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Growth and Uptake of Sodium and Potassium in Broad Bean (*Vicia faba* L.) under Salinity Stress

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Vicia faba L. (broad bean or faba bean), a food crop of worldwide importance, is moderately tolerant of saline conditions, such as are increasingly common in Mediterranean countries and in Turkey. Our objective was to determine the influence of two salinity levels [50 and 100 mM sodium chloride (NaCl)] and two potassium salts, potassium nitrate (KNO₃) (N1 and N2) or potassium acetate (CH₃COOK) (A1 and A2), on the development of seedlings of two cultivars of broad bean (cvs. Eresen 87 and Filiz 99) grown in pots of perlite under controlled greenhouse conditions. Flame photometer (FP) analysis of tissues from roots, stems, and leaves of 3-month-old seedlings showed significant differences in growth, internodal length, and potassium (K⁺)/sodium (Na⁺) ratios. The FP analyses revealed that Na⁺ was the ion most responsible for inhibition of growth parameters seen in both cultivars and salt treatments. K⁺ contents were consistently higher in cv. Filiz 99 than in cv. Eresen 87. Possible correlations between these data and the tolerance to salinity of these cultivars are discussed.

Keywords Broad bean, salinity, *Vicia faba* L.

Introduction

Salinity is one of the stress factors that can damage soil structure and cause reduction of crop yield. According to Alam (1999), Jacoby (1999), Güneş, Inal, and Alpaslan (1996), and Cornillon and Palloix (1997), plant growth is inhibited by osmotic stress, nutritional imbalance, and specific ion toxicity.

Worldwide, about a third of the irrigated land has been affected by salinity to varying degrees (Jacoby 1999). In Turkey, an area of approximately 1,513,645 ha is facing salinity and alkalinity in most of the agricultural areas because of mismanagement and changing environmental conditions (Özcan et al. 2000).

Studies of plant-growth responses to soil salinity over the whole plant lifecycle are important. Water deficit, ion excess, and nutrient imbalance are accepted as major constraints for plants grown in saline substrates (Koyro and Huchzermeyer 1999). Many papers on the effect of salt stress on plants have focused on the growth and development of various parts

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of plants, as well as nutrient changes (Bernstein et al. 2001; Rodriguez et al. 2005; Muscolo, Panuccio, and Sidari 2003; Shiyab, Shibli, and Mohammad 2003; Zhu 2003; Alpaslan et al. 1998; Cordovilla, Ligeró, and Lluch 1999; Viegas, Silveira, and Junior 2001).

All soils contain a wide range of soluble salts, some of which are essential for growth and development. Calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) are the most common cations and chloride (Cl^-), sulfate (SO_4^-), and bicarbonate (HCO_3^-) are the anions associated with soil salinity (Grattan and Grieve 1999). Most commonly, Na^+ (Irshad et al. 2002; Özcan et al. 2000; Weimberg, Lerner, and Poljakoff-Mayber 1984), Cl^- (Özcan et al. 2000; Subbarao et al. 1990), or both Na^+ and Cl^- (Özcan et al. 2000; Ashraf and O'Leary 1995; Banuls, Legaz, and Primo-Millo 1990) are reported to account for damage to crop plants grown under saline conditions. According to Tucker (1999), Na^+ and Cl^- are thought to be necessary for some plants. Neumann, Van Volkenburgh, and Cleland (1988) concluded that Na^+ toxicity symptoms are leaf burn, necrotic spots, and limited expansion in sensitive plants, when they contain approximately 0.25% Na^+ on a dry-weight basis.

Broad bean is an important nutritious vegetable all over the world, containing 20–36% protein for human and animal consumption. While in Turkey 47,000 tonnes of dry broad bean has been produced, total world production is up to 4 438 510 tonnes (Anonymous 2005). The broad bean (*Vicia faba* L.) is a member of the *Leguminosae* family (*Fabaceae*) whose growth shows mild sensitivity to salt stress (Katerji et al. 2000). Since there appears to have been no study on ion uptake by this plant species, grown in perlite under controlled greenhouse conditions, the following investigation has been carried out on two selected cultivars grown in perlite-filled pots.

In this research, we hypothesized that the two salinity levels with two additional salts could have a negative effect on biomass production of all parts of salt-sensitive broad bean seedlings of both cultivars. The second hypothesis was that changes in the Na^+ /potassium (K^+) ratio might be related to the characteristics of salinity tolerance of those cultivars.

In the present study, we have measured growth parameters of seedlings of two broad bean cultivars, growing in a saline environment [via addition of sodium chloride (NaCl)], to test the effects on developmental traits of two different potassium salts: potassium nitrate (KNO_3) [presented in Hoagland-Arnon (1950) solution] and potassium acetate (CH_3COOK). Thus, we investigated how allocation to growth and development are affected in relation to changes in growth media. We were also interested in knowing whether salt-stress-resistance mechanisms might develop in broad bean seedlings living under salinity and how that response may be influenced.

Materials and Methods

The experiments were conducted with two broad bean cultivars in the plant physiology laboratory of the Department of Biology, Marmara University, during the period from October 2006 to November 2007. The seeds were obtained from the Agricultural Research Institute, Izmir. The cultivars, Filiz 99 and Eresen 87, were germinated in petri dishes containing the solutions formulated in Table 1. The plantlets were transferred into plastic containers filled with perlite. According to Kabaş et al. (2005) and Şeniz (1998), perlite is the best and most convenient growth medium or soil regulator for Turkey. Perlite was obtained from Taşper Perlite Ltd., Turkey.

