Soil & Environ. 28(1): 81-87, 2009
www.se.org.pk

Effect of seed priming with NaCl on salinity tolerance of hot pepper (*Capsicum annuum* L.) at seedling stage

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Abstract

An experiment was conducted to improve the hot pepper seed performance under salinity stress conditions. The effects of priming with an optimized dose (1 mM) of sodium chloride (NaCl) were assessed for improving seedling vigour and salt stress tolerance in seedlings of the hot pepper. Seeds primed with NaCl solution (1 mM) were examined at different salinity levels [0, 3, 6 and 9 dS m^{-1}] in relation to early growth stage. Priming with NaCl was effective in alleviating the adverse effects of salinity. Significant increase in germination percentage, germination index and germination speed, vigour index, plumule and radicle length, and dry weight of the seedlings as compared to control was recorded. On the other hand, mean germination time, time to reach 50% germination and fresh weight of seedlings were non-significant against control. In this experiment, it was concluded that seed priming with NaCl has been found to be better treatment as compared to non-primed seeds in case of hot pepper for improving the seedling vigour and seedling establishment under salt-stressed conditions.

Key words: Seed priming, sodium chloride, salinity tolerance, seedling vigour, hot pepper

Introduction

Among the abiotic stresses, salinity is a major limiting factor in the crop productivity all over the world. Salinity affects almost every aspect of the physiology and biochemistry of plants which in turn significantly reduces yield (Ashraf, 1994; Parida and Das, 2005; Munns and Tester, 2008) being a major environmental factor (Ashraf and McNeilly, 1987; Munns, 2002). It has been estimated that 20% of cultivated area of the world and half of the world's irrigated lands are affected by the salinity (Szabolcs, 1994; Flower and Yeo, 1995; Chinnusamy *et al.*, 2005). In Pakistan, almost 6.3 m ha of land is affected by salinity, which is estimated to be 14% of irrigated land and this causes a yield loss of 64% (Afzal *et al.*, 2005).

Hot pepper (*Capsicum annuum* L.), commonly known as chilli, is an important vegetable as well as spice crop of Pakistan. The chilli production is estimated to be 120.46 thousand tons from an area of 59.4 thousand hectares with an average yield of 2.028 tons per hectare (Govt. of Pakistan, 2006). The yield difference is much higher between Pakistan and other chilli growing countries. In Pakistan, chilli production is mainly affected by high salinity levels in Sindh province, which is main chilli producing province. In addion to that, chilli is much sensitive to salinity (Haman, 2000; Kanber *et al.*, 1992). Seed germination and early seedling growth are the most sensitive stages which are stressed by salinity in most of the crops (Sivritepe *et al.*, 2003; Ashraf and Foolad, 2005). Salinity adversely affects seeds and seedlings and greatly contributes in establishment of poor stand and eventually poor production of vegetable crops (Grassbaugh and Bennett, 1998). It has also been reported that chilli seed germination and emergence is slow and non-uniform under normal as well as stressed conditions (Demir and Okcu, 2004). Soil salinity, if not properly managed, can become a limiting factor for chilli stand establishment (Flynn *et al.*, 2003) causing decrease in germination rate and germination percentage of pepper seeds (Chartzoulakis, 2000). Furthermore, chilli yield is reduced upto 14% for every increase in unit of salinity above its threshold (Rhoades *et al.*, 1992).

