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THE EFFECT OF NACL PRIMING ON EMERGENCE, GROWTH AND YIELD OF FENUGREEK UNDER SALINE CONDITIONS

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ABSTRACT. Salinity is considered as a major abiotic stress affecting crop production in arid and semi-arid regions in the world. Poor germination and decrease of seedling growth are major results of poor crop establishment and failure. Many research studies have shown that seed priming is an efficient method for increasing plant growth and yield in saline condition. For this reason, this experiment was conducted to evaluate the effects of NaCl priming on the emergence and seedling growth of Tunisian fenugreek (*Trigonella foenum-graecum* L.) under salinity conditions. Seeds of fenugreek were primed with NaCl (4 g L^{-1}) for 36 h in continuous 25°C. Primed (P) and non-primed (NP) seeds were sown in plastic pots and placed in greenhouse for 4 months. Experiments were conducted using various NaCl concentrations (0, 4, 6, 8 and 10 g L^{-1}). Results showed that emergence percentage of primed seeds was greater than non-primed seeds. Roots length, plant height, leaf area, fresh and dry weight and yields of plants derived from primed seeds were higher compared with non-primed seeds. Na^+ content of plants derived from primed seeds was lower than that of primed ones. In the other side, K^+ and Ca^{2+} content of plants derived from primed seeds was higher compared with plants derived from non-primed seeds. These results suggest that NaCl priming of fenugreek seeds increased salt tolerance of seedlings by reducing Na^+ and promoting K^+ and Ca^{2+} accumulation.

Key words: Emergence; Fenugreek; Growth; NaCl priming; Salt Tolerance; Yield.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a flowering annual plant, with autogamous flowers. This crop is native to an area extending from Iran to northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. (Petropoulos, 2002). In Tunisia, it is especially cultivated in the regions of the North (Marzougui *et al.,* 2007) and it is commonly used as a condimentin food preparation for its nutritive and restorative properties and has been used in folk medicine

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for centuries for a wide range of diseases including diabetes (Eidi *et al.,* 2007). Fenugreek is a very useful legume crop and can be incorporated into short-term rotation (Moyer *et al.,* 2003), for hay and silage (livestock feed), and for soil fertility (fixation of nitrogen) (Sadeghzade *et al.,* 2009).

Like many other leguminous crops, the production of this crop is affected by environmental stress such as: drought, salinity and heat (Almansouri *et al.,* 2001). In fact, in arid and semi-arid lands, which cover a third of the globe surface, soil and irrigation water salinity is one of the major factors affecting plant growth and crop yield (Zid and Grignon, 1991). Properly, this can be resulted from a low osmotic potential of soil solution (osmotic effect), specific ion effects (salt stress), nutritional disequilibrium (nutritional stress), or a combination of the three effects (Ashraf, 1994; Zhu, 2002).

Seed priming is a successful method that has been proved to improve seed germination and emergence of seedlings. It is a controlled hydration treatment at low water potential that allows pre-germinative metabolism to proceed, but prevents radicle emergence (Bradford, 1986). Seed priming improved germination and emergence in seeds of many vegetable under normal as well as stress condition and many substances are used as osmotic solution which lower the water potential such as PEG, NaCl, KNO_3, $ZnSO_4$ and $CaCl_2$ (Barasa *et al.,* 2005; Ehsanfar *et al,.* 2006; Esmaielpour *et al.,* 2006; Neamatollahi *et al.,* 2009; Sivritepe *et al.,* 2003). There are reports that seed priming permits early DNA replication, increase RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites (McDonald, 2000). This technique helped seedlings to grow in stressed conditions (Welbaum *et al.,* 1998; Ashraf and Foolad, 2005). Seed priming, especially with NaCl, have improved germination and growth of many crops under stressed conditions (Sivritepe *et al.,* 2003; Omami, 2005; Basra *et al.,* 2005). Seed priming with NaCl improved growth and yield of tomato plants (Cano *et al.,* 1991), asparagus plants (Pill *et al., 1991*) and cucumber plants (Passam and Kakouriotis, 1994), which were exposed to different, salt treatments. Therefore, the aim of the present study was to examine the effect of NaCl priming on salt tolerance of fenugreek and to improve growth and productivity of this plant under saline conditions.

