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Full Length Research Paper

Salinity exposure modifies nutrient concentrations in fenugreek (*Trigonella foenum graecum* L.)

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Salinity is one of the most important abiotic factors limiting plant growth and yield. Salinization occurs naturally in arid and semiarid regions where evaporation is higher than rainfall. In this study, the effect of different concentrations of NaCl (0, 50, 100, 150, 200 and 250 mM) on mineral ions content in various plant organs of fenugreek (*Trigonella foenum graecum* L.) was investigated. Fenugreek plant was divided into root, shoot, leaf and pod parts for nutrient (Ca, Na, K, Cl, P, Fe, Mn, Cu and Zn) content measurements. Results indicated that the nutrient concentrations in plant tissues like leaves, roots, shoots and pods of fenugreek was strongly affected by all salt treatments. The chloride and sodium ions increased significantly in various parts of the plant depending on salinity increase. Specifically, increased salt concentration caused an increase in some nutrient contents (Na, Cl, P, Fe, Mn, Cu and Zn), while same factor caused a decrease in some nutrient contents (Ca and K). In general, an evident increase in nutrient composition in plant organs was observed mainly at the higher level of salt concentration when compared to control.

Key words: Fenugreek (*Trigonella foenum-graecum* L.), salt stress, nutrient concentration.

INTRODUCTION

Fenugreek (*Trigonella foenum graecum* L.) is widely grown in Mediterranean countries, India and China (Ahmad et al., 2005). It is commonly used as a condiment and seasoning in food preparations; is assumed to possess nutritive and restorative properties and has been used in folk medicine for centuries for a wide range of diseases including diabetes to decrease the blood glucose level (Eidi et al., 2007).

Seeds of fenugreek are used locally as a yellow dye, for cosmetics and medicinal purposes. Fenugreek is a good soil renovator and has widely been used as a green manure (Abdelgani et al., 1999). Fenugreek seeds and leaves are rich in minerals, proteins and carbohydrates, but low in oil (Gad et al., 1982). The seeds are used as spice worldwide, whereas the leaves are used as green leafy vegetables in diets. Fenugreek seeds are bitter to taste and are known for a long time for their medicinal qualities. Its seeds have been in use for over 2500 years (Srinivasan, 2006).

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In Turkey, fenugreek is widely cultivated as a spice crop for a long time. Production area of fenugreek is about 850 ha and an annual seed production is about 1000 ton. Average seed yield is about 1180 kg ha^{-1} in 2004 (Bayram et al., 2010). It is one of the most important industrial and export crops in Turkey, as well. Fenugreek is also used to produce a semisolid paste which consists of some natural flavors (garlic-flavored sausage) known as çemen. Çemen is used as an edible coating material in the production of traditional meat product called Pastırma. Fenugreek is warm-climate crop and it cannot survive in winter. Fenugreek can be cultivated as summer crop in cool climates, as winter in the warm climates. It is durable temperature and drought. But, growth of plant has declined with increasing of drought. Fenugreek has no special soil requirements. But, optimum soil properties for high yield and quality in fenugreek are loamy and good drainage, and low lime content.

Soil salinity is one of the serious environmental problems that adversely affects plant metabolism and growth of crop (Rueda-Puente et al., 2007). Salinity affects plant physiology through changes in the water and ionic status of the cells (Sultana et al., 1999; Hasegawa et al., 2000). Ionic imbalance occurs in the cells due to excessive accumulation of Na and Cl which reduces the uptake of other mineral nutrients including Ca, Mg, Mn and K (Cramer and Nowak, 1992; Khan et al., 1997; Grattan and Grieve, 1999; Lutts et al., 1999).

Salinity problem mostly seen in arid and semiarid regions may also be a problem in irrigated land. It was estimated that approximately one third of irrigated land has been affected by salinity problem (Shannon, 1984). Approximately 300,000 ha irrigated lands lose their productivity because of mis-irrigation every year in the world (Harrison, 1993). It is estimated that about one-third (400 to 950 million ha) of irrigated lands (Pakistan 14%, China 15%, India 27 to 60%, Egypt 30%, Iraq 50% and Turkey 50%) has been affected by salinity problem in the world (Hasegawa ve ark. 1986).

