



**Original Article**

**Salinity tolerance of dhaincha genotypes at seed germination and seedling growth**

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**ABSTRACT**

To evaluate the germination capability and seedling growth of dhaincha accessions under salt stress, the experiment was conducted at the Plant Physiology Laboratory of the Department of Crop Botany, Bangladesh Agricultural University. The germination test was carried out in Petri dishes following two factorial CRD with four replications. Twenty-five accessions of dhaincha and three salt levels *viz.*, 0 (control), 6 and 12 dSm<sup>-1</sup> were used as experimental treatments. The germination percentage, plant height, fresh weight and biomass yield and different stress tolerance indices were recorded and/or calculated to screen the accessions for salt tolerance. Salinity adversely affects the germination behaviour and seedling growth descriptors of dhaincha genotypes; the seed germination was relatively less affected by salt concentrations compared to other growth descriptors. A significant variation among the dhaincha accessions in response to salt stress was also observed. Only germination test was not good enough to screen germplasms for salt tolerance; however, seedling growth especially plant height and biomass accumulation could be a reliable and efficient method for screening dhaincha germplasms for salt tolerance. Based on germination behaviour and biomass yield, five accessions *viz.* #5, 7, 59, 82 and 85, were selected; these accessions can be used for further breeding programmes and/or cultivation in coastal saline-prone areas with further investigation.

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**Introduction**

Salinity is one of the most detrimental factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigour and crop yield (Munns and Tester, 2008). A soil may be rich in salts because the parent rock from which it was formed contains salts. Seawater is another source of salts in low-lying areas along the coast. A very common source of salts in irrigated soils is the irrigation water itself. The total amount of salinity affected land in Bangladesh has been increasing day by day. For example, 83.3 m ha in 1973, 102 m ha in 2000 and the amount has risen to 105.6 m ha in 2009 and continues to increase (SRDI, 2010). In the last 35 years, salinity increased around 26 per cent in the country, spreading into non-coastal areas as well. Soil salinization was also caused and influenced by climate change events (Anon., 2018). First, ocean temperatures are rising, and warmer water takes up more space. Ice sheets and glaciers are melting and flowing into the oceans. This process pushes salty water onshore along coastlines. Moreover, the salinity of seawater is increasing at an alarming rate (Boutin *et al.*, 2019). Climate change also causes heat stress, which will deplete groundwater resources and increase saline

contamination of soils inland. Development of salt-tolerant crop varieties and farming methods is one of the possibilities that can help coastal farms remain viable as sea levels rise (Anon., 2018). Besides case-specific optimization of irrigation and drainage management, combinations of soil amendments, conditioners, and residue management can contribute to significant reductions of soil salinity while significantly increasing crop yields (Cuevas *et al.*, 2019). The increase in soil salinity would hamper the agro-based economy in other ways. It may cause internal migration in Bangladesh that would increase by 25 per cent if all coastal locations had to contend with the highest soil salinity content currently observed (Anon., 2018; Chen and Mueller, 2018). In total, some 200,000 Bangladeshi coastal farmers per year could migrate inland to seek new livelihoods. The reclamation of saline soil by adding organic matter might be one of the options to rehabilitate degraded lands into productive croplands (Shirale *et al.*, 2018).

Dhaincha (*Sesbania* spp.), the miracle plant, is one of the most important green manure crops with multiple uses, *viz.* mulch for moisture conservation, weed suppression, ground cover, firewood, fuel, fibre and bioenergy sources, providing

