ECOLOGIA BALKANICA
2020, Vol. 12, Issue 2 December 2020 pp. 87-96

The Role of Seed Zinc Priming on Tolerance and Ionic Ratios of Green Bean under Salinity

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Abstract. The effects of salt applications of different concentrations (50, 100, and 150 mM NaCl) on two green bean genotypes ['Şeker Fasulye' (salt-tolerant) and 'Local Genotype' (salt-sensitive)] grown from seeds with zinc (Zn) and without Zn priming were investigated. In order to determine which part of the green bean plant involved in salt tolerance of genotypes, the tolerance ratio (TR) were calculated on the basis of root, stem, first true leaf (FTL) and trifolia leaflet (TL) dry weights. Also, the ability of maintain in equilibrium for potassium (K)/sodium (Na) and calcium (Ca)/Na ratio in the root, stem, FTL and TL parts of plants were considered. According to TR values based on dry weight; it is thought that stem is the organ of green bean genotypes which plays an important role in salt tolerance. When considering all parts of both genotypes, K/Na ratio of 'Şeker Fasulye' was determined to be affected positively in plants from Zn primed seeds. Additionally, Zn application decreased the Ca/Na ratio in all parts of both green beans. In conclusion, the results of the current study suggest that the use of Zn priming of seeds could be an effective strategy to increase the salt tolerance of green bean genotypes.

Key words: dry weight, *Phaseolus vulgaris* L., salt tolerance, K/Na, Ca/Na.

Introduction

Salt stress prevents the growth and development of the plant as a result of osmotic and ion stress (Zhao et al., 2020). Osmotic stress is the first stress that occurs when a plant is exposed to soil salinity, and it affects plant growth instantly (Horie et al., 2012). The decrease in the amount of useable water causes the cell expansion to diminish and sprout development to slow down (Dadkhah & Grrifiths, 2006). Salinity negatively affects all development periods of the plant and promotes premature leaf fall, causing severe decreases in yield (Flowers et al., 2010). Ion toxicity then occurs when the salt level reaches a certain threshold, and after that point the plant is unable to maintain ion homeostasis and growth balance (Munns & Tester, 2008). Ion toxicity and osmotic stress are primary stresses that can cause oxidative stress and a number of secondary stresses (Liang et al., 2018). During the ion stress phase that occurs subsequent to the osmotic stress, denutrition or nutritional imbalance takes place in plants as the increasing sodium (Na^+) and chlorine (Cl^-) ions in the environment compete with necessary nutrition elements such as potassium (K^+), calcium (Ca^{2+}) and nitrate (NO_3^-) (Bazihizina et al., 2019).

Studies of plant tolerance to salt stress cover many aspects of the influences of salinity on plant behavior, including alterations at the morphological, physiological and molecular levels (Dajic, 2006). Since salt stress has very different effects on plants, many mechanisms can tolerate this stress in plants. Plants are able to reduce ionic toxicity by reducing the accumulation of toxic ions (Na^+ and Cl^- exclusion) in leaf blade and/or by increasing their ability to tolerate salts they cannot exclude from shoots (Roy et al., 2014). There are many strategies to reduce the negative effects of salt stress. Seed coating (Song et al., 2017) and seed priming is an effective strategy for decreasing the impacts of salt stress on plants, particularly at seedling stage (Ghassemi-Golezani and Nikpour-Rashidabad, 2017). Effects of priming or pre-treatment of seed persist under sub-optimum field conditions, such as salinity (Wahid et al., 2007).

One of the protective or assisting applications used in cultivation on salty fields is zinc (Zn) application to the soil or the seed (Saleh et al., 2009; Gulmezoglu et al., 2016). Alpaslan et al. (1999) informs that Zn application on salty soil diminishes the negative effects of Na and Cl on plants. Zinc is taken in by plants in the form of Zn^{+2} and becomes a part of various metabolic occurrences in plants (Gautam & Dubey, 2018). In addition to the plant variety, Zn intake of plants are affected by many conditions such as soil pH, organic substance of the soil, useful phosphorus substance, water substance, soil temperature and salinization by other elements. Soil salinization causes the Zn concentration in the soil compound to decrease (Khoshgoftar et al., 2004). This occurrence decreases the Zn intake as a result of a strong competition of salt ions with Zn^{+2} on the root's surface (AbdEl-Hady, 2007). In a case of Zn deficiency, transmissivity of the stem cell membrane increases (Gautam & Dubey, 2018) and consequently intake amount of other nutrition elements drops with the increasing Na^+ and Cl^- amounts in the roots of plants that grow in saline areas, therefore, Zn application decreases the negative impacts of salinization (Eker et al., 2013).

