

Full Length Research Paper

The role of seed priming in improving seed germination and seedling growth of maize (*Zea mays* L.) under salt stress at laboratory conditions

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Salinity is considered as a major abiotic stress affecting germination, seedling growth and crop production in arid and semi-arid regions. Many techniques are used to improve tolerance to salinity. Priming is believed to be an effective technique that increases germination, plant growth and improve yield of several vegetables and crops under saline soil condition. The objective of this study was to see the effect of seed priming with 5 g/L NaCl on germination and seedling growth of maize (*Zea mays* L.) exposed to five salinity levels under laboratory conditions. Maize seeds were soaked in solutions of NaCl (5 g/L) for 12 h at room temperature. Primed and unprimed seeds were sown in Petri dishes and were irrigated with saline solutions of five concentrations (0, 2, 4, 6 and 8 g/l NaCl). Priming seeds with NaCl significantly improved ($P < 0.05$) germination and growth of maize. As a result, fresh and dry weights of shoot and root were improved. Seed priming alleviated the inhibitory effect of salt stress on germination and seedling growth of maize under salt stress. Thus, seed priming with 5 g/l NaCl could be used to improve germination and early growth. Further, this study needs to be continued if performance of mature maize plants could also be improved and yield increased by sowing primed seeds in saline soils.

Key words: Maize, salinity, seed germination, seed priming, seedling growth.

INTRODUCTION

Salinity affects 6% of the world's total land area which is approximately 800 million hectares (FAO, 2008). Salinization is more spreading in irrigated lands because of inappropriate management of irrigation and drainage. Rain, cyclones and wind also add NaCl into coastal agricultural lands (FAO, 2008). Salinity has an adverse effect on seed germination of many crops, by creating an osmotic potential outside the seed inhibiting the absorption of water, or by the toxic effect of Na^+ and Cl^- (Khajeh-Hosseini et al., 2003). Osmotic and saline stresses are responsible for the inhibition and hindrance of germination and plant growth (Almansouri et al., 2001).

Water uptake during the imbibitions stage decreases and salinity induces an excessive absorption of toxic ions by the seed.

Seed priming or osmoconditioning is one of the physiological methods which improves seed performance and provides faster and synchronized germination. It is an easy, low cost and low risk technique, which is recently being used to overcome the salinity problem in agricultural lands (Neto and Tabosa, 2000). It entails the partial germination of seed by soaking in either water or in a solution of salts for a specified period of time, and then re-drying them just before the radicle emerges

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(Neto and Tabosa, 2000). Seed priming stimulates many of the metabolic processes involved in the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously, and perform better in adverse conditions (Cramer, 2002). Some of the factors that affect seed priming response are solution composition and osmotic potential (Chinnusamy et al., 2005). It has been shown that NaCl seed priming could be used as a solution to improve salt tolerance of seeds (Cayuela et al., 1996; Elouaer and Hannachi, 2012).

Ethiopia is reported to possess over 11 million hectares of unproductive naturally salt affected wasteland (PGRC, 1996). These areas are normally found in the arid and semi-arid lowlands and in rift valley and other areas that are characterized by higher evapotranspiration rates in relation to low precipitation. According to the study of Hawando (1994) salt-affected lands are increased from 6 to 16% of the total land area of Ethiopia in the last years of the 20th century and 9% of the population lived in these areas. About 44 million ha (36% of the country's total land areas) are potentially susceptible to salinity problems. Reclaiming back these salt affected areas and using them for agricultural production is very costly and time consuming.

Maize (*Zea mays*) production in Ethiopia ranges from large-scale commercial farms to smallholders and subsistence farmers. It is consumed as main food, with its crop residues and by-products commonly fed to Livestock and used as source of fuel. Hence, priming of maize seeds with NaCl could be important in improving the growth and yield of maize in areas that are potentially susceptible to salinity problems and totally unproductive salt affected lands. Such studies were not done before with maize in the Ethiopian agricultural landscapes. Therefore, this study investigated the effect of priming on improving seedling growth of maize under different salinity levels at laboratory conditions.