Seed priming is a technique of seed enhancement that improves germination or seedling growth and rate or uniformity of the seedling establishment (Taylor *et al.*, 1998). Seed priming improves seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination and emergence in different crops (Pill, 1995; Warren and Bennet, 1997; Powell *et al.*, 2000; Cantliffe, 2003). This also helps seedlings to grow in stressed conditions (Welbaum, *et al.*, 1998; Ashraf and Foolad, 2005; Carbineau and Come, 2006). Seed priming with different salts, especially NaCl, have shown to improve germination and growth of many crops under stressed conditions (Sivritepe *et al.*, 1999; Sivritepe *et al.*, 2003; Omami, 2005; Basra *et al.*, 2005; Esmaielpour *et al.*, 2006; Sivritepe and Sivritepe, 2007). Seed priming with NaCl also showed improvement in growth and yield of mature tomato plants when salt treatments were applied with seed sowing (Cano *et al.*, 1991) and similarly in asparagus and tomatoes (Pill *et al.*, 1991), and with cucumber (Passam and Kakouriotis, 1994). Improvement of germination in pepper plant by priming with water and NaCl has also been reported (Smith and Cobb, 1991). Keeping in view the role of NaCl in

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improving vigour, increasing salt tolerance and beneficial effects of seed priming in various crops, a study was conducted to improve the vigour and salt stress alleviation in hot pepper cultivar Hot Queen.

Materials and Methods

The proposed study was conducted in the Vegetable Seed Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, to determine the effect of different pre-sowing seed treatments on germination and seedling vigour of hot pepper (*Capcicum annuum* L.) cultivar Hot Queen under saline conditions. The seeds were obtained from Yousuf Seed Corporation Registered, Faisalabad, Pakistan. The initial seed moisture was 8% (dry weight basis).

Priming Treatment

Seeds were surface sterilized by dipping in sodium hypochlorite (5%) solution for 5 min. and dried on filter paper. These surface sterilized seeds were primed in aerated solution of sodium chloride (NaCl) at concentrations of 1.0 m*M* for 48 h at 25±2 ^{o}C under dark conditions. After respective priming treatments for specific period, seeds were washed with distilled water (Khan, 1992). The seeds were then dried at room temperature on filter paper for 24 h upto their original moiture contents (Bennett and Waters, 1987). Dried seeds were then packed in polythene bags and stored in a refrigerator (5±2 ^{o}C) for further use.

Emergence Test

Primed and un-primed seeds were sown in plastic trays filled with washed sand and irrigated with half-strength Hoagland's nutrient solution containing different salinity levels [0 (Control), 3, 6 and 9 dS m^{-1}], kept at 25$\pm$2 ^{o}C under 16 hours photoperiod in an incubator.

Data Collection

Seeds with visible radicle were considered as emerged. Data were recorded on daily basiss for emergence for 14 days following seedling evaluation protocol given in the handbook of the Association of Official Seed Analysis (1990).

The time taken to 50% emergence [E_{50}] was calculated according to the following formula of Coolbear *et al.* (1984), as modified by Farooq *et al.* (2005):

$$E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where N is the final number of emerged seeds and n_i, n_j are the cumulative number of seeds emerged by adjacent seed count at times t_i and t_j, respectively when $n_i < N/2 < n_j$. Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981):

$$MET = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds emerged on day D, the number of days counted from the beginning of emergence.

Final emergence percentage was calculated at the end of each experiment. Emergence index (EI) was calculated (Association of Official Seed Analysts, 1983) as the following formula:

$$\mathrm{EI} = \frac{\text{No.of emergedseeds}}{\text{Daysof firstcount}} + - - - - - + \frac{\text{No.of emergedseeds}}{\text{Daysof finalcount}}$$

Ten normal seedlings from each replication were taken at random at final count and plumule and radicle length was measured from collar region with the help of measuring tape after 14 days of sowing and averaged to obtain mean shoot and root length. Seedling fresh weight was obtained using ten normal seedlings from each replication after 14 days of sowing and dry weight was obtained by drying at 105 ^{o}C for 24 hours in oven.

Statistical Analysis

The experiment was arranged according to completely randomized design with four replicates, each replicate having 50 seeds. Data recorded were analyzed statistically using Fisher's analysis of variance technique and Duncan's Multiple Range Test at 5% probability level to compare the differences among treatment means (Steel *et al.*, 1997).

