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RESEARCH ARTICLE

Hydroponic and *in vitro* screening of wheat varieties for salt-tolerance

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Abstract

Salt-tolerant wheat cultivars are essential for sustainable wheat production and global food security. The present study aimed to establish a reliable screening protocol as well as successfully isolated the potential salt-tolerant wheat varieties by discerning morpho-physiological parameters with multivariate analysis. Seventeen wheat varieties were evaluated at 1, 12, 15 and 18 dSm^{-1} salinity levels in a hydroponic culture system at the seedling stage. Moreover, *in vitro* callusing responses of four selected varieties were determined to clarify the salt tolerance capability at 0, 9, 12 and 15 dSm^{-1} salt treatments. The seedling growth of most wheat varieties was highly interrupted and reduced by the toxic effects of salinity, however, some varieties such as *BARI Gom-32*, *BARI Gom-33*, *BARI Gom-31*, *BARI Gom-30*, and *BARI Gom-28* showed the lowest reduction under all salinity stress conditions. The total salt tolerance index (TSTI) showed that the cultivar *BARI Gom-33* was the most salt-tolerant followed by *BARI Gom-32* and *BARI Gom-30* whereas *BARI Gom-25* was identified as the most sensitive. These results were strongly supported by the principal component analysis (PCA) and Ward's Methods Euclidean based clustering. *In vitro* results revealed that the lowest reduction of callus induction was recorded in *BARI Gom-33* which might show the greatest tolerance to salinity by improving morpho-physiological characteristics against salt stress. Therefore, the identified genotypes might be employed as donor parents to develop salt-tolerant and high-yielding cultivars in the wheat breeding program.

Keywords

Callogenesis, PCA, salinity, seedlings growth, *Triticum aestivum*, TSTI

Introduction

Salinity is a common environmental condition that inhibits plant growth and development, resulting in reduced agricultural productivity (1, 2). Salinity induced desertification occurs in arid and semi-arid areas across the world when agricultural land is lost over time (3). In saline soils, sodium ions are numerous, with chloride and sulphate anions dominating, resulting in increased ionic conductivity (>4 dSm^{-1}) (4). Around 800 million hectares or more than 6% of the world's lands are affected by salinity (5), wreaking havoc on more than 20% of present agriculture (6). Salinity affects approximately 20% of net agricultural land in Bangladesh's coastal area at varying levels (7). The amount of saline impacted areas is gradually growing due to the consequences of rising sea levels, coastal erosion, increased tidal influence and continuous reduction of river flow, especially during dry seasons

(8, 9). Saline conditions create an acute osmotic pressure and subsequent toxicity as a result of the ion concentration (10). Abiotic stressors such as salinity are responsible for more than half of the yield loss (11). Moreover, in the next 30 years, the total population is estimated to increase to 9.6 billion people (12). Thus, the world's food production might be enhanced by more than 70% by 2050 to ensure food and nutritional safety.

Wheat (*Triticum aestivum* L.), a member of the Poaceae family, became one of the most important food crops all over the world (13). It ranks second in position in terms of grain production in Bangladesh next to rice and is usually cultivated in the winter season (14). It is cultivated on approximately 17% of the arable lands and contributes globally to about 30% of total grain cereals production (15). Wheat is an important source of calories and proteins for people and has an important role in economic growth, food security and supply and human nutrition (16). Bangladesh is able to produce around 1.0 million tons of wheat from 0.40 million hectares of cultivable lands, compared to the national requirement of 3.0-3.5 million tons which necessitates imports of roughly 2.0-2.5 million tons of wheat grain to compensate for our demand every year (17). Wheat cultivation must be strengthened to feed Bangladesh's increasing population. Furthermore, there is no method to increase agricultural lands to improve agricultural production in Bangladesh because it is one of the world's most populated countries. Salinity-affected coastlines in Bangladesh's south might provide an alternative supply of agricultural land that is now barren. As a result, a critical necessity is to produce salt-tolerant genotypes that can maintain maximum production levels to meet the never-ending demand for food (18). Producing salt-tolerant and high-yielding cultivars may be the best method to develop these areas (19).

