Research Journal of Biological Sciences 4 (11): 1152-1155, 2009
ISSN: 1815-8846

Investigation of Salinity Stress on Seed Germination of *Trigonella foenum-graecum*

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Abstract: This study was conducted to determine seed germination on the mechanisms of salinity stress in *Trigonella foenum-graecum* in 2009. The type of experiment was a completely randomized design with 7 salinity treatment and four replications was used. Salinity treatment consisted of 0 (control), 50, 100, 150, 200, 250 and 300 mM NaCl. The experiment was carried out under 70% relative humidity at 25°C with a photoperiod of 8:16 h light/dark. The germinated seeds were counted every day and germination percentage and seed germination rate were measured. The data were analyzed by one-way ANOVA and LSD multiple comparison test.

Key words: *Trigonella foenum-graecum*, salinity stress, germination, seed germination, ANOVA

INTRODUCTION

Seven percent of the land's surface and 5% of cultivated lands are affected by salinity (Flowers *et al*., 1997). The two major environmental factors that currently reduce plant productivity are drought and salinity (Serrano *et al*., 1999a). Salinity is one of the major obstacles to increasing production in crop growing areas throughout the world. In spite of this extensive literature there is still a controversy with regard to the mechanism of salt tolerance in plants (Neumann, 1995). Salinity in soil or water is one of the major stresses and especially in arid and semi arid regions, can severely limit crop production (Shannon, 1998).

Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield (Greenway and Munns, 1980). The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts (Volkmar *et al*., 1997). These concentrations fluctuate because of changes in water source, drainage, evapotranspiration and solute availability (Volkmar *et al*., 1997). Seedling establishment is a critical stage in crop production and considerably depends on biochemical and physiological structures of seed. In order to obtain fast and good establishment of seedling, high vigor seed is needed to provide essential nutrients for seedling until it becomes established and can photosynthesize independently (Derek-Bewley and Black, 1994). Seed germination, seedling emergence and early survival are particularly sensitive to substrate salinity (Baldwin *et al*., 1996). Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease (Roundy, 1987). These salts interfere with seed germination and crop establishment (Fowler, 1991). Germination and seedling characteristics are the most viable criteria used for selecting salt tolerances in plants (Boubaker, 1996). Salinity stress can affect seed germination through osmotic (Welbaum *et al*., 1990). The present study was undertaken to evaluate the effect of salinity tolerance of *Trigonella foenum-graecum* in salt tolerance during seed germination and early seedling growth and also to find the relation between salinity and growth.

MATERIALS AND METHODS

Seeds of *Trigonella foenum-graecum* were used in this investigation. This experiment was conducted to evaluate the influence of different NaCl concentrations on germination, germination rate and root and shoot length of the seedlings. The type of experiment was a completely randomized design with 7 salinity treatment and four replications was used. Salinity treatment consisted of 0 (control), 50, 100, 150, 200, 250 and 300 mM NaCl. Seeds were incubated at 4°C for a few fays and then surface-sterilized with 5% sodium hypochlorite. The experiment was carried out under 70% relative humidity at 25°C with a photoperiod of 8:16 h dark/light. Plastic Petri dishes (100 mm diameter, 15 mm height) with a tight-fitting lid were used for the experiment. Twenty five seeds for each of the six NaCl treatments as well as control were used. Seeds were hand sorted to eliminate broken and small seeds. Seed were allowed to germinate in laboratory condition on filter paper (Whatman No. 2) in Petri dishes soaked in a solution of the respective salt concentration. The seed germination was evaluated after every 12 h. Seed germination was started after 48 h (seeds were considered to be germinated with the emergence of the radical). The germinating seeds were counted at daily intervals. The lengths of root and shoot of the germination seeds which were >2 mm in length were measured and recorded after 15 days of sowing. In all treatments a continuous increase in the number of germinating seeds as well as in the lengths of roots and shoots was observed during the subsequent days of germination.

The data were analyzed by one-way ANOVA and LSD (Least Significant Difference) multiple comparison test ($p<0.05$). Linear regression equations were developed by using SPSS version 12.0 statistical software package.

