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# Responses of barley genotypes for salt tolerance under *in vitro* culture condition

MR Mallik<sup>1</sup>, AJ Swarna<sup>1</sup>, A Mobaswera<sup>2</sup>, ABMMM Khan<sup>1</sup>, MHK Howlader<sup>1\*</sup>

<sup>1</sup>Department of Agricultural Botany, <sup>2</sup>Department of Soil Science, Patuakhali Science and Technology University, Patuakhali, Bangladesh.

#### **Abstract**

An experiment was undertaken at Agricultural Botany Laboratory, Patuakhali Science and Technology, to screen out salt tolerant barley (*Hordeum vulgare* L.) genotypes. The evaluation was done based on germination percentage, shoot length, root length and shoot: root ratio. Five barley genotypes including BARI barley 2, BARI barley 3, BARI barley 4, BARI barley 5 and BARI barley 7 were used in the screening program. The barley genotypes were grown in eight levels NaCl solution like 0(Control), 20, 40, 60, 80, 100 and 120 mM. The present study shows that salinity reduced germination percentage of barley and the extent of reduction increased with the increase in the concentration of salinity in the growth medium. Shoot dry weight (1.40 mg), root dry weight (0.21 mg), shoot moisture content (80.0%), root elongation rate (1.81), vigor index (21.30), speed of germination (7.91) and root length (21.67 cm) were obtained from the variety BARI barley 4 with 80 mMNaCL (V3T4). Based on the growth attributes the genotypes BARI barley 7 and BARI barley 4 were found tolerant to salt stress as they grown up to 60 mM and 80 mMNaCl solution respectively. The genotypes BARI barley 4 was therefore selected to be grown in south coastal saline soil of Bangladesh.

**Key words:** Salinity, barley genotypes, salt tolerance, in vitro culture

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\*Corresponding Author: hhkabir63@gmail.com

#### Introduction

Salinity is a major abiotic constraint to crop productivity throughout the arid and semi-arid regions of the world (Foolad and Lin, 1997). In Bangladesh, there are approximately 2.85 million ha of coastal land which occurs in the southern parts of the Ganges tidal floodplain, in the young Meghna estuarine floodplain and in tidal areas of the Chittagong coastal plain and offshore islands. About one million ha of land of these coastal and offshore areas are seriously affected by varying degrees of salinity (SRDI 2010). The average cropping intensity (%) in the coastal areas has not increased as much compared to flood plain agriculture. About 30-50% of net cropped areas remains fallow in

Rabi and *Kharif-1* seasons in the coastal region, mainly due to soil and water (irrigation) salinity, high tidal flooding depth in monsoon season (June-October), late draining, direct inundation by saline water, and upward or lateral movement of saline ground water during dry season (November-May), heavy soil consistency, poor soil fertility status, high osmotic pressure causing reduction in absorption of water and nutrients, poor soil structure and cyclonic storm surges. The salinity increases in dry months showing a peak in March-April and decreases in wet months with the minimum in July-August. Annual precipitation does not exceed evapotranspiration (Rokonuzzaman et al., 2018),

soluble salts tend to accumulate and build-up in the soil of coastal regions, instead of being leached, and can reach the levels inhibitory to plant growth and development. Intensive irrigation with light saline surface water in such areas further complicates the problem, leaving behind huge salt deposits after evaporation, leading to secondary salinization and alkalization (Roychoudhury and Chakraborty 2013).

The scope of increasing food production in Aus and Aman season is limited due to high water depth, where HYV rice could not persist. Rabi season is the most important season where there have highest scope to increase food production in the vast fallow lands of this areas. Introduction of barley, a high value nutritious cereal crop, in the coastal saline soils could be most profitable approach to improve food production as well as income of the farmers. In this context it is very important to know what extent of salinity barley can tolerate. This information will help to make decision in which areas of the coastal region will be suitable for barley. Seed germination is a major factor limiting the establishment of plants under saline conditions (Carpici et al. 2009). Salinity may cause significant reductions in the rate and percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields (Foolad et al., 1999).