Wheat plants' reaction to salinity stress is a multifaceted phenomenon that affects morphological and physiological features. Salt stress also hinders plants' growth by causing water deficiencies, oxidative stress, ion instability and nutrient and metabolic imbalance (20-22). Plant responses to ameliorate the harmful effects of salt include ion exclusion, the deposition of organic osmolytes, antioxidant production and changes in mineral and nutrient uptake (22, 23). As a result, having a full understanding of several morpho-physiological mechanisms of plant response to salinity stress is required to select salt stress-tolerant genotypes. Phenotypic parameters such as seedling development have been used directly to select salt stress-tolerant cultivars (24). Screening at the field level is difficult due to soil heterogeneity, climatic factors, and other environmental factors that may influence the plants' physiological processes. Hence, screening in a laboratory environment is necessary such as the hydroponic system is considered to be preferable to field testing (25). Besides, the seedling stage of wheat crop development is usually the most sensitive (26-28). Several investigations were previously made on the screening of salt-tolerant wheat genotypes under hydroponic culture conditions at the seedling stage (14, 19, 28, 29).

In vitro culture, a tissue culture approach is frequently identified as a salt-tolerant crop and was employed to find salt-tolerant cell lines of rice (30, 31), barley (32), potato (7, 33), sugarcane (34) and tomato (35). Key criteria for success in the *in vitro* selection technique for salt tolerance are believed to be a large variety of cells, easy salt administration and an *in vitro* selection approach for the tolerant cell. Apart from this, when attempting to increase wheat's salinity tolerance, the reaction of wheat callus to salt stress is an essential issue to consider. Although several reports have been described worldwide, this is the first study from Bangladesh on *in vitro* screening for salt tolerance in wheat. We established a reliable screening approach as the morphological study combined with the *in vitro* technique to be fruitful to isolate the candidate wheat genotypes for salt tolerance. This research aimed to assess morphological characters in seedlings to find prospective salt-tolerant wheat cultivars. Moreover, *in vitro* callusing responses of selected varieties were also observed to clarify salt tolerance capability at the cellular level.

Materials and Methods

Phenotyping wheat seedlings for salinity tolerance

Plant materials and experimental site

The experimental plant materials were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur (Table 1). The research was carried out at the Glasshouse of the Plant Breeding Division, Bangla-

Table 1. List of 17 wheat genotypes utilized in the morphological investigation

Sl. No.	Variety	Released year	Feature	Pedigree
1	BARI Gom-20	1998	HYV	TURACO/CHIL
2	BARI Gom-21	2000	HYV	MRNG/BUC//BLO/PVN/3/PJB-81
3	BARI Gom-22	2005	HYV	KAN/6/COQ/F61.70//CNDR/3/OLN/4/ PHO/5/ MRNG/ ALDAN//CNO
4	BARI Gom-23	2005	HYV	NL297*2/LR25
5	BARI Gom-24	2005	HYV	G-162/BL-1316//NL-297
6	BARI Gom-25	2010	HYV	ZSH 12/HLB 19//2*NL297
7	BARI Gom-26	2010	HYV	ICTAL123/3/RAWAL87//VEE/HD2285
8	BARI Gom-27	2012	HYV	Waxwing*2/Vivitsi
9	BARI Gom-28*	2012	HYV	CHIL/2*STAR/4/BOW/CROW//BUC/ PVN/3/2*VEE#10
10	BARI Gom-29	2014	HYV	-
11	BARI Gom-30*	2014	HYV	-
12	BARI Gom-31	2017	HYV	-
13	BARI Gom-32*	2017	HYV	-
14	BARI Gom-33*	2017	HYV	-
15	BINA Gom-1	2016	HYV	-
16	BWMRI-1	2019	HYV	-
17	BWMRI-2	2020	HYV	-

HYV = High Yielding Variety
Adopted from Haque et al 2020 and Hasanuzzaman et al 2021
The genotypes with asterisks (*) were used in *in vitro* study

desh Institute of Nuclear Agriculture (BINA) in Mymensingh. The experiment was run in a dual-factor, completely randomized design with three replications.