live support fencing wood, raw materials for industrial uses, and in traditional agroforestry systems, animal feed and fodder and medicinal importance (Chanda *et al.*, 2019). Being a member of the family Fabaceae, it fixes atmospheric N in the soil through Legume-*Rhizobium* symbiosis. The genus *Sesbania* showed a luxuriant growth in soil with a high electrical conductivity up to 10 mS cm<sup>-1</sup>, and some of the *Sesbania* spp. have been recommended for reclamation of saline and sodic soils (Chavan and Karadge, 1986). Long time cultivation of dhaincha would combat desertification of marginal lands, e.g., char land, saline area, etc., and rehabilitate degraded lands into productive croplands for intensive food crop agriculture (Carroll and Somerville, 2009). Bangladesh, although, has a long history of cultivation of dhaincha as a green manure crop (Prain, 1903 and older references therein), information on the use of dhaincha plant for saline soil reclamation or salinity tolerance of dhaincha plant in Bangladesh condition is scanty (Tinne, 2018). The experiment was, therefore, conducted to study the effect of salinity on seed germination and seedling growth descriptors of dhaincha plant. This is a very first step to identify salt-tolerant dhaincha accession(s) for cultivation in the saline-prone area to improve soil fertility and productivity.

### Materials and Methods

The experiment was conducted at the Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University. Seeds of 25 accessions of dhaincha plants were collected from Laboratory of Plant Systematics of the same Department (detail collection information available upon request). A two factorial experiment was set following a completely randomized design (CRD) with four replications following Islam *et al.* (2019). The experimental factors were i) dhaincha accessions (twenty-five) and ii) salt stress (three levels; 0, 6 and 12 dSm<sup>-1</sup>). Twenty-five dhaincha accessions were randomly assigned to twenty-five Petri dishes (9 cm diameter of which each Petri dish contained 25 seeds of each genotype following four replicates). Therefore, a total of 300 Petri dishes were required for the experiment. Two salt concentrations, *viz.* 6 and 12 dSm<sup>-1</sup>, were obtained by dissolving common natural salt (NaCl) in the solution until the treatment level reached to the desired EC. An EC meter was used to check the desired EC regularly. The control (0 dSm<sup>-1</sup>) was maintained using distilled water only. Seeds were sterilized with 5% sodium hypochlorite for 30 min and washed thoroughly with distilled water. The seeds were then soaked in water and imbibed for 24 h and then placed in Petri dishes containing filter paper to allow them for germination. In control, 4 mL of distilled water was added to the Petri dish. Filter papers were moistened with 4 mL of respective salt solutions to develop the respective level of salt treatments (6 dSm<sup>-1</sup> and 12 dSm<sup>-1</sup>). Seeds were allowed to germinate at around 25°C room temperature and kept them for ten days for observation. The number of germinated seeds (radicle emergence) was counted every day. The final count was done on day ten and germination percentage (GP) was calculated using the following formulae.

$$\text{Germination Percentage (GP)} = \frac{\text{total number of seed germinated}}{\text{total number of seed taken}} \times 100$$

Seedling fresh weight was recorded immediately after the harvest on day ten. Plant biomass was recorded after drying at 72°C to a constant weight. Different physiological indices

like Promptness Index (PI), Germination Stress Tolerance Index (GSTI), Plant Height Stress Tolerance Index (PHSI), Fresh Weight Stress Tolerance Index (FWSI), Biomass Stress Tolerance Index (BSI) and Vigor Stress Tolerance Index (VSI) were calculated according to the following formula described by Ashraf *et al.* (2006):

$$\text{PI} = \text{nd1} (1.00) + \text{nd2} (0.75) + \text{nd3} (0.50) + \text{nd4} (0.25)$$

Where: nd1, nd2, nd3 and nd4 = Number of seeds germinated on the 2nd, 4th, 6th and 8th day, respectively.

$$\text{GSTI} = \frac{\text{PI of stressed seeds}}{\text{PI of control seeds}} \times 100$$

$$\text{PHSI} = \frac{\text{Plant height of stressed plants}}{\text{Plant height of control plants}} \times 100$$

$$\text{FWSI} = \frac{\text{Fresh weight of stressed plants}}{\text{Fresh weight of control plants}} \times 100$$

