (Khadri *et al*., 2006).

Seeds were irrigated with 20 ml of deionized water daily until the fully developed true leaf at third node (V1) emerged. Following this, seedlings were irrigated with saline water (50; 100; and 150 mM NaCl), except in the control plot which continued to receive deionized water. The duration of treatment was two weeks.

Leaf relative water content (RWC) and loss of turgidity (LOT): Three discs from different fresh plants per replicate were taken and weighed at the end of two weeks in each treatment. To obtain the turgid weight, 1.5 cm leaf discs were floated on distilled water in a petri dish for 4 hours at room temperature. After incubation, the leaf discs were removed from the petri dish, the surface was blotted and weighed immediately. The leaf discs were then placed in a new dry petri dish with a lid and located in an oven at 70°C for 48 hours. After incubation, the leaf discs were weighed (Gulen and Eris 2003). The RWC and LOT are calculated as follows:

RWC (%) = [(*Wf -Wd)/(Wt -Wd*)]*100 and
LOT (%) = [(*Wt -Wf)/ Wt*]*100

where, Wf is the fresh weight, Wd is the oven dry weight and Wt is the turgid weight.

Plant mineral analysis: At the end of experiments, root, stem, first true leaf and trifoliolate leaflets were separated and dried for 48 hours at 70°C to determine their dry weight. Dried samples were grinded and turned into ash at 500 °C for 5 hours. Samples were then dissolved in 3.3% HCl and analyzed for Zn, Na, K and Ca by atomic absorption spectrometer (Analytic-Jena novAA 350, Germany). To check the related elemental measurements, reference tomato leaf samples from National Institute of Standards and Technology (Gaithersburg, MD, USA) were used.

Statistical analysis: Data was analyzed for variance using the IBM SPSS Statistics 20.0 program and mean separation was accomplished using the least significant difference (LSD) test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Fresh and dry weight: A significant difference was observed in the Zn concentrations in fresh parts of green beans with exception of fresh root biomass. Zinc priming increased the fresh weight of trifolia leaflet (Table 1). A significant difference in genotypes was noticed in the fresh weight of stem. Fresh weight of 'Şeker Fasulye' genotype's stem was higher than the 'Local Genotype' (Figure 1a and b). Fresh weights of root, stem and trifoliolate leaflet decreased with increasing salt application, while control was registered the highest fresh weight (Table 1).

Zinc priming did not result in a significant variation in the root fresh weight but, it positively influenced fresh weight of stem. Zinc priming did not register any compensation in the fresh weight of the first true leaves under salt stress. Weisany *et al*. (2012; 2014) also reported that NaCl applications to soybean decreased the fresh weights of root and the stem significantly while Zn application decreased the negative effects of NaCl.

Zinc priming significantly affected dry weight of all parts of the seedling (Table 1). Zinc priming had an adverse effect on dry weight of root, stem and trifoliolate leaflets. Dry weight of stem differed significantly between genotypes. The 'Şeker Fasulye' genotype recorded more dry matter in stem and first true leaf than the 'Local Genotype' (Table 1). Increased NaCl concentrations caused a decrease in dry weight in all plant parts of both genotypes (Figure 1c and d). Zn x NaCl interaction was significant at $p<0.05$ on the dry weight of trifoliolate leaflet; however, no difference was found between the genotypes (Table 1).

Weisany *et al*. (2012; 2014) have also found that salt application decreased the dry weight of stem and root in soybean while Zn application has a positive effect on eliminating the detrimental effects of NaCl on dry matter. In present study, decrease in the leaf dry weight on increasing NaCl concentrations can result either from osmotic dehydration generated by osmotic stress due to the presence of salt in the plants or from an increase in transpiration. When osmotic dehydration takes place, it decreases osmotic and water potential of cell and causes cell volume and expansion ratio to decrease. As a result of a decreasing transpiration, dryness that occurs in stem and leaves of the plant leads to plant weight loss (Gazanchian *et al*., 2007).