Hydroponic system setup and salt treatment

The testing was carried out in a glasshouse at temperatures of 30/20 °C (day/night) and relative humidity above 50% throughout the day. Rectangular plastic trays (4×30×35 cm) were selected for the experiment. Each tray had a capacity of 12 L in which the exterior surface was painted to discourage algae development by creating a dark condition inside the tray. Nylon net and rectangle-shaped Styrofoam with 10×10 holes were inserted at the start of each trial. Peters water-soluble fertilizer containing Urea, TSP, MP (20:20:20) and ferrous sulphate ($FeSO_4.7H_2O$) were blended in plastic containers as a nutrient solution.

Per litre of tap water, 1.0 g peter fertilizer and 200 mg ferrous sulphate were mixed together to form the nutrient solution. A pH meter was used to keep the pH between 5.1 and 5.3 (Hanna HI 2211, Nasfalau, Romania) using HCl and NaOH if necessary. Seeds were heat-treated for two days at 48 °C in a convection oven before being soaked in tap water for 24 hrs to break the dormancy. Before being placed on Petri Dishes and germinating for 48 hrs at 30 °C, the seeds were washed and rinsed in tap water. For 3 days, ten germinated seeds of each type were placed on the seedling float with only water and pre-folding paper. Then, the nutrient solution was added to the 6 day aged pre-seedlings and kept for 4 days for seedling establishment. Salt treatment was applied at 10 days old seedling (2-3 leaf stage) by adding crude seashore salt containing sodium chloride with the nutrient solution in a plastic container. The electrical conductivity was measured using an EC meter (WTW 2FD45C, Weilheim, Germany) and adjusted the solution at 0, 12, 15 and 18 dSm^{-1} salinity treatments. The electrical conductivity was measured using an EC meter (WTW 2FD45C, Weilheim, Germany) and salinity treatments (12 dSm^{-1}, 15 dSm^{-1} and 18 dSm^{-1}) were adjusted accordingly.

Evaluation of salt stress symptoms and data collection

To detect the optical indications of salt stress, the IRRI's modified Standard Evaluation System (SES) was applied (Table 2). This grading method identified tolerant (T), moderately tolerant (MT), and susceptible (S) genotypes.

Table 2. Modified SES of visual salt injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Almost normal growth, but leaf tips of few leaves whitish and rolled	tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry: some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

The first scoring was done 15 days after salinization and the final score was done 21 days afterwards. Three samples of each genotype were obtained from each replication after 21 days of salinization and data on plant shoot length (SL), root length (RL), shoot fresh and dry weight (SFW, SDW), and root fresh and dry weight (RFW, RDW) were recorded. The fresh weight of the seedling was taken using electric balance. After recording, the fresh mass of each seedling was dried for 3 days using an oven and the dry weight of the seedling was recorded using electric balance.

Salt tolerance indices (STI)

The sum of cumulative salt tolerance index (CSTI) under-tested salinity levels was used to calculate a genotype's total salt tolerance index (TSTI).

$$TSTI = CSTI_{(12\ dSm\text{-}1)} + CSTI_{(15\ dSm\text{-}1)} + CSTI_{(18\ dSm\text{-}1)}$$

Under 12 dSm^{-1} salt stress, CSTI (12 dSm^{-1}) was determined as the sum of every individual salt tolerance index (ISTI) of each parameter. Here, ISTI was computed by dividing the value of a parameter under stress for a certain genotype by the value of the same parameter under the control condition at a specific salinity level.

$CSTI_{(12\ dSm\text{-}1)}$ = Σ (value of the parameter at low salinity divided by the value of the parameter at the control)

Similarly, the $CSTI_{(15\ dSm\text{-}1)}$ and $CSTI_{(18\ dSm\text{-}1)}$ were calculated using ISTI.

Statistical analysis

The data were statistically evaluated using analysis of variance (ANOVA) and shown in Duncan's multiple range test at the 5% and 1% probability levels using MSTAT-C software (36). Microsoft Excel was used to calculate the salinity tolerance indices (STI). To demonstrate the correlations among analyzed genotypes based on recorded attributes, PCA biplots were generated separately for the stressed and controlled circumstances using XLSTAT.