The cylindrical containers were set up in a completely randomized block (Mead and Curnow 1983) in a greenhouse at $23 \pm 2^\circ\text{C}$ (Eriş and Şeniz 1997), $55 \pm 5\%$ relative

Table 1
Preparation of control solution

Full strength of Hoagland nutrient solution	
Macronutrients (Hoagland and Arnon 1950)	
5 ml $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 5 ml KNO_3 , 2 ml $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 ml KH_2PO_4 , 2 ml FeEDTA	
Micronutrients	
1 ml from the stock solution of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, H_3BO_3 , K_2MoO_4	

Table 2
Preparation of salt treatments

Treatments	Experimental groups	
	Cultivar eresen 87	Cultivar filiz 99
Control 1	(C)	(C)
50 mM NaCl (N1)	+ KNO_3 (N1)	+ KNO_3 (N1)
100 mM NaCl (N2)	+ KNO_3 (N2)	+ KNO_3 (N2)
Control 2	(C)	(C)
50 mM NaCl (A1)	+ CH_3COOK (A1)	+ CH_3COOK (A1)
100 mM NaCl (A2)	+ CH_3COOK (A2)	+ CH_3COOK (A2)

humidity, and exposed to 4000–4200 lux light intensity for 14/10 h day and night periods respectively. For germination the seeds from two cultivars were treated separately with two salts (KNO_3 and CH_3COOK), similar to the controls, for 2 weeks (Table 1). The seeds were then transferred into cylindrical plastic pots containing equal amounts of perlite. Seedlings were watered with the appropriate solutions (see Table 2) at 2-day intervals for a month, until the second leaves had expanded. After this point, the six replicate seedlings for each treatment, arranged in a randomized manner for both salts and controls, were continued with the assigned treatments for a further month before harvesting.

At the harvesting time, plant height (PH), number of leaves (NL), number of internodes (NoI), internodal length (IL), leaf fresh weight (LFW), leaf dry weight (LDW), stem fresh weight (SFW), and stem dry weight (SDW) of the seedlings were recorded and measured by the methods of Roberts et al. (1993), Beadle (1993), and Mackey and Neal (1993). The separated parts of the plants were finally oven dried at 75°C for 12 h and kept in a desiccator until weighing for dry-weight determination.

Na⁺/K⁺ Analyses

The roots, stem, and leaves of broad bean were prepared for nutrient analyses according to the method of wet ashing described by Kaçar (1972). The dried samples were crushed into powder using mortar and pestle and transferred to individual Erlenmeyer flasks. To the powder in each flask were added 6 mL of nitric acid + perchloric acid solutions. The samples were digested for 30 min in a water bath at 40 °C and then heated at 150–180 °C

until the extracts were reduced to 1 ml. This residue was dissolved in distilled water and made up to 100 ml in standard flasks. The samples for Na⁺ and K⁺ were analyzed using flame photometer (FP) (Jenway, UK) and evaluated statistically.

Statistical Analysis

The data obtained from the experiments were subjected to SPSS (13.0 for Windows; SPSS, Chicago, Ill.) for two-sample T with 5% significance level for differences between means. Means are indicated with standard error (\pm SE).

Results

This study revealed significant differences between the two cultivars of broad bean grown at two levels of salt concentrations and under two different K⁺ sources, with significant effects on growth. Although there have been many reports regarding the effects of NaCl on leguminous (*Fabaceae*) plants, KNO₃ and CH₃COOK were used in this study to investigate the effect of these other salts on NaCl uptake.

The two salts and two different NaCl concentrations of modified Hoagland solutions differed in their effects on growth parameters, as shown in Table 3. Plant growth measurements for this study included stem, leaf, and root fresh and dry weights, plant height, number of internodes, and internodal lengths. Table 3 shows statistical comparison of growth parameters in different cultivars and at different salt-stress treatments. Cultivar Filiz 99 gave no response to KNO₃, while CH₃COOK significantly affected nearly all growth parameters, except root fresh weight (RFW) and root dry weight (RDW), which remained unchanged. Plant height decreased under both salt-stress treatments, but potassium acetate significantly decreased PH as 32% and NoI 36% ($P = 0.023$, $\alpha = 0.05$; $P = 0.003$, $\alpha = 0.05$) respectively compared to controls, while NL was significantly decreased (89%) in A2 compared to A1 seedlings ($P = 0.036$, $\alpha = 0.05$).

Unlike the reduction in PH, the other growth parameters increased in seedlings of cultivar Eresen 87 under salinity, and the seedlings took up KNO₃ (50 and 100 mM). The pH values of both N1 and N2 were less than controls, the latter significantly. However, CH₃COOK caused an increase in pH in A1 and A2 with the former significantly greater than controls (Table 3). While NoI in both salt treatments (N1 and N2) was significantly different from controls ($P = 0.006$, $\alpha = 0.05$; $P = 0.042$, $\alpha = 0.05$), for LDW, N1 treated seedlings differed significantly from both control and N2 seedlings ($P = 0.013$, $\alpha = 0.05$; $P = 0.028$, $\alpha = 0.05$). With CH₃COOK, nearly all growth parameters were decreased. In the cases of NoI and LDW, the decrease was significant compared to controls and A1, respectively ($P = 0.035$, $\alpha = 0.05$; $P = 0.029$, $\alpha = 0.05$), whereas pH increased in both salt concentrations, where the increase from A1 treatment was significant compared to the control ($P = 0.012$, $\alpha = 0.05$).