MATERIALS AND METHODS

The study was conducted in Bahir Dar city at Bahir Dar University (Peda campus). Bahir Dar city is the capital of the Amhara National Regional State in the Federal Democratic Republic of Ethiopia. It is located at 11°37'30"N and 37°27'30"E on the southern side of Lake Tana with an altitude of 1801 m above the mean sea level. The annual temperature of Bahir Dar city ranges from a minimum average of 12.6°C to a maximum average of about 27.0°C, and the mean annual rainfall is about 1406.98 mm (NMABDBO, 2013).

Seed materials and priming

Maize seeds (Melkasa 4) were obtained from the certified seed supplier of Ethiopian seed enterpriser (ESE) store located in Bahir Dar, Ethiopia. The seeds were surface sterilized (disinfected) with 5% sodium hypochlorite (Na₂HCl) solution for 3 min and then thoroughly washed for 5 min with distilled water. Subsequently, the seeds were primed by soaking with 5 g/L NaCl solution for 12 h at room temperature under shade and the ratio of seed weight to solu-

tion volume was 1/5 (g/ml). After priming, seeds were removed and washed with tap water and then rinsed three times in distilled water. Finally, seeds were left in air between two filter papers to re-dry to their original moisture level (Afzal et al., 2008).

Experimental design

The study was carried out from October 20, 2012 to March 16, 2013. A complete random design (CRD) study was used to study the effect of seed priming on germination and seedling establishment of maize under salt stress. This part of the experiment was carried out in the laboratory in 40 Petri dishes that is, 20 for NaCl primed seeds and 20 for unprimed (control) seeds. The experiment was replicated four times. All petri dishes were sterilized and labeled for various treatments (0, 2, 4, 6 and 8 g/L) of NaCl.

Primed and unprimed seeds were placed in 15 cm diameter Petri dishes on a layer of filter paper (Whatman # 1). Thirty seeds were placed in each Petri dish. Petri dishes were irrigated with five different saline solutions (0, 2, 4, 6 and 8 g/L concentrations of NaCl) 10 ml per Petri dish daily. The Petri dishes were placed at room temperature. The experiment was a factorial with two factors which are salinity at 5 levels (0, 2, 4, 6 and 8) g/L and priming with 2 levels (unprimed and NaCl primed seeds), arranged in a completely randomized design with four replications and thirty seeds per replicate. Seed germination was recorded daily up to day 14 after the start of the experiment. A seed was considered as germinated when the radicle emerges by about 2 mm in length (Ahmadvand et al., 2012).

Physiological parameters such as seed germination percentages, mean germination time, germination index and coefficient velocity of germination were determined. Shoot and root length of five randomly selected seedlings was measured using transparent ruler from each petri dish after the seedlings were separated carefully from their roots. Then, shoot and root fresh weight was measured immediately. To determine the dry weight of these seedlings, the separated shoots and roots were dried to 70°C for 12 h (Ahmadvand et al., 2012) and then weighed.

Computing measurements of physiological parameters

Total germination (TG) was measured in the 14th day after sowing using the formula

$$TG(\%) = \frac{n}{N} \times 100$$

Where, n is the total germinated seeds and N is the total seeds sowed. Mean germination time (MGT) was calculated according to the formula used by Fuller et al. (2012).

$$MGT = \frac{\sum(T_i n_i)}{\sum n_i}$$

Where, n_i is the number of germinated seeds on the ith day and T_i is the rank order of day i (number of days counted from the beginning of germination). Vigour index (VI) was measured using the formula of Elouaer and Hannachi (2012) and it is calculated as follows.

$$VI = \frac{TG(\%) \times \text{Seedling Length}(cm)}{100}$$

Table 1. Analyses of Variance of seed germination of Maize seedlings under laboratory conditions.

Source	TG (%)	MGT	SL	RL	R/S	VI	SFW	SDW	RFW	RDW
Priming	3180.319*	92.045*	44.796*	21.433*	0.206*	38.796*	1.197*	0.316*	0.632*	0.060*
Salinity level	9142.315*	75.312*	433.215*	362.430*	0.928*	416.314*	27.341*	1.810*	9.269*	0.277*
Priming× salinity level	260.137*	8.229*	3.624*	7.142*	0.030*	5.175*	0.138	0.033	0.084	0.014
Error	59.352	0.107	1.084	0.55	0.003	0.907	0.157	0.027	0.066	0.006

*Significant at 0.05 according to Post Hoc LSD test.