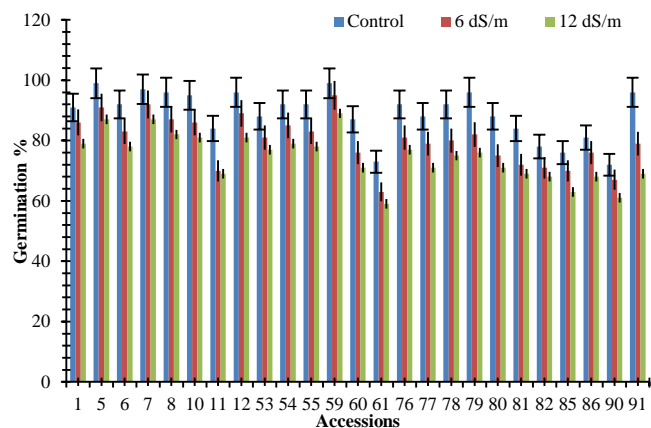
$$\text{BSI} = \frac{\text{Biomass yield of stressed plants}}{\text{Biomass yield of control plants}} \times 100$$

$$\text{VSI} = \frac{\text{Vigour index of stressed plants}}{\text{Vigour index of control plants}} \times 100$$

The statistical software 'R' was used to evaluate the variations of parameters among the treatments and genotypes.

### Results and Discussion

Germination is one of the most important stages in plant life histories. Seed germination reduces by soil salinity in salt stress condition i.e. seed germination decreased with the increase of salinity level (Ravelombola *et al.*, 2017). Germination percentage (GP) and other seedling growth descriptors of dhaincha genotypes were greatly hampered in presence of high NaCl concentration, although seed germination was relatively less adversely affected by NaCl (Figure 1, Table 1). The germination was directly related to the amount of water absorbed and the delay in germination to the salt concentration of the medium. In control (0 dSm<sup>-1</sup>) condition, the GP varied from 72 to 100 (Figure 1). On the contrary, the GP varied from 63 to 95 at 6 dSm<sup>-1</sup> and 57 to 89 at 12 dSm<sup>-1</sup>. The decrease in GP due to salinity stress might affect the germination process in two ways – i) salts in the medium decrease the osmotic potential to such a point which retard or prevent the uptake of water necessary for mobilization of nutrient required for germination, and ii) the salt constituents or ions may be toxic to the embryo (Kaymakanova, 2009). The germination process may be delayed or inhibited due to the toxicity of Na<sup>+</sup> (Kazemi and Eskandari, 2011).



**Figure 1.** Per cent germination of twenty-five dhaincha accessions under different salinity levels (0, 6 and 12 dSm<sup>-1</sup>). Vertical bars represent SEM (n = 4).

The decrease in germination rate particularly under drought and salt stress conditions may be because seeds seemingly develop an osmotically enforced “dormancy” under water stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring the proper establishment of the seedlings (Gill *et al.*, 2003). The negative effects of high salinity may be due to ion toxicity on seed germination, as a consequence of a coincident increase in anion and cation (Panuccio *et al.*, 2014). The GSTI significantly differed in different dhaincha accessions examined in both salinity levels. In 6 dSm<sup>-1</sup>, the maximum value for germination stress tolerance index (99.33) was observed in accession #1 and 59 followed by accessions #5 and 7 having GSTI value 98.67 (Table 1). The lowest value for GSTI (83.11) was observed in accession #61. In 12 dSm<sup>-1</sup>, the value for GSTI followed a similar trend and varied from 94.02 to 80.21 (Table 1).

**Table 1.** Relative values (% of control) of some morphological parameters of twenty-five dhaincha accessions grown under control and salt stress (6 dSm<sup>-1</sup> and 12 dSm<sup>-1</sup>) condition. GSTI Germination Stress Tolerance Index; PHSI Plant Height Stress Tolerance Index; FWSI Fresh Weight Stress Tolerance Index; BSI Biomass Stress Tolerance Index; VSI Vigor Stress Tolerance Index.