These findings are supported by the reports of Irshad et al. (2002) with corn; Ramoliya and Pandey (2002) with the salt-tolerant plant *Salvadora oleoides*; Romero-Aranda, Soria, and Cuartero (2001) with tomato; Noaman and El-Haddad (2000) with six halophytic plants; Caines and Shennan (1999) with tomato; Carvajal et al. (1998) with melon; Morabito et al. (1996) with *Eucalyptus microtheca*; and Cachorro, Ortiz, and Cerda (1993) with *Phaseolus vulgaris*.

In both cultivars, LFW showed a slight increase in the experimental groups that were treated with 100 mM KNO₃, compared to controls (Table 3). On the other hand, potassium acetate caused decreases in LFW of both cultivars, with the reduction being significant in

Table 3
Growth parameters of two cultivars under two salts

Cultivar	Salt	Treatment	pH	Nol	NL	LFW	LDW	SFW	SDW	RFW	RDW
FILJZ 99	KNO ₃	C	51.92 ± 4.23	9.50 ± 0.67	21.83 ± 2.41	2.84 ± 0.25	0.32 ± 0.03	3.88 ± 0.28	0.38 ± 0.03	2.66 ± 0.23	0.21 ± 0.02
		N1	49.92 ± 3.92	9.17 ± 0.70	19.67 ± 1.09	2.59 ± 0.29	0.29 ± 0.03	4.08 ± 0.34	0.39 ± 0.03	2.97 ± 0.23	0.22 ± 0.01
		N2	48.58 ± 4.14	8.83 ± 1.11	23.00 ± 5.00	2.90 ± 0.30	0.32 ± 0.04	4.53 ± 0.56	0.48 ± 0.09	3.65 ± 0.46	0.24 ± 0.03
	CH ₃ COOK	C	52.92 ± 3.42	8.83 ± 0.48	22.00 ± 3.68	3.56 ± 0.22	0.41 ± 0.03	5.01 ± 0.36	0.52 ± 0.04	3.55 ± 0.40	0.28 ± 0.04
		A1	46.33 ± 1.48	7.67 ± 0.56	15.83 ± 1.22	2.06* ± 0.19	0.25* ± 0.02	3.55* ± 0.36	0.36* ± 0.05	3.06 ± 0.27	0.23 ± 0.03
		A2	40.17* ± 2.35	6.50* ± 0.34	11.67** ± 0.80	1.92* ± 0.60	0.22* ± 0.02	3.13* ± 0.25	0.30* ± 0.03	3.62 ± 0.29	0.26 ± 0.02
ERESEN 87	KNO ₃	C	61.5 ± 2.9	6.7 ± 0.4	15.3 ± 1.7	2.48 ± 0.24	0.23 ± 0.02	5.48 ± 0.40	0.38 ± 0.03	3.28 ± 0.45	0.18 ± 0.02
		N1	56.2 ± 2.6	8.5* ± 0.3	19.8 ± 1.7	3.01 ± 0.22	0.37* ± 0.03	5.75 ± 0.41	0.42 ± 0.03	3.78 ± 0.40	0.24 ± 0.03
		N2	50.1* ± 2.9	7.5* ± 0.4	19.2 ± 1.4	2.55 ± 0.18	0.29** ± 0.03	5.16 ± 0.47	0.42 ± 0.05	3.80 ± 0.25	0.24 ± 0.02
	CH ₃ COOK	C	46.3 ± 1.3	7.8 ± 0.3	16.7 ± 1.7	2.66 ± 0.22	0.28 ± 0.03	4.80 ± 0.42	0.33 ± 0.04	3.81 ± 0.38	0.24 ± 0.03
		A1	59.8* ± 1.8	7.0 ± 0.6	15.0 ± 1.1	2.25 ± 0.22	0.26 ± 0.03	4.86 ± 0.16	0.40 ± 0.03	3.50 ± 0.28	0.25 ± 0.02
		A2	53.9 ± 3.9	6.2* ± 0.4	15.0 ± 1.6	1.79 ± 0.22	0.19** ± 0.02	4.43 ± 0.68	0.34 ± 0.05	3.28 ± 0.34	0.22 ± 0.03

Notes. PH, plant height; NL, number of leaves; LDW, leaf dry weight; SDW, stem dry weight; RDW, root dry weight;

Nol, no. of internodes; LFW, leaf fresh weight; SFW, stem fresh weight; RFW, root fresh weight.

*Significantly different from C; ** +Significantly different from C and A1.

**Significantly different from A1 or N1.

cultivar Filiz 99 in both CH₃COOK concentrations compared to controls. For Eresen 87, 50 mM CH₃COOK caused a slight increase in LFW, but 100-mM treatment caused a slight, but nonsignificant, decrease. These results are in agreement with the findings of Heuer and Nadler (1995) and Lopez and Satti (1996), who reported decreasing LDW in potato and tomato under saline conditions. The reductions in LFW in broad bean might be related to the inhibitory effect of salinity on leaf growth and development, resulting from reduction of assimilation in the leaves.

