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ORIGINAL ARTICLE

Effect of NaCl Priming on Seed Germination of Tunisian Fenugreek (*Trigonella foenum-graecum* L.) Under Salinity Conditions

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Salinity is one major problem of increasing production in crop growing areas throughout the world. The objective of this research was to evaluate the effect of NaCl priming on seed germination of Tunisian fenugreek (*Trigonella foenum-graecum* L.) under salinity conditions. Seeds of fenugreek were primed with NaCl (4g/l) for 36 h in continuous 25°C. Experimental factors were included 2 priming treatments (NaCl and non-priming as control) and five salinity solution (4,6,8,10 and 12 gl^{-1}). Results showed that seed priming increased final germination percentage, germination speed and radicle length over the non-primed treatment. At the lowest levels of salinity, there were no notable differences between primed and non-primed seeds, but with increasing salinity levels, primed seeds showed the better performance than non-primed seeds. These results indicated that NaCl priming significantly improved seed performance under salinity conditions.

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Many medicinal plants need to be cultivated in order to satisfy their increasing demand, but salinity and other forms of abiotic stress represent serious threats to the growth and crop yields (Qureshi *et al*. 2005). In fact, salinity is a major environmental problem to crop productivity throughout the arid and semi-arid regions of the world (Foolad and Lin, 1997). So, salt tolerance at germination stage is an important factor and has detrimental effects on germination of seeds (Sharma *et al*., 2004).

In this context, 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 condiment in food preparations for its nutritive and restorative properties and has also been used in folk medicine 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 *et* Grignon, 1991). In fact, this can be resulted from a low osmotic potential of the 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 it prevents radicle emergence (Bradford, 1986).

Seed priming improves germination and emergence of seeds for many vegetable under normal as well as stress condition and many substances are used as osmotica 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).

In fact, Demir and Mavi (2004) found watermelon seed priming with KNO_3 solution, effectively improved germination and seedling growth of the seeds under salinity compared to non-primed seeds. In addition, priming seeds improves germination, seedling emergence and seedling growth of tomato plants under saline conditions (Cayuela *et al*., 1996). Higher salt tolerance of plants from primed seeds seems to be the results of a higher capacity of osmotic adjustment (proline or carbohydrate synthesis) in leaves than plants from non-primed seeds (Sivritepe *et al.*, 2003).

Also, there are reports showing 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).

Farhoudi *et al*. (2007) suggested that seed priming with NaCl improves salinity tolerance in canola plants by limiting the destruction of cell membranes due to salinity and increasing proline concentration in seedlings. Moosavi *et al*. (2009) added that seed priming with an osmotic solution increase germination and the activity of antioxidant enzymes compared to non-priming seeds.

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 germination of fenugreek seeds under saline conditions.

MATERIALS AND METHODS

This study was carried out at the Department of Agronomy, Higher Institute of agronomy, Chott-Mariem, Tunisia. Seeds of fenugreek (*Trigonella foenum graecum L*.), obtained from a local cultivar cultivated in north of Tunisia. These seeds were superficially sterilized with 1% Sodium hypochlorite solution for 3 min and then thoroughly washed for 5 min with distilled water. Then they were primed with 4 g/l of NaCl solution for 36 h at 25°C. Following this treatment, seeds were washed three times for 5 min with distilled water and dried in room temperature for 48 h at 25°C. Later, dry seeds from each of the treatments (primed and non-primed seeds) were placed in 90-mm-diameter Petri dishes between two layers Watman filter paper and then moistened with six NaCl concentration (0, 4, 6, 8, 10, 12 gl^{-1}).Seeds were kept at room temperature (25°C ± 1°C) under normal light for germination. The experimental design was two factors factorial arranged in a completely randomized design. The first factor was salinity concentration (0, 4, 6, 8, 10 and 12 gl^{-1}) and the second factor was priming treatment (NaCl priming and non-primed seeds). Each treatment includes 5 Petri dishes which contains 150 healthy and homogenous seeds (30 seeds/ Petri dish). The number of germinated seeds was counted daily for 7 days after which no further seed germination was occurred. The appearance of 2 mm or more of radicle was considered as germination. Germination percentage (%): N_i / N × 100 where N_i: number of germinated seed till i^{th} day and N: Total number of seeds. Parameters of germination based on the final germination values, kinetics and the length of radicles for fenugreek seedlings were obtained and 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 germination