In vitro callusing for salt-tolerant wheat

Plant material and explants sterilization

In vitro study on assessing the efficient callusing of three selected wheat cultivars (four most tolerant genotypes based on morphological study) was conducted in the Plant Tissue Culture Laboratory of Bangladesh Agricultural University (BAU) in Mymensingh. The seeds were washed three times with autoclaved water and with 70% ethanol for 1 min. Then the mature embryos were rinsed with autoclaved distilled water and afterwards treated with 10% sodium hypochlorite solution for 15 min. The experimental seeds were finally rinsed with 0.1% $HgCl_2$ for 1 min), washed again with water and dried on autoclaved filter papers in sterilized Petri plates (37).

Salt treatment and *in vitro* callus induction

The seeds of each cultivar were surface sterilized and aseptically packed with forceps before being put on an MS-based callus induction medium (MS + 30 gL^{-1} sucrose + 3 gL^{-1} 2, 4-D + 6 gL^{-1} gelrite). Before sterilizing by autoclave at 121°C and 15 $lbs/inch^2$ for 25 min, the pH of the medium was adjusted to 5.7-5.8. Four concentrations of NaCl (1, 9, 12 and 15 dSm^{-1}) are added to the optimized callus induc-

tion medium to investigate the effect and sensitivity of NaCl salt on wheat callus growth and development. Explants were cultured for 14 days at 25±2 °C in dark intervals.

Experimental Design, Data collection and statistical analysis

A completely randomized design was used, with three replications per genotype treatment. The callus induction % was measured as (the number of calli induction/ the number of seeds inoculated) X 100. The data were analyzed utilizing the MSTATC statistical software program at different statistical significance levels using ANOVA.

Results and Discussion

Morphological screening for salt tolerance in wheat

Visual scoring of salt injury at the seedling stage

In a hydroponic system, wheat cultivars were evaluated for salinity tolerance using the modified standard evaluating score (SES) of the salt damage approach. Out of 17 wheat genotypes, 7 genotypes (*BWMRI-2*, *BARI Gom-21*, *BARI Gom-24*, *BARI Gom-32*, *BARI Gom-29*, *BARI Gom-25* and *BINA Gom-1*) were found as tolerant and 6 genotypes (*BWMRI-1*, *BARI Gom-20*, *BARI Gom-30*, *BARI Gom-33*, *BARI Gom-27* and *BARI Gom-31*) were moderately tolerant. On the other hand, the rest of the 4 genotypes (*BARI Gom-22*, *BARI Gom-23*, *BARI Gom-26* and *BARI Gom-28*) genotypes were found as susceptible (Table 3). Salinity stress had a deleterious impact on leaf life in addition to shoot development, which is consistent with earlier findings of (38). In all wheat cultivars, leaf mortality increased as salt stress increased during the early seedling development phase (28). A higher concentration of sodium ions in the root area causes the suppression of simplistic xylem loading and results in stunted shoots in plants (39). Leaf rolling, drying and the brownish and yellowish hue of the leaf tip produced a loss in shoot length in all genotypes under high-salinity conditions, however, tolerance genotypes retain a lower reduction by implementing defense mechanisms based on SES scoring susceptibility according to respective SES scoring levels (Table 3). Researchers conducted screening experiments and found substantial variations in damage rates across genotypes under salt stress (10, 40).

Table 3. Performance of wheat cultivars grown in a hydroponics system under salinized conditions (EC 12, 15, and 15 dSm^{-1})

Variety	SES score	Salinity tolerance
BARI Gom-21, BARI Gom-24, Gom-32, BARI Gom-29, BARI Gom-25, BINA Gom-1, BWMRI-2	3	Tolerant
BARI Gom-20, BARI Gom-30, BARI Gom-33, BARI Gom-27, BARI Gom-31, BWMRI-1	5	Moderately tolerant
BARI Gom-22, BARI Gom-23, BARI Gom-26,BARI Gom-28	7	Susceptible

Growth performance of wheat seedlings in response to saline

Salinity has a detrimental effect on plant growth and development (e.g. wheat) by reducing shoot and root biomass, height and root length (40). SL, RL