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Comparative study of salt tolerance between two soybean varieties *(Glycine max.* L*)* at germination stage

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Abstract

In December 2019, a research work on seed germination was carried out in Plant Physiology and Biochemistry Laboratory, Jahangirnagar University, to determine the salt tolerance level and compare the salt stress response between two soybean varieties at early seedling stage. Seeds of soybean varieties *viz* Shohag and BARI Soybean-5 were grown in Petri dishes and treated with 0, 50, 100, 150, 200, 250, and 300 mM NaCl for 14 days. Salinity had adverse effects on germination percentage, germination index, germination speed and growth parameters *viz* shoot length, root length, fresh weight of shoots, fresh weight of roots and dry matter production in shoot and root for early seedling growth. The results illustrate that germination percentage and germination index of Shohag were more than 80% at up to 200 mM NaCl concentration, while both of these variables for BARI Soybean-5 were more than 80% at up to 100 mM NaCl treatments. Although the germination speed of BARI Soybean-5 was faster than Shohag at moderate salinities, it was much slower for BARI Soybean-5 than Shohag at high salinities. Results showed that the growth of both varieties slowed significantly with increased salinity. However, Shohag outperforms BARI Soybean-5 in terms of growth at high salt concentration. As a result, it can be stated that BARI Soybean-5 can tolerate moderate salt. It can be hypothesized that Shohag could be used for field experiments due to its superior germination performance.

Key words: Salinity, Germination percentage, Germination index, Germination speed.

INTRODUCTION

Nowadays, increasing soil salinity is a major problem worldwide. In Bangladesh, salinity affects around 1.5 million hectares of coastal and offshore land out of a total of 2.85 million hectares (Polash *et al*., 2018). Critical geographical location, sea level rise, natural disasters, backwater impacts, rainfall, shrimp production and higher salinity in groundwater are possible causes of salinity in Bangladesh (Mahmuduzzaman *et al*., 2014). Germination is a crucial part of plant history. The ability of seeds to germinate in soils with high salt concentrations is therefore crucial for the survival and reproduction of plant species (Akhtar *et al*., 2021). It is a comprehensive physiological mechanism that oxidizes the seed's lipids and carbohydrates while breaking down stored proteins to obtain energy and amino acids for plant development (Berhanu and Berhane, 2014). Salinity affects plant growth by distributing the water relation, causing water imbalance in plant nutrition, and affecting plant physiological and biochemical processes (Afrin *et al*.,

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2021b; Akhtar *et al*., 2013a). Soil salinity affects germination by altering the osmotic component, leading to a depletion of soil water available to plants and causing ionic toxicity (Afrin *et al*., 2021a; Khanam *et al*., 2018). As a result, dehydration of the plant's cellular cytoplasm occurs due to water deficiency (Akhtar *et al.* 2013b; Berhanu and Berhane, 2014). Although salt stress usually reduces germination rates and delays the onset of germination, its effects are modified by interactions with other environmental factors such as temperature and light. Soybeans are grown worldwide primarily as an oil-producing crop that often suffers from salinity problems. Therefore, it is necessary to find out the soybean variety that can withstand severe salinity during germination and early seedling stage. This information will be helpful in identifying salt-tolerant varieties and/or genotypes and in developing saline soil management strategies. The aim of our study was to investigate the effects of different salinity levels on germination and to compare salt tolerance between two soybean varieties.

MATERIALS AND METHODS

Plant materials and experimental design: Seeds of soybean varieties (Shohag and BARI Soybean-5) were collected from Bangladesh Agricultural Research Institute (BARI) and used as plant materials.