Treatment	GSTI		PHSI		FWSI		DSI		VSI	
	6 dS/m	12 dS/m	6 dS/m	12 dS/m	6 dS/m	12 dS/m	6 dS/m	12 dS/m	6 dS/m	12 dS/m
Accession #1	99.33 a	93.36 a	35.89 e	20.36 e	52.83 c	33.91 e	48.90 e	38.61 d	36.78 e	20.25 e
Accession #5	98.67 a	89.24 b	40.42 d	26.81 d	47.87 d	34.65 e	61.52 b	43.74 c	41.48 d	23.87 d
Accession #6	85.51 c	88.00 b	35.11 e	20.23 e	46.06 d	33.45 e	47.78 e	37.62 d	35.87 e	19.55 e
Accession #7	98.67 a	92.56 a	52.16 b	27.81 c	47.60 d	45.98 b	57.07 c	47.68 b	52.27 ab	25.92 c
Accession #8	84.67 c	81.22 d	34.58 e	18.55 f	42.56 e	33.02 e	45.67 f	31.05 e	30.13 f	15.55 f
Accession #10	86.12 c	81.12 d	30.98 f	20.11 e	42.45 e	30.01 f	41.11 g	35.65 d	30.87 f	14.49 f
Accession #11	85.54 c	85.88 c	33.45 e	20.02 e	42.67 e	31.01 f	47.89 e	37.50 d	35.99 e	19.02 e
Accession #12	84.59 c	85.34 c	35.67 e	17.78 f	40.56 e	33.05 e	47.67 e	33.50 e	30.33 f	19.45 e
Accession #53	87.61 c	85.11 c	31.56 f	18.67 f	42.11 e	33.01 e	46.54 e	32.77 e	36.11 e	19.33 e
Accession #54	86.11 c	85.56 c	30.28 f	20.01 e	37.35 f	32.01 e	42.45 fg	36.60 d	30.33 f	16.56 f
Accession #55	86.11 c	84.56 c	35.56 e	20.05 e	37.45 f	30.06 f	41.11 g	37.75 d	33.56 f	20.02 e
Accession #59	99.33 a	94.02 a	54.37 a	34.56 a	60.78 a	51.95 a	69.16 a	55.95 a	53.67 a	33.56 a
Accession #60	88.11 c	85.34 c	31.76 f	20.11 e	45.12 d	32.32 e	46.56 e	38.33 d	30.30 f	20.12 e
Accession #61	83.11 d	80.21 d	29.99 f	16.34 f	36.67 f	29.01 f	40.23 g	30.63 e	28.02 g	13.59 f
Accession #76	88.67 c	85.56 c	31.67 f	20.05 e	45.11 d	32.33 e	46.67 e	38.34 d	30.31 f	20.11 e
Accession #77	87.15 c	86.55 c	31.74 f	17.79 f	44.32 d	30.50 f	45.79 f	37.02 d	36.34 e	18.34 e
Accession #78	86.64 c	84.11 c	35.12 e	20.21 e	37.67 f	33.44 e	45.64 f	32.45 e	30.79 f	15.50 f
Accession #79	83.23 d	83.88 c	31.45 f	19.78 ef	36.76 f	33.12 e	41.31 g	31.34 e	33.65 e	15.23 f
Accession #80	86.78 c	85.58 c	35.55 e	18.98 f	46.67 d	31.01 f	41.11 g	33.65 e	34.89 e	20.07 e
Accession #81	85.79 c	81.11 d	31.12 f	19.67 f	45.11 d	33.67 e	41.13 g	37.76 d	31.12 f	20.32 e
Accession #82	95.40 b	88.65 b	52.63 b	32.31 b	56.89 b	42.12 c	51.81 d	46.58 b	51.33 b	30.01 b
Accession #85	94.59 b	87.55 b	46.74 c	31.59 b	56.76 b	38.84 d	62.77 b	41.10 c	43.75 c	28.74 b
Accession #86	87.67 c	84.56 c	35.33 e	20.11 e	42.21 e	33.11 e	41.12 g	32.00 e	30.91 f	15.66 f
Accession #90	88.10 c	82.54 c	30.65 f	18.88 f	42.24 e	31.12 e	47.67 e	35.27 d	29.34 c	19.45 e
Accession #91	88.01 c	86.34 c	30.87 f	17.78 f	37.23 f	32.21 e	45.78 f	36.30 d	35.12 e	19.35 e
CV (%)	1.47	1.43	1.92	1.28	1.53	1.69	1.46	2.2	2.01	3.34