As in many other plants, the effects of salt stress on green bean were examined in terms of morphological, physiological and biochemical properties (Taïbi et al., 2016). Although various authors have described green bean as sensitive to salinity (Levitt, 1980), the differences between varieties also were determined in green bean as in many other crops (Assimakopoulou et al., 2015; Gulmezoglu et al., 2016; Torche et al., 2018). For instance, according to dry weights and andioxidative response, Tema showed better protection against salinity than Djadida genotype (Taïbi et al., 2016). Torche et al. (2018) also showed that there was a genetic variability between four green bean genotypes for salinity tolerance based on yield traits, total phenolic and flavonoid content. Even though there are considerable studies on the effects of salinity on green bean plants, there is a lack of information about the strategies to cope with salt stress. Therefore, this study, aims to determine the alleviative effects of seed Zn priming on variations in tolerance, K/Na and Ca/Na ratios of green bean genotypes at different salt concentrations.

Material and Methods

Zinc priming: Seeds of two green bean genotypes ('Şeker Fasulye' and 'Local Genotype') were used in the experiment. For Zn priming, the seeds were soaked in a solution of zinc sulphate ($ZnSO_4.7H_2O$) prepared to contain 0.05% zinc (Zn) before planting (Harris et al., 2008). After the seeds were soaked in the Zn solution, they were washed with deionized water and air dried. The seeds without Zn were washed only with deionized water and dried.

The trial: Randomized blocks are arranged in 3 repeats and 10 plants per repeat according to the trial pattern. The seeds were sown into seedling trays filled with a mixture of peat: perlite: vermiculite (2:1:1) and placed in a plant growth chamber (DAIHAN WGC-1000), in which temperatures were 26/18 ºC (day / night), with approximately 70% RH and a light intensity of 450 molμm^{-2}s^{-1} (Khadri et al., 2006). The water needs of the plants were started as 20 mL of ultrapure water daily until the first true leaves (FTL) were emerged, and then the salt applications [0 (control), 50, 100 and 150 mM NaCl] were started and treated for two weeks.

Determination of ions: At the end of experiment, the aboveground parts of the plants [FTL, trifolia leaflet (TL), and stem] and root parts were separated, and were washed with tap water and then with deionized water. The plant parts were dried in the drying cabinet at 65 °C until it reached a constant weight. After the weights of the aboveground parts of the dried plant samples were determined, they were milled in the tungsten coated plant-grinding mill. 0.2 g of the milled plant samples were ashed at 550 °C according to the dry ashing method and dissolved in 3.3% HCl (20 mL) and filtered with blue tape filter paper. Potassium, Ca and Na in the strainer were determined in the flame photometer (Thermo Aquamete-2000E) (Richards, 1954).

Tolerance Ratio (TR): Tolerance ratio was used to compare green bean genotypes based on reactions to different NaCl concentrations (Chandler et al., 1986). Tolerance ratio was calculated based on root, stem, FTL, and TL dry weights for each genotype and each salt concentration with and without Zn pre-treatment according to the formula below. Thus, genotypes were not compared to real figures determined in saline environment, but with their proportional development against control in an environment with a certain concentration of salt.

$TR = T_x / T_0$

T_x = dry weight gain on x mM NaCl,

T_0 = dry weight gain on 0 mM NaCl,

The ability of keeping the balance for K/Na and Ca/Na ratios in the aboveground and root parts of the plants were also considered as the other parameters to compare green bean genotypes reaction to salinity.

The results obtained were evaluated using the "IBM SPSS Statistics 20" statistics program. The difference between applications was compared with the Tukey's multiple comparison test at $p < 0.01$ and 0.05 significance levels.

Results and Discussion

Tolerance Ratio (TR): The TR values of aboveground and root parts of Zn priming and Zn non-priming two green bean genotypes under saline conditions are shown in Table 1. According to the average values, Zn priming increased the TR values only in concerning the TR calculated on the basis of FTL dry weight. However, no statistically significant differences were found between genotypes and salt applications in terms of the TR calculated on the basis of FTL dry weight. Besides, the data showed that, 'Şeker Fasulye' (TR=0.91) was more tolerant to salinity than 'Local Genotype' (TR=0.77) exclusively according to TR values calculated on the stem dry weight. Additionally, it is clear from the data, the TR values generally decreased on the basis of the dry weight calculated in all parts of the plants in both genotypes depending on the increases in NaCl concentrations.