The germination experiment was conducted in Plant Physiology and Biochemistry Laboratory, Jahangirnagar University to determine the salt tolerance between soybean varieties at seedling stage. The experiment was conducted in a randomized block design with three replications. The seeds of both varieties were surface sterilized with 0.1% sodium hypochlorite for three minutes and then rinsed thoroughly with distilled water. Ten seeds based on seed uniformity were placed with blotting paper in a 9 cm diameter Petri dish, moistened with 10 ml of saline each, and distilled water, which was considered as control. For the experiment, there were seven treatments with three replicates with different NaCl doses: 0, 50, 100, 150, 200, 250 and 300 mM. These treatment solutions were applied separately and regularly to each Petri dish. The Petri dishes were kept under laboratory conditions with the temperature varying between 23 and 25°C. The number of germinated seeds was recorded at 24-h intervals for 14 days. The emergence of redicle (root) and plumule (shoot) of both varieties from each petri dish was assessed regularly after sowing. The germination percentage (GP), germination index (GI) and germination speed (SG) were calculated according to the following formula:

Germination Percentage (GP) = $\frac{\text{No.of germinated seeds}}{\text{Total no.of seed sown}} \times 100$ (Kandil *et al*., 2012)

Germination Index (GI) = $\frac{\text{Germination \% in each treatment}}{\text{Germination \% in the control}} \times 100$ (Karim *et al*., 1992)

Speed of Germination (SG) = $\frac{\text{No.of seedlings}}{\text{1st count on day 1}} + \cdots + \frac{\text{No.of seedlings}}{\text{Final count on day 14}}$ (Mohammad *et al*., 1989)

Shoot and root lengths were recorded on day 14 after seed placement for germination. When harvested on day 14, seedlings were kept in the oven and dried at 72°C until they reached a constant weight. Data on germination behavior and seedling characteristics for each NaCl treatment were compared with those of the control treatment.

Data analysis: Statistical analysis of the collected data was performed using Analysis of Variance (ANOVA). All statistical analyzes were performed using the SPSS statistical package (SPSS program 16.00). Data were presented as means for each treatment and means were compared using Duncan's test at 5% probability.

RESULTS AND DISCUSSION

Germination percentage (GP) and Germination index (GI): Increasing salt concentrates is found to delay seed germinates. In the control treatment, germination began on the day-2 after the seeds were laid out for germination. At 300 mM NaCl, BARI Soybean-5 required the longest time (7 days) whereas Shohag needed the shortest time (4 days) to germinate. The mean values of germination percentage and germination index of two soybean varieties affected by different salinities are shown in Table 1. The germination percentage of two varieties was significantly different. Shohag showed the highest germination rate in all treatments. In our experiment, Shohag showed statistically similar germination percentage at NaCl concentrations from 0 mM to 250 mM, while BARI Soybean-5 showed statistically similar germination percentage at NaCl concentrations from 0 mM to 150 mM. In treatments with 0, 50, 100, 150, and 200 mM NaCl, the germination percentage of Shohag was more than 80%. For treatments with 0, 50, and 100 mM NaCl, it was more than 80% for BARI Soybean-5. Compared to control, the highest salinity level significantly reduced the germination percentage by 43.33% for Shohag and 26.66% for BARI Soybean-5. Germination index of varieties Shohag decreased from 100.00 to 43.33 as NaCl concentrations increased from 0 to 300 mM, while GI decreased from 100.00 to 29.62. Therefore, the highest GI was found in Shohag and the lowest was found in BARI Soybean-5. Similar result was also obtained by many scientists in different crops *viz* soybean (Kondetti *et al*., 2012, Farhoudi & Tafti, 2011) and pea (Berhanu & Berhane, 2014).

Speed of Germination: Salinity significantly reduced germination rate (SG) in all treatments. The results showed that the germination speed of Shohag decreased from 6.86 in the control to only 3.69 at 300 mM NaCl (Table 1), while the germination speed of BARI Soybean-5 decreased from 6.51 in the control to only 3.31 at 300 mM NaCl (Table 1). At 150 mM NaCl concentration, the germination speed of BARI Soybean-5 was greater compared to Shohag, but for BARI Soybean-5 it was reduced at higher salinity.