RESULTS

The results revealed that the germination of *Trigonella foenum-graecum* was strongly affected by all salt treatments. Increased salt concentration caused a decrease in germination. Strong reduction was observed mainly at the higher level of salt concentration compared to control. Statistical analysis of variance showed that there were highly significant differences ($p<0.01$) among all the salt treatment for germination, root and soot length (Table 1). The results showed that germination response of *Trigonella foenum-graecum* at 50, 100, 150 and 200 mM were not significantly different from control treatment but 250 and 300 mM treatments were significantly different ($p<0.01$) from control and 50 mM treatments. The results revealed that the germination percentage at 100, 150 and 200 mM treatments in comparison with 250 and 300 mM ($p<0.05$, $p<0.01$, respectively) showed difference. Figure 1 and 2 indicated that germination and germination rate delayed as the level of salinity increased.

Experiment was prolonged to investigate the effect of salinity (NaCl) on seedling vigor of germinating seeds. It was investigated that an increased salinity level caused delayed emergence of root and shoot as compared to controls. The increase in length of root and shoot was continuously observed in frequent hours of germination in *Trigonella foenum-graecum* in the control as well as salt treatments.

The data on the average length (Fig. 3 and 4) of root and shoot of root and shoot shows that *Trigonella foenum-graecum* showed a strong inhibition with the increasing level of salt solution particularly at high salt levels (200, 250 and 300 mM). The results presented in Fig. 3 and 4 indicated that great reduction of shoot growth and particularly in root growth occurred with NaCl

Table 1: Analysis of variance on the salt treatment for germination, root and soot length

Length	Sum of squares	df	Mean square	F	Sig.
Root					
Between groups	226.257	6	37.710	27.752	0.000
Within groups	19.023	14	1.359		
Total	245.280	20			
Shoot					
Between groups	31.622	6	5.270	11.358	0.000
Within groups	6.496	14	0.464		
Total	38.118	20			
Germination					
Between groups	0.547	6	0.091	24.917	0.000
Within groups	0.051	14	0.004		
Total	0.598	20			

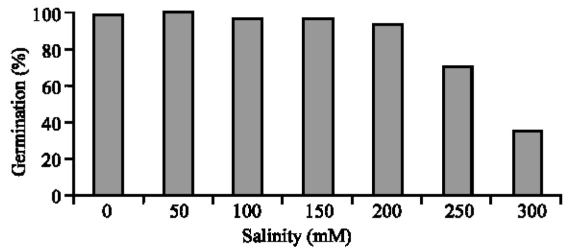

Fig. 1: Effect of different treatments of NaCl stress on germination of *Trigonella foenum-graecum*

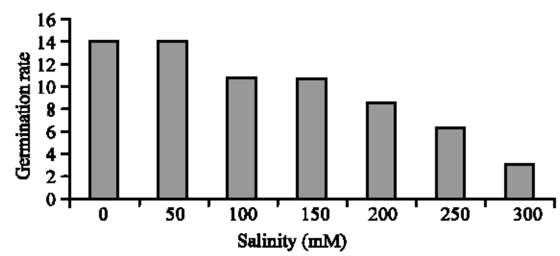

Fig. 2: Effect of different treatments of NaCl stress on germination rate of *Trigonella foenum-graecum*

treatments. The results showed that root and shoot length of *Trigonella foenum-graecum* at 50 and 100 mM were not significantly different from control treatment but 150 mM treatment was significantly different ($p<0.05$) from control and 200, 250 and 300 mM treatments ($p<0.01$) showed difference.

Linear regression equations were developed to find the relationship between salinity and final germination, germination rate, root and soot length of *Trigonella foenum-graecum* (Table 2). Linear regression revealed a significant negative relationship between salinity and final germination, germination rate, root and shoot length and plant. Linear regression also revealed a strong ($R^2 = 0.95$, $p<0.001$) negative significant relationship between salinity and germination rate. There was also a weak ($R^2 = 0.67$, $p = 0.024$) negative significant relationship between salinity and germination (Table 2).

