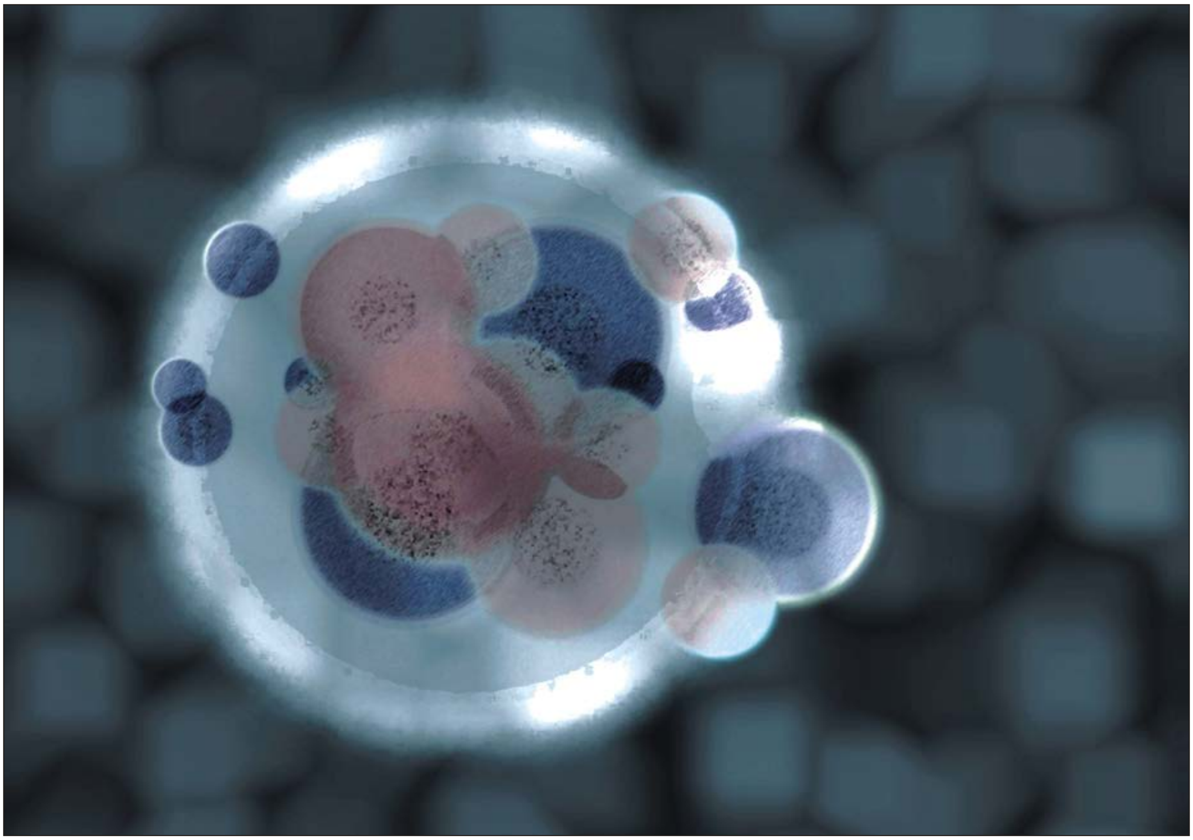

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Effects of Salt Stress on Germination Percentage and Seedling Growth in Sweet Sorghum Cultivars

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Abstract: In this study, the response of sweet sorghum genotypes to salinity during germination and seedling growth stages were evaluated as possible indicators of the salinity tolerance of sweet sorghum cultivars. The degree of salinity tolerance among four sweet sorghum cultivars (Soave, Rio, Sofra and Keller) was evaluated, at seed germination and seedling growth stages, using four different salt concentrations (0, 100, 200 and 300 mM NaCl). The results showed that in all cultivars as the salt concentration increased, seed germination decreased significantly. For all salt concentrations, Soave had the highest germination percentage. Sofra did not germinate at 200 and 300 mM NaCl. Therefore, at germination stage, Soave has the highest tolerance to salt while Sofra has the lowest resistance to salinity. In addition, results were similar at both the seedling and germination stages. Salt concentration decreased fresh seedling weight. At the seedling stage, Soave and Sofra had the lowest and the highest percentage reduction of fresh seedling weight, respectively. Tests indicate that for the best results, at seedling germination and growth stages, Soave is the cultivar to be recommended for soils with high salinity.