Several authors reported the presence of considerable genetic variation in salinity tolerance among barley varieties (Bogemans *et al.*, 1990 and Duczek, 1993). Many group of scientists reported on awareness building and perception on natural disasters, air pollution and water pollution and metal pollution and management but limited documents were available on salinity stress and agriculture (Mondol et al., 2021a, b; Hanif et al., 2020; Hasan et al., 2020; Sarker et al., 2018; Rahman et al., 2018). Therefore, it is possible to improve the heritable tolerance to salinity by adopting various breeding methods and cultivation could be made possible by cultural manipulation of soils. With such thinking, among the cereals the barley having high level of tolerance to salinity can be taken as an

advantage to introduce this crop in the southern coastal region of the country, where salinity is prevailing in various intensity.

Salt tolerance at germination stage is important factor, where soil salinity is mostly dominated at surface layer. High concentration of salt has detrimental effects on germination of seeds (Kayani and Rahman, 1987; Rahmanet al., 2000; Sharma et al., 2004; Saboora and Kiarostami, 2006). Plant growth is ultimately reduced by salinity stress (Haqueet al. 2014). It is very important to know what extent of salinity, a variability can tolerate at germination and early seedling growth. This information will help to identify salt tolerant varieties and/or genotypes and to develop saline soil management strategies.

#### **Materials and Methods**

The experiment was conducted at the laboratory of the Department of Agricultural Botany, Patuakhali Science and Technology University, Dumki, Patuakhali during the period of November 2018. The experimental field located at 22.37° N latitude and 89.10° E longitude at Ganges Tidal Floodplains and falls under Agroecological Zone "AEZ 13". The study locations also lie under Ganges Tidal Floodplain AEZ (AEZ No. 13) in coastal non-saline zone of Bangladesh. This region occupies an extensive area of tidal floodplain land in the south—west of the country. The area lies at 0.9 to 2.1 meter above mean sea level.

Treatments: Nine different salinity concentrations were used in the experiment. Each concentration was considered as an experimental treatment. The different salinity concentrations were obtained by dissolving required amount of crude salt (collected from seashore) in distilled water. Before weighing salts were oven dried to remove moisture. The control (0Mm NaCl) was maintained using distilled water only. The experiment was designed following 2 factor completely randomized design (CRD). The treatments were as follows (Table 1).

**Table 1**. Different treatments with salt concentration.

Salt concentration				
0 mMNaCl (Control)				
20 mMNaCl				
40 mMNaCl				
60 mMNaCl				
80 mMNaCl				
100 mMNaCl				
120 mMNaCl				

Experimental set up: The experiment was carried out in Petri dishes of 12 cm diameter. A thin layer of cotton was set at the bottom of the Petri dishes. Twentyfive seeds were placed on cotton bed in a circle pattern. Ten milliliter of treatment solutions of different salinity concentrations were poured in each Petri dish to immerse the seeds partially for ensuring proper aeration. Three replications were maintained for each salinity concentration. The

Petri dishes were placed on a table in the laboratory. The seeds were allowed to germinate at room temperature (25±2°C). Required amount of distilled water was added to each Petri dish every day to maintain same level of water as in initial date. Seeds were considered germinated when radicles measured 2 mm size. Partial view of the experiment is shown in plate-1.

Observation and statistical analysis: The number of seeds germinated was recorded starting from 2 days after sowing (DAS) to 12DAS. The results obtained in each day were converted into percentage. Data recorded on crop characters were subjected to statistical analysis through computer statistical program Mstat-C (Michigan State University, East Lansing, Ml, USA) following the basic principles, as outlined by Gomez and Gomez (1984). Significant effects of treatments were determined by analysis of variance (ANOVA) and treatment means were compared at 5% level of significance by Duncan's Multiple Range Test (DMRT).