High salt concentrations in the irrigation water result in reduced plant growth, limiting leaf expansion and changing the relationship between the aerial and root parts (Acosta-Motos et al., 2017). In this study, although the TR values calculated on the basis of root and TL dry weights were found higher in 'Local Genotype' than those in 'Şeker Fasulye' genotype, 'Şeker Fasulye' was more tolerant than 'Local Genotype' to salinity according to the TR values calculated on the basis of stem dry weight. In our previous work it has been found that

'Şeker Fasulye' genotype has more fresh and dry weight in all sampling organs (except dry weight of FTL) than 'Local Genotype' thus was found to be relatively more salt tolerant according to these parameters (Gulmezoglu et al., 2016). Therefore, it is suggested that the higher TR values calculated on the basis of stem in green bean plants could possibly be one of the factors involved in conferring salt tolerance. There have been no reports demonstrating this subject in green bean plants. However, it was determined that the highest TR values calculated on the basis of leaf and root dry weight in cv. Camarosa which was more salt tolerant strawberry cultivar than cv. Chandler (Turhan & Eris, 2007). Besides, it is a fact that different results can occur between species or even varieties depending on the salt concentrations and the duration of salt exposure (Acosta-Motos et al., 2017).

K/Na ratio: The significant differences in K/Na ratio of green beans'parts on Zn, salt and genotype were shown in Table 2. Seed Zn priming had only a significant effect ($p<0.01$) on K/Na ratio in stem. Therefore, K/Na ratio was not affected by Zn in the other parts of the green beans genotypes. However, salt application was found significant ($p<0.01$) on K/Na ratio of all parts of the green bean. While K/Na ratio in stem was found to be significant on genotypes, there was no difference that of root, FTL and TL. Salt × Zn interaction was significant on K/Na ratio in the all parts of green bean plants. The interactions of Zn × Genotype, Salt × Genotype and Zn × Salt x Genotype were significant ($p<0.05$ or 0.01) only on K/Na ratio of stem and TFL.

The Zn × Genotype interaction among the studied parts of the plants, the least amount of K/Na ratio was seen in the root (Fig. 1). The K/Na ratio of 'Local Genotype' was higher in root, TFL and TL without-Zn priming but K/Na ratio in stem had higher both without-Zn priming and with-Zn priming. The K/Na ratio in stem of 'Şeker Fasulye' genotype had no big difference according to Zn priming. The K/Na ratio at without-Zn priming in TL of 'Şeker Fasulye' was lower than that of 'Local Genotype'; however, Zn priming caused the K/Na ratio of 'Şeker Fasulye' to increase and that of 'Local Genotype' to decrease. When considering all parts of both genotypes, K/Na ratio of 'Şeker Fasulye' was affected positively in plants from Zn priming seeds (Fig. 1). K/Na ratio in FTL and TL of 'Şeker Fasulye' had higher than 'Local Genotype' in plants from Zn primed seeds. The K/Na ratio under salt stress is an important data and K/Na was affected significantly with Na^+ and K^+ concentrations. These elements showed changes genotypically negative and positive directions when Zn applied to seeds (Fig.1). Also, Zn × Genotype interaction was found to be significant and similar findings were found in tomato (Alpaslan et al., 1999), wheat (Singh et al., 2015) and mung bean (Samreen et al., 2017).