Results

On the basis of previous experiments (data not shown), best dose of priming agent (NaCl) was selected for this experiment to observe the effects of seed priming on performance of hot pepper under different salinity levels. Results for final emergence percentage (FEP) were found to be significant for all treatments under saline conditions (Figure 1). Salinity reduced the final emergence percentage (FEP) of both the primed and non-primed seeds with more intensity as salinity levels were increased. However, priming reduced the adverse effects of salinity on hot pepper emergence as compared to non-primed seeds. Salinity increased the time required for emergence of hot pepper seeds. Non-significant (P<0.05) results were found for all treatments under saline conditions for both of mean emergence time (MET) (Figure 2) and time taken to reach

50% emergence (E_{50}) (Figure 3) but it was observed that priming with NaCl decreased the time required for emergence by early emergence of hot pepper seeds. Both the parameters presented better performance of NaCl priming with respect to salinity for improving the emergence of the hot pepper seeds. Similarly, emergence index (EI) was also found to be much enhanced in primed seeds as compared to non-primed seeds at all the salinity levels (Figure 4). Emergence speed (ES) was also accelerated by priming treatment, even though, salinity had adverse effects on emergence speed (Figure 5). Plumule length of primed seeds was boosted by NaCl priming as compared to non-primed seeds at all the salinity levels. However, it was observed that both the treatments showed highest plumule length at 3 dS m^{-1}, although, there was also a treatment having no salinity imposed (Figure 6). Similarly, results at 6 and 0 dS m^{-1} were same presenting that there were no adverse effects of salinity up to 6 dS m^{-1} regarding plumule length. Radicle length was also improved by the NaCl priming as compared to non-primed seed and gradually decreased with increase in salinity levels (Figure 7). Non-significant results were found for fresh weight of the seedlings (Figure 8) but it was observed that NaCl priming partially alleviates the adverse effects of salinity by increasing fresh weight of the seedlings at all salinity levels. In addition, dry weight of the seedlings was significant but there was a negligible effect of NaCl priming on fresh weight of the hot pepper seedlings (Figure 9). There was mixed behaviour of the treatments for dry weight of the seedlings. Vigour index (VI) was highly boosted by NaCl priming as compared to non-priming treatments (Figure 10) which indicated that priming with NaCl has improved the vigour of the seedlings and showed better results.

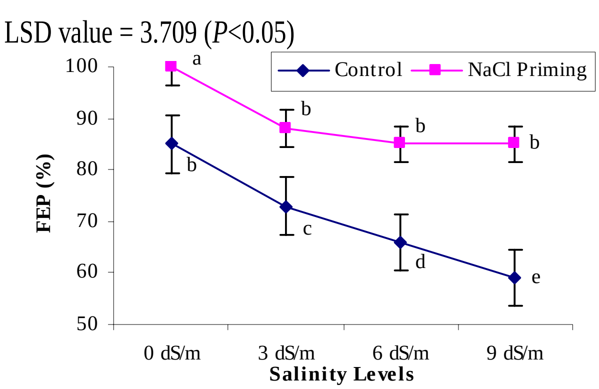

Figure 1. Effect of NaCl priming on final emergence (%) of hot pepper under saline conditions

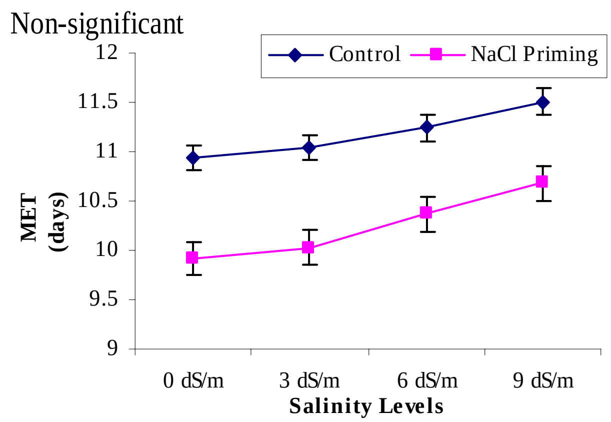

Figure 2. Effect of NaCl priming on mean emergence time of hot pepper under saline conditions