Effect of salinity on total germination percentage of fenugreek is presented in Figure 1. When salt was absent (0 gl^{-1}), all seeds from non-primed and primed seeds have germinated. As a result, the percentage of germination was 100 %. But, increasing NaCl salinity decreased significantly (p <0.05) total germination of seeds derived from primed (P) and non-primed (NP) seeds. Indeed, with the highest concentration (12 gl^{-1}), we have the lowest rate of germination (10 and 50 % respectively in NP and P seeds). However, total germination percentage in primed seeds was higher compared with those derived from the non-primed seeds for all salinity levels. In addition, the effect of priming and concentration are highly significant and the positive effect of priming begins to appear clearly for NaCl concentration above 6 gl^{-1}. The difference in germination rate between the two types of seeds increases depending on the concentration, it reaches 40 % at 12 gl^{-1}.

Kinetics of germination

The effect of NaCl priming and salinity on germination kinetics of fenugreek seeds was also examined (Figure 2). In non-primed seeds, germination started the second day of imbibition for concentrations 0 and 4 $gl^{-1}$of NaCl. But with the presence of higher concentrations of this salt, the germination is delayed and the period of the delay increases with the concentration. So, with the presence of high concentrations of NaCl (10, 12 gl^{-1}), radicles of the seeds appeared on the 4^{th} day (Figure 2). In primed seeds, germination started one day after the imbibition until the concentration of 8 gl^{-1} of NaCl. But beyond this concentration, the seeds have germinated on the third day. Salinity not only decreased the germination but also delayed it.

Radicle length

The effects of salinity stress on radicle length have been showed in Figure 3. Comparison of radicle length means in different salinity levels showed that when salinity level increase, seedlings radicle length decrease. In fact, when salt was absent (0 gl^{-1}), radicle length was almost 8, 5 cm in control seeds (non-primed) as well as in primed seeds. With the presence of NaCl (4 to 12 gl^{-1}), salt seems to have two opposing effects, a stimulatory effect and an inhibitor effect. Indeed, levels 4 and 6 gl^{-1} stimulated the length of the radicle, which grows to 11 cm (non-primed seeds) and 11.5 cm (primed seeds). It means an elongation of 35 % compared to the radicles of non-primed seeds. But, above 6 g/l, the salt had an inhibitor action and the length of the radicle is being shortened in depending on the concentration of NaCl. The most reduction in radicle length related to 12 gl^{-1} (5,5 and 8,5 cm respectively in NP and P groups). But, for all salinity levels, reduction was significantly higher in non-primed group than in primed group which seems to be more resistant to salt (Figure 4).