The Salt × Genotype variation of K/Na ratio in stem of 'Local Genotype' was higher than that of 'Şeker Fasulye' according to salt ratio increased. However, K/Na ratio in TL was higher in 'Şeker Fasulye' than that of 'Local Genotype'. Trifoliate leaflet of both genotypes was affected positively by 150 mM salt concentration which K/Na ratio was the highest. However, 'Şeker Fasulye' also showed a positive effect on transportation of K/Na from root to young leaves (TL) under salt condition. It is known that the high K/Na ratio in the plant is directly proportional to salt resistance (Wei et al., 2017). There is a positive correlation between the amount of K^+ content in the plant leaves and the increase in plant resistance under saline conditions and high K/Na ratio are directly proportional to salt resistance (Wei et al., 2017). It was pointed out by tolerance rate that 'Şeker Fasulye' were more tolerant than the 'Local Genotype' to salt stress. The increase that occurs in the Na^+ amount usually disturbs the osmotic regulation and nutrition balance, therefore enters into the specific ion toxicity, and starts competing with K^+ ion due to the similarities in their ionic diameters and electric charges. Consequently, this increase in Na^+ prevents the intake of K^+ ion (Levitt, 1980). Romero et al. (1997) assert that the increasing Na^+ amount in the leaves might cause K^+ deficiency as a result of the antagonistic impact of Na^+ and K^+ ions. As a result, Zn application from seed decreased the Na^+ intake to the cell, and increased K/Na ratio in the plant parts with less Na^+ intake. Similar results were found by some researchers (Saleh et al., 2009; Shabala et al., 2010; Singh et al., 2015) as well.

The Salt × Zn interaction was significant on all parts of green bean. The highest K/Na ratio in all parts of green bean genotypes was obtained without salt applications and this increase decreased with increasing ratio of salt. However, Zn priming of seeds changed increasingly K/Na ratio with increasing salt ratio. The K/Na ratio of TFL was positively affected by Zn priming. The decreases in K/Na ratio in all parts of green beans with increasing salt concentrations were observed in different plants (Eker et al., 2013). Zinc is a very important on strength of cell membranes. When Zn is deficient in plants, membrane permeability increases, especially under stress conditions (Cakmak, 2000). This may increase uptake of Na^+ to a toxic level for plants (Chakraborty et. al., 2018).

The Salt x Genotype x Zn interaction was found to be be statistically significant on K/Na rate of stem and TL (P<0.01) (Table 2). Besides K / Na ratio showed differences according to genotypes in organs determined to be statistically significant. In plants from Zn primed seeds of 'Şeker Fasulye' had higher K/Na ratio at 150 mM salt application than that of 'Local Genotype' (Fig. 1).

Ca/Na ratio: The variance analysis results of Ca/Na ratio of belong to root, stem, FTL and TL of two green bean genotypes are shown in Table 2.

Table 1. Mean and analysis of variance of TR of different parts of two green bean genotypes from with or without zinc primed seeds under salinity conditions. Legend: (*) Significant within column at p<0.05, (**) Significant within column at p<0.01, (ns) - non-significant.

Trait	Tolerance ratio (TR)			
	Root	Stem	FTL	TL
		Zinc (Zn)		
(-) Zn	0.67a	0.89a	0.89b	0.94a
(+) Zn	0.52b	0.79b	1.17a	0.61b
		Genotype (G)		
Local Genotype	0.66a	0.77b	1.07	0.83a
Şeker Fasulye	0.52b	0.91a	0.99	0.72b
		Salinity (S) (mM)		
0	-	-	-	-
50	0.72a	0.97a	1.13	0.94a
100	0.64a	0.85b	0.99	0.72b
150	0.42b	0.70c	0.97	0.67c
		***F* test**		
Zn	**	*	**	**
G	**	**	ns	*
ZnxG	**	**	ns	**
S	**	**	ns	**
ZnxS	**	ns	ns	ns
GxS	ns	*	ns	ns
ZnxGxS	**	*	ns	ns

Table 2. Analysis of variance belongs to K/Na and Ca/Na ratio of different parts of green bean genotypes from with or without zinc primed seeds under salinity conditions. Legend: see Table 1.

	K/Na ratio				Ca/Na ratio			
Trait	**Root**	**Stem**	**FTL**	**TL**	**Root**	**Stem**	**FTL**	**TL**
Zinc (Zn)	ns	**	ns	ns	**	**	**	**
Salt (S)	**	**	**	**	**	**	**	**
Genotype (G)	ns	**	ns	ns	ns	**	**	**
Zn × G	ns	**	ns	*	ns	**	ns	*
S × G	ns	**	ns	**	ns	**	*	**
S × Zn	**	**	**	**	**	**	**	**
S × G× Zn	ns	**	ns	**	ns	**	*	**

A significant variance ($p < 0.01$) was determined for Ca/Na ratio of the root, stem, FTL and TL in terms of Zn and salt. Genotypic difference was not found only Ca/Na ratio of root. The Zn × Salt interaction was significant on Ca/Na ratio of root, stem, FTL and TL. The Zn × Genotype interaction was found significantly on Ca/Na ratio in stem and TL while Genotype × Salt and Zn × Genotype × Salt interactions were not significant only root in terms of Ca/Na ratio.