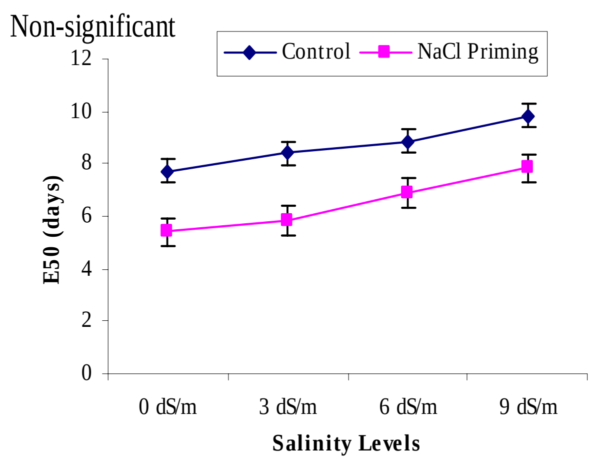

Figure 3. Effect of NaCl priming on time to 50% emergence of hot pepper under saline conditions

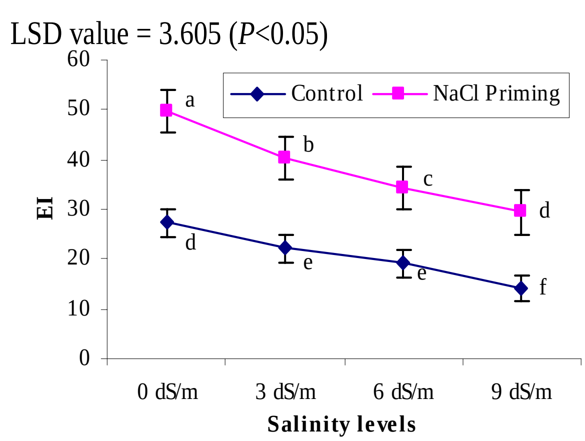

Figure 4. Effect of NaCl priming on emergence index of hot pepper under saline conditions

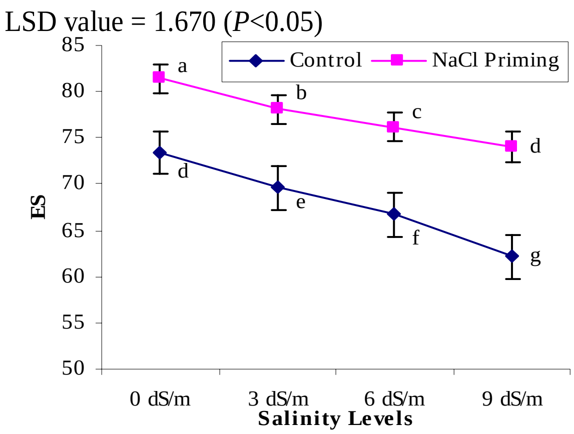

Figure 5. Effect of NaCl priming on emergence speed of hot pepper under saline conditions

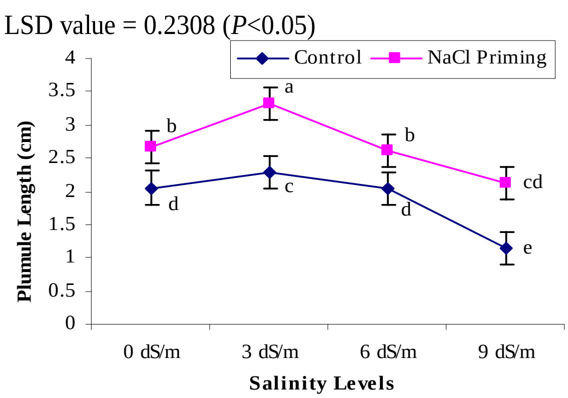

Figure 6. Effect of NaCl priming on plumule length of seedlings of hot pepper under saline conditions