In a column, figures bearing uncommon letters differ significantly at 5% level of significance

Based on only germination test, germplasms cannot be screened for salt tolerance. The accessions, which exhibited higher values for GSTI, appeared to be medium in performance under saline conditions (Ashraf *et al.*, 2006). Although the decrease of GP was a common trend, the GP of seeds (of some accessions) enhanced by the NaCl salinity treatments. Seeds of some dhaincha accessions with relatively lower germination percentage (< 60%) in the control condition performed better in NaCl salinity treatments. The salt tolerance of plants varies with the type of salt and osmotic potential of the medium (Kayani and Rahman, 1988). Dhaincha accessions with exceptional germination behaviour

might be a topic of further studies and could be cultivated in the saline-prone areas.

Root and shoot growth were also reduced with the increase of salinity and ultimately reduction in biomass occurred. It may be due to lack of water uptake and ion toxicity which hampers the photosynthetic system. Plant height, fresh weight and biomass yield and different tolerance indices, *viz.* plant height stress tolerance index (PHSI), fresh weight stress tolerance index (FWSI), biomass stress tolerance index (BSI) and vigour index stress tolerance index (VSI), are very important indicators for any species to be adaptive in saline condition. Salinity caused a significant reduction on root

length and shoot length ultimately seedling height and biomass of dhaincha accessions. Different dhaincha accessions responded differently for these descriptors (Table 1). At 6 dSm<sup>-1</sup>, the value of PHSI was more than 50% in 3 dhaincha accessions (Table.1). The accession #59 was the best performer with the highest PSTI value of 54.37% (Table. 1). At 12 dSm<sup>-1</sup> salinity, the value of PHSI was decreased and the largest was 34.56% also in the accession #59. However, the FWSI and BSI were responded differently. At 6 dSm<sup>-1</sup>, the FWSI value more than 50% in 4 accessions with the highest (60.78%) in accession #59 and the BSI more than 50% in only 5 accessions with the largest (69.16%) in accession #59 (Table 1). At 12dSm<sup>-1</sup> salinity, the highest value of FWSI (51.95%) and BSI (55.95%) was observed in accession #59. The maximum values of VSI were found in accession #59 in both 6 and 12 dSm<sup>-1</sup> salinity (Table 1). Although accession #59 was the best performer and accession #61 was the worst for all the descriptors studied, other accessions did not occupy any specific position. Their positions were variable depending upon descriptors (Figure 1, Table 1). Similar observations of reduction of growth under salinity were observed for several plant species such as *S. grandiflora* (Chavan and Karadge, 1986), cowpea (Islam *et al.*, 2019), and *S. sesban* (Daba *et al.*, 2020). Salinity affects the seedling growth of plants by slow or less mobilization of reserve foods, suspending the cell division, enlarging and injuring hypocotyls (Kaymakanova, 2009). Reduction in FW at high salinity might be due to poor absorption of water from the growth medium due to physiological drought (Munns and Tester, 2008). Decrease of growth in root and shoot can be related to NaCl toxicity and disproportion in nutrient absorption by seedlings. Salt stress caused a decrease in the fresh weight and dry weight of shoot and root (Nasri *et al.*, 2015). In dhaincha accessions, the maximum value of BSTI was higher than that of FWSI (Table 1). This might be due to salinity caused a clear reduction in tissues water content (Nasri *et al.*, 2015). The positive correlation between plant height and biomass accumulation suggest that seedling growth could be a reliable and efficient method for screening dhaincha germplasm for salt tolerance (Ashraf *et al.*, 2008). The accessions having higher biomass stress tolerance index and plant height stress tolerance index produced a higher yield. It may be concluded that salinity adversely affects the germination behaviour and seedling growth descriptors of dhaincha genotypes. Some dhaincha accessions showed resistance to salinity at the germination stage. Based on germination behaviour and biomass yield at 12 dSm<sup>-1</sup> salinity, five accessions, viz. #5, 7, 59, 82 and 85, were selected for further studies.

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