Leaf relative water content and loss of turgidity: Results given in Table 1 and Figures 1 e-f - g-h reveal that though Zn priming was effective on neither the first true leaf nor the trifoliolate leaflet RWC or LOT in green beans is considered. Zn x G interaction was significant on the first true leaf. The leaf RWC of the 'Local Genotype' on Zn application was higher than that of 'Şeker Fasulye' genotype at the first true leaf (Figure 1 e). NaCl application was found to have significant effect on RWC of both leaves, however, salinity decreased the RWC values.

Research made on rosemary plant revealed that 2 μM Zn application decreased leaf RWC value significantly under salt stress conditions (Mehrizi *et al*., 2011). In strawberry plant, salt stress conditions also decreased leaf RWC values; however, these negative effects were eliminated on foliar Zn applications (Saadati and Moallemi, 2011).

Table 1: Main effects of Zn priming and NaCl on fresh, dry weight of plants, leaf relative water content and loss of turgidity of green bean genotypes

	Fresh weight (g)				Dry weight (g)				RWC (%)		LOT (%)	
Trait	**Root**	**Stem**	**FTL**	**TL**	**Root**	**Stem**	**FTL**	**TL**	**FTL**	**TL**	**FTL**	**TL**
Zinc (Zn)												
(-) Zn	1.43	0.15a	0.25a	0.60b	0.09a	0.15a	0.25a	0.07a	79.47	70.95	19.97	26.87
(+) Zn	1.36	0.12b	0.16b	1.21a	0.06b	0.12b	0.16b	0.12b	80.80	69.46	19.00	28.35
$LSD_{0.05}$	*0.22*	*0.02*	*0.03*	*0.15*	*0.01*	*0.02*	*0.03*	*0.02*	*2.33*	*2.02*	*1.06*	*2.16*
Genotype (G)												
Local	1.38	0.12b	0.20	0.90	0.07	0.12b	0.20	0.10	80.98	68.79b	18.96	29.87a
Seker	1.44	0.15a	0.22	0.92	0.07	0.15a	0.22	0.10	79.29	71.62a	20.00	25.35b
$LSD_{0.05}$	*0.22*	*0.02*	*0.03*	*0.15*	*0.01*	*0.02*	*0.03*	*0.02*	*2.33*	*2.02*	*1.06*	*2.16*
Salinity (S) (mM)												
0	1.83a	0.15a	0.21	1.35a	0.10a	0.15a	0.21	0.13a	88.29a	87.23a	10.93d	11.70d
50	1.41b	0.15a	0.23	1.00b	0.07b	0.15a	0.23	0.11a	80.69b	72.62b	17.77c	24.87c
100	1.23bc	0.13ab	0.20	0.68c	0.06bc	0.13ab	0.20	0.08b	78.47b	63.02c	21.84b	34.36b
150	1.09c	0.11b	0.19	0.60c	0.05c	0.11b	0.20	0.08b	73.10c	57.94d	27.40a	39.50a
$LSD_{0.05}$	*0.32*	*0.03*	*0.05*	*0.21*	*0.019*	*0.029*	*0.048*	*0.024*	*3.293*	*2.856*	*1.356*	*4.98*
***F* test**												
Zn	ns	*	**	**	**	**	**	**	ns	ns	ns	ns
G	ns	*	ns	ns	ns	*	ns	ns	ns	**	ns	**
ZnxG	ns	*	ns	ns	ns	*	ns	ns	**	ns	**	ns
S	**	*	ns	**	**	**	ns	**	**	**	**	**
ZnxS	ns	ns	ns	**	ns	ns	ns	*	*	ns	**	ns
GxS	ns	ns	ns	ns	ns	ns	ns	ns	*	**	**	**
ZnxGxS	ns	ns	ns	ns	ns	ns	ns	ns	**	**	**	**

*, **: significant at $p < 0.05$ and $p < 0.01$ respectively; ns: not significant. †: Means followed by the same letter(s) in each column are not significantly different at $p < 0.05$. RWC: Leaf relative water content, LOT: Loss of turgidity, FTL: First true leaf, TL: Trifoliolate leaflet.