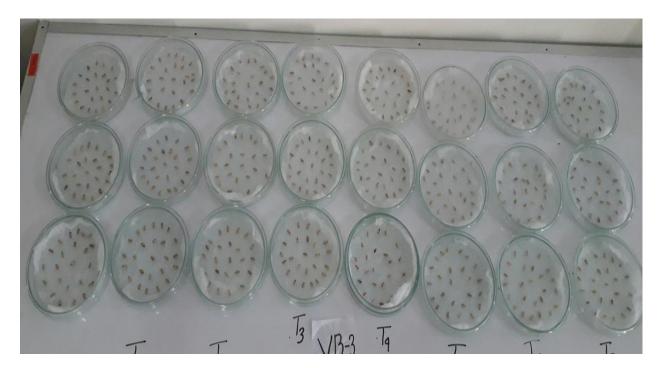


Plate-1: Experimental design

#### **Results and Discussion**

Effect of variety: Analysis of variance data on shoot fresh weight significantly influence by the Barley varieties. From Table 2 it is obvious that highest shoot fresh weight (5.57 mg) was recorded from BARI barley 4. The lowest shoot fresh weight (2.04 mg) was in BARI barley 3. BARI barley 4 produced the highest root dry weight (0.16 mg) while BARI barley 5 recorded the lowest root dry weight (0.11mg). Increasing salt concentration reduced the shoot moisture content at every sampling date. Maximum shoot moisture content at 12 DAS was recorded from BARI barley 2 (83.14) and BARI barley 7 (80.14) respectively. Minimum shoot moisture content was found in BARI barley 5 (74.14). It was observed from

the table 4.8 that shoot elongation rate of seedlings of barley was maximum (1.85mm day-1) in variety BARI barley 4 and the minimum shoot elongation rate (1.30mm day<sup>-1</sup>) was observed in variety BARI barley 5. Shoot elongation rate was varied among the genotypes with the progress of time. Among the varieties, the variety BARI barley 4 produced the root elongation rate (1.46) at 12 DAS and the lowest root elongation rate (1.06). BARI barley 4 produced the highest Vigor Index (26.16) while BARI barley 5 recorded the lowest Vigor Index (23.33). It is obvious that highest speed of germination (26.16) was recorded from BARI barley 4. The lowest speed of germination (23.33) was in BARI barley 5. BARI barley 4 produced the highest root length (17.52cm) while BARI barley-3 recorded the lowest Root length (11.31cm).

**Table 2.** Effects of variety on barley.

Variety	Shoot dry weight (mg)	Root dry weight (mg)	Shoot moisture content	Root elongation rate	Vigor Index	Speed of Germination	Root length
BARI Barley-2	0.58 d	0.13ab	83.14 a	1.07 c	24.80 c	8.33 b	12.71 c
BARI Barley-3	0.54 e	0.12ab	74.14 c	0.94 d	24.05 d	7.09 d	11.31 d
BARI Barley-4	1.11 a	0.16 a	80.14 b	1.46 a	26.16 a	8.76 a	17.52 a
BARI Barley-5	0.61 c	0.11 b	79.86 b	1.08 c	23.33 e	7.91 c	12.74 с
BARI Barley-7	0.77 b	0.15 a	80.00 b	1.36 b	25.37 b	8.44ab	16.29 b
%CV	3.89	3.48	2.44	4.56	2.55	4.19	4.52
Significance level	***	***	***	***	***	***	***
SE (±)	0.006901	0.009759	0.4238	0.01380	0.1377	0.09181	0.1392

\*\*\* = Significant at 1% level of probability.