Essential cause of salinity is inadequate rainfall and high evaporation in arid and semiarid regions (Carpıcı et al. 2011). In Turkey, the areas affected by salts are about 2 million ha, but it may increase if proper management systems are not considered (Doğan et al., 2009). Salinity can seriously alter plant metabolic activities such as assimilation of mineral nutrients (Grattan and Grieve, 1999), stomata conductance (Wilson et al., 2006), carbon metabolism or efficiency of photosynthetic enzymes (Parida and Das, 2005). High concentration of salts causes ion imbalance and hyper-osmotic stress in plants (Molina et al., 2002).

It is a frequent constraint to agriculture in arid regions. Irrigation with poor quality water is one of the main factors resulting in salt accumulation and decrease of agricultural productivity. The plants exposed to salt stress adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa et al., 2000).

The plant growth is ultimately reduced by salinity stress but plant species differ in their salinity tolerance (Munns and Termaat, 1986). In addition, Salinity is well known to delay or reduce germination and inhibit seedling growth, however, detailed studies on the time course of events during germination are generally lacking. Salinity has been reported to inhibit first phase of germination which beings with hydration of seeds (Uhvitz, 1946). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri et al., 2001).Calcium is a particularly important nutrient in plants exposed to salt stress because of its role in membrane function cell wall extension, and cellular stress recovery (Lynch and Lauchli, 1985). Salinity dominated by Na salts not only reduces Ca availability but also reduces Ca^{2+} transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs. Calcium has been shown to ameliorate the adverse effects of salinity on plants (Ehret et al., 1990).

Fenugree k (*T. foenum-graecum* L.) is one of the most important medical and spice plants in the world. Also saline soils and saline irrigation waters present potential hazards to production (Bybordi and Tabatabael, 2009). Fenugreek is sensitive to salinity, like many other leguminous crops. But, it has been known that increasing NaCl concentrations significantly reduced the germination capacity, radicle and plumule lengths, and fresh and dry matter yields of seeds (*T. foenum-graecum* L.), especially at high salinity levels (120 and 175 mM) (Heidari, 2009; Amzallag, 1994). Soil salinity is a major limitation to legume production in many areas of the world.

The objective of this study is to investigate the effect of salinity on nutrient contents in various plant organs of fenugreek under salinity stress.

MATERIALS AND METHODS

This study was conducted in pots filled with soil in the greenhouse of the Horticulture Department of Agriculture Faculty of Yüzüncü Yil University, Van (Turkey) during April and June 2009. The experiment was carried out using a complete randomized design containing of non salinity and sodium chloride (NaCl) application (0, 50, 100, 150, 200 and 250 mM) with 3 replications. Fenugreek plant was used as experimental material. The daily air temperature ranged from 30°C (maximum during the daytime) to 10°C (minimum during the night), with the daily average temperature being about 25°C. Relative humidity fluctuated between 30 and 85%; the average value was about 60%.Ten seeds in each plastic pot containing 4 kg of field soil were directly sown. Thinning was carried out 15 days after planting, leaving four plants in each pot. Surface soil was collected from an agricultural field and passed through a 2-mm mesh screen. The texture of the soil based on sand clay silt, total organic matter 1.96%, total salt 0.035%, pH 7.30, total nitrogen 0.9%, available phosphorus 8.92 ppm in dry soil, exchangeable potassium 480 ppm in dry soil.All pots were fertilized with urea as a nitrogen fertilizer equivalent to 150 kg N ha^{-1} and triple- super phosphate (80 kg P_2O_5 ha^{-1}) were incorporated into the soil before seeding. Non-salt-treated plants were kept as controls and salt-stressed plants were subjected to different salinity levels (50, 100, 150, 200 and 250 mM) of NaCl 30 days after sowing and all plants, including controls, were then sampled. The salinity treatments were maintained until final harvest. The pots were randomly arranged in a greenhouse. Immediately after sowing, soils were watered and watering was carried out regularly every two days during experiment (45 days) and NaCl applications (50, 100, 150, 200 and 250 mM) were given together with water. Plants were irrigated until saturated, with the excess solution allowed to drain into collection pans. All genotypes were harvested 45 days after planting. Samples were washed in distilled water to remove salts from the tissue surfaces, all green parts were weighed. Roots, shoots, leaves and pods of the fenugreek plants were separated for nutrient (Ca, Na, K, Cl, P, Fe, Mn, Cu and Zn) contents measurements.