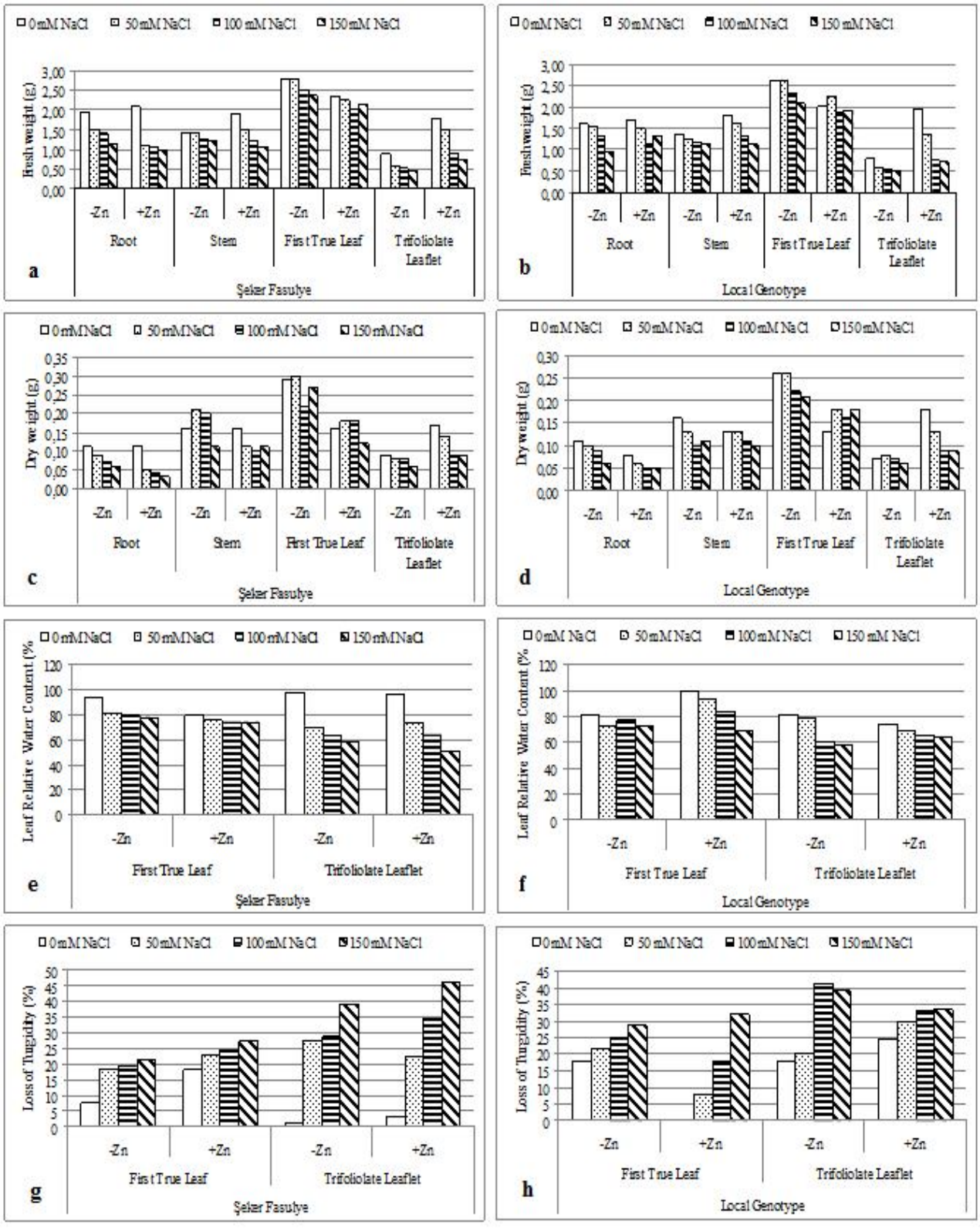

Fig 1: The effect of Zn priming and NaCl treatments on fresh and dry weight of plants, leaf relative water content and loss of turgidity of green bean genotypes.