In Eresen 87 treated with KNO₃, LDW measurements were significantly increased by both N1 and N2 relative to controls (Table 3). In all other treatments of both cultivars, LFW and LDW were either unchanged or significantly decreased. Dry weight of leaves of Filiz 99 remained similar to control values with KNO₃ but decreased in experimental groups with potassium acetate, which caused more severe reduction in growth parameters of both cultivars than KNO₃ (Table 3). Our findings support other results for the salt-tolerant plant *Salvadora oleoides* (Ramoliya and Pandey 2002). The reduction in LDW may relate to decreasing carbon dioxide (CO₂) uptake due to inhibition of stomatal regulation accompanying decreases in leaf expansion and assimilation by the leaf cells and resulting in loss of weight.

Stem weight measurements varied with salt treatments and cultivars. Potassium acetate showed a relatively greater effect on both cultivars. Under potassium acetate treatment, both SFW and SDW of cv. Filiz 99 decreased significantly. Under the two potassium acetate treatments, SFW decreased by 41% and 60% and SDW decreased by 44% and 73%, compared to control values (Table 3). These findings agree with the reports of SDW decrease by Ashraf, Nazir, and McNeilly (2001), Kaya, Kirnak, and Higgs (2001), Caines and Shennan (1999), Adams (1988), and Satti and Al-Yahyai (1995) for tomato; Leidi and Saiz (1997) for cotton, and Bar-Tal et al. (1991) in corn. According to Çakır (2004), the decrease in SDW in corn might be related to a decrease in leaf surface, causing a reduction of assimilation ratio and shorter stems, resulting from elevated salinity. This reduction may also be related to the inhibition of uptake of essential elements such as K⁺, Ca²⁺, and Mg²⁺ resulting in decreased photosynthetic assimilation and plant development. However, SFW in Eresen 87 showed a slight decrease whereas SDW increased slightly under both salts and concentrations.

The results showed no significant differences for fresh weights of root growth, which increased with 100 mM CH₃COOK in cultivar Filiz 99 and in both 50 and 100 mM KNO₃ in cultivar Eresen 87 (Table 3). The RFW remained almost unchanged in cultivar Filiz 99 but was slightly decreased by CH₃COOK treatment in Eresen 87 but without any significant difference.

There were no significant variations in RDW among the cultivars. Nevertheless, for both cultivars the results revealed a slight increase under KNO₃ treatments but a slight decrease under CH₃COOK compared to control values. The decreasing of RDW is consistent with the previous reports from Ashraf, Nazir, and McNeilly (2001) for *Brassica*, Caines and Shennan (1999) for tomato, and De Herralde et al. (1998) for *Argyranthemum coronopifolium*. The effect of salt causing RDW loss is thought to relate to reduction of the root number and length as well as root and root hairs being weakened and dying under prolonged exposure to salt by the time of harvest.

This decrease in biomass confirms the findings of Lopez and Satti (1996), who postulated that salt stress causes weight loss. In the current experiment, we suggest that RFW loss is related to a decrease in root number and inhibition of root development as well salt causing loss of root hairs. As a reason for unchanged or increased growth of roots, it might

be suggested that perlite particles have the advantage of not tightening up as the structure of salinized soil is affected.

This study revealed a significant difference between two cultivars of broad bean in terms of the effects on growth of two different salt concentrations and two different sources of K^+ . Although there are many reports regarding the effects of NaCl on *Leguminous* (*Fabaceae*) plants, KNO_3 and CH_3COOK were used in this study to assess whether these salts influence NaCl uptake by plants.

We observed that internodal length in control plants gradually increased, starting from the first one in Filiz 99. In cultivar Filiz 99, the internodal lengths in N1 and N2 under KNO_3 treatment showed no significant differences compared to controls or between the treatments (Figure 1). Increased salt concentration caused increasing internodal length until the eighth node but internode reduction after the ninth one. In the same cultivar, the lower level of salt (50 mM) increased the length of internodes 1, 4, 5, 6, 7, and 8, while lengths of internodes 2, 3, 9, 10, and 11 decreased. Potassium nitrate affected the lengths of internodes 5, 6, and 7.

Potassium acetate (CH_3COOK) effects on internodal length varied in cultivar Filiz 99, and whether the second internode increased in A2 compared to controls; however, the seventh internode was decreased significantly in A2 compared to controls and A1 (Figure 2). The findings on internodal length decrease were consistent with the research on sugarcane by Lingle and Wiegand (1997) and reports of Rizk and Normand (1969). According to Parida, Das, and Mitra (2004), the reasons for the reduction of internode length were the prevention of uptake of NO_3 and some specific nutrients, and inhibition of production of growth regulators such as gibberellic acid and cytokinin.

In Eresen 87, KNO_3 treatment was found to be more effective in N1 and N2, especially in internodes 2 and 3 ($P = 0.002$, $\alpha = 0.05$ and $P = 0.005$, $\alpha = 0.05$), 4 and 6 ($P = 0.013$, $\alpha = 0.05$ and $P = 0.021$, $\alpha = 0.05$), which were significantly decreased compared to controls (Figure 3). The decreasing of the sixth and eighth ($P = 0.036$, $\alpha = 0.05$ and $P = 0.036$, $\alpha = 0.05$) internodal lengths were significant compared to N1 treatment. Potassium acetate did not have any negative influence on A1 and A2 internodal lengths.