Key words: Sweet sorghum, salinity, seed germination, seedling growth

INTRODUCTION

Worldwide, the effects of salinity on plant development are increasingly problematic. Saline soils are estimated to cover about 5-10% of the world's arable land (Szabolcs, 1994) and the area affected by salinity is increasing steadily, in part due largely to mismanaged irrigation (Ghassemi *et al*., 1995). Salt-affected soils cover about 25.5 mha in Iran (Siadat *et al*., 1997). The effect of salinity on plant growth is a complex syndrome that involves osmotic stress, ion toxicity and mineral deficiencies (Hasegawa *et al*., 2000; Roy *et al*., 2005). Sorghum [*Sorghum bicolor* (L.) Moench], is characterized as moderately tolerant to salinity (Igartua *et al*., 1994). The presence of large genotypic variation for tolerance to salinity is reported in sorghum (Maiti *et al*., 1994). This report offers a good scope for integrating tolerance characteristics into appropriate breeding programs to improve crop productivity in saline soils. Soil salinity can lead to reduced emergence and poor crop establishment, thus diminishing yield potential. Reduced germination in saline soils can be a consequence of either the direct toxic effects of salts, or the general delay of the germination process caused by osmotic stress, resulting in longer exposure of seedlings to biotic and abiotic hazards (Igartua *et al*., 1994). Therefore plant breeding programs addressing soil salinity stress should aim at finding materials showing tolerance throughout the whole growing period of the crop, which must include finding vigorous emergence ability. Sorghum seems to offer a good potential for selection, as intraspecific variation for germination under saline conditions or in the presence of other osmotic agents has already been reported (Igartua *et al*., 1994). The advancement of salinity tolerance during the early stages of sorghum growth been successfully accomplished through selection. (Igartua *et al*., 1994). Azhar and McNeilly (1988) found that, for salinity tolerance of young sorghum seedlings, both additive and dominant effects were involved, the latter being of greater importance. Attempts have been made to evaluate salt tolerance at the germination and emergence stages in sorghum (Igartua *et al*., 1994) and large genotypic differences were reported, but this early evaluation appears to have little relation to overall performance under saline conditions (Munns, 2002). Therefore, there is a need to identify traits associated with salinity tolerance and simple, highly efficient, repeatable screening methods to evaluate large number of genotypes. In fact, the variation in whole-plant biomass responses to salinity was considered to provide the best means of initial selection of salinity tolerant genotypes (Krishnamurthy *et al*., 2007). In the present study, the responses of sweet sorghum genotypes to salinity during germination and seedling growth stages were evaluated as possible indicators of the degree of salinity tolerance of sweet sorghum cultivars.

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MATERIALS AND METHODS

Experiment location and plant material: This experiment was conducted in the greenhouse at the Isfahan University in 2006. Seeds of four sweet sorghum cultivars (Rio, Keller, Sofra and Soave) were provided by the University of Isfahan Research Station.

Seed germination: Washed grains of sorghum were surface sterilized with 20% hypochlorite sodium solution for 20 min followed by 70% ethanol for 5 sec. Seeds were thoroughly rinsed with distilled water and imbibed for 24 h. Seeds were germinated at 80% humidity via the Top of Paper method in a Petri dish at 26°C in the light for a 14 h photoperiod and at 21°C in the dark for a 10 h photoperiod in a growth chamber (Model E15, Conviron-Canada). After imbibitions 100 seeds were placed in a Petri dish containing sterile filter sheets, moistened with 20 mL of distilled water (control 0 mM NaCl) or 20 mL salt solutions of 100, 200 and 300 mM NaCl. The experimental design was factorial having two factors (different cultivars and salinity levels) with 4 replications. The number of seeds-germinated was counted 7 days after seed treatment and the germination percentage was determined.

Seedling growth: Seeds of the above cultivars were imbibed for 24 h in distilled-water. They were then transferred to 500 mL square plastic containers containing vermiculite and Hoagland's solution. The seedlings were grown under hydroponic conditions in a greenhouse: humidity was 65% during the light period; the photoperiod was 16 h light and 8 h darkness; temperature was 26°C in the light and 21°C in the dark at the base of the leaves (the growth zone). Each container held two seedlings. Hoagland's solution was added to the container every 4 days. Fourteen days after germination, sodium chloride was added to the Hoagland's solution to make saline treatments of 100, 200 and 300 mM. One week after the seedlings were grown under stress conditions, reduction percentages in the fresh weight of the seedlings were determined. The experimental design was factorial having two factors (different cultivars and salinity levels) with 6 replications. Statistical analyses were performed using the SAS computer program. The means were compared according to the Duncan multiple range test.