NaCl applications significantly influenced the loss of turgidity in leaf samples of both bean genotypes. Differences between genotypes were also found to be significant at the $p<0.01$ level if LOT of trifoliolate leaflets was considered. Approximate LOT of trifoliolate leaflets between genotypes was observed of 'Şeker Fasulye' genotype. It was approximately 25.3%, which was clearly lower than 'Local Genotype' (29.9%) (Figure 1 g-h).

It can be concluded that NaCl application aggravated the LOT in the bean genotypes, but application of Zn was not sufficient in eliminating this negative transition while 'Şeker Fasulye' genotype was found to have better salt tolerance than 'Local Genotype'. This study is important from point of view that there is no study on the impact of Zn application on leaf turgidity under salt stress conditions.

Mineral nutrient concentrations: The effects of Zn priming (on seed) and varying concentrations of Zn, Na, K and Ca on the root, stem, first true leaf and trifoliolate leaflets of two different bean genotypes grown under salt stress revealed a significant difference in Zn concentrations in the root (Table 2, Figure 2). The effects of Zn priming, NaCl application and their interactions were also found significant between genotypes. Zinc concentration in the roots for both the green bean genotypes depicted an increase on Zn priming of seeds. In the roots of plants that were not subjected to Zn priming, the highest Zn concentration was found at 50 mM NaCl treatment whereas increasing salt concentration with Zn priming increased the Zn concentration (Figure 2a and b).

Significant differences were observed in the Zn concentrations in the stem after Zn priming and salt application. Zn priming and NaCl applications increased Zn concentration in the stem of green bean plants. A decrease in Zn concentration was observed in the stem of 'Şeker Fasulye' genotype that had not received Zn priming but had received the salt treatment. Zinc concentration in the stem was found to be highest in 'Şeker Fasulye' genotype exposed to Zn at 150 mM salt application whereas Zn concentration was lowest in the control plants of 'Local Genotype' without Zn application. Zinc concentration of stem was found lower than root but it was higher than the first true leaf and trifoliolate leaflet.

Zinc, NaCl applications and their interactions were significant ($p<0.01$ or 0.05) for Zn concentration in the first true leaf which varied according to the Zn concentration in the genotypes. Zinc concentration in the first true leaf of the 'Local Genotype' was less than 'Şeker Fasulye' on increasing salt concentration and Zn priming. Zinc concentration in the trifoliolate leaflet was affected only by Zn application ($p<0.01$). Zinc concentration in the trifoliolate leaflet did not show an increase on salt application and Zn priming had no effect on Zn accumulation in the tissue.

Zinc concentration in all parts of green bean plants increased with Zn priming since salinization of soil causes a decrease in Zn concentration of soil solution (Alloway, 2008). Alpaslan *et al*. (1999) found that Zn application in saline areas decreases the negative effect of Na^+ and Cl^- ions on plants and increase the tolerance of plants against salinity. Under saline conditions, the relationship between Na^+ and Cl^- ions and Zn appears to decrease the Zn intake (Alpaslan *et al*., 1999). In this study Zn priming enable green bean plants to react positively to salt conditions and increase Zn concentration in plant parts. Similar results have also been reported by Aktas *et al*., 2006 and Eker *et al*., 2013.

Sodium concentrations in the stem, first true leaf and trifolia leaflet of the green bean genotypes were affected significantly ($p<0.01$) by Zn priming (Table 2) but there was no effect on root Na concentration. Further, Na concentrations in the root and the trifoliolate leaflet were not affected by salt applications.

A decrease was observed in Na concentration in all tissues of various parts of 'Şeker Fasulye' and 'Local Genotype' on Zn priming (Figure 2c and d) at increasing rates of NaCl. The highest Na concentration in both genotypes was noticed in the first true leaves of control plants on Zn priming. Sodium concentration in the studied parts of the green bean genotypes increased with salt concentrations (without Zn conditions) whereas Na concentration decreased on Zn priming.