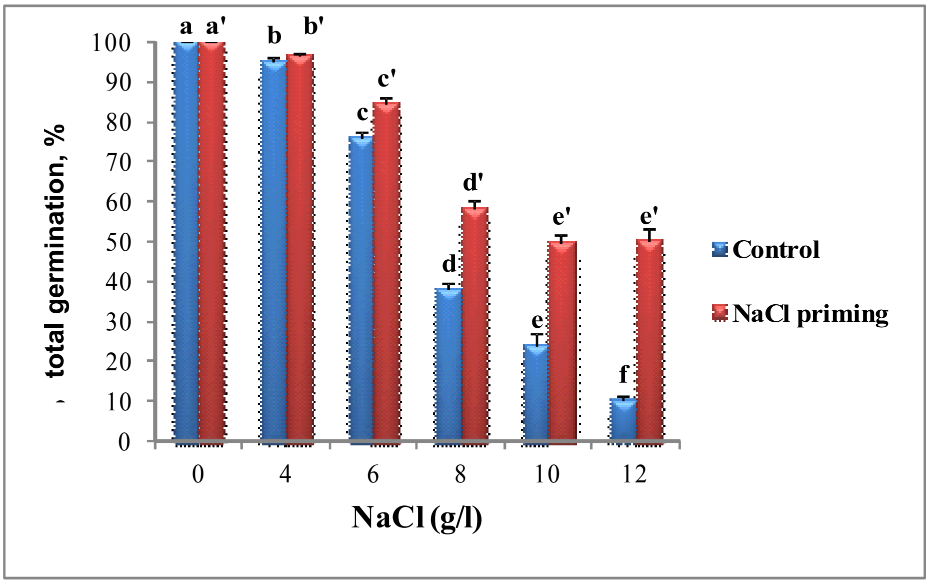

Figure 1: Effects of NaCl on germination of fenugreek seeds (Non-primed and primed seeds). Means followed by the same letters are not significantly different according to Duncan test at 5% level.

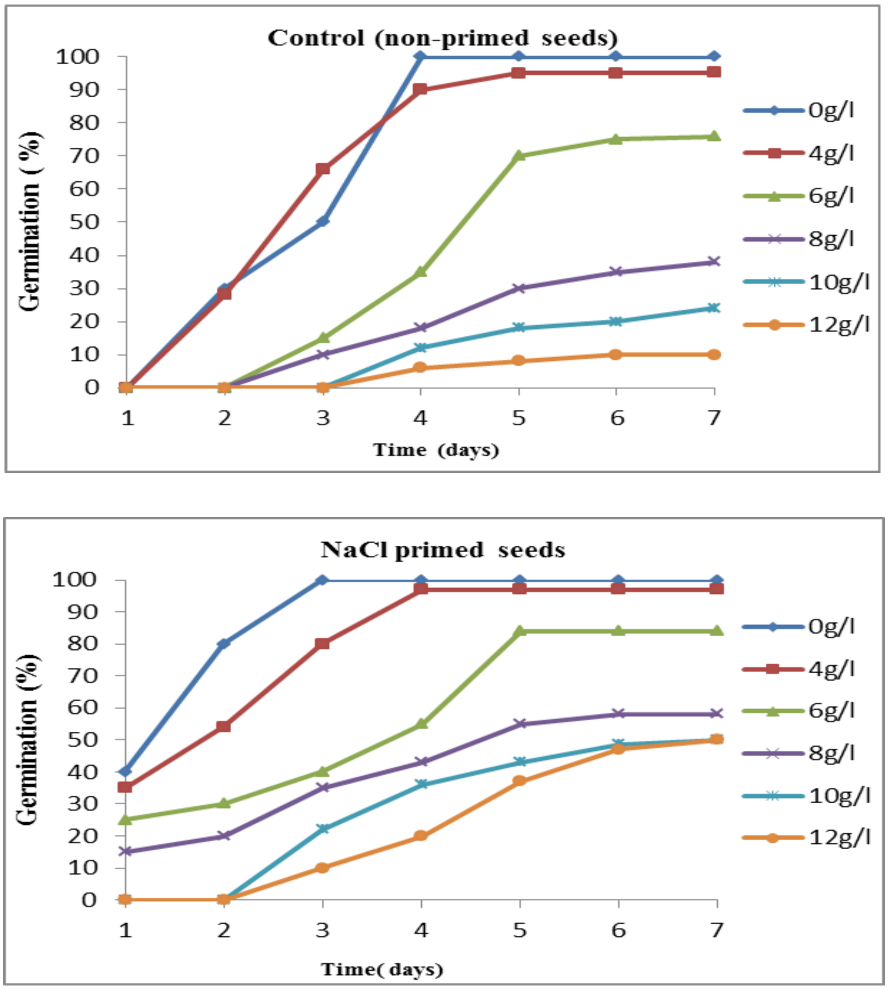

Figure 2: Kinetics of germination under salt stress (NaCl) for non-primed and primed seeds