Chemical analysis for nutrients

For ion determination, fresh samples of roots, shoots, leaves and pods were extracted in 0.1N nitric acid. Na, K, Ca, Cl, Fe, Mn, P, Cu and Zn contents of the fenugreek plants samples were determined by flame photometry.

Relative ion accumulation (Na, K, Ca, Cl, Fe, Mn, P, Cu and Zn) in whole plant (WP) was calculated as described by Taleisnik and Grunberg (1994). Cl content was determined by the silver ion-titration method with an automatic chloridometer (Buckhler–Cotlove

Table 1. The effects of salinity levels on nutrients concentrations in various plant parts of fenugreek.

Salt treatments	Plant organs	Ca	Na	K	Cl	P	Fe	Mn	Cu	Zn
	Leaf	5.21	1.06	27.32	4.36	0.15	61.15	93.15	4.05	50.52
	Shoot	0.48	1.00	22.58	2.23	0.09	75.04	48.60	4.14	113.46
S0	Root	1.06	2.10	29.02	3.54	0.14	87.11	101.56	4.35	47.62
	Pod	0.86	0.47	28.23	2.07	0.11	36.74	36.47	4.22	49.13
S0 Means		1.90[a]	1.16[e]	26.79[a]	3.05[e]	0.12[ab]	65.01[f]	69.94[e]	4.19[f]	65.18[f]
	Leaf	4.77	1.33	21.22	5.93	0.10	73.92	100.48	4.23	66.13
	Shoot	0.48	1.95	22.50	3.49	0.10	85.03	56.25	4.68	129.05
S50	Root	0.99	2.17	20.87	3.62	0.11	96.07	115.26	4.68	57.16
	Pod	0.81	0.72	16.75	3.74	0.15	56.17	43.18	4.31	62.16
S50 Means		1.77[b]	1.54[e]	20.34[b]	4.19[d]	0.11[b]	77.79[e]	78.79[d]	4.47[e]	78.62[e]
	Leaf	4.65	1.66	20.63	5.91	0.14	97.06	106.15	4.28	74.12
	Shoot	0.48	2.28	22.05	4.65	0.14	99.63	62.41	4.72	140.16
S100	Root	0.82	2.28	19.82	4.35	0.11	101.37	117.08	4.77	64.21
	Pod	0.73	0.74	15.39	5.23	0.13	64.40	48.13	4.33	70.14
S100 Means		1.67[c]	1.74[d]	19.47[c]	5.04[c]	0.13[ab]	90.62[d]	83.44[c]	4.52[d]	87.15[d]
	Leaf	4.59	2.12	19.75	9.85	0.13	90.95	112.25	4.36	78.02
	Shoot	0.44	2.21	21.18	5.65	0.12	102.50	66.24	4.82	151.03
S150	Root	0.76	2.65	13.37	6.14	0.13	106.51	124.14	4.88	70.13
	Pod	0.73	1.53	13.88	5.42	0.11	101.13	69.12	4.37	74.64
S150 Means		1.63[cd]	2.13[c]	17.05[d]	6.76[b]	0.12[ab]	102.41[c]	92.94[b]	4.60[c]	93.45[c]
	Leaf	4.53	2.44	13.67	13.87	0.10	103.51	120.06	4.44	81.01
	Shoot	0.45	2.53	14.24	10.30	0.11	113.01	72.15	4.96	157.16
S200	Root	0.68	2.78	14.40	8.76	0.12	115.48	131.89	5.12	77.17
	Pod	0.70	1.56	12.88	8.79	0.16	103.61	72.16	4.37	80.52
S200 Means		1.59[d]	2.33[b]	13.80[e]	10.43[a]	0.12[ab]	108.90[b]	98.85[a]	4.72[a]	98.96[a]
	Leaf	4.56	2.49	12.85	12.98	0.14	103.24	121.89	4.44	80.18
	Shoot	0.42	2.63	12.82	11.13	0.11	112.54	70.13	4.96	157.02
S250	Root	0.63	2.94	13.45	8.67	0.16	112.65	132.24	5.01	77.13
	Pod	0.71	2.17	12.06	8.75	0.13	110.92	71.27	4.36	79.14
S250 Means		1.58[d]	2.56[a]	12.80[f]	10.38[a]	0.13a	109.84[a]	98.88[a]	4.69[b]	98.37[b]
	Leaf	4.72[a]	1.85[c]	19.24[a]	8.81[a]	0.13[a]	89.73[c]	109.00[b]	4.30[d]	71.66[b]
Plant Organs	Shoot	0.46[d]	2.10[b]	19.23[a]	6.24[b]	0.11[b]	97.96[b]	62.63[c]	4.71[b]	141.31[a]
Means	Root	0.82[b]	2.49[a]	19.49[b]	5.85[c]	0.13[a]	103.20[a]	120.22[a]	4.80[a]	65.57[d]
	Pod	0.76[c]	1.20[d]	16.53[c]	5.67[c]	0.13[a]	78.83[d]	56.72[d]	4.32[c]	69.29[c]