MATERIALS AND METHODS

The experiment was carried in the field research of High Institute of Agriculture, Chott Mariem, Tunisia. Tunisian fenugreek seeds were primed with 4 g L^{-1} NaCl solution for 36 hours, at 25° C. After priming, primed and non-primed seeds (control seeds) were washed with tap water for 3 min. and then rinsed with distilled water. Later, seeds were dried between two filter papers and set to germinate. Primed (P) and non-primed seeds (NP), were sown in plastic pots (12 cm diameter and 22 cm height) filled with black fertilized peat and placed in an unheated greenhouse where temperature ranged between 18-30° C and humidity ranged between 65 - 75%. Throughout their vegetative cycle (4 months), plants from primed and control seeds were irrigated daily with five saline solutions which derived from tap water by the use of NaCl. The five salinity levels were 0 (control), 4, 6, 8 and 10 g L^{-1}. The experiment was arranged in a factorial completely randomized design with two factors; which are priming treatment (NaCl primed seeds and control seed) and salinity levels (0, 4, 6, 8 and 10 g L^{-1} of NaCl) with three replications and 15 plants per replicate.

Germination boxes were inspected daily and seedling emergence recorded as the appearance of the cotyledons. Total number of emerged seedlings in each replicate was determined as percentage in calculation of total emergence. At the end of 4 months and when pods were ripped, seedlings (shoots, roots, pods) were harvested and evaluated for their response to NaCl stress.

Parameters measured were: root length (cm), plant height (cm), leaf area (cm^2) which measured by planimeter (Area Meter 3100), fresh weight (g) for both shoot and root were measured, number of pods per plant and number of seeds per pod.

In order to determine dry weight (DW) and analyses ions concentrations of seedlings, plant materials, dried in an oven at 80°C for 48 h.

K^+, Na^+ and Ca^{2+}, root and shoot content, were analyzed using Eppendorf Elex 6361 model flame photometry following nitric-perchloric acid digestion as described by Miller (1998). Growth and yield parameters of fenugreek were evaluated with analysis of variance (ANOVA) and Duncan multiple range test ($p<0.05$) using the SPSS (13.0) System. Differences were considered significant at the 5% level (means followed by different letters).

RESULTS

Total emergence

Effect of salinity on total emergence percentage of fenugreek was presented in *Table 1*. Increased NaCl salinity decreased significantly ($p<0.05$) total emergence of seedlings derived from P and NP seeds. However, total emergence percentages in P groups were higher compared with those derived from the NP groups. The most inhibiting salinity level for total emergence was 10 g L^{-1} (24% and 49.8%, respectively in NP and P groups). But, for all salinity levels, total emergence percentages were significantly higher in P than in NP groups.

Root length

Root length significantly decreased by increasing salinity levels in both primed and non-primed seeds. However, root length of primed seeds was longer than unprimed ones. In fact, mean root length varied between 32.5 and 9.5 cm in non-primed group and the lowest root length was obtained when non-primed seeds were watered with 10 g L^{-1} NaCl. On the other side, mean root length varied between 33.5 and 12 cm in primed group (*Table 1*).

Plant height

Increasing salinity levels had significantly ($p< 0.05$) reduced plant height. This reduction was more important in non-primed seed compared with primed seed. In fact, NaCl priming had a positive effect on plant height of seedlings of fenugreek cultivars. For example, the increase in salinity up to 10 g L^{-1} in non-primed groups caused a 66.6% reduction in plant height compared to control. However, for primed seedlings, this reduction was only 51.83% compared to control.

Leaf area

Analysis of variance showed that salinity significantly affected leaf area (*Table 1*). Indeed, significant interactions of salinity levels and priming treatments showed that the highest leaf area (4.75 and 4.71 cm^2) obtained when the plants were watered with normal water (control). For plants derived from primed seeds, NaCl salinity affected leaf area for concentrations above 6 g L^{-1} NaCl.

Table 1 - The effects of NaCl salinity on total emergence, root length, plant height and leaf area of fenugreek seedlings derived from NP and P seeds.