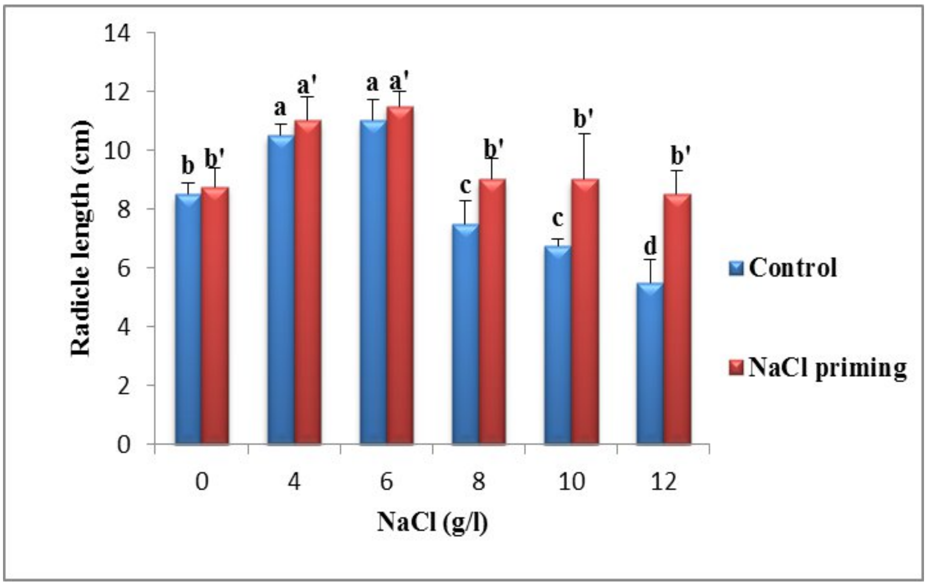

Figure 3: Effects of NaCl on radicle length of fenugreek seeds (Non-primed and primed seeds). Means followed by the same letters are not significantly different according to Duncan test at 5% level.

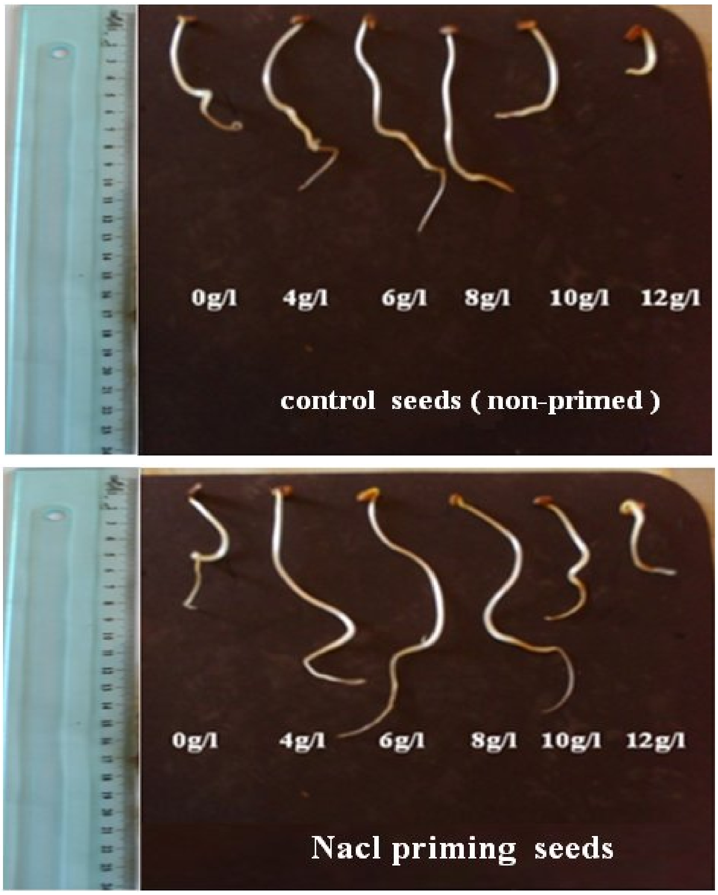

Figure 4: Effect of salt stress and seed priming on radicle length of fenugreek seedlings

DISCUSSION

The reduction in total germination 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).