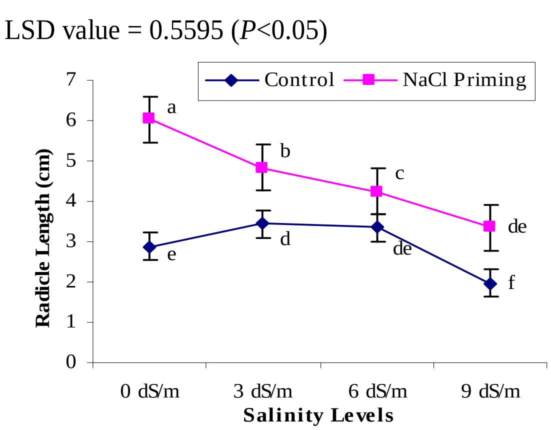

Figure 7. Effect of NaCl priming on radicle length of seedlings of hot pepper under saline conditions

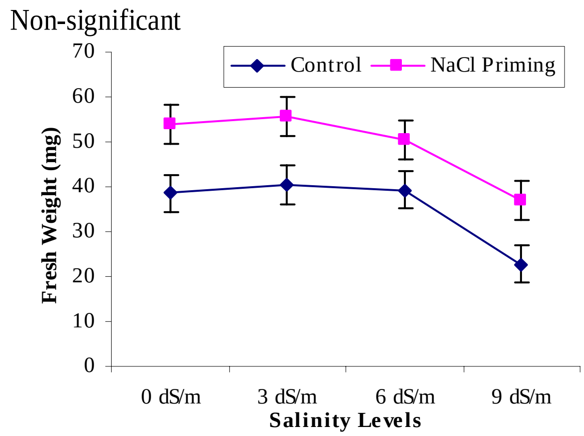

Figure 8. Effect of NaCl priming on fresh weight of seedlings of hot pepper under saline conditions

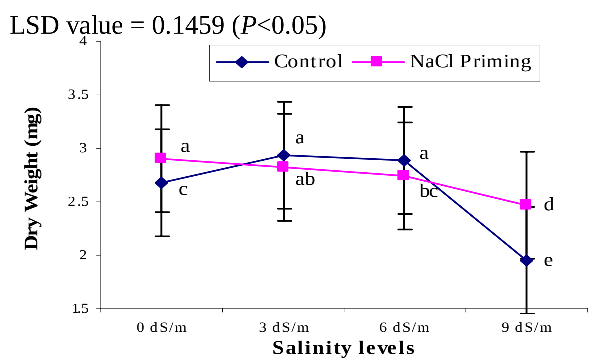

Figure 9. Effect of NaCl priming on dry weight of seedlings of hot pepper under saline conditions

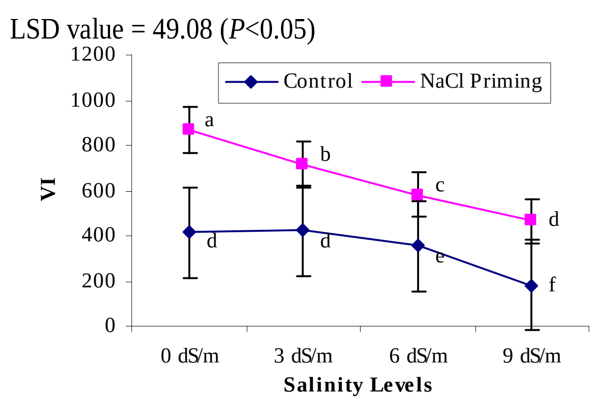

Figure 10. Effect of NaCl priming on vigour index of seedlings of hot pepper under saline conditions

