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The Effect of Zinc on Growth and Shoot Concentrations of Sodium and Potassium in Pepper Plants under Salinity Stress

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Abstract: The effect of increasing concentrations of zinc (Zn) on NaCl toxicity was studied in pepper (*Capsicum annuum* L. cv. Kahramanmaras-3) plants grown in a growth chamber under controlled conditions. Plants were grown in severely Zn-deficient soil with increasing Zn (0, 2, and 10 mg Zn kg^{-1} soil) and NaCl (0%, 0.5% and 1.5% NaCl in irrigation water) treatments. After 46 days of growth, the plants were harvested and the shoots were analyzed for dry matter production, concentrations of Zn, sodium (Na), potassium (K), and phosphorous (P), and K/Na ratios. The results showed that Zn deficiency in soil significantly reduced shoot growth, particularly under the highest salt treatment. As expected, increasing the application of NaCl reduced shoot dry matter production; however, this decrease was greater in the 2 mg Zn kg^{-1} soil compared to the 10 mg Zn kg^{-1} soil. Increases in Zn application from 2 to 10 mg kg^{-1} soil reduced shoot concentration of Na and elevated K concentration. Consequently, K/Na ratios of plants were highest in the highest Zn application condition. The results of the present study indicated the importance of the Zn nutritional status of plants in improving salt stress tolerance. Possibly, by affecting the structural integrity and controlling the permeability of root cell membranes, adequate Zn nutrition reduces excess uptake of Na by roots in saline conditions. Adequate Zn nutrition is, therefore, important for the maintenance of good growth and yield under saline conditions.

Key Words: *Capsicum annuum*, salinity stress, zinc deficiency, membrane integrity

Çinko Uygulamasının Tuz Stresi Altındaki Biber Bitkisinde Büyüme ve Yeşil Aksam Sodyum ve Potasyum Konsantrasyonu Üzerine Etkisi

Özet: Kontrollü iklim odalarında yetiştirilen biber bitkilerinde artan çinko uygulamalarının NaCl toksisitesi üzerine etkisi araştırılmıştır. Bitkiler çinko eksikliğine sahip bir toprakta artan Zn (0, 2 ve 10 mg Zn/kg toprak) ve NaCl (% 0, % 0.5 ve % 1.5 sulama suyu içinde) uygulamalarında yetiştirilmiştir. Bitkiler 46 günlük olunca hasat edilmiş ve yeşil aksam kuru madde üretimi, çinko (Zn), sodyum (Na), potasyum (K) ve fosfor (P) konsantrasyonları ve K/Na oranları belirlenmiştir. Sonuçlar, topraktaki çinko eksikliğinin özellikle yüksek tuz uygulaması altında yeşil aksam büyümesini önemli ölçüde etkilediğini göstermiştir. Beklenildiği gibi, artan NaCl uygulaması yeşil aksam kuru madde üretimini azaltmıştır, fakat bu azalma 2 mg Zn kg toprak uygulamasında 10 mg Zn kg toprak uygulamasına göre daha fazla olmuştur. Çinko uygulamasının 1 kg toprak başına 2 mg'dan 10 mg'a arttırılması, yeşil aksam Na konsantrasyonunu azaltmış ve K konsantrasyonunu ise arttırmıştır. Bu etkilerin bir sonucu olarak, yüksek Zn uygulamasında bitkilerin K/Na oranları da yüksek olmuştur. Bu çalışmadan elde edilen sonuçlar, tuz stresine karşı toleransın arttırılmasında bitkilerin Zn beslenme statüsünün önemine işaret etmektedir. Olasılıkla, tuzlu koşullar altında yeterli Zn beslenmesi, kök hücre membranlarının yapısal bütünlüğünü ve membran geçirgenliğini kontrol ederek fazla sodyum alınmasını azaltmaktadır. Bu nedenle, yeterli bir Zn beslenmesi tuzlu koşullarda iyi büyüme ve verim açısından önem taşımaktadır