Table 1. Effects of different concentrations of NaCl on germination percentage, germination index and germination speed of two soybean varieties

Treatments	Germination percentage (%)		Germination index (%)		Germination speed	
	Shohag	BARI Soybean-5	Shohag	BARI Soybean-5	Shohag	BARI Soybean-5
0 mM NaCl	100.00 ± 0.00 a	90.00 ± 10.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	6.86 ± 0.40 a	6.51 ± 0.28 a
50 mM NaCl	93.33 ± 11.54 a	86.66 ± 5.77 a	93.33 ± 11.54 a	96.29 ± 6.41 a	6.57 ± 0.16 a (95.77)	6.39 ± 0.35 a (98.15)
100 mM NaCl	93.33 ± 5.77 a	83.33 ± 15.27 a	93.33 ± 5.77 a	92.59 ± 16.97 a	6.11 ± 0.39 ab (89.06)	6.07 ± 1.10 a (93.24)
150 mM NaCl	90.00 ± 10.00 a	70.00 ± 20.00 ab	90.00 ± 10.00 a	77.78 ± 22.22 a	5.45 ± 0.62 bc (79.44)	5.73 ± 0.31 a (88.01)
200 mM NaCl	83.33 ± 5.77 a	50.00 ± 10.00 bc	83.33 ± 5.77 a	55.56 ± 11.11 b	5.09 ± 0.29 c (74.19)	4.38 ± 0.51 b (67.28)
250 mM NaCl	76.66 ± 11.50 a	46.66 ± 5.77 cd	76.66 ± 11.50 a	51.84 ± 6.42 b	4.65 ± 0.49 c (67.78)	4.38 ± 0.38 b (67.28)
300 mM NaCl	43.33 ± 25.20 b	26.66 ± 5.77 d	43.33 ± 25.20 b	29.62 ± 6.41 c	3.69 ± 0.80 d (53.79)	3.31 ± 0.70 b (50.84)
Cultivar Mean	82.50	64.76	82.50	58.72	5.49	5.25
CV%	25.06%	38.32%	25.06%	38.02%	20.78%	24.09%
LSD	10.80	10.11	10.80	10.61	0.43	0.51

*Average value of nine plants in each treatment.

*Means in a column followed by the same letter do not differ significantly at 5% level by ANOVA test, followed by Duncan's multiple-range test and ± means standard deviation.

* Values within parenthesis indicate percentage relative to the control.

Shoot and Root length: Shoot and root length of soybean were recorded 14 days after seed germination. The shoot and root length data of both varieties show a decreasing trend at all salinities (Table 2). The shoot length of Shohag and BARI Soybean-5 in the control was 16.06 cm and 14.82 cm, respectively, which was reduced to 1.03 cm for Shohag and 0.86 cm for BARI Soybean-5 at 300 mM NaCl. At the highest salinity, the relative seedling height value was 6.41 and 5.80 for Shohag and BARI Soybean-5, respectively. The root length of Shohag and BARI Soybean-5 in the control was 12.20 cm and 8.48 cm, respectively, which was reduced to 0.76 cm for Shohag and 0.43 cm for BARI Soybean-5 at 300 mM NaCl. At the highest salinity, the relative value of root length was 6.23 and 5.07 for Shohag and BARI soybean-5, respectively. BARI Soybean-5 showed improved shoot and root growth at 100-150mM NaCl concentrations compared to Shohag, which was drastically reduced in BARI Soybean-5 at 200-350 mM NaCl. However, Shohag generally showed higher salt tolerance than BARI Soybean-5. The decreasing pattern of seedling growth was consistent with the results of earlier researchers (Kumar, 2017; Khan *et al.*, 2016; Kondetti *et al*., 2012).