Means values indicated by the same letter are not significant different ($P < 0.05$).

chloridometer) according to Bozcuk (1970).

Data were analyzed by an analysis of variance using SAS (1985) software to test the significance of the main effects. Means were compared using LSD multiple range tests. Terms were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The effects of six salt levels on the nutrient compositions in different plant organs of fenugreek are shown in Table 1. According to the result of variance analysis the accumulation of nutrient compositions in fenugreek plant parts was significantly influenced by salt stress ($P < 0.01$).

Ca contents in all of the plant parts of fenugreek were affected differently by NaCl treatment (Table 1). Increase in salinity levels decreased Ca contents of plant organs. It was observed in this study that the Ca concentration in leaves, shoots, roots and pods of fenugreek under salinity were slightly lower than those of control plants. Considering mean values of all organs, the highest contents of Ca was determined in control applications (1.90 µg/mg), and the lowest Ca contents (1.58 µg/mg) in 250 mM NaCl applications. There were no statistical differences between 200 and 250 mM NaCl applications in terms of Ca contents in the plant parts. Similar results were reported by Lynch and Lauchli (1985), Sharifi et al. (2007), Nedjimi and Daoud (2009), Alhammadi and Edward (2009) and Saleh et al. (2009) that increasing salt levels caused a decrease in Ca concentrations.

Significant differences were determined between plant parts for Ca content. In point of plant parts of fenugreek the highest Ca accumulation was determined in leaves and the lowest in shoot parts. Also, it has been reported by Nedjimi and Daoud (2009), concentrations of Ca decreased in shoots and roots in the presence of NaCl stress. Calcium is well known to have regulatory roles in metabolism (Epstein, 1998) and Na ions may compete with Ca ions for membrane binding sites. Therefore, it has been suggested that high calcium levels can protect the cell membrane from the adverse effects of salinity.

Sodium chloride applications increased Na accumulation in different plant part of fenugreek and this increase was statistically significant depending on salinity level. Na contents in plant parts showed significant differences with salinity level comparing to the control (0 mM NaCl). In this study, the highest Na accumulation was obtained from roots parts of fenugreek; while the lowest Na accumulation was determined in pods. As similar to our findings, Nedjimi and Daoud (2009) noted that sodium concentration increased in shoots and roots in two Atriplex species in the presence of NaCl stress.