NaCl salinity (g L^{-1})	NaCl priming	Total emergence (%)	Root length (cm)	Plant height (cm)	Leaf area (cm²)
0	NP	100 a	32.50 a	60.00 a	4.75 a
	P	100 a	33.50 a	61.25 a	4.71 a
4	NP	94.20 b	28.50 b	57.00 b	3.75 b
	P	97.60 b	31.00 b	60.25 b	4.50 a
6	NP	76.00 c	18.50 c	42.50 c	3.40 c
	P	84.40 c	25.50 c	52.50 c	4.20 b
8	NP	38.00 d	12.50 d	32.50 d	3.10 d
	P	58.40 d	15.00 d	43.50 d	4.05 b
10	NP	24.00 e	9.50 e	20.00 e	2.95 d
	P	49.80 e	12.00 e	29.50 e	3.50 c

*Means with the same letters in each column are not significantly different at 0.05 according to Duncan test.

Biomass

Fresh and dry weights of shoots and roots of fenugreek plants grown in 0 to 10 g L^{-1} NaCl are presented in *Table 2*. Significant reductions in fresh and dry weight of fenugreek seedlings (roots and shoots) were observed under saline conditions. Nevertheless, the seedlings of the P group had higher values. For non-primed group, at 10 g L^{-1}, the reduction in dry weight was 58.8% and 40% for shoot and root respectively compared to control (12.25 and 6.5 g). But, for the primed group, at 10 g L^{-1}, the reduction in dry weight was 55.2% and 22% for shoots and roots, respectively, compared to control (12.5 and 6.47 g). Similar results were found by other authors (Korkmaz, 2005; Nascimento, 2003).

Table 2 - The effects of NaCl salinity on plant biomass of fenugreek seedlings derived from NP and P seeds.

NaCl salinity ($g\ L^{-1}$)	NaCl priming	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
0	NP	30.50 a	15.00 a	12.25 a	6.50 a
	P	31.10 a	14.75 a	12.50 a	6.47 a
4	NP	28.25 b	13.50 b	9.20 b	6.05 b
	P	30.50 b	14.10 b	10.50 b	6.35 a
6	NP	20.15 c	13.00 b	7.80 c	5.80 c
	P	24.50 c	14.05 b	8.50 c	6.18 a
8	NP	16.50 d	12.05 c	6.75 d	4.90 d
	P	20.75 d	13.75 c	7.65 d	5.72 b
10	NP	8.00 e	4.5 d	4.90 e	3.90 e
	P	12.75 e	6.75 d	5.60 e	5.05 c

*Means with the same letters in each column are not significantly different at 0.05 according to Duncan test.

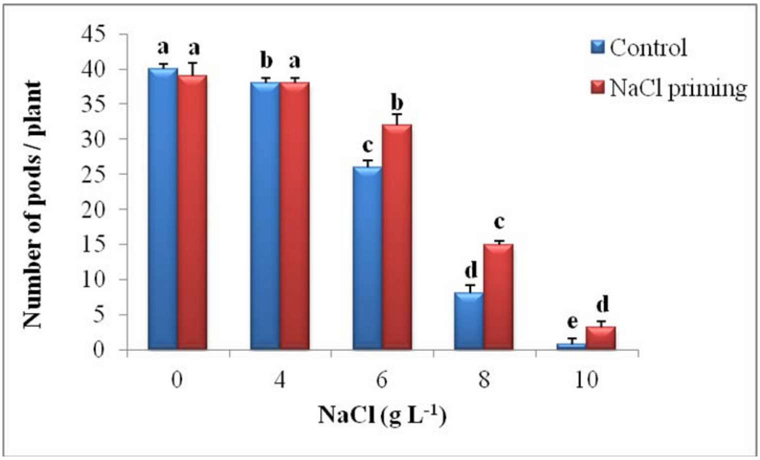
Number of pods

Mean number of pods per plant (*Figure 1*) showed a decrease with the increase of NaCl salinity. At highest NaCl concentration (10 $g\ L^{-1}$), fenugreek plant didn't produce more than 1 pod for non-primed groups, which correspond to 97.5% decrease compared to control (40 pods per plant). However, mean number of pods per plant in primed group was significantly higher in 6, 8 and 10 $g\ L^{-1}$ than in non-primed group. For example, at 10 $g\ L^{-1}$, fenugreek plant in primed group produced more than 3 pods per plant, which only correspond to 91.8 % decrease compared to control (39 pods per plant).