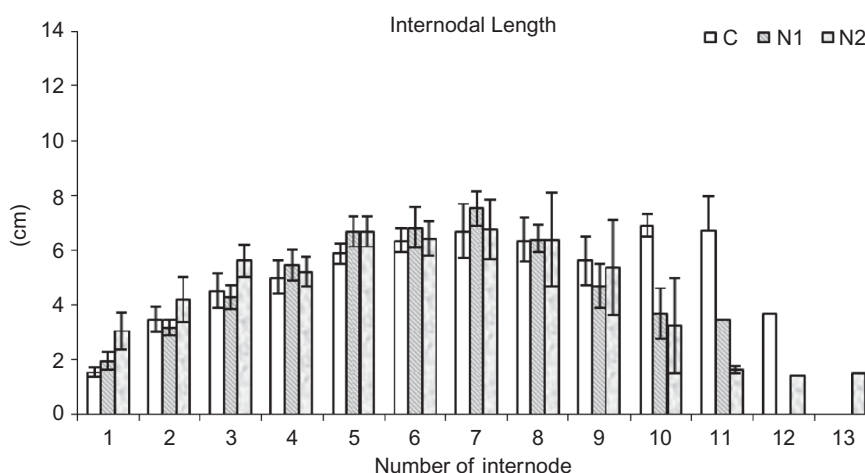


Figure 1. Internodal measurements of cv. Filiz 99 under KNO_3 treatment. C, control; N1, 50 mM; N2, 100 mM.

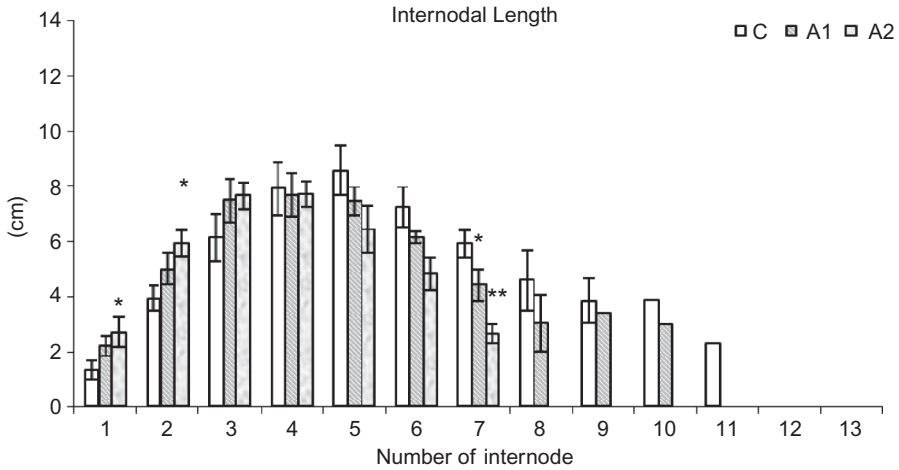


Figure 2. Internodal measurements of cv. Filiz 99 under CH_3COOK treatment. C, control; A1, 50 mM; A2, 100 mM. *Significantly different from control. **Significantly different from A1.

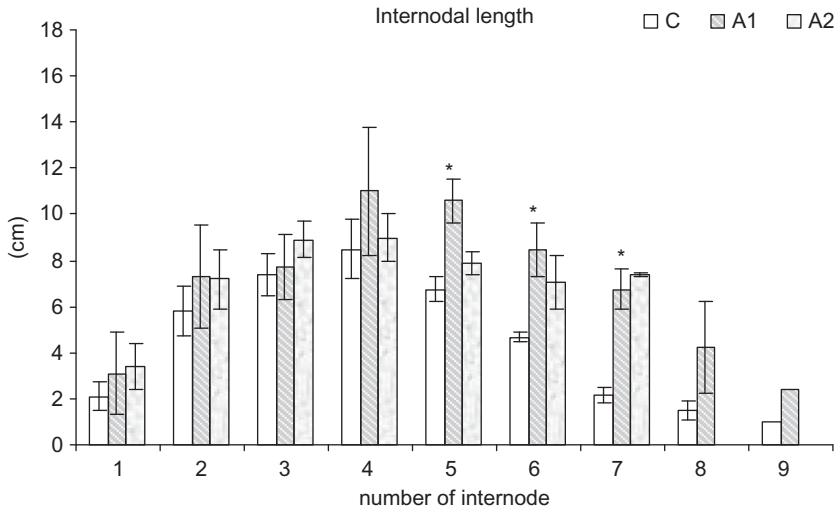


Figure 3. Internodal measurements of cv. Eresen 87 under KNO_3 treatment. C, control; N1, 50 mM; and N2, 100 mM. *Significantly different from control.

In contrast, with potassium nitrate, the fifth, sixth, and seventh internodal lengths were significantly longer than those in control plants.

In the same cultivar, with CH_3COOK the increasing salt concentration caused clear increases in all internodal lengths (Figure 4). With 50 mM concentration, internodes 5, 6, and 7 were significantly different compared to control internodal lengths ($P = 0.039$, $\alpha = 0.05$; $P = 0.021$, $\alpha = 0.05$; $P = 0.004$, $\alpha = 0.05$), respectively.