When the mean values of all organs was considered, the highest Na content was seen in 250 mM NaCl (2.56 µg/mg), and the lowest Ca content (1.16 µg/mg) was obtained from control applications. There were no statistical differences between 0 and 50 mM NaCl applications. Lynch and Lauchli (1985), Glenn and Brown (1998), Sharifi et al. (2007), Alhammadi and Edward (2009), Nedjimi and Daoud (2009) reported that increasing salt levels were increased Na concentrations. High Na content generally disrupts the nutrient balance, thereby causing specific ion toxicity despite disturbing osmotic regulation (Greenway and Munns, 1980; Grattan and Grieve, 1999).

The results showed that, K accumulation in the various plant parts of fenugreek was significantly affected by salt concentrations. Different level of salinity had significant effect on K content of the plant parts of fenugreek and increasing salt levels decreased K contents. When compared to control plants, salt treatment caused significant decreases in K content of all of the plant parts. While the highest K accumulation was obtained from the control application, the lowest K accumulation was determined in 250 mM NaCl application. Many researchers have reported similar results (Glenn and Brown, 1998; Sharifi et al., 2007; Alhammadi and Edward, 2009; Nedjimi and Daoud, 2009; Saleh et al., 2009). Significant differences were determined between plant parts for K content. In the study, it was determined that K accumulation in roots of fenugreek was higher than other plants parts. In terms of plant parts, the lowest K contents were determined in pods. As similar to our findings, Sharifi et al. (2007) determined that the highest K accumulation was in the roots of soybean plants.

The results obtained in the study showed that, different levels of salinity have significant effect on Cl contents of different plant parts. The highest and the lowest Cl contents of plant parts were observed in leaves and pods of fenugreek, respectively. The Cl ions concentration increased in all of the plant parts with increased NaCl applications. The transport of Cl ions occurs mainly in the transpiration stream, which explains the high concentrations of Cl ions in leaves (El-Sidding and Luedders, 1994). It has revealed that Cl ions accumulate in higher amounts in the leaves (Wahome, 2003) and this was found to be in good harmony with our results.

The highest Cl contents were obtained from 200 mM NaCl applications while the lowest were obtained from controls. There were no statistical differences between 200 and 250 mM NaCl applications for Cl contents in the plant parts. With regard to salinity levels, increasing salt levels increased the Cl content of all plant parts of fenugreek. Our study demonstrated that high levels of salinity increased directly the Cl ratios in plants parts. These findings are in good agreement with the results of Alhammadi and Edward (2009).

The data in Table 1 indicate that, there are significant differences between the plant parts of fenugreek in terms of P contents as a result of salinity applications. Increased salinity caused a significant change in P contents in plant parts. Increased salt concentration also affected the P content in leaves, roots, shoots and pods tissues. But, there were no statistical differences among leaf, root and pod parts for P contents. The increase was observed mainly at the higher level of salt concentration compared to control. There were no statistical differences between 0, 100, 150 and 200 applications in terms of P contents in the plant parts. The lowest P content was observed in case of 100 mM NaCl salinity treatments while the highest P content was observed in 250 mM NaCl salinity treatments. Differently from our findings; Sharifi et al. (2007) noted that P contents in plant organs reduced with increasing NaCl treatments and in similar findings the highest P accumulation was in the roots of plant in this study.

NaCl applications increased Fe accumulation in different plant part of fenugreek and this increase was statistically significant depending on salinity concentration increase. Fe contents of salinity applied plant parts showed significant differences comparing to the control (0 mM NaCl).

In point of Fe content in the leaves, shoots, roots and pods, significant differences were found for six-salt levels. The Fe contents in all of the plant organs significantly increased with NaCl treatment (Table 1). The highest Fe accumulation was determined in the roots and the lowest in the pods of plants. When compared to control applications, salt treatment caused significant increases in Fe content for all of the plant parts. Under salt stress, the least Fe content retained from control applications. The highest Fe content was obtained from 250 mM NaCl applications. Similar results were reported that Fe concentration increased in some plants with increasing salinity levels (Maas et al., 1972; Martinez et al., 1987). However, Strogonov (1964) determined that there was a reduction in Fe contents in all of the plant parts under salt stress.