A decrease was observed in Na concentrations in the studied parts of green bean genotypes on Zn priming which correlate with findings of Aktas *et al.*, 2006 and Weisany *et al*., 2014. This decrease showed that the plant has a tolerance for NaCl toxicity on Zn application at a sufficient level; because Zn protects the structural and functional configuration of stem cell's membrane and Zn helps in the transfer of Na ions to the plasma membrane (Daneshbakhsh *et al*., 2013). Similarly, some researchers assert that Zn has a protective role in the membrane of the stem cell (Cakmak, 2000).

A significant effect for Zn priming and salt applications on K concentration in the root and the stem of the bean was found (Table 2). Potassium concentration in the root and stem of the plants increased with Zn application. NaCl application significantly influenced the K concentration in the root, stem, first true leaf and trifolia leaflet. In both genotypes, K concentrations in the root, stem, first true leaf and trifoliolate leaflet decreased with the increase in level of salt application regardless of Zn priming (Figure 2e-f). Potassium concentration in the trifoliolate leaflet was found to be higher in the 'Şeker Fasulye' genotype than 'Local Genotype'. A decrease in K concentration in all parts of the plant due to salt applications was observed and was found not to be dependent on Zn priming.

Table 2: Main effects of Zn priming and NaCl concentrations on Zn, Na, K and Ca concentrations in various parts of common green bean genotypes

	Zn concentration (mg kg^{-1})				Na concentration (%)				K concentration (%)				Ca concentration (%)			
Trait	**Root**	**Stem**	**FTL**	**TL**	**Root**	**Stem**	**FTL**	**TL**	**Root**	**Stem**	**FTL**	**TL**	**Root**	**Stem**	**FTL**	**TL**
Zinc (Zn)																
Without Zn	88.71b	55.05b	28.67b	64.58b	0.79	0.72a	1.172b	0.71a	1.52b	2.37b	2.85	2.68	0.70a	1.82a	2.78a	1.27a
With Zn	209.86a	115.49a	75.21a	88.91a	0.79	0.70b	1.561a	0.62b	1.73a	2.52a	2.95	2.83	0.50b	1.26a	1.66b	1.19b
$LSD_{0.05}$	*4.13*	*4.52*	*3.26*	*12.2*	*0.06*	*0.02*	*0.15*	*0.04*	*0.17*	*0.12*	*0.22*	*0.19*	*0.03*	*0.03*	*0.07*	*0.04*
Genotype (G)																
Local	176.65a	84.45	47.03b	80.587	0.88a	0.79a	1.19b	0.682	1.80a	2.64a	2.75b	2.52b	0.60	1.19b	2.36a	1.17b
Seker	121.93b	86.10	56.85a	72.908	0.71b	0.62b	1.53a	0.654	1.45b	2.25b	3.05a	2.98a	0.60	1.89a	2.08b	1.29a
$LSD_{0.05}$	*4.13*	*4.52*	*3.26*	*12.1*	*0.06*	*0.02*	*0.15*	*0.04*	*0.17*	*0.12*	*0.22*	*0.19*	*0.03*	*0.03*	*0.07*	*0.04*
Salinity (S) (mM)																
0	105.18d	70.35c	44.00c	73.15	0.83	0.775a	1.717a	0.67	2.38a	3.03a	3.46a	3.55a	0.82a	2.01a	3.39a	1.86a
50	142.71c	83.51b	51.52b	83.80	0.79	0.645c	1.630a	0.62	1.64b	2.63b	2.96b	2.74b	0.63b	1.94b	2.92b	1.44b
100	156.87b	91.97a	55.87ab	80.69	0.81	0.702b	0.989b	0.66	1.34c	2.18c	2.71bc	2.52b	0.55c	1.70c	2.06c	1.01c
150	192.40a	95.26a	56.37a	69.35	0.74	0.729b	1.129b	0.70	1.14c	1.94d	2.47c	2.20c	0.39d	0.51d	0.52d	0.61d
$LSD_{0.05}$	*5.84*	*6.39*	*4.61*	*17.2*	*0.08*	*0.03*	*0.21*	*0.06*	*0.24*	*0.17*	*0.32*	*0.28*	*0.04*	*0.04*	*0.11*	*0.05*
***F* test**																
Zn	**	**	**	**	ns	*	**	**	*	*	ns	ns	**	**	**	**
G	**	ns	**	ns	**	**	**	ns	**	**	*	**	ns	**	**	**
ZnxG	**	ns	*	ns	ns	**	**	**	**	*	ns	**	**	**	ns	**
S	**	**	**	ns	ns	**	**	ns	**	**	**	**	**	**	**	**
ZnxS	**	**	ns	ns	**	**	**	**	ns	ns	ns	ns	**	**	**	**
GxS	**	**	**	ns	ns	**	*	**	ns	**	*	ns	ns	**	*	**
ZnxGxS	**	**	*	ns	**	**	ns	**	ns	**	ns	ns	**	**	**	**