Data analysis

All the data obtained from the experiments were subjected to an analysis of variance (Two way ANOVA) using the Statistical Package for the Social Sciences (SPSS) (version 20.0) software and the difference between means were compared by Fisher's Post Hoc LSD tests ($P < 0.05$).

RESULTS AND DISCUSSION

Examination of variance showed that both salinity level and priming have significant effects on the studied parameters. Moreover, the interaction of salinity level and priming with salt solution had significant effect on all parameters tested at 5% significant level (Table 1). Priming with NaCl increases the germination parameters (TG (%), MGT) and growth parameters (RL, SL, SFW, SDW, RFW, RDW, R/S and VI) of maize, as compared to unprimed seeds, under different levels of saline conditions.

Total germination percentage (TG (%)) and mean germination time (MGT)

Figure 1a and 1b shows the effect of NaCl priming on maize TG (%) and MGT at different salinity concentrations, respectively in 14 days. TG (%) from both primed and unprimed seeds decreased significantly ($P < 0.05$) with increasing NaCl salinity level (Figure 1a). However, this reduction in TG (%) was significantly higher for unprimed seeds compared to primed seeds. The data in Table 2 indicated a reduction of 83.75% on TG (%) due to an increase in salinity level from 0 to 8 g/L. Although salinity stress significantly ($P < 0.05$) increase MGT for both primed and unprimed maize seeds, the primed seeds had significantly lower MGT compared to unprimed seeds. Such variation is shown in Figure 1b. Generally, increasing salinity causes a decrease in maize germination; this might be due to the toxic effects of Na^+ and Cl^- in the process of germination (Khajeh-Hosseini et al., 2003). It alters the imbibitions of water by seeds due to lower osmotic potential of germination media, causes toxicity which changes the activity of enzymes of nucleic acid metabolism, changes protein metabolism, interrupts

hormonal balance, and reduces the utilization of seed reserve food (Gomes-Filho et al., 2002).

Primed seeds of maize might have better competency for water absorption from the growing media that enabled metabolic activities in seeds during germination process of a start much earlier than radicle and plumule appearance (Elouaer and Hannachi, 2012). Similarly increased solubilization of seed storage proteins like the beta subunit of the globulin and reduction in lipid peroxidation and enhanced antioxidative activity in primed seeds facilitated germination. This faster germination was due to the synthesis of DNA, RNA and protein during priming (Afzal et al., 2008).

Vigor index

Based on the analysis of variances, salinity level, seed priming and their interaction have significant ($P < 0.05$) effect on vigor index (Table 1). Increasing salinity causes a significant decrease ($P < 0.05$) in maize vigor index for both unprimed and primed seeds (Table 2 and Figure 2). This decrease was from 17.684 at 0 g/L to 0.184 at 8 g/L (Table 2). However, the relative decrease for primed seeds was significantly ($P < 0.05$) different when compared to seedlings from unprimed seeds. Vigor index was increased in primed compared to unprimed maize seeds (Figure 2). Similar results were found by Ruan et al. (2002a) working on rice seeds, priming showed higher vigour index than non-primed ones. The significant improvement in seedling length and total germination percentage may be a result of earlier induced germination by primed seeds over unprimed seeds (Farooq et al., 2005), which resulted in vigorous seedlings with more root and shoot length than the seedlings from unprimed seeds.

Shoot and radicle length

Salinity had a significant inhibitory effect on shoot length for both primed and un-primed seeds. However, this effect was significantly ($P < 0.05$) less pronounced in seedlings from NaCl primed seeds compared to the unprimed seeds (Figure 3a). Similarly, salinity affected