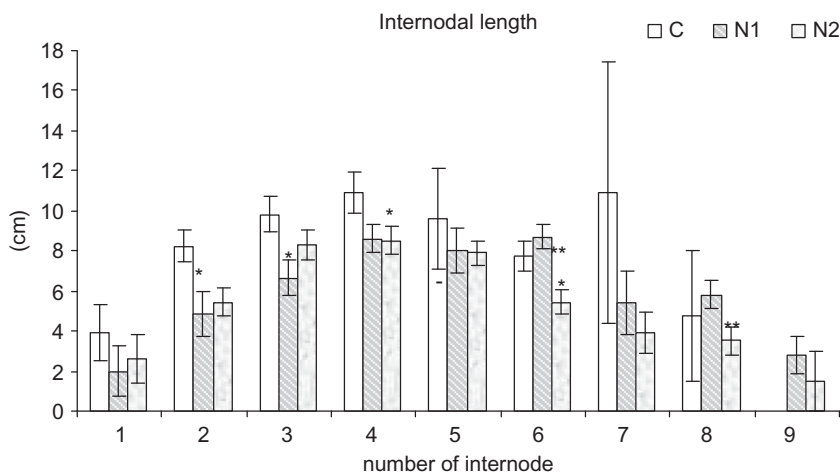


Figure 4. Internodal measurements of cv. Eresen 87 under CH_3COOK treatment. C, control; N1, 50 mM; N2, 100 mM. *Significantly different from control. **Significantly different from N1.

Na⁺/K⁺ Relations in the Plant Organs

In cultivar Eresen 87 under KNO_3 treatment, the K^+ content of leaves decreased significantly in N1 and N2, compared to controls (Table 4). In the leaves of seedlings treated with CH_3COOK , the K^+ content increased significantly, unlike with potassium nitrate, in both concentrations of 50 and 100 mM, compared to control values. During KNO_3 treatment, the K content of the stem in N1 was significantly different from control, whereas N2 content did not show any significant difference. The K^+ content of the stem also decreased in both concentrations, being significantly different in A2 compared to N1 under CH_3COOK treatment. Root K^+ content was increased in both salts and concentrations. Under KNO_3 treatment, the increase in N2 was significantly different from N1, whereas the increase was significant in both A1 and A2 compared to control K^+ content under CH_3COOK treatment.

The results of nutrient analyses indicated that the concentration of K^+ was greater than Na^+ in all organs of both cultivars. In cultivar Filiz 99, the K^+ content of the leaves showed a slight decrease in potassium nitrate, but remained unchanged under potassium acetate treatment. In the stem of Filiz 99, K^+ content decreased under 100 mM KNO_3 treatment and was significantly different from N1 and C. In the same cultivar, the apparent decrease in both CH_3COOK concentrations was not significant at the level $\alpha = 0.05$. Under KNO_3 treatment, K^+ content in the roots decreased slightly, whereas it increased at 50 mM CH_3COOK (61%) and decreased (15%) again to the control level without any significant differences (Table 4).

The concentration of Na in Filiz 99 leaves showed significant differences in both salts between the cultivars. The greatest concentration was in N2, which was significantly different from N1, with a 124% increase. Similarly the Na^+ content of leaves was significantly greater in A2 compared to control values, with an increase up to 430%. Na^+ stem concentrations in N1 and N2, unlike leaves, decreased significantly compared to control values by 5.8% and 47% respectively. Potassium acetate significantly increased the Na^+ content in A1- and A2-treated seedlings respectively by 647% and 1249% compared to controls ($P = 0.040$, $\alpha = 0.05$; $P = 0.000$, $\alpha = 0.05$). On the other hand, when KNO_3 was used,

Table 4
Na-K content in both cultivars under two salts

Cultivars/Na-K content (mg/g in dry weight)	Root						Stem					
	KNO ₃			CH ₃ COOK			KNO ₃			CH ₃ COOK		
	C	N1	N2	C	A1	A2	C	N1	N2	C	A1	A2
ERESEN 87												
Na	7.27 ± 1.4	19.18* ± 1.0	19.37 ± 1.7*	7.36 ± 0.6	20.12 ± 1.0*	23.77* ± 2.4	1.76 ± 0.2	13.68* ± 0.8	22.32** ± 3.1	1.57 ± 0.1	17.75* ± 1.3	26.43* ± 2.8
K	20.26 ± 1.6	20.20 ± 0.6	23.92** ± 0.4	21.55 ± 1.9	33.14* ± 1.7	34.41* ± 2.1	24.80 ± 1.3	20.07* ± 1.0	20.49 ± 3.0	23.14 ± 0.7	20.54 ± 1.3	16.52** ± 1.1
FILIZ 99												
Na	10.17 ± 0.6	19.36* ± 1.9	23.9* ± 3.3	26.56 ± 9.1	25.79 ± 6.0	36.13 ± 6.0	22.68 ± 3.3	21.44* ± 7.6	15.43* ± 2.4	3.31 ± 0.9	24.74* ± 7.0	44.67* ± 7.1
K	42.17 ± 2.0	38.85 ± 2.9	36.91 ± 5.1	59.30 ± 9.8	95.24 ± 37.3	51.62 ± 6.3	42.74 ± 8.9	33.95 ± 3.5	19.13** ± 1.5	44.15 ± 6.1	27.75 ± 2.9	25.46 ± 2.3
Cultivars/Na-K content (mg/g in dry weight)	Leaf											
	KNO ₃			CH ₃ COOK								
	C	N1	N2	C	A1	A2						
ERESEN 87												
Na	2.09 ± 0.2	5.67* ± 0.5	12.46** ± 1.7	0.62 ± 0.1	9.82* ± 1.1	14.83* ± 1.8						
K	21.12 ± 1.0	15.29* ± 0.4	13.96* ± 1.0	15.56 ± 1.1	18.36* ± 0.8	21.09* ± 0.9						
FILIZ 99												
Na	16.64 ± 10.3	17.11 ± 2.6	38.41** ± 4.2	4.67 ± 3.2	12.28 ± 3.7	24.77* ± 5.2						
K	41.37 ± 4.1	35.01 ± 1.4	33.77 ± 2.0	41.35 ± 5.5	41.50 ± 8.2	40.40 ± 12.8						

*Significantly different from C.