Number of seeds per pod

Number of seeds per pod significantly decreased with salinity in primed and non-primed groups. But, the decrease was higher in nom-primed group especially above 6 $g\ L^{-1}$ (*Figure 2*). In general, the decrease in number of seeds per pod was lower in primed group than in non-primed group. For example, at highest NaCl concentration (10 $g\ L^{-1}$), the reduction in non-primed group was 90% compared to control (14 seeds per pod) but it was only 74.28% in primed group compared to control (14 seeds per pod).

Na^+ concentration significantly increased with salinity in primed and non-primed groups (*Table 3*). Generally, the increase in Na^+ concentration was lower in primed groups compared to non-primed groups and Na^+ content in shoots was lower than Na^+ content in roots for the two groups of plants (non-primed and primed). Increased NaCl salinity caused significant decreases in K^+ concentration especially for 8 and 10 $g\ L^{-1}$ (shoots and roots). However, K^+ concentration was lower in non-primed groups than in primed groups. Similar to general results obtained in K^+ concentration, increased NaCl salinity caused a decrease in Ca^{2+} concentration (shoots and roots) both in non-primed and primed groups. However, plants derived from primed group accumulated higher Ca^{2+} concentration than plants, which derived from non-primed groups. The present result was in agreement with the work of Mezni *et al.* (2002) in medicago, Kaya *et al.* (2002) in strawberry and Sivritepe *et al.* (2003) in melon.



*Means with the same letters in each column are not significantly different at 0.05 according to Duncan test.

Figure 1 - The effects of NaCl salinity on number of pods per plant of fenugreek seedlings derived from NP and P seeds.

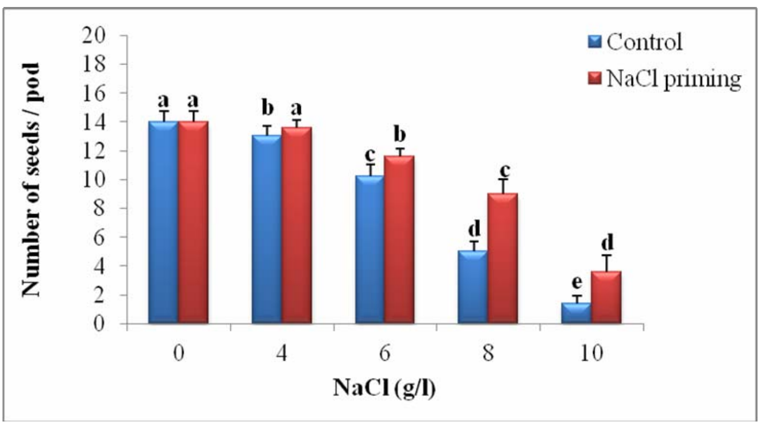


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Figure 2 - The effects of NaCl salinity on number of seeds per pod of fenugreek seedlings derived from NP and P seeds.

Table 3 - The effects of NaCl salinity on changes in Na, K and Ca concentration (meq/g DW) of fenugreek seedlings derived from NP and P seeds.

NaCl salinity (g L^{-1})	NaCl priming	Shoots Na^+	Roots Na^+	Shoots K^+	Roots K^+	Shoots Ca^{2+}	Roots Ca^{2+}
0	NP	0.40 e	0.64 e	5.53 a	5.27 a	5.65 a	4.80 a
	P	0.37 e	0.61 e	5.60 a	5.22 a	5.62 a	4.89 a
4	NP	0.89 d	1.11 d	5.12 b	5.02 a	5.00 b	4.65 b
	P	0.77 d	0.86 d	5.40 b	5.19 a	5.52 b	4.78 b
6	NP	1.27 c	1.88 c	4.85 c	4.66 a	4.71 c	4.35 c
	P	1.05 c	1.32 c	5.12 c	5.05 b	5.20 c	4.61 c
8	NP	2.85 b	3.37 b	4.20 d	4.04 b	4.44 d	4.12 d
	P	2.15 b	2.57 b	4.88 d	4.70 c	4.92 d	4.45 d
10	NP	4.28 a	4.36 a	3.80 e	3.60 b	3.90 e	3.81 e
	P	3.40 a	3.67 a	4.56 e	4.44 d	4.65 e	4.11 e

*Means with the same letters in each column are not significantly different at 0.05 according to Duncan test.