Effects of different levels of salinity: Shoot dry weight of seedlings was significantly affected by different levels of salinity (Table 3). Shoot dry weight ranged from 0.28 to 1.37 mg. The highest dry weight of shoot (1.37 mg) was obtained in control treatment while barley grown in 120 Mm NaCl concentrations accumulates minimum photosynthates in shoot that enhanced lowest dry weight of shoot (0.28 mg). The

dry weight of shoot declined respectively over control. The result conforms to the findings of Abbasi *et al.* (2014) who found that salt stress significantly decreased the plant shoot dry weight. He stated that reduction in shoot fresh and dry weight of maize hybrids in the presence of NaCl was attributed to ion toxicity as excess Na+ resulted in nutritional and metabolic imbalances. Maximum root fresh weight of

1.18 mg was obtained in control treatment while the lowest root fresh weight of 0.27 mg was obtained in 120 mM NaCl concentration that was might be due to shortest roots. Highest shoot moisture content obtained from 40 mM NaCl concentration. Beyond 120 mM NaCl concentration increasing salt concentration reduced shoot moisture content. At 6 DAS, longest (1.03cm) shoot of barley was obtained in control and the length was shortened with the increasing level of salinity. The shortest shoot elongation rate was 31cm at 120 mM NaCl. At 9 DAS, maximum shoot elongation rate 1.82 mm was obtained in control treatment while minimum (.69 mm) shoot height was found in 120 mM NaCl. At 12 DAS, maximum shoot elongation rate 1.89 cm was obtained in control treatment while minimum (1.07 cm) shoot height was found in 120 mM NaCl. The 0 mM NaCl concentration had highest and identical vigor index (36.95). When vigor index value was compared with control treatment it was found that the

20, 40, 60, 80, 100 and 120 Mm. Speed of germination was gradually decreased with the increasing of salinity levels. The reduction of 4.32, 10.22, 15.70, 19.18, 21.92 and 30.45% was estimated in 20, 40, 60, 80, 100 and 120 mM NaCl concentration, respectively over control treatment. Shoot length gradually decreased with the increasing of salinity. The length of barley root decreased to 7.98, 17.39, 23.78, 29.25, 44.15 and 51.97% in 20, 40, 60, 80, 100 and 120 mM NaCl concentration, respectively over control. Longest root length (18.80 cm) was obtained in control treatment while smallest root was in 120 mM NaCl concentration (9.03 cm). In 120 mM NaCl concentration, the root length reduced drastically that might be due to deformed, twisted and squized root. The result was in alignment with the findings of Ratnakara and Raib (2013) who found that salinity had significant effect on root length.

**Table 3.** Effects of different levels of salinity on barley.

Treatment	Shoot dry weight (mg)	Root dry weight (mg)	Shoot moisture content	Root elongation rate	Vigor Index	Speed of Germination	Root length
$T_0$	1.37 a	0.25 a	78.40 b	1.58 a	36.95 a	9.49 a	18.80a
$T_1$	1.10 b	0.21ab	81.00 a	1.45 b	31.69b	9.08 b	17.30b
$T_2$	0.77 с	0.15 b	77.80 b	1.31 c	27.31 с	8.52 c	15.53c
T <sub>3</sub>	0.63 d	0.12bc	81.00 a	1.19 d	24.32d	8.00 d	14.33d
$T_4$	0.54 e	0.11 cd	79.60 ab	1.11 e	19.69e	7.67 e	13.30e
T <sub>5</sub>	0.35 f	0.06 de	79.00 ab	0.87 f	18.58f	7.41 f	10.50f
T <sub>6</sub>	0.28 g	0.05 e	79.40 ab	0.76	14.65g	6.60 g	9.03 g
%CV	3.89	3.48	2.44	4.56	2.55	4.19	4.52
Significance level	***	***	***	***	***	***	***
SE (±)	0.008165	0.01155	0.5014	0.01633	0.1629	0.108	0.1647

\*\*\* = Significant at 1% level of probability.