**Significantly different from N1 or A1.

*** ± Significantly different from C and N1.

the Na^+ content of roots increased, by 90% and 135% respectively, but under potassium acetate treatment, Na^+ content increased in A2 but showed a slight decrease in A1.

In cultivar Eresen 87, Na^+ concentration was lower than in Filiz 99 in all organs. In leaves, Na^+ content increased significantly by 171% in N1 compared to controls. On the other hand, for N2, the Na^+ content of the same cultivar under KNO_3 treatment significantly differed from C and N1 values, increasing by 495% and 119% respectively. In cv. Eresen 87, potassium acetate treatment also increased Na^+ content significantly in the leaves in both concentrations by 1484% and 2292%, respectively. In the same organs, the significant Na^+ increase in KNO_3 treatment was 171% and 496% greater in N1 and N2 respectively compared to controls. Stem Na^+ content increased significantly in the presence of both salts compared to control values; nevertheless the Na^+ content in N2 was also significantly greater than N1 by 63%. When CH_3COOK was used in A1 and A2 treatments, Na^+ increased by 1006% and 1550%, respectively, compared to controls (Table 4).

Discussion

Salinity is an important growth-limiting factor for most plants. The results presented here and in the literature confirm that many growth parameters are affected. The reports state that salt stress causes reductions in plant height, number of leaves, leaf extension and expansion (assimilation area), thickness of epidermal and mesophyll layers, and CO_2 uptake as well as difficulties in stomatal regulation and transpiration rate. The available studies also record decreases in chlorophyll content, number and length of internodes and nodes, and, related to this, number of flowers and reduction in weight of plant organs such as leaves, shoots, and roots. The development of roots has been decreased, and inhibition of lateral root development also occurred (Parida, Das, and Mitra 2004; Kaya et al. 2003; Singh et al. 2003; Ramoliya and Pandey 2002; Sairam, Rao, and Srivastava 2002; Bolarin et al. 2001; Katerji et al. 2000; Carvajal et al. 1998; Rodriguez et al. 2005; Turan et al. 2009).

However, not all of our results were consistent with the existing literature. Decreasing LNs under salinity have been recorded by Muscolo, Panuccio, and Sidari (2003) in *Pennisetum clandestinum*; Romero-Aranda, Soria, and Cuartero (2001) in tomato; Ramoliya and Pandey (2002) in salt-tolerant *Salvadora oleoides*; and Lopez and Satti (1996), in tomato seedlings. In the present study, NL of both cultivars was decreased in the seedlings treated with CH_3COOK , which in A2 was significant, while others showed a slight increase. Potassium acetate had stronger effects on this and other growth parameters than potassium nitrate. The reduction of the number of leaves seems to parallel the inhibition of plant height, which decreased 31% in Filiz 99. According to Muscolo, Panuccio, and Sidari (2003), the reason for reduction of leaves was related to leaves not being produced as fast as they are lost.

In cultivar Filiz 99, LFW of the cultivars showed significant differences in both concentrations of CH_3COOK , with the reduction up to 46% in 100-mM treatment compared to controls. Similarly, potassium acetate caused a slight reduction in the 100-mM treatment in cultivar Eresen 87. KNO_3 treatment in this experiment did not influence shoot weight significantly, which showed slight increase and decrease in cultivars Filiz 99 and Eresen 87 respectively.

In this context, Navarro, Martinez, and Carvajal (2000) and Viegas, Silveira, and Junior (2001) found that plants reduce their shoot weights when grown under saline conditions. The results of the current experiment suggest that the LFW reduction was

accompanied by decreased plant heights, as a result of restriction of uptake of certain nutrients that contribute to plant growth and development.

In the present study, salinity had a pronounced effect on number of leaves (NL), leaf fresh weight (LFW), and leaf dry weight (LDW), which were significantly ($P < 0.05$) more decreased under potassium acetate. CH_3COOK affected LFW, LDW, SFW, and SDW, which were significantly ($P < 0.05$) decreased in both salinity concentrations (A1 and A2) compared to control plants.