Table 2. Effects of different concentrations of NaCl on shoot and root length of two soybean varieties

Treatments	Shoot length (cm/plant)		Root length (cm/plant)	
	Shohag	BARI Soybean-5	Shohag	BARI Soybean-5
0 mM NaCl	16.06 ± 3.89 a	14.82 ± 4.40 a	12.20 ± 3.44 a	8.48 ± 4.03 a
50 mM NaCl	10.56 ± 2.77 b (65.75)	9.66 ± 3.59 b (65.18)	9.80 ± 4.06 b (80.33)	7.76 ± 3.30 a (91.50)
100 mM NaCl	4.66 ± 1.21 c (29.01)	3.66 ± 1.28 c (24.69)	5.29 ± 1.89 c (43.34)	6.94 ± 3.83 ab (81.83)
150 mM NaCl	3.03 ± 0.49 cd (18.86)	3.77 ± 1.12 c (25.43)	4.12 ± 3.39 c (33.77)	5.05 ± 2.62 b (59.55)
200 mM NaCl	2.62 ± 0.81 de (16.31)	2.25 ± 0.79 cd (15.18)	2.77 ± 1.00 cd (22.70)	0.86 ± 0.51 c (10.10)
250 mM NaCl	1.27 ± 0.36 de (7.90)	1.66 ± 0.26 cd (11.20)	0.86 ± 0.89 de (7.02)	0.65 ± 0.98 c (7.67)
300 mM NaCl	1.03 ± 0.40 e (6.41)	0.86 ± 0.38 d (5.80)	0.76 ± 0.47 e (6.23)	0.43 ± 0.39 c (5.07)
Cultivar Mean	5.61	5.25	5.11	4.31
CV%	99.06%	99.79%	93.03%	97.48%
LSD	1.56	15.32	2.07	10.75

*Average value of 9 plants in each treatment.

*****Means in a column followed by the same letter do not differ significantly at 5% level by ANOVA test, followed by Duncan's multiple-range test and ± means standard deviation.

***** Values within parenthesis indicate percentage relative to the control.

Fresh weight of shoot and root: The salt content leads to a reduction in the fresh weight of shoots and roots in both varieties. The fresh weight of shoots and roots decreased with increasing salinity (Table 3). The fresh weight of the shoots (average value) of Shohag and BARI Soybean-5 in the control was 0.32 g and 0.29 g, respectively, whereas 0.03 g at 300 mM NaCl for Shohag and 0.02 g for BARI Soybean-5. At the highest salinity, the relative value for shoot fresh weight was 9.37 for Shohag and 6.89 for BARI Soybean-5. The root fresh weight (mean value) of Shohag and BARI Soybean-5 in the control was 0.051 g and 0.027 g, respectively, which was reduced to 0.001 g for Shohag and 0.0009 g for BARI Soybean-5 at 300 mM NaCl. At the highest salinities, the relative value for root fresh weight was 1.96 for Shohag and 3.33 for BARI Soybean-5. Both varieties showed a reduction in root fresh weight. Shohag produced roots with a relatively high fresh weight, BARI Soybean-5 had the lowest roots. Compared to control, the relative fresh weight was higher in Shohag than BARI Soybean-5 at maximum salinity. The reduction in shoot and root fresh weight of the seedling was reported by many scientists in different soybean varieties (Kumar, 2017; Kondetti *et al*., 2012; Farhoudi & Tafti, 2011). Soil salinization could reduce the fresh weight of shoots and roots due to a lack of metabolites in young growing tissues (Das and Islam, 2018). According to Farhoudi & Tafti (2011), weight loss is one of the major consequences of excessive transpiration in salt-stressed seedlings.