RESULTS AND DISCUSSION

Effect of salinity on seed germination: The choice of single selection criteria for salt tolerance at the germination stage is complicated by two factors. First, the mechanisms for salt damage during germination are not fully understood. Bewley and Black (1994) explained how soil salinity might affect the germination of seeds in two ways: (a) by creating an osmotic potential to prevent water uptake and/or (b) by providing conditions for the entry of ions that may be toxic to the embryo or developing seedling. Second, but no less important, the germination process comprises two distinct phases: the first is imbibition, mainly dependent on the physical characteristics of seeds (Igartua *et al*., 1994) and the second is a heterotrophic growth phase between imbibition and emergence. The nature of salt damage could be different in both phases, as suggested by Bliss *et al*. (1986). The results show the effect of different salt concentrations on sorghum cultivars germination percentage were significant at the 1% level (Table 1). Interactions between salt concentrations and sorghum cultivars on germination percentage were significant at the 1% level. Means comparisons of sorghum cultivar germination percentages are presented in Table 2. At different salt concentrations, cv. Soave and Sofra had the highest and lowest germination percentage (69.50 and 23.07%), respectively. Rio had the second highest germination percentage (36.05%). The germination percentage of Sofra and Keller was not significantly different. It seems that Soave demonstrates a better tolerance to salt stress with respect to other cultivars at the germination stage. At the germination stage, the difference in tolerance to salinity may be due to genetic diversities and heredity differences between cultivars of a species (Azhar and McNeilly, 1988). Maas *et al*. (1986) reported that sorghum cultivars were significantly more tolerant at germination than at later stages of growth. Many reports show that salt-stress decreases seed germination (Igartua *et al*., 1994; Hossain and Al-aswad, 1997; Gill *et al*., 2002; Roy *et al*., 2005). For example, Amzallag (2000) reported that in comparison with the

Table 1: Analysis of variance for germination percentage of four sweet sorghum cultivars

Sources	df	Mean squares
Replications	4	356.597
Treatments	8	331.875**
Cultivars	3	9526.597**
Salt concentrations	3	14029.657**
Cultivars×salt concentrations	9	3669.856**
Error	32	3797.171

**: Significant at 1% level

Table 2: Mean comparisons* among sweet sorghum cultivars for germination percentage

Cultivars	Germination (%)
Soave	69.50a
Rio	36.05b
Keller	23.50c
Sofra	23.07c

*: Values (a-c) by the same letter(s) are not significantly different at $p<0.05$

control, germination decreased markedly under NaCl. Exposure of 8-day-old seedlings of *Sorghum bicolor* (L. Moench) to 150 mM NaCl for 3 weeks induces an ability to grow at 300 mM NaCl, a lethal concentration for untreated plants. Also, Gill *et al.* (2002) reported that under stressful conditions germination decreased markedly, whereas the control at the same time reached its maximum germination of 99%. Present results are in agreement with the Gill *et al.* (2002). There, the control (0 mM NaCl) had the highest germination percentage (71.02%) and as the salt concentration increased, germination percentage decreased, until the germination percentage at 300 mM NaCl was only 9.93% (Table 3). A higher germination percentage of cultivars at (0 mM NaCl) were due to absence of salt in the medium. Increased salt concentration in the nutrition solution decreased germination percentage due to the slowing down or stopping of germination processes as a result of contact with high concentrations of Na^+ and Cl^- ions (Igartua *et al.*, 1994). High concentration of NaCl in the nutrition solution increases its osmotic potential. In addition, high absorption of Na and Cl ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination and can also decrease germination percentage (Taiz and Zeiger, 2002). Mean interaction between salt concentrations and sorghum cultivars on germination percentage is presented in Table 4. Cv Soave had the highest germination percentage at control (94%), 100 mM NaCl (89%), 200 mM NaCl (66%) and 300 mM NaCl (29%). Mean Germination percentage of Soave in control (0 mM NaCl) and 100 mM NaCl was not significantly different. Sofra did not germinate at 200 and 300 mM NaCl (Table 4). Soave had more tolerance to salinity with respect to other cultivars at the germination stage, because its seeds had the ability to germinate at high salinity (300 mM NaCl). Sofra and Keller cultivars were recognized to be more sensitive to salinity with respect to other cultivars during germination, because their seed did not germinate at high salinity (300 mm NaCl) Following Soave, Rio had the highest tolerance to salt concentrations. It germinated at 200 and 300 mm NaCl. On the other hand Sofra and Keller cultivars did not germinate at the above-mentioned salt concentrations. Sofra had the highest germination percentage under all conditions, followed by Rio.