DISCUSSION

The present study investigated the effect of salinity and seed priming on the growth and yield of fenugreek. In general, increased NaCl level, reduced percentage of emergence, root length, plant height, leaf area, fresh and dry weight and yields. However, in the present study, it was concluded that NaCl priming diminished inhibiting effects of salinity on seed emergence and seedlings growth. In fact, all results obtained showed that plants derived from primed seeds had higher performance than plants derived from non-primed seeds. The reduction in total emergence was higher for non-primed seeds, compared to primed seeds. Therefore, primed seeds maybe had better efficiency for water absorption and faster metabolic activities in seed during germination process (Hopper *et al.,* 1979). Root length of primed seeds was greater than that of non-primed ones. This can be the result of salt deposit in root‘s cells, which could be a reason for physiological drought, the reduction of cell division and ultimately reduced root growth (Munns, 1993).

High levels of salinity reduced leaf area, plant height, fresh and dry weight and yields. This is probably due to the reaction of salinity, which reduces the contribution of phytohormones in the biosynthesis (Cuartero *et al.,* 2006). Furthermore, reduced leaf area leads to a decrease of photosynthetic activities and consequently lowers plant production and biomass (Afkari *et al.,* 2009). In addition, salinity disturbs mineral nutrient absorption and ion balance in the plant. Thus, leaf and stem length reduction can be attributed to mineral nutrient deficiency. It was also reported that the primed plants display a faster and stronger activation of the various defense responses that are induced following attack by pathogens, insects and various abiotic stress (Conrath *et al.,* 2006).

Results showed also that NaCl salinity causes increase in Na^+ concentration and decrease in K^+ and Ca^{2+} concentration in the different part of fenugreek seedlings (roots and shoots). These results support the previous findings of Sivritepe *et al.* (2003) working on melon and Bajehbaj (2010) working on sunflower. In fact, plants derived from primed seeds showed higher adaptation capacity to salinity because of less Na^+ but more K^+ and especially Ca^{2+} accumulation. As a result, Na^+/Ca^{2+} balances of seedlings derived from the primed seeds were lower than those of the non-primed seeds under similar salinity levels.

There are many reports demonstrated that increasing K^+ and Ca^{2+} concentration in seeds of different crops significantly increased seed emergence and enhancement of seedling growth under saline conditions (Cramer *et al.,* 1990). In addition, potassium has an important action involving the maintenance of ionic balance in cell and has an effect in some enzymes like the pyruvate kinase which is essential in respiration and carbohydrate metabolism (Aisha *et al.,* 2007) and this could probably be the reason for improving growth and yield in plants derived from primed seeds. Recently, many studies indicated that an increase in the concentration of Ca^{2+} in plants exposed to salinity could ameliorate the inhibitory effects on growth (Navarro *et al.,* 2000; Kaya *et al.,* 2002). Furthermore, higher Ca^{2+} accumulation capacity under salinity provides the sustainability of Na^+/Ca^{2+} balance, which is responsible for the semi-permeability of cell membranes (Greenway and Munns, 1980). Cayuela *et al.* (1996) have concluded that higher salt tolerance of plants derived from primed seeds seems to be the result of a higher capacity for osmotic adjustment since plants from primed seeds have more Na^+ and Cl^- in roots and more sugars and organic acids in leaves than plants from non-primedseeds. This osmoregulation can occur in plants by active uptake of inorganic ions (such as Na^+, K^+ and Cl^-) or synthesis of organic solutes such as sugars, organic acids, free amino acids and proline (Demir and Kocacaliskan, 2001; Cayuela *et al.,* 1996).

CONCLUSIONS

NaCl priming is a technique, which has many advantages, such as feasibility and low cost and has been shown to improve plant establishment under saline conditions for different crops. However, the mechanisms for seed priming that trigger the changes in the processes of germination and seedling growth are not fully understood. So, NaCl priming on salt tolerance of fenugreek seeds still requires more investigations and more biochemical and molecular researches before applying the method to other crops.

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