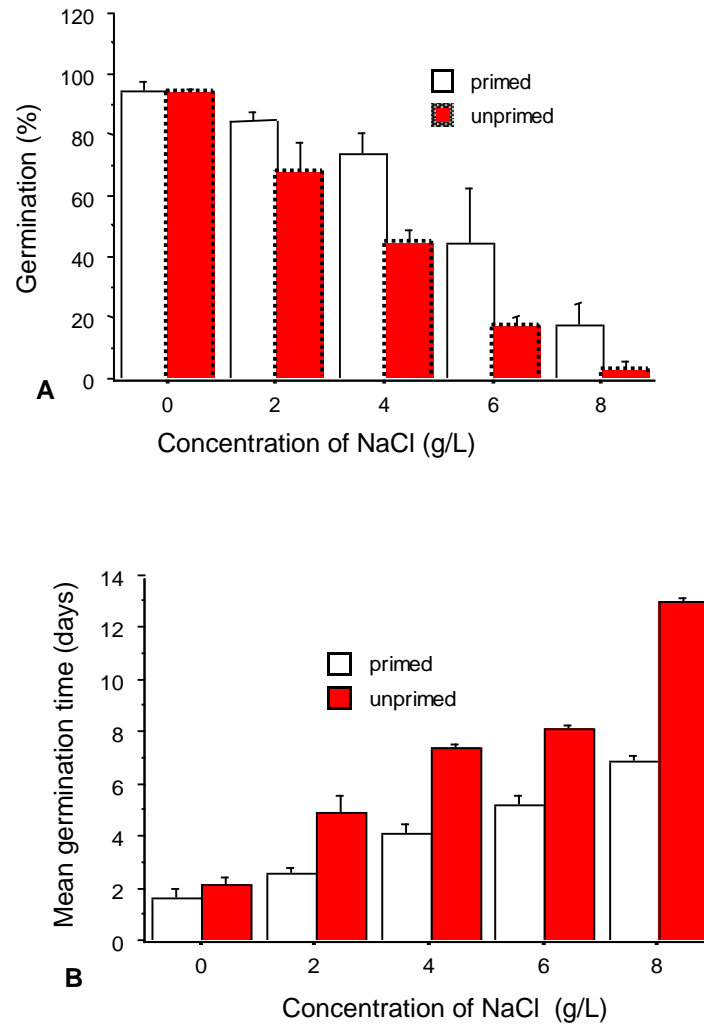


Figure 1. Effect of different salinity levels on germination percentage (a) and mean germination time (b) of primed (NaCl) and unprimed seeds of maize under laboratory conditions in 14 days. (Error bars ± 1 standard deviation).

Table 2. Means comparison of the traits under different salinity levels after 14 days of germination time.

Salinity (g/L)	TG (%)	MGT (Day)	SL (cm)	RL (cm)	R/S	VI	SFW (g)	SDW (g)	RFW (g)	RDW (g)
0	94.583 ^a	1.900 ^a	18.700 ^a	16.350 ^a	0.896 ^a	17.684 ^a	4.675 ^a	1.185 ^e	2.604 ^a	0.451 ^a
2	76.667 ^b	3.811 ^b	7.362 ^b	4.285 ^b	0.536 ^b	5.813 ^b	2.325 ^b	0.413 ^a	0.812 ^b	0.108 ^b
4	59.583 ^c	5.780 ^c	4.262 ^c	1.965 ^c	0.255 ^c	2.750 ^c	1.325 ^c	0.288 ^b	0.249 ^c	0.046 ^c
6	31.250 ^d	6.705 ^d	1.586 ^d	0.667 ^d	0.153 ^d	0.619 ^d	0.420 ^d	0.044 ^c	0.114 ^c	0.024 ^c
8	10.833 ^e	10.01 ^e	0.345 ^e	0.054 ^e	0.053 ^e	0.184 ^e	0.066 ^d	0.014 ^c	0.015 ^c	0.003 ^c

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

seedlings' radicle length of both primed and unprimed seeds of maize with increasing salinity level. But this influence was highly prominent in shoot (Figure 3a) and radicle (Figure 3b) lengths from unprimed seeds when compared to the primed ones. When the concentration of

salinity increase, the growth of root and shoot becomes very slow and mostly the roots died after some days.