Anahtar Sözcükler: *Capsicum annuum*, Tuz stresi, Çinko eksikliği, Membran stabilitesi

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Introduction

In practical agriculture, several compounds are being applied to ameliorate the Na toxicity problem by inhibiting its uptake by plant roots. Application of Ca-containing compounds like gypsum is highly effective in alleviating Na-dependent cell damage in plants (Rengel, 1992; Shabala et al., 2006). According to Shabala et al. (2006), Ca ameliorates Na toxicity in plants by reducing Na uptake and improving K nutrition of plants. The protective effects of Ca against Na toxicity in plants are ascribed to the role of Ca in maintaining the membrane integrity of root cell membranes (Rengel, 1992; Epstein, 1999; Shabala et al., 2006).

By regulating membrane integrity and controlling permeability, Ca prevents excessive Na uptake by roots. Like Ca, Zn also greatly affects the structural and functional integrity of cell membranes (Welch et al., 1982; Cakmak and Marschner, 1988). In Zn-deficient plants, loss of membrane integrity and increase in membrane permeability are very common in different crop species (Cakmak, 2000). Loss of membrane integrity under Zn deficiency may affect the uptake and accumulation of Na at toxic levels in plants. Norvell and Welch (1993) showed that low Zn supply enhanced Na accumulation in barley plants. Similarly, Alpaslan et al. (1999) found that a sufficient Zn supply could reduce Na accumulation and contributed to salt tolerance in tomato plants. Zinc deficiency is a significant nutritional problem in soils worldwide (White and Zasoski, 1999), as well as in Turkey (Cakmak et al., 1999), and is generally associated with salinity problems found in the soils of Australia and Central Anatolia (Cakmak et al., 1996; Genc et al., 2005). Therefore, improving the Zn nutritional status of plants could greatly improve their salt stress tolerance.

In the present study we used a severely Zn-deficient soil to study the effect of increasing NaCl treatment on growth and ion accumulation in a pepper (*Capsicum annuum* L.) genotype in different Zn conditions. Pepper is known to be very sensitive to salt stress (Sonneveld, 1988; Shannon and Grieve, 1999) and, therefore, it is important to study the effect of Zn on the salt stress tolerance of pepper plants.

Materials and Methods

Pepper seeds (*Capsicum annuum* var. Kahramanmaras-3) were provided by Çukurova University, Faculty of Agriculture, and the Horticulture Department Seed Bank. Seeds were sown in plastic germination boxes filled with perlite. Germination was conducted in a computer-controlled growth chamber under total dark conditions at 28 °C. On day 8, the germination boxes were uncovered and preculture conditions were set to a 16h/8h day/night regime (light intensity: ~400 µmol $m^{-2} s^{-1}$ at plant height) at 26 °C/22 °C and 60%/70% relative humidity. Perlite medium was sufficiently moistened with concentrated $CaSO_4$ solution during the germination and preculture periods. Following 7 days of preculture in perlite, uniformly germinated seedlings were selected and transplanted to plastic pots filled with 3000 g of air-dried soil at a rate of 2 seedlings per pot.

The Zn deficient soil used in the experiment was collected from the Central Anatolia region and had the following properties as measured according to Jackson (1958): texture: clay (C); $CaCO_3$: 14.9%; pH: 8.04; organic matter: 0.69%; total soluble salts: 0.08%; DTPA-extractable Zn: 0.09 mg kg^{-1} soil. A basal treatment of 200 mg N kg^{-1} soil as $Ca(NO_3)_2$, 100 mg P kg^{-1} soil as KH_2PO_4, and 2.5 mg Fe kg^{-1} soil as Fe-EDTA was applied to all plants. Zinc was applied at the rate of 0, 2, and 10 mg kg^{-1} soil as $ZnSO_4.7H_2O$. Plants were grown in a greenhouse (lat 37°03'21"N, long 35°21'19"E) with an average day/night temperature of 27 °C /20 °C (± 3 °C). Salt treatment by irrigation with NaCl solutions of 0%, 0.5%, and 1.5% (w/v) was started 17 days after transplanting to soil. At harvest (46 days after sowing), shoots were excised, dried at 70 °C for 48 h, and weighed to determine dry matter production. All samples were then ground and ashed in a muffle furnace at 550 °C for 4 h. The ashed samples were dissolved in 3.3% (v/v) HCl and analyzed for Na, K, and Zn with an atomic absorption spectrometer (Varian Spectra AA 220 FS). Phosphorus was determined with spectrophotometry (Hitachi U-2000), according to Barton (1948).