Effect of salinity on seedlings growth: Amzallag (2000) reported that germination, growth, respiration and other related processes can be affected in seeds that are subjected to environmental stress. Sorghum, a moderately salt-tolerant species, shows decreased growth under saline conditions (Bernstein *et al.*, 1995). Igartua *et al.* (1994) reported that during imbibition, the effect of salt was merely osmotic until a hydration threshold was surpassed. After this, salt had combined toxic and osmotic effects that could be lethal at high concentrations. These effects may result in differences between the germination and emergence patterns of a species under saline conditions. Therefore, experiments covering both phases should be carried out to achieve a good understanding of the effect of salt on emergence (Igartua *et al.*, 1994). The results show the effect of cultivars and salt concentrations on the reduction percentage of fresh seedling weight were significant at 5% (Table 5). Table 6 showed that Soave and Rio had the lowest reduction percentage of fresh seedling weight (24.88 and 27.36%, respectively) while Sofra had the

Table 3: Mean comparisons* among salt concentrations on germination percentage of sweet sorghum cultivars

Salt concentrations (mM)	Germination (%)
Control	71.02a
100	45.94b
200	25.23c
300	9.93d

*: Values (a-d) by the same letter(s) are not significantly different at $p<0.05$

Table 4: Comparison of seed germination percentage among salt concentrations on sweet sorghum cultivars

Treatments	Seed germination (%)
V_1C_1	64.05bc
V_1C_2	28.25d
V_1C_3	0.01e
V_1C_4	0.01e
V_2C_1	71.03b
V_2C_2	38.53d
V_2C_3	24.93d
V_2C_4	9.72e
V_3C_1	55.00c
V_3C_2	28.00d
V_3C_3	10.00e
V_3C_4	1.00e
V_4C_1	94.00a
V_4C_2	89.00a
V_4C_3	66.00bc
V_4C_4	29.00d

Values by the same letter(s) are not significantly different at $p<0.05$

Table 5: Analysis of variance for the reduction percentage of seedlings fresh weight

Source of variations	df	Mean squares
Replications	5	952.32
Treatments	8	2328.87*
Cultivars	3	6245.42*
Salt concentrations	3	33666.65*
Cultivars×salt concentrations	9	1457.60
Error	40	3797.50

*: Significant at 5%

Table 6: Mean comparisons* among sweet sorghum cultivars on the reduction percentage of seedlings fresh weight

Cultivars	Reduction % of seedlings fresh wt.
Sofra	59.20a
Keller	44.22b
Rio	27.26c
Soave	24.88cd

*: Values (a-d) by the same letter(s) are not significantly different at $p<0.05$

Table 7: Mean comparisons* among salt concentration on the reduction percentage of seedlings fresh weight

Salt concentrations (mM)	Reduction % of seedlings fresh wt.
300	77.70a
200	63.50b
100	14.00c
Control	0.01d

*: Values (a-d) by the same letter(s) are not significantly different at $p<0.05$

highest (59.20%). Therefore, it seems that Soave and Rio have demonstrated a better tolerance to salt stress with respect to other cultivars at the seedling stage. Sofra is more sensitive to salinity at that stage. The genetic basis of salt (NaCl) tolerance at the seedling stage was examined in *Sorghum bicolor* (L. Moench) which differed in relative NaCl tolerance (Krishnamurthy *et al*., 2007). So, it seems that in sweet sorghum cultivars, difference in tolerance to salinity at the seedling stage may be due to genetic diversities and heredity differences between cultivars (Azhar and McNeilly, 1988). The results show that in comparison to the control the reduction of fresh seedling weight at 100, 200 and 300 m NaCl was 14.00, 63.50 and 77.70%, respectively (Table 7). There is a direct relation between salt concentration and reduction of fresh seedling weight. As salinity levels increased, the amount of seedling fresh weight decreased (Graifenberg and Paola, 1996; Gill *et al*., 2002). Some plants are sensitive to salinity at the seedling stage, because the mechanism of tolerance to salinity is not yet fully developed. However some other plants may also show tolerance to salinity at this stage (Roy *et al*., 2005). It seems that Soave has some mechanism of tolerance to salinity at the seedling stage and its reduction was 24.88%, whereas Sofra does and its reduction was twice that of Soave at 59.20%. Based on the results, since Soave has the mechanism to tolerate salinity at both seed germination and seedling growth stages it is highly recommended for planting in saline soils.

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