Salinity has both osmotic and specific ionic effects on seedlings growth. Likewise, toxic ion accumulation (Na^+ and Cl^-) negatively affect plant metabolism. It has also

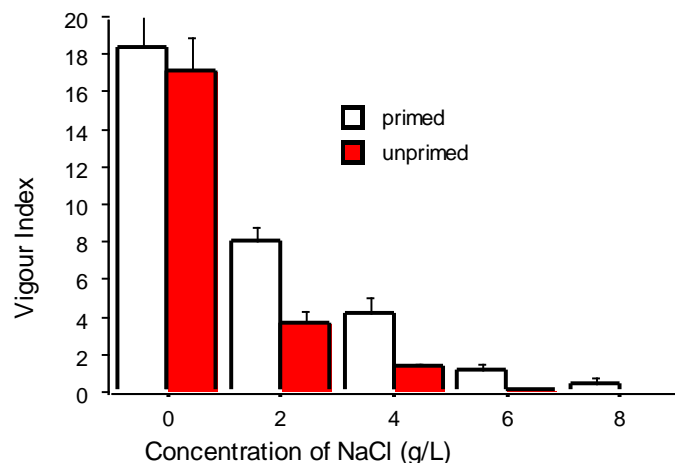


Figure 2. Effect of different salinity levels on Vigor Index of primed and unprimed Maize seeds under laboratory conditions. Error bars ± 1 standard deviation.

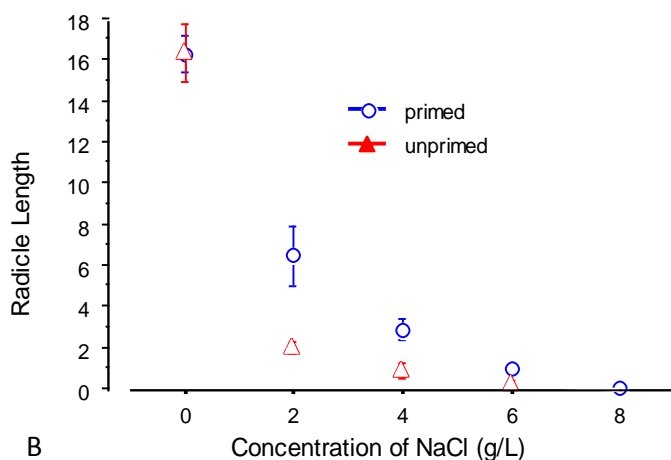
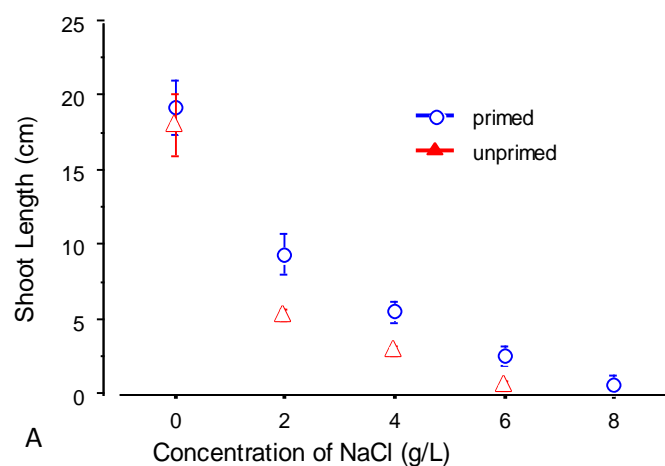


Figure 3. Effect of different salinity levels on shoot length (A) and radicle length (B) of primed and unprimed maize seeds under laboratory conditions. Error bars ± 1 standard deviation.

been reported that salinity suppresses the uptake of essential nutrients like P and K which could adversely affect seedlings growth (Nasim et al., 2008). In this study, seed priming significantly improved maize seedling growth at different salinity levels. Significant improvement in root and shoot length in the primed seeds may be attributed to earlier germination induced by priming (Farooq et al., 2005). During priming, the embryo expands and compacts the endosperm. The compaction force of the embryo and hydrolytic activities on the endosperm cell walls may change the tissues to have their flexibility upon dehydration, producing free space and facilitating root and seedling fast projection after rehydration (Mohammadi, 2009). This resulted in vigorous seedlings with more roots and shoot length than the seedlings from unprimed seeds. The present results confirm the findings of Nawaz et al. (2012) in tomato (*Lycopersicon esculentum* Mill.) and in Canola (*Brassica napus* L.) where priming of the seeds significantly improved shoot and radicle length.