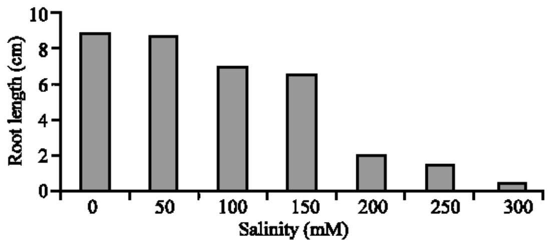

Fig. 3: Effect of different treatments of NaCl stress on root length of *Trigonella foenum-graecum*

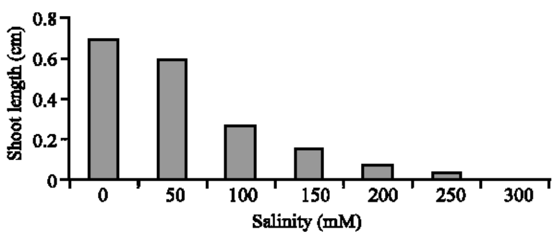

Fig. 4: Effect of different treatments of NaCl stress on shoot length of *Trigonella foenum-graecum*

Table 2: Relationship between salinity and germination percentage, germination rate, root length and soot length

Parameters	Linear regression equation (Salinity (mM)	Regression coefficient (R^2)	Probability
Germination	111.29-0.1819X	0.67	0.024
Germination rate	15.008-0.036X	0.95	0.000
Root length (cm)	9.6921-0.0316X	0.93	0.001
Shoot length (cm)	0.6179-0.0024X	0.89	0.002

DISCUSSION

Seed germination is important growth stage often to high mortality rates. Salinity inhibits the germination and germination rate of *Trigonella foenum-graecum* as the salt treatment increased. It is also assumed that in addition to toxic effects of ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (Maas and Nieman, 1978). It appears that a decrease in germination is related to salinity induced disturbance of metabolic process leading to increase in phenolic compounds (Ayaz *et al*., 2000). The results obtained in this study are similar in line with Dianati *et al*. (2008), Ramazani *et al*. (2009), Khan and Gulzar (2003), Kader and Jutzi (2004) and Mauromicale and Licandro (2002). They found that salinity increase results in decrease in germinability and delayed rate of germination. Growth and survival of plants at high salinity depends on adaptation to both low water potentials and high sodium concentrations, with high salinity in the external solution of plant cells producing a variety of negative concentrations. Salt stress causes ionic imbalance (Zhu *et al*., 1997), with excess sodium and chloride ions having a deleterious effect on many cellular systems (Serrano *et al*., 1999b), therefore plant survival and growth depends on adaptations to re-establish homeostasis. High salinity also inflicts hyper osmotic shock on plants, as chemical activity of water is decreased, causing a loss of cell turgor. Salt induced reduction in chloroplast stromal volume and generation of Reactive Oxygen Species (ROS) also plays an important role in decreasing plant photosynthetic capacity and therefore growth (Price and Hendry, 1991).

The root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress (Jamil and Rha, 2004). Salt stress inhibited the seedling growth (root and shoot length) but root length was more affected then shoots length (Fig. 3 and 4). Inhibition of plant growth by salinity may be due to the inhibitory effect of ions. The reduction in root and shoot development be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. It may be due to the ability of the root system to control entry of ions to the shoot is of crucial importance to plant survival in the presence of NaCl (Hajibagheri, 1989). High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Werner and Finkelstein, 1995) may be another reason for this decrease.

Linear regression revealed a significant negative relationship between salinity and final germination, germination rate, root length and shoot length and plant (Table 2). A negative relation of salinity on germination has been reported in several studies (Greenway and Munns, 1980; Jamil and Rha, 2004; Khan and Gulzar, 2003). Salinity reduced total plant biomass by negatively affecting root, stem and leaf mass (Greenway and Munns, 1980). Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to ability of seeds for best germination under unfavorable conditions, so necessity of evaluation of salt resistance plant species are important at primary growth stage.

CONCLUSION

The results indicated that the effect of various salinity levels on seed germination was significant at 1%. Salinity levels at 250 and 300 mM had significantly different effects on seed germination. Totality, seed germination rate, percent of germination, root and shoot lengths decreased with increasing salinity concentration.

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