As seen from Table 1, the effects of different salinity levels on Mn contents in plant parts of fenugreek were significant. In terms of Mn contents the highest values were obtained from 250 mM NaCl treatments while the lowest from controls. There were no statistical differences between 200 and 250 mM NaCl applications for Mn contents in the plant parts. Similar results were reported (Maas et al., 1972; Martinez et al., 1987; Alpaslan et al., 1998) that increasing salt levels caused an increase in Mn contents. Increased salt concentration also affected the Mn content in leaves, roots, shoots and pods. In terms of different plant parts the most abundant Mn contents were obtained from pods of fenugreek, while the least from roots of plant. Increasing salt levels increased Mn content in all of the plant parts of fenugreek.

The results showed that, different levels of salinity had significant effect on Cu contents of different plant parts of fenugreek. Sodium chloride application increased Cu accumulation up to 200 mM NaCl applications in different plant parts of fenugreek and this increase was found to be statistically significant at different salinity concentrations. The Cu ratio increased significantly up to 200 mM NaCl application and after that somewhat declined (Table 1). Similar results, (Maas et al., 1972; Martinez et al., 1987 and Alpaslan et al., 1998) reported that, increasing salt levels resulted to increased Cu concentrations.

Cu contents in plant parts showed significant differences relating to salinity level compared to the control treatments. Salinization treatment was increased Cu accumulation in plant parts. In the present study, the highest Cu accumulation was determined in root parts of fenugreek; while the lowest Cu accumulation was obtained from leaves of fenugreek plant.

NaCl applications increased Zn accumulation in various plant parts of fenugreek and this increase was found to be statistically significant. The highest and the lowest Zn contents of plant parts were observed in shoots and roots of fenugreek, respectively. The Zn concentration increased in all of the plant parts with increased level of NaCl application up to 200 mM. Afterward, it decreased slightly in 250 mM NaCl applications. Relating to salt level the highest Zn contents were obtained from 200 mM NaCl applications while lowest were obtained from controls. There were statistically significant differences between 0, 50, 100, 150 and 200 and 250 mM NaCl applications in terms of Zn ratios in the plant parts. As similar to this study results, it was determined by Sharifi et al. (2007) that increased salinity levels decreased Zn content of plant after a certain point (150 mM NaCl).

The highest Zn content was observed in the shoots of fenugreek plants, while it was lowest in the pods of plants. In the other studies; Mass et al. (1972), Khoogar et al. (1999), Taborin et al. (2004), reported that the higher salinity levels the higher concentration of Zn were observed. Ravikovitch and Navrot (1976) noted that salinization had little effect on Zn concentration in tomato plant

Conclusion

Salt stress significantly ($P < 0.01$) influenced the concentrations of nutrient elements in all of the plant parts. Different levels of salinity had different effects on nutrient compositions (Ca, Na, K, Cl, P, Fe, Mn, Cu and Zn) in the plant parts like leafs, shoots, roots and pods of fenugreek. For example, while the concentrations of nutrient elements like Na, Cl, P, Fe, Mn, Cu and Zn increased in all of the plant parts in case of high salinity level applications, contents of nutrition elements like Ca and K decreased.

In this study it was observed that nutrient content changed in different plants parts. The results obtained from this experiment showed that salt stress caused an accumulation of nutrient elements like Na, K, P, Fe, Mn and Cu in the roots of plants at the highest level, while same applications caused higher accumulation of Cl and Ca in the leaves and Zn in the shoots. However, the lowest accumulation occurred in the shoots for Ca and P; in the pods for Na, K, Cl, Fe and Mn; in the leaves for Cu and in the roots for Zn. The results indicated that there were significant changes in nutrient contents and distribution in the plant tissues as a result of salt stress.

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