The Zn × Genotype interaction was examined that Zn priming to seed decreased the Ca/Na ratio in all parts of plant (Fig. 1). The Ca/Na ratio from the highest to the lowest was changed between sampled organs and arranged in order of FTL > stem >TL >root from the highest to the lowest, respectively. The content of Ca decreased in the roots, stems, FTL and TL of both genotypes due to Zn priming. Priming of seeds by Zn in this study may affected negatively on Ca concentrations of green bean plants. The Ca/Na ratio in root and stems of 'Şeker Fasulye' was affected much more by Zn priming than 'Local Genotype' but Ca/Na ratio was found lower in FTL and TL and 'Şeker Fasulye' than that of 'Local Genotype'.

Increasing salt concentrations decreased Ca/Na ratio all parts of green bean genotypes. The Salt × Genotype interaction was not efficient on root. The mean of Ca/Na ratio in 'Şeker Fasulye' was higher than that of 'Local Genotype' in all organs. Thus, Ca/Na ratio in 'Local Genotype' was affected negatively. The Ca/Na ratio in organs decreased in order; FTL > stem> TL> root. Roots were damaged firstly from salt because of being contact organ. Salt tolerance of most plants at high concentrations depends on being impermeable to salt (Chakraborty et al., 2018). However, for the cell to preserve its selective permeability, it depends on the balance of the monovalent (K^+, Na^+) and divalent (Ca^{2+}) cations (Chakraborty et al., 2018).

In this study, it has been shown that the Ca/Na ratio decreased as a result of higher Na ion uptake under salt conditions. It is expected that a plant which is protected from salt by passively keeping the salt away from its body, will have low permeability against Na salts at fairly high salt concentrations. In addition, Ca^{2+} is the main cation in order for the aforementioned balance in permeability to be protected (Tuna et al., 2007).

Depending on Salt x Zn, Ca and Na concentration has dropped as the Ca/Na ratio in the parts of the plant increased slightly with Zn priming (Fig. 1). It has been observed that Na concentration is high in parts of the plant with the salt application, whereas the Ca/Na ratio is low in plants in which Zn priming has not been applied. It was showed that Zn application to green bean seeds restrained to uptake of Ca. The Ca/Na ratio in organs due to Zn priming was lower when compared to without-Zn priming and it was on the decline in order; FTL > stem> TL> root. There were contrary results for Ca/Na ratio of some plants studied Zn applications under salt stress.