According to Gill and Singh (1985) germination, growth, respiration, and other related processes can be affected in seeds that are subjected to salt stress changes in any one of these processes can affect other metabolic activities, particularly the carbohydrate metabolism that plays an important role in germination (Prado *et al*.,2000).

The reduction in final germination percentage can be explained by the increase of external osmotic pressure which affects the absorption of water by the seed and can be also due to the accumulation of Na^+ and Cl^- in the embryo which may lead to an alteration in the metabolic processes of germination and causes cells death in the embryo (Hajlaoui *et al*., 2007).

More specifically, this reduction in the germination rate, which was more pronounced in non-primed seeds, is related to the specific ionic salt stress during the second phase of germination that corresponds to the activation of embryo growth using reserve metabolites (Nonogaki *et al*., 2010).

In this study, the osmotic solution used as a priming agent (NaCl) was effective in reducing the effect of salt stress on germination. Indeed, priming improved the final germination percentage of fenugreek seeds throughout different concentrations of NaCl (4-12 gl^{-1}). This improvement is clearly noticeable for 12 gl^{-1} when the final percentage of germination obtained was close to 50 % compared to 10 % for non-primed seeds.

These findings are in full agreement with the work of Sivritepe *et al*. (2003) on melon showing that osmopriming improves the rate and uniformity of germination and further development of plants under salt stress. Increased emergence percentage of primed seeds under salt stress may be due to reduced uptake of NaCl by the germinating seeds or due to increased uptake of oxygen, and the efficiency of mobilizing nutrients from cotyledons to the embryonic axis (Kathiresan *et al.*, 1984).

Salt also delays germination and slows its speed. This delay could be due to the alteration of some enzymes and hormones which are present in the seed (Botia *et al.*, 1998).Gill *et al.* (2003) added that the germination delay can be due to a problem of seed hydration following a high osmotic potential which cause the inhibition of some mechanisms leading to radicle emergence.

The shortening of the latency time observed in primed seeds is probably the result of faster seed imbibitions resulting of aquaporin activation (Boursiac *et al*., 2005).

Faster emergence after priming may be due to increased rate of cell division in the root tips of seedlings from primed seeds as reported in wheat (Bose and Mishra, 1992) and tomato (Farooq *et al.*, 2005). Seedling growth was suppressed under saline conditions, which is strongly in accordance with the work of Cicek and Cakirlar (2002) who reported that salinity reduced shoot length of maize seedlings. Moreover, under moderate saline conditions (4 and 6 g/l) primed and non-primed seeds performed better than under 0 g/l. The stimulatory effect of low concentrations of NaCl on growth has been observed in other species like *Eucalyptus camaldulensis* (Rawat and Banerjee, 1998) and wheat (Drihem and Pilbeam, 2002). Radicle length of primed seeds was greater than that of non-primed ones. Improvement in radicle length was increased due to earlier germination induced by NaCl priming treatment. These results are in accordance with Stofella *et al.* (1992) who reported that priming significantly improved root length in pepper seeds. Similarly, Liu *et al.* (1996) described that osmopriming improves radicle and plumule length in treated tomato seeds.

CONCLUSION

Results suggest that priming could ameliorate the destructive effect of salinity on germination. In fact, osmopriming with NaCl proved enhancing the seed performance under normal as well as under saline conditions. This technique has many advantages, such as feasibility and low cost. 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 for seeds still requires more investigations and more biochemical and molecular researches.

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