*, ** significant at $p < 0.05$ and $p < 0.01$ respectively; ns: not significant. †: Means followed by the same letter(s) in each column are not significantly different at $p < 0.05$.
FTL: First True Leaf, TL: Trifoliolate Leaflet.

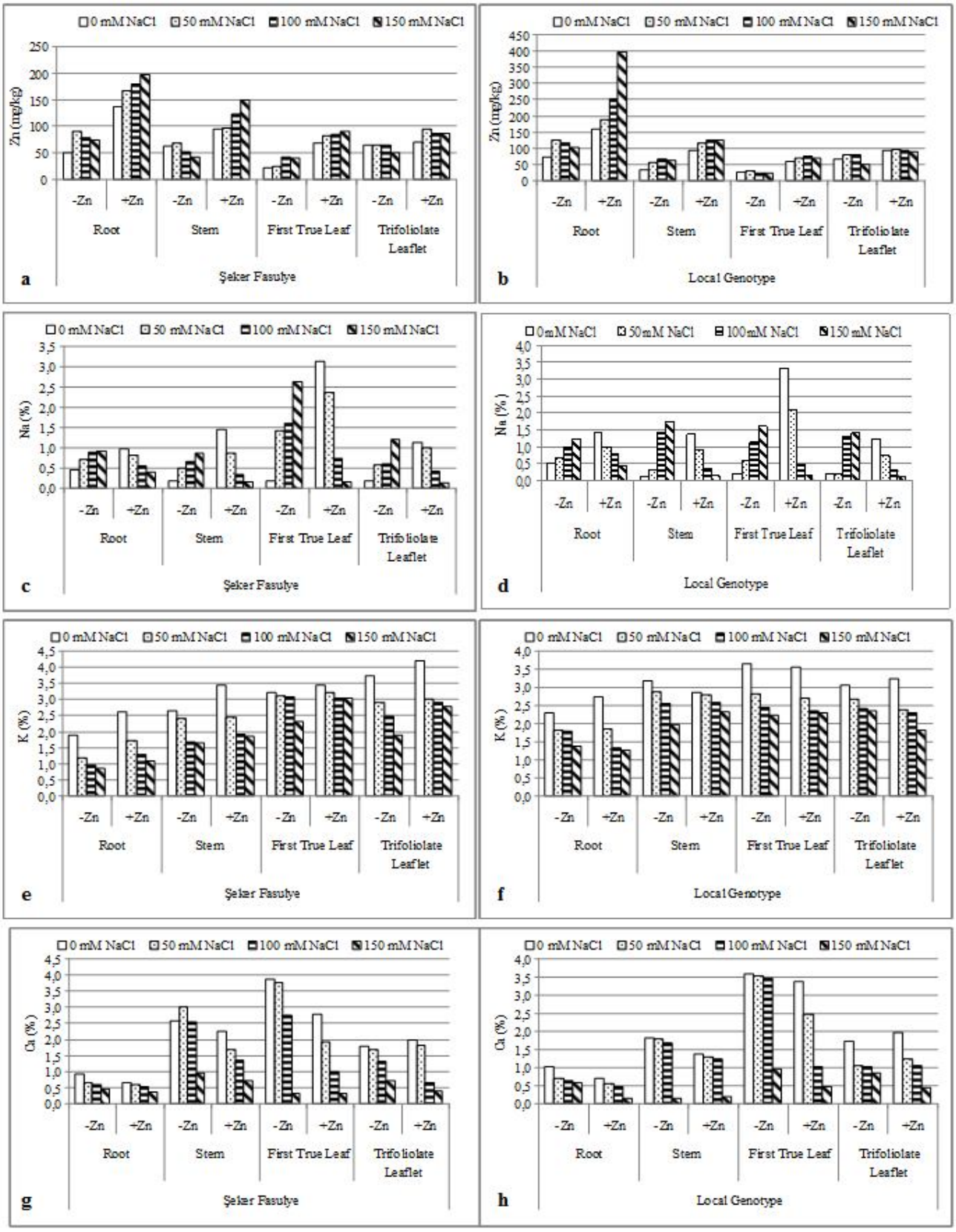

Fig 2: The effect of Zn priming and NaCl treatments on Zn, Na, K and Ca concentrations of green bean genotypes.

Potassium intake by plant roots depends on mass flow and diffusion but diffusion has a bigger role than mass flow. Water potential decreases in direct proportion to Na concentration in irrigation water and consequently the plant water uptake is less and K^+ intake decreases significantly. Sarwar and Ashraf (2003) reported that this decrease in the corn plant was a result of the antagonism between Na^+ and K^+. Generally, it is assumed that plants cannot hold K ions in their roots when subjected to salt stress and as a result they send the ions to the green part to maintain the osmotic balance of photosynthetic apparatus. In this study, it was observed that in both genotypes, the least amount of K concentration accumulated in the root which is well in accordance with findings of Eker *et al*. (2013).

Zinc priming had a significant but a decreasing effect on Ca concentration in the roots, stems, first true leaves and trifoliolate leaflets of both the green bean genotypes (Table 2). NaCl application significantly affected the Ca concentration in the root, stem, first true leaf and trifoliolate leaflet. Calcium concentration in all plant parts of both the genotypes decreased with increasing NaCl concentrations and Zn priming (Figure g-h). Ca concentration in the bean genotypes was highest in the first true leaf and lowest in the root. Calcium concentration in the plants' root, stem and first true leaf was higher on Zn priming than plants raised under control (Figure 2g-h).