Discussion

To improve salt tolerance of the plants, especially horticultural commodities, at different stages of their lifecycle is highly desirable in the present day of food shortage. So, we need more methods for improving the crops under salt stressed condition by simple, un-expensive and environment friendly ways. Seed priming is one of such type of methods and NaCl priming have shown improved germination and growth of many crops under stressed conditions (Sivritepe *et al.*, 2003; Basra *et al.*, 2005; Esmaielpour *et al.*, 2006; Sivritepe and Sivritepe, 2007). Priming with NaCl also showed improvement in growth and yield of mature tomato plants when salt treatments were applied with seed sowing (Cano *et al.*, 1991). Similar findings were reported in pepper (Smith and Cobb, 1991) asparagus and tomatoes (Pill *et al.*, 1991), and cucumber (Passam and Kakouriotis, 1994).

In this study, NaCl priming significantly enhanced the FEP, EI, ES, plumule length, radicle length, seedling dry weight and vigour over control in hot pepper while improvement in MET, E_{50} and fresh weight was found to be non-significant. In case of FEP, NaCl priming was shown to alleviate the adverse effects of salinity. The results for FEP confirmed the findings of Warley and Fernando (2004) who reported that priming improved the germination percentage and germination rate of un-aged and aged seeds of muskmelon. Similarly, aerated hydration treatment of aubergine and pepper significantly increased final germination of both, but greater on aubergine, over a wide range of temperature (18-35 ^{o}C) (Demir and Okcu, 2004). In previous studies, it has been reported that aerated hydration treatment of *Solanum melongena* and *Capsicum annum* decreased the mean germination time (Demir and Okcu, 2004). This significant reduction in mean emergence time could be the result of shortening of the lag phase during priming. The lower mean emergence time could be a result of additive effect of priming treatments. Moreover, Thronton and Powell (1995) concluded that priming reduced T_{50} in *Brassica oleracea* seeds. Early reserve breakdown and early reserve mobilization might be the cause of significant reduction in E_{50}. The germination time was, on an average, reduced by 2 to 3 days by primed seeds of pepper and tomato as described by Andreoli and Khan (1999). The earlier and synchronized germination in the osmoprimed seed is related to increased metabolic activities (Alvardo and Bradford, 1987; Liu *et al.*, 1996). The results for emergence index are in accordance with Rao *et al.* (2005) who found enhanced germination index in tomato with priming. Higher rate of germination index might be due to the result of recovered development of genetic repair mechanisms during the priming operations (Bradford, 1986).

Improvement in plumule length was increased due to earlier germination induced by NaCl priming treatment. These results are in accordance with Mavi *et al.* (2006) who reported that priming treatments increased seedling size. Andreoli and Khan (1999) reported that priming was much efficient in improving germination and stand establishment of pepper and tomato seeds and our results also confirmed it. Stofella *et al.* (1992) 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. It is apparent that early germination induced by NaCl priming has resulted in vigorous roots. Bose and Mishra (1992) reported faster emergence rate after osmopriming due to an increased rate of cell division in the root tips of wheat. Furthermore, Farooq *et al.* (2007) stated that improved seedling fresh and dry weights might be due to increased cell division within the apical meristem of seedling roots, which caused an increase in plant growth. In earlier studies, it was observed that seedlings from primed tomato seeds maintained greater mean plant dry weights than untreated seedlings throughout the pre-flowering period (Alvardo and Bradford, 1987) and we found the same results. Basra *et al.* (2005) reported higher shoot and root lengths, seedling dry weight in wheat seedlings by hydropriming and halopriming with NaCl. Farooq *et al.* (2005) concluded in their study that seeds subjected to NaCl priming resulted in improved germination and seedling vigour by dormancy breakdown as compared to untreated seeds. Seed treatments not only improved the germination rate and time but also enhanced the seedling vigour as indicated by increased plumule and radicle length and seedling fresh and dry weights.

From our study, it may be concluded that seed priming can improve emergence, seedling vigour and overall seedling establishment under salinity. Osmopriming with NaCl proved superiority over control in enhancing the seed performance under normal as well as under saline conditions.

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