The soil salinity levels (EC) following salt treatments were determined at harvest in saturated paste extract, according to the method described by Jackson (1958), and were classified according to the USDA Salinity Lab Staff (1964). Measurements of Na, K, Zn, and P were checked against reference leaf samples from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Data presented are mean values obtained from

4 independent replications (± SD). The differences among the means were compared by the Duncan multiple range test at the 0.05 probability level.

Results

Treatment of the experimental plants with water containing varying concentrations of NaCl (0%, 0.5%, and 1.5%) began when the plants were 17-days-old and continued for the next 20 days. The treatment of plants with such different amounts of NaCl over 20 days resulted in different levels of soil salinity in the pots (Figure 1). Regardless of salt treatment concentration, growing plants in Zn-deficient soil without Zn treatment greatly reduced shoot dry matter production (Figures 2 and 3). As shown in Figure 3, the reduction in shoot growth caused by Zn deficiency became more severe at the highest concentration NaCl treatment. Adequate addition of Zn into soil (e.g., 2 or 10 mg Zn kg^{-1} soil) enhanced shoot dry matter production by nearly 3-fold at the nil NaCl treatment (Figure 3). Treatment of the plants with increasing amounts of NaCl reduced shoot dry matter production at each Zn treatment. Under sufficient Zn conditions (e.g., at 2 and 10 mg kg^{-1} soil), increasing NaCl reduced the shoot growth of plants in Zn 2 mg kg^{-1} soil conditions at greater rates than the plants in Zn 10 mg kg^{-1} soil (Figure 3). These results indicated the protective role of high Zn supply against salt stress-dependent decreases in growth.

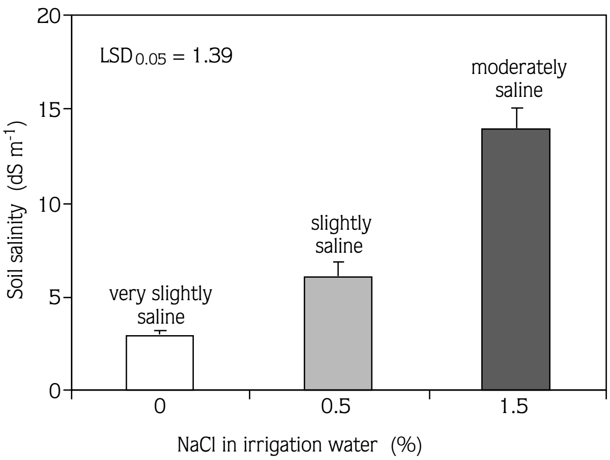

Figure 1. The effect of NaCl in irrigation water on soil salinity at harvest. Soil salinity level was classified according to USDA (Salinity Lab Staff, 1964). Data presented are mean values obtained from 4 independent replications (± SD).