Interaction effects of variety and different levels of salinity: The interaction of variety and different levels of salinity showed a significant effect on Shoot dry

weight (Table 4). Shoot dry weight ranged from 0.14 to 1.73 mg. The highest Shoot dry weight (1.73 mg) was obtained from BARI barley 4 with control treatment

 $(V_3T_0)$  and the lowest  $(0.14\ mg)$  was recorded in BARI barley 2 with 120 mM NaCl  $(V_1T_6)$ . It was found the highest value  $(0.28\ mg)$  was obtained from the variety BARI barley 4 with control treatment  $(V_3T_0)$  and lowest value  $(0.04\ mg)$  was found from BARI barley 3 with 120 mM NaCl  $(V_2T_6)$  that was statistically similar to BARI barley 2 with 120 mM NaCl  $(V_1T_6)$ . It was found the highest value (1.37) was obtained from the

variety BARI barley 4 with control treatment ( $V_4T_0$ ) and lowest value (0.85) was found from BARI barley 2 with 120 mM NaCl ( $V_2T_6$ ). The highest root elongation rate (88%) was obtained from the variety BARI barley 1 with control treatment ( $V_1T_6$ ) and lowest root elongation rate (68%) was found from BARI barley 2 with 120 mM NaCl ( $V_2T_6$ ).

Table 4. Interaction effects of variety and different levels of salinity on barley.

Treatment x Variety	Shoot dry weight (mg)	Root dry weight (mg)	Shoot moisture content	Root elongation rate	Vigor Index	Speed of Germination	Root length
$V_1T_0$	1.25 e	0.26abc	79.00 f	1.40 g	38.33 c	9.56 b	16.33 g
$V_1T_1$	1.15 h	0.24bcd	79.00 f	1.34 h	32.93 f	8.75 d	15.83 h
$V_1T_2$	0.56 о	0.12 h	86.00 b	1.15 j	28.50 ij	8.33 e	13.83 ј
$V_1T_3$	0.41 p	0.10hij	86.00 b	1.05 k	23.90 о	8.33 e	12.50 k
$V_1T_4$	0.33 q	0.08ijklm	80.00 e	0.981	21.23 q	7.94 f	11.831
$V_1T_5$	0.21 t	0.06klmn	84.00 c	0.84 o	16.43 v	7.91 f	10.17 o
$V_1T_6$	0.14 v	0.04 n	88.00 a	0.72 s	12.23 y	7.50 g	8.50 s
$V_2T_0$	1.09 i	0.23bcde	74.00 h	1.31 i	33.20 e	8.75 d	15.67 h
$V_2T_1$	0.86 j	0.20def	86.00 b	1.30 i	30.93 g	8.33 e	15.50 h
$V_2T_2$	0.65 m	0.18fg	64.00 k	1.06 k	24.83 n	7.50 g	12.83 k
$V_2T_3$	0.34 q	0.09ijkl	79.00 f	0.83 op	18.83 t	7.08 h	10.00 op
$V_2T_4$	0.32qr	0.07jklmn	78.00 g	0.75 r	15.20 w	7.08 h	9.00 r
$V_2T_5$	0.30 r	0.05mn	70.00 i	0.69 t	25.13 m	6.66 i	8.33 s
$V_2T_6$	0.19 u	0.04 n	68.00 j	0.65 u	20.20 r	4.25 j	7.83 t
$V_3T_0$	1.73 a	0.28 a	79.00 f	1.91 a	40.60 b	10.00 a	23.00 a
$V_3T_1$	1.23ef	0.20efg	80.00 e	1.68 c	30.50 h	10.00 a	20.17 c
$V_3T_2$	1.29 d	0.18fg	80.00 e	1.52 e	28.73 i	9.67 b	18.33 e
$V_3T_3$	0.85 j	0.10hij	80.00 e	1.36 h	27.97 k	8.34 e	16.33 g
$V_3T_4$	1.40 b	0.21 de	80.00 e	1.81 b	21.30 q	7.91 f	21.67 b
$V_3T_5$	0.68 kl	0.07jklmn	81.00 d	1.04 k	18.83 t	7.91 f	12.50 k
$V_3T_6$	0.58 n	0.06lmn	81.00 d	0.89 n	15.20 w	7.50 g	10.67 n
$V_4T_0$	1.37 c	0.20efg	80.00 e	1.44 f	30.50 h	9.16 c	17.33 f
$V_4T_1$	1.08 i	0.18fg	80.00 e	1.31 i	28.73 i	8.75 d	15.67 h