Root length to shoot length ratio (R/S)

Analysis of variance indicated that salinity level, priming and their interaction have significant effects on root to shoot ratio (Table 1). It was decreased significantly with increasing salinity level from 0 g/L (0.896) to 8 g/L (0.053) (Table 2), but the effect was more pronounced in unprimed seeds (Figure 4). This study showed that salt stress inhibits radicle growth more than primary shoot growth. Decreased R/S with increasing salinity level could be due to the first exposition of the root to salinity, rapidly affected before the shoots (Akram et al., 2010). In saline conditions, NaCl priming increased root to shoot ratio of maize seedlings. This may in turn have the advantage of increased ratio of water up take by seedlings. High root to shoot ratio in primed seeds under salt stress may be considered important for salt tolerance of growth attributes (Alian et al., 2000). Thus, high root to shoot ratio induced by maize seeds primed with NaCl appears to be an adaptation (solution) to salinity.

Shoot and root fresh and dry weights

Increasing salinity significantly ($P < 0.05$) decreased maize seedlings fresh and dry weight for both primed and unprimed seeds. In actual fact, the increase in salt concentration in culture medium reduced shoot (Figure 5a) and root (Figure 5c) fresh weight from 4.675 g at 0 g/L to 0.066 g at 8 g/L and from 2.604 g at 0 g/L to 0.015 g at 8 g/L, respectively (Table 2). However, primed seeds showed better performance than unprimed seeds. The difference in performance was significant at 95% confidence level. In the same way seed priming with NaCl significantly ($P < 0.05$) enhanced maize shoot dry weight (Figure 5b) and root dry weight (Figure 5d) as

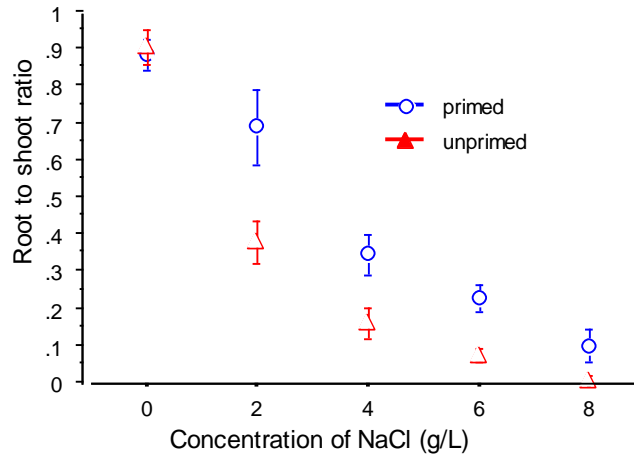


Figure 4. Effect of different salinity levels on root to shoot ratio of primed and unprimed maize seeds under laboratory conditions. Error bars ± 1 standard deviation.