In plants, maintaining Ca and membrane integrity is an important element in ensuring selectivity in ion intake and transport. Excessive salt application subjects plants to salt stress and as a result the transpiration ratio drops. Since, transportation of Ca in a plant depends mostly on transpiration, a decrease was detected in Ca concentration (Gilliham *et al*., 2011). In this study, a higher accumulation of Ca in the first true leaf seems indicate that NaCl application process began at the same time the first leaf was observed. A severe decrease in Ca concentration in all parts of genotypes was detected at the highest NaCl application (150mM). Tavori *et al*. (2004) also reported that NaCl concentration in high amounts decreased Ca accumulation in the stem and the leaves of chickpea. Mateos-Naranjo *et al*. (2008) found that Zn reduced the tissue Ca concentration of *S. densiflora*.

CONCLUSION

The results of this study conclude that salt concentration decreased the fresh and dry weights of plants in both green bean genotypes and Zn priming has compensatory effects. Leaf RWC was decreased with NaCl application with the LOT increased, thus, the compensatory effect of Zn applications was not observed 'Şeker Fasulye' genotype was found to be relatively more tolerant to salt than the 'Local Genotype'. Based on salt and Zn applications, genotypes showed different reactions. Zinc priming decreased Na concentration in different plant organs.

Zinc concentrations in the bean plants increased with Zn priming while Na concentration decreased on Zn priming at increasing NaCl concentrations. Zinc priming had a significant impact on root and stem K concentration while NaCl had a similar significant impact on all parts of the plant. A decrease in K concentrations was observed at increasing NaCl concentrations. Calcium in all plant parts of the genotypes decreased with Zn priming and NaCl applications. However, Zn priming improved Ca intake under NaCl conditions. Finally, it can be stated that green bean seeds will have lower Na and higher Zn concentration following Zn priming and it will reduce the salt damage.

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