Table 3. Effects of different concentrations ofNaClon shoot and root fresh weight of two soybean varieties

Treatments	Shoot fresh weight (gm/plant)		Root fresh weight (gm/plant)	
	Shohag	BARI Soybean-5	Shohag	BARI Soybean-5
0 mM NaCl	0.32 ± 0.13 a	0.29 ± 0.08 a	0.051 ± 0.03 a	0.027 ± 0.02 a
50 mM NaCl	0.29 ± 0.10 a (90.62)	0.23 ± 0.05 b (79.31)	0.050 ± 0.02 a (98.04)	0.025 ± 0.006 a (92.59)
100 mM NaCl	0.17 ± 0.07 b (53.12)	0.14 ± 0.05 c (48.28)	0.037 ± 0.02 ab (72.55)	0.018 ± 0.009 a (66.67)
150 mM NaCl	0.13 ± 0.04 b (40.63)	0.10 ± 0.02 cd (34.48)	0.032 ± 0.01 ab (62.75)	0.016 ± 0.009 ab (59.25)
200 mM NaCl	0.08 ± 0.02 bc (25.00)	0.06 ± 0.01 de (20.69)	0.007 ± 0.007 b (13.72)	0.004 ± 0.002 bc (14.81)
250 mM NaCl	0.04 ± 0.01 c (12.50)	0.03 ± 0.009 e (10.34)	0.004 ± 0.003 b (7.84)	0.002 ± 0.001 c (7.40)
300 mM NaCl	0.03 ± 0.003 c (9.37)	0.02 ± 0.004 e (6.89)	0.001 ± 0.00 b (1.96)	0.0009 ± 0.00 c (3.33)
Cultivar Mean	0.14	0.14	0.029	0.015
CV%	80.49%	73.02%	88.97%	97.40%
LSD	0.05	0.04	0.06	0.009

*Average value of 9 plants in each treatment.

*****Means in a column followed by the same letter do not differ significantly at 5% level by ANOVA test, followed by Duncan's multiple-range test and ± means standard deviation.

***** Values within parenthesis indicate percentage relative to the control.

Dry weight of Shoot and Root: The shoot dry weight (mean value) of both varieties was 0.029 g in the control, which was reduced to 0.003 g in Shohag and 0.001 g in BARI Soybean-5 at 300 mM NaCl. The relative value of shoot dry matter at 300 mM NaCl was 10.34 for Shohag and 3.70 for BARI Soybean-5 (Table 4). The root dry weight (mean value) of the Shohag and BARI Soybean-5 varieties in the control was 0.009 and 0.008, respectively, was reduced to 0.0006 g in Shohag and 0.0002 in BARI Soybean-5 at 300 mM NaCl. The relative value of root dry matter at 300 mM NaCl was 6.67 for Shohag and 2.50 for BARI soybean-5 (Table 4). Typically, tolerant varieties have a higher root mass compared to salt-sensitive varieties. In this experiment, Shohag had higher root mass than BARI Soybean-5. Similar results of reduction in root dry weight under saline conditions have been reported in soybean (Zhou *et al*., 2023; Kondetti *et al*., 2012; Farhoudi & Tafti, 2011), sunflower (Chowdhury *et al*., 2018) and also in pea (Berhanu & Berhane, 2014).

Table 4. Effects of different concentrations of NaClon shoot and root dry weight of two soybean varieties