Previous studies have demonstrated that the reasons for growth inhibition under salt stress include the following: (1) The presence of Na^+ prevents uptake of Na^+ , Ca^{2+} , K^+ , Mg^{2+} (through antagonistic or competitive effects), and Cl^- affects uptake of NO_3^- (so the presence of high amounts of Na^+ or Cl^- may cause low levels of Ca^{2+} , K^+ , Mg^{2+} , and NO_3^- in roots and leaves). (2) Electrolyte loss in roots due to Na^+ causes damage in the root tissues. (3) Reduction of the amount of water and osmotic effects of soluble nutrients. (4) Inhibition of water transportation to the stem because of reduction of water-transport capacity of the roots. (5) Decrease in synthesis of DNA, RNA, and protein, increase of ABA, followed by decreasing of gibberellic acid and cytokinin (Lorenzen, Aberle, and Plieth 2004; Kaya et al. 2003; Adiku et al. 2001; Katerji et al. 2000; Khan, Ungar, and Showalter 2000; Rodriguez et al. 1997; Marschner 1995; Botella, Cerda, and Lips 1994; Kuchenbuch, Claassen, and Jungk 1986; Kafkafi, Valoras, and Letey 1982; Tal 1977; Helal, Koach, and Mengel 1975).

According to Parida, Das, and Mitra (2004) and Khan, Ungar, and Showalter (2000), one of the main reasons for inhibition of growth and development under salinity is the reduction of water uptake and NO_3^- , decreasing the total amount of N available as basic nutrient.

Increase of NoI in Eresen 87 was found only during application of KNO_3 , with significant increases in both experimental groups compared to control values (Figure 3). The NoI decreased in N1 and N2 with both salt treatments for Filiz 99 and was significant with potassium acetate. Similarly the same salt reduction was significant in A2 compared to control values (Table 3). The observed reduction in broad bean of the number of nodes is in agreement with the study by Al-Tahir and Abdulsalam (1997), which stated that node number was decreased because of salt.

The available literature records that salt stress in various plants leads to reductions in growth parameters such as inhibition of plant height; reduction in number of leaves; inhibition of leaf extension and development (assimilation surface); thinner epidermal and mesophyll layers in leaves; decreased rate of CO_2 uptake; difficulties of stomatal regulation; reduction of transpiration rate, chlorophyll content, and cell division and development; reduction of internode numbers and internodal length; reduction of dry weights of plant organs, leaves, stems, and roots; and inhibition of lateral roots (Parida, Das, and Mitra 2004; Kaya, Erol, and David 2003; Singh et al. 2003; Ramoliya and Pandey 2002; Sairam, Rao, and Srivastava 2002; Bolarin et al. 2001; Katerji et al. 2000; Carvajal et al. 1998; Rodriguez et al. 1997; Katerji et al. 1994; Matsuda and Riazzi 1981; Rizk and Normand 1969).

Internodal measurements indicated variations in internodal length with some of these being significant differences. The measurements were evaluated from top to the bottom statistically. Internodal lengths of cultivar Eresen 87 were found cumulatively to be longer than those of Filiz 99.

Depending on plant species and age, the type and quantity of salts present in soil, and the exposure time, salinity affects plant development and growth parameters at different rates and, in addition, affects mineral uptake in many cases. Studies on soybeans

(Elsheikh and Wood 1995), chickpeas (Elsheikh and Wood 1990), peas and faba bean (Delgado, Ligeró, and Lluch 1994) showed reduced shoot growth in these plants when they were treated with NaCl concentrations of 0.05 mol L⁻¹ and 0.1 mol L⁻¹. This reduced shoot growth was based on reduction in nodule number and mass, percentage of N, and dry tissue mass. The results from the plant organ analysis suggested that K⁺ absorption continued successfully, because organic acid concentrations increased K⁺ transportation. However, the acetate ion caused a reduction in plant growth parameters. This reduction was significantly related to increasing Na⁺ concentration in both cultivars while under potassium acetate treatment.

Conclusions

The study revealed that when modified Hoagland nutrient solution was supplemented with two salts (potassium acetate and potassium nitrate), several growth parameters of broad bean seedlings, including internodal measurements, were altered. Potassium acetate had a pronounced effect on growth parameters of cv. Filiz 99, which, except for root weights, were decreased significantly by both concentrations.

As a cause of salinity, Na has an important negative effect on salt-sensitive plants such as broad bean. Plant heights (PH), NoI, and LDW of the two cultivars (Eresen 87, Filiz 99) showed significantly differing responses under treatment with 100 mM salt concentration.

We can conclude that Na⁺ is mostly responsible for inhibition of growth parameters. However, the addition of potassium acetate had further pronounced effects on growth parameters of cultivar Filiz 99 and caused significant reduction of growth in both concentrations. In cultivar Filiz 99, potassium acetate with 100 mM NaCl increased sodium content in roots, stems, and leaves by 36%, 1250%, and 430% respectively compared to controls. In Eresen 87, Na⁺ concentration increased with potassium acetate in roots, stems, and leaves by 222%, 1583%, and 2291%, respectively in seedlings treated with 100 mM NaCl. Such extreme variations in percentage values for Na content suggest high levels of physiological damage of tissues under these treatments.

Potassium nitrate was found to have less effect on Na⁺ accumulations in both cultivars, although Na⁺ values in Eresen 87 were relatively greater than those in Filiz 99. Sodium concentration was increased in root, stem, and leaves of Eresen 87 by 166%, 1168%, and 496% respectively in 100 mM NaCl treatment compared to control values, while in Filiz 99 Na⁺ content in root and leaves increased by 131% and 38%, respectively, but decreased in stems by 47%.

Potassium ratios were greater in cv. Filiz 99 than Eresen 87 in both K sources. The K⁺ content in Filiz 99 declined without showing any significant differences, whereas K⁺ content fluctuated in Eresen 87 under both K sources supplied as nitrate and acetate.

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