Treatment x Variety	Shoot dry weight (mg)	Root dry weight (mg)	Shoot moisture content	Root elongation rate	Vigor Index	Speed of Germination	Root length
$V_4T_2$	0.69 k	0.11 hi	79.00 f	1.31 i	28.33 j	8.34 e	14.33 i
$V_4T_3$	0.34 q	0.09hijkl	80.00 e	1.04 k	27.96 k	7.90 f	12.50 k
$V_4T_4$	0.30 r	0.07klmn	80.00 e	0.94 m	21.30 q	7.50 g	11.33 m
$V_4T_5$	0.28 s	0.06lmn	80.00 e	0.79 q	15.20 w	7.08 h	9.50 q
$V_4T_6$	0.21 t	0.05 n	80.00 e	0.71st	11.30 z	6.66 i	8.50 s
$V_5T_0$	1.39 b	0.26ab	80.00 e	1.82 b	42.13 a	9.98 a	21.67 b
$V_5T_1$	1.19 g	0.21 de	80.00 e	1.61 d	35.33 d	9.56 b	19.33 d
$V_5T_2$	0.67 lm	0.17 g	80.00 e	1.53 e	26.131	8.75 d	18.33 e
$V_5T_3$	1.22 f	0.23cde	80.00 e	1.69 c	22.93 p	8.33 e	20.33 c
$V_5T_4$	0.34 q	0.09hijk	80.00 e	1.05 k	19.43 s	7.91 f	12.67 k
$V_5T_5$	0.30 r	0.08klmn	80.00 e	1.001	17.30 u	7.50 g	12.001
$V_5T_6$	0.27 s	0.05mn	80.00 e	0.81 pq	14.33 x	7.08 h	9.67 pq
%CV	3.89	3.48	2.44	4.56	2.55	4.19	4.52
Significance	***	***	***	***	***	***	***
level							
SE (±)	0.005345	0.007559	0.1895	0.006172	0.06157	0.04106	0.1078

\*\*\* = Significant at 1% level of probability.

The highest vigor Index (42.12) was obtained from the variety BARI barley 7 with control treatment (V<sub>5</sub>T<sub>0</sub>) and lowest vigor Index (11.30) was found from BARI barley 5 with 120 mM NaCl (V<sub>4</sub>T<sub>6</sub>). From table 3, it was found the highest speed of Germination (10.00) was obtained from the variety BARI barley 4 with control treatment (V<sub>3</sub>T<sub>0</sub>) that is statistically similar to BARI barley 4 with 20 mM NaCl concentration (V<sub>3</sub>T<sub>1</sub>), BARI barley 7 with control treatment  $(V_5T_0)$  and lowest value (4.25) was found from BARI barley 3 with 120 mM NaCl (V<sub>3</sub>T<sub>6</sub>). Root length had statistically significant due to the effect of interactions of variety and different levels of salinity where as it was varied from 7.83 to 23.00 cm. From the above variation, it was found the highest value (23.00) was obtained from the variety BARI barley 4 with control treatment (V<sub>3</sub>T<sub>0</sub>) and lowest value (7.83cm) was found from BARI barley 3 with 120 mM NaCl (V<sub>2</sub>T<sub>6</sub>).

## Conclusion

In conclusion, from above observation of the present study it may be concluded that seed germination and growth of barley varied according to the change in NaCl levels. The increase of salt stress decreased all studied parameters on germination and seedling growth. Salinity reduced germination percentage of barley and the extent of reduction increased with the increase in the concentration of salinity in the growth medium. NaCl upto 80 mM could be identified as safe for BARI barley 4 for seed germination. Up to 60 mM NaCl concentration salinity might considered for seedling growth of BARI barley 7. At higher concentration like 120 mM NaCl, root was more affected than shoot.

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