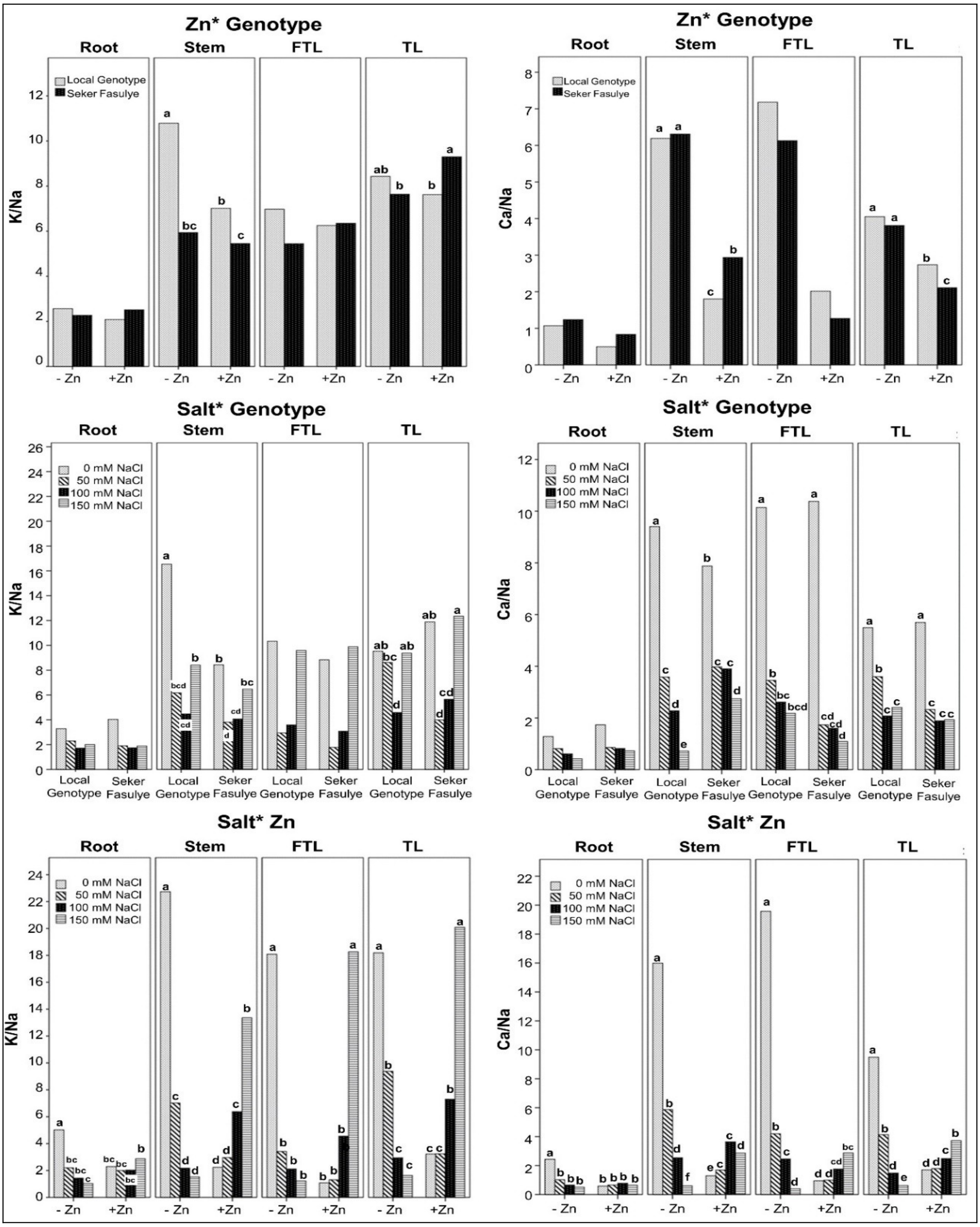

Fig. 1. The interactions (Zn x Genotype, Salt x Genotype and Salt x Zn) of NaCl on K/Na and Ca/Na ratio of different parts of green bean genotypes from with or without zinc primed seeds. Different letter(s) represented on each bar show(s) statistically significant differences at $p < 0.05$.

Researchers attributed that Ca/Na ratio in plant parts increase with-Zn application but it was decreased by salt treatments (Eker et al., 2013; Hejazi-Mehrizi et al., 2011).

The Salt x Genotype x Zn interaction was found to be significant on Ca/Na rate of stem, FTL and TL (Table 2), besides in plants from Zn primed seeds of 'Şeker Fasulye' had higher Ca/Na ratio at 150 mM salt application than that of 'Local Genotype' (Fig. 1). Higher Ca/Na and K/Na ratios in the leaf organs of 'Şeker Fasulye' were found to be effective in salt tolerance.

It has been identified that there is a significant difference ($p<0.01$) between applications and genotypes, and their interactions to the ratio of the stem and Ca/Na concentration. The Ca/Na ratio of stem has reached the highest value after the Ca/Na ratio of FTL and increased in 'Local Genotype' with the application of Zn and 100 mM NaCl. The Ca/Na concentration of FTL has been determined to be higher in without Zn application. The Ca/Na ratio in FTL of both genotypes under Zn condition has decreased as the salt ratio increased.

Conclusion

In conclusion, the higher TR values calculated on the basis of dry weight indicate that tolerance of stems could possibly be one of the factors involved in conferring overall salt tolerance in green bean plants. Also, this research has shown the importance of Zn priming of seeds to alleviate the effects of salt stress on green bean plants by increasing K/Na and Ca/Na ratio. Positive react of genotypes to Zn priming was observed at 'Şeker Fasulye'. It is recommended to examine the effect of Zn priming to seed of green beans on salt tolerance, K/Na and Ca/Na ratios under the field conditions.

Acknowledgements

The authors thank to the Eskisehir Osmangazi University Research Foundation for supporting this study (Project No: 201323D15) financially.

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Received: 03.06.2020
Accepted: 12.09.2020