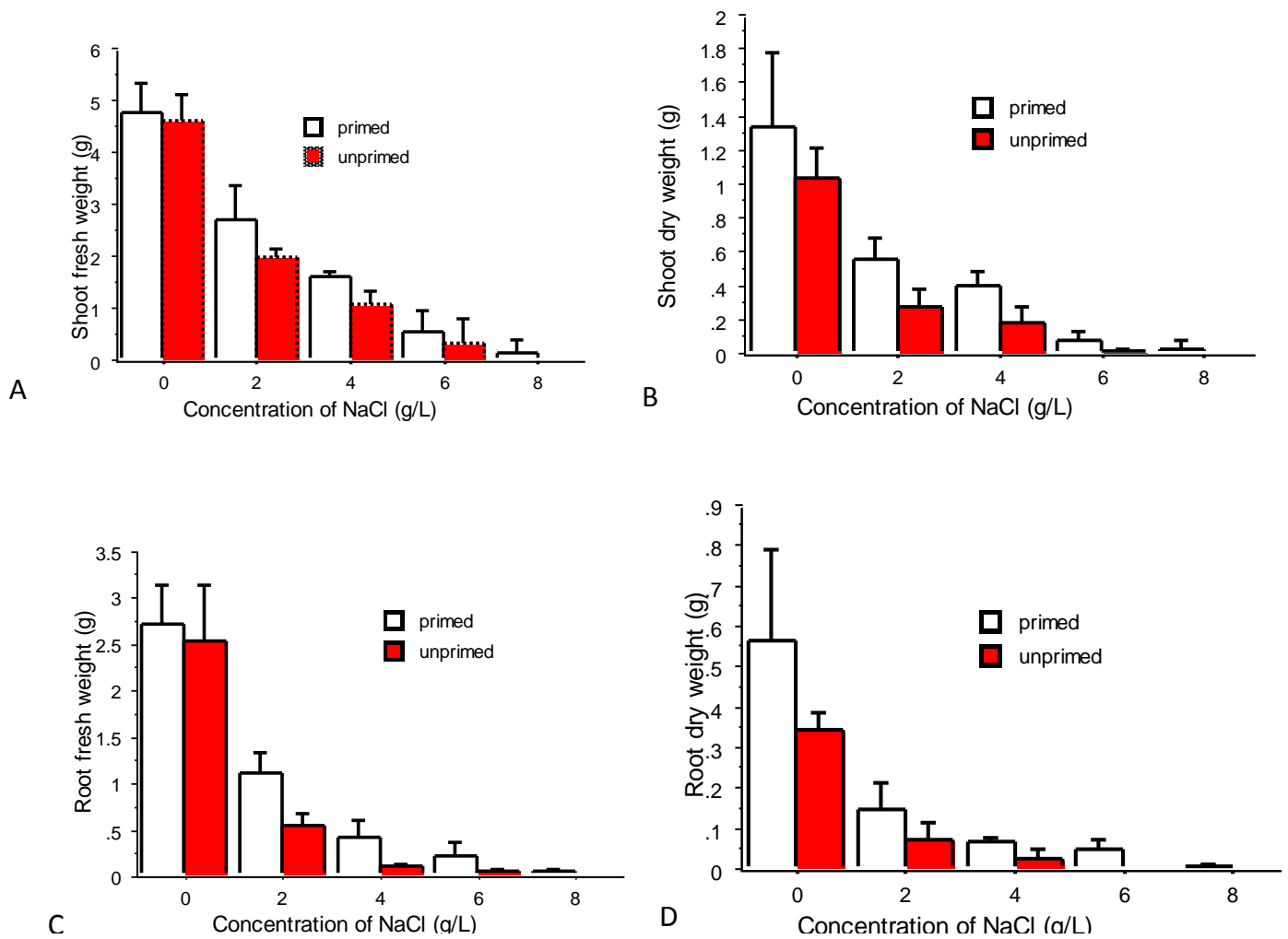


Figure 5. Effect of different salinity levels on shoot fresh weight (A), shoot dry weight (B), root fresh weight (C) and root dry weight (D) of primed (NaCl) and unprimed maize seeds under laboratory conditions. Error bars ± 1 standard deviation.

compared to the unprimed seeds.

The results in this study are similar to that found by Achakzai et al. (2010) where they found that salinity can rapidly inhibit seedling and root growth and this might be due to the ability of inhibiting uptake of water and essential mineral nutrients from culture media. Also increment of the toxic effect of sodium at high salt level caused physiological effects that resulted in strong reduction in photosynthesis, enzymatic process and protein synthesis, which resulted in limited growth and poor leaf area development. Increment of shoot and root fresh and dry weights were observed in primed seeds in contrast to the unprimed maize seeds. This might be as a result of improvement of plant water status and minimizing the toxic effects of Na⁺ resulted in maximum fresh and dry weights (Tahir et al., 2006).

Conclusion

Generally, salinity inhibits germination and early seedling growth of maize seeds. Roots were affected more than shoots as evidenced from the root to shoot ratio. Priming is an effective method to meet the demands of farmers during the installation of the culture in conditions of salt stress. Seed priming with NaCl improved germination and early growth of maize seeds. Primed seeds should be sown in saline soils to increase germination and early growth of maize seeds. Further study is required to investigate the effects of seed priming on later growth and yield stages of this plant. Additional molecular research is needed to explore priming induced alteration in physiological and biochemical attributes both at seed and whole plant levels in maize.

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