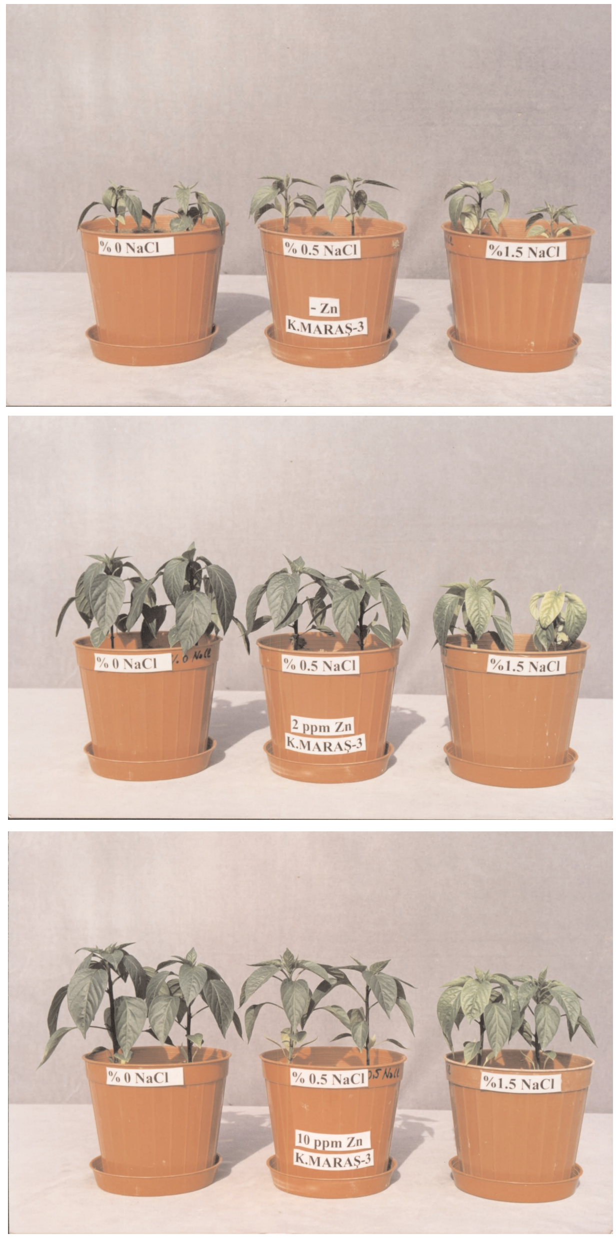

Figure 2. The effect of increasing Zn (0, 2, and 10 mg Zn kg^{-1} soil) and NaCl (0%, 0.5%, and 1.5% in irrigation water) on the shoot growth of 45-day-old pepper plants grown in severely Zn-deficient soil.

Shoot Zn concentrations of the plants were much lower at the nil Zn treatment than the plants with Zn treatments (Table 1). At each salt treatment, increasing Zn accordingly increased shoot Zn concentration of the plants. The greatest increases in shoot Zn concentration were found at the highest NaCl treatment, possibly due to the concentration effect on reduced plant growth.

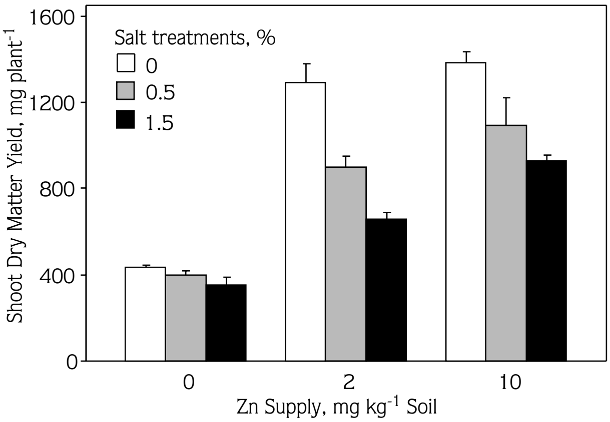

Figure 3. The effect of increasing Zn and NaCl treatments on the shoot dry matter production of 45-day-old pepper plants grown in severely Zn-deficient soil. Each value represents the mean of 4 independent replications.