Treatments	Shoot dry weight (gm/plant)		Root dry weight (gm/plant)	
	Shohag	BARI Soybean-5	Shohag	BARI Soybean-5
0 mM NaCl	0.029 ± 0.005 a	0.029 ± 0.007 a	0.009 ± 0.002 a	0.008 ± 0.002 a
50 mM NaCl	0.028 ± 0.006 a (96.55)	0.026 ± 0.007 a (89.66)	0.008 ± 0.002 a (88.89)	0.007 ± 0.004 a (87.50)
100 mM NaCl	0.019 ± 0.006 b (65.52)	0.016 ± 0.005 b (55.17)	0.007 ± 0.003 ab (77.78)	0.006 ± 0.002 a (75.00)
150 mM NaCl	0.012 ± 0.004 c (41.38)	0.010 ± 0.002 c (34.48)	0.004 ± 0.003 bc (44.44)	0.003 ± 0.002 b (37.50)
200 mM NaCl	0.008 ± 0.001 cd (27.59)	0.005 ± 0.003 cd (17.24)	0.002 ± 0.001cd (22.22)	0.002 ± 0.003 b (25.00)
250 mM NaCl	0.005 ± 0.001 d (17.24)	0.004 ± 0.002 d (13.79)	0.001 ± 0.001d (11.11)	0.0004 ± 0.001 b (5.00)
300 mM NaCl	0.003 ± 0.0007 d (10.34)	0.001 ± 0.001 d (3.10)	0.0006 ± 0.0004 d (6.67)	0.0002 ± 0.0001 b (2.50)
Cultivar Mean	0.016	0.015	0.005	0.004
CV%	65.33%	78.91%	72.50%	90.00%
LSD	0.003	0.004	0.002	0.002

*Average value of 9 plants in each treatment.

*Means in a column followed by the same letter do not differ significantly at 5% level by ANOVA test, followed by Duncan's multiple-range test and ± means standard deviation.

*Values within parenthesis indicate percentage relative to the control.

The seed germination stage is the initial stage of plant growth and development; it is one of the most sensitive stages to salt stress. In this experiment, the germination percentage reduced in both varieties with increasing salinity. The decrease in germination rate is most likely related to the degree of osmotic potential, leading to lower water uptake. Furthermore, a reduction might have occurred due to inactivation of hydrolyase enzymes during seed germination under high NaCl salt levels (Maas & Hoffman, 1977). It is observed in *Hexaploid triticale* that the germination index (GI) decreased as the NaCl level increased, and a negative correlation coefficient was obtained between the NaCl concentration and GI (Karim *et al*., 1992). Salt stress severely affects the osmotic balance in cells, resulting in decreased osmotic potential and increased water potential. According to Akbar and Ponnamperuma (1982), the inhibition of seed germination may occur due to the osmotic effects of salinity. It is assumed that germination percentage and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions (Kumar, 2017). Our study stated that, the average germination speed is higher in Shohag than BARI soybean-5. It suggests that seeds germination time of Shohag was shorten at the concentration of 250 mM NaCl, but higher level NaCl concentration elongated the germination time of BARI soybean-5. The difference of germination time between two varieties could be due to the diversity of genetic potential.

In plants, roots are the first organs that come into direct contact with the medium, absorb water and nutrients and release them to the shoots. It stated that salt stress hindered the root growth by damaging redicle during germination. The inhibition of radicle growth under salt stress may be due to the reduction in the turgor of the radicle cells (Li, 2008). According to Tesfaye *et al*. (2014), excessive salt accumulation in the elasticity of the cell wall is probably the cause of the reduction of shoot length, leading to the formation of a secondary cell wall that prevents cell wall enlargement and ultimately slows shoot growth. Chowdhury *et al*. (2018) found that the plant needs to produce appropriate metabolites to adapt osmotically, which requires additional energy. As a result, the growth process suffers greatly. In addition, the longer roots have the advantage of absorbing more water than shorter roots. In this experiment, Shohag showed longer roots than BARI Soybean-5. So, Shohag could probably use more water than BARI Soybean-5. The reduction in dry weight may be due to the toxic effect of Na^+ on the rate of photosynthesis at higher concentrations. Furthermore, excessive salt levels can induce a decrease in the transport rate of important ions like NO_3^-, which reduces N-containing molecules and, as a result, inhibits plant development and biomass accumulation (Hamid *et al*., 2008).

Conclusion: From our study, higher salinity had negative effects on soybean seed germination and both varieties can withstand moderate salinity stress. These results also indicated that Shohag soybean had relatively better tolerance to higher salinity than BARI Soybean-5. It is suggested that Shohag can be grown in saline areas of Bangladesh, but genetic improvement of BARI soybean-5 is required.

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