Shoot concentrations of Na were increased by elevated levels of NaCl at each Zn treatment (Table 1). The increases in Na concentration were greater at the lower (2 mg Zn kg^{-1} soil) than the higher (10 mg Zn kg^{-1} soil) Zn application (Table 1). In the case of K, increases in Zn application increased shoot K concentration. This Zn effect was found at each NaCl treatment. Shoot P concentrations were significantly higher in the Zn-deficient plants than in the plants grown in 2 and 10 mg Zn kg^{-1} soil conditions (Table 1). Applying NaCl elevated the P concentration of Zn-deficient plants. At 2 and 10 mg Zn kg^{-1} soil applications, increasing NaCl treatments did not result in significant changes in P concentration in the shoots (Table 1).

In accordance with the increasing effect of Zn on K concentration and its reducing effect on Na concentration in shoots, K/Na ratios generally increased along with increased Zn application (Figure 4). At the low NaCl treatment (0.5%), the effect of Zn treatment on K/Na ratio was very distinct, especially at the highest Zn treatment.

Discussion

The treatment of plants with increasing amounts of NaCl significantly reduced shoot growth (Figures 2 and 3). The decreases in shoot growth due to NaCl became less distinct when the growth of the plants was already depressed by Zn deficiency (Figure 2). This indicated that under the Zn-deficient conditions, Zn deficiency was a greater limiting factor than NaCl toxicity in reducing growth. Similar results were also reported by Genc et al. (2005) in studies with wheat plants.

When the plants were adequately supplied with Zn (e.g., 2 and 10 mg kg^{-1} soil), the reductions in shoot growth due to NaCl treatments were more evident at the lower (2 mg kg^{-1} soil) than the higher (10 mg kg^{-1} soil) doses of Zn. For example, at the 2 mg Zn kg^{-1} soil condition, increasing NaCl treatment from 0% to 5% and from 0.5% to 1.5% reduced shoot dry matter production by 50% and 27%, respectively, while these values were 32% and 15% for the plants treated with 10 mg Zn kg^{-1} soil (Figure 2). These results suggested that Zn was protective against NaCl toxicity in plants.

Table 1. The shoot Zn, Na, K, and P concentration of 45-day-old pepper plants grown with different Zn levels under different NaCl treatments (mean ± SD; n: 4).

Zinc	NaCl	Zn	Na	K	P
(mg kg^{-1} soil)	(%)	(mg kg^{-1} dry wt.)	(mg g^{-1} dry wt.)		
0	0	2.5 ± 0.4 e	1.9 ± 0.2 e	17.7 ± 0.3 e	4.0 ± 0.2 b
	0.5	3.0 ± 0.8 e	6.0 ± 0.4 de	17.2 ± 0.5 e	4.5 ± 0.3 a
	1.5	4.3 ± 0.2 e	14.4 ± 1.2 c	20.8 ± 0.9 de	4.5 ± 0.1 a
2	0	12.4 ± 0.7 d	3.0 ± 0.4 e	36.3 ± 1.9 a	2.9 ± 0.1 c
	0.5	17.9 ± 1.0 c	8.5 ± 0.9 d	25.1 ± 2.8 c	2.7 ± 0.0 c
	1.5	21.4 ± 1.6 c	26.7 ± 2.0 a	20.5 ± 1.3 de	2.6 ± 0.1 c
10	0	51.3 ± 3.0 b	0.5 ± 0.1 de	39.8 ± 1.1 a	2.5 ± 0.0 c
	0.5	71.3 ± 3.0 a	5.1 ± 0.8 de	29.4 ± 1.2 b	2.6 ± 0.1 c
	1.5	72.3 ± 2.4 a	20.0 ± 3.3 b	22.4 ± 0.7 cd	2.5 ± 0.1 c

* Values not associated with the same letter are significantly different (P < 0.05).

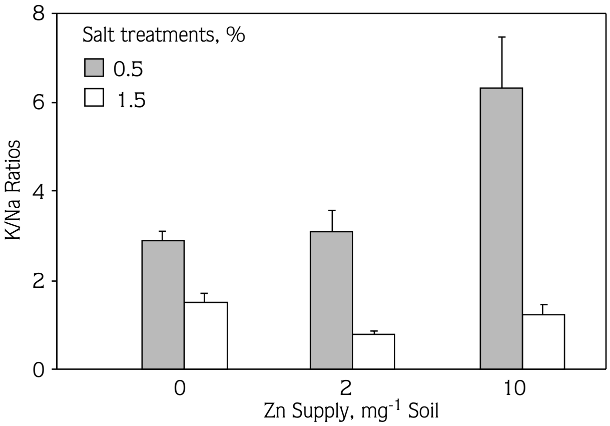

Figure 4. The effect of increasing Zn and NaCl treatments on the K/Na ratio in the shoots of 45-day-old pepper plants grown in severely Zn-deficient soil.

The physiological mechanisms by which Zn contributes to the alleviation of salt stress are not clear and need to be investigated in detail in future studies. The results in Table 1 suggest that Zn deficiency possibly alters homoeostasis between K and Na concentrations in shoots. Zinc deficiency also resulted in a high accumulation of P in shoots, which is very typical for Zn-deficient plants (Cakmak, 2000). At each NaCl treatment, increasing Zn enhanced the K concentration of the plants. With regard to Na concentration, the effect of Zn was the opposite. Increasing Zn from 2 to 10 mg kg^{-1} soil reduced Na concentration in the shoots at each NaCl treatment (Table 1). Due to very severe decreases in shoot growth in Zn-deficient soil at the nil Zn treatment (Figure 1 and 2), Na concentrations of shoots grown in this condition were not compared with those grown with sufficient Zn treatments. Very low Na concentrations in the shoots of Zn-deficient plants may have been a consequence of the substantial decrease in growth (e.g., root growth) and the consequent restricted root uptake of ions (Table 1).

An increase in Zn treatment from 2 to 10 mg kg^{-1} soil was effective in increasing K and reducing Na concentrations. The decline in shoot Na concentration due to Zn supply was also reported by Alpaslan et al. (1999) in wheat plants grown in a soil treated with 1.75 g NaCl kg^{-1} soil. According to Alpaslan et al. (1999), adequate Zn supply could be important for high plant tolerance to NaCl toxicity. By maintaining the structural and functional integrity of root cell membranes, Zn possibly controls the influx and efflux of Na across the plasma membranes. It has been well documented that Zn is a critical micronutrient in maintaining the integrity of root cell membranes, thus controlling membrane permeability (Welch et al., 1982; Cakmak, 2000). Under Zn-deficient conditions, root cell membranes are very leaky, resulting in increased passive uptake of several ions, such as P and Fe (Cakmak and Marschner, 1988; Cakmak 2000). It is possible that Zn deficiency promoted uptake of Na due to its adverse effects on membrane integrity. A similar conclusion was also made by Alpaslan et al. (1999) and Cakmak (2000). Previously, Shukla and Prasad (1974), and Norvell and Welch (1993) reported that adequate supply of Zn is important in controlling root uptake and shoot accumulation of Na. Lower concentrations of K in Zn-deficient plants might be the result of increased leakage of K from root cells. Cakmak and Marchner (1988) showed that Zn deficiency is associated with marked increases in K efflux from roots into growth medium.

As a consequence of enhanced K uptake and reduced Na accumulation, plants with the highest Zn supply generally had greater K/Na ratios than the plants with lower Zn supply at 0.5% and 1.5% NaCl treatments (Figure 4). High K/Na ratio is very often reported as a good indicator of a high tolerance to salt stress conditions (Zhu, 2003; Munns et al., 2006).

In conclusion, the results of the present study indicated the importance of plant Zn nutritional status in increasing tolerance to salt stress. In many cases, Zn deficiency is widespread in areas where soil salinity is also an important problem. Improving the Zn nutritional status of plants under such saline soil conditions could be important for maintaining higher yields. Similarly, it has been reported that in Zn-deficient soils, plants can absorb higher amounts of B leading to B toxicity, and adequate Zn supply is, therefore, of great importance for reducing the B toxicity problem in plants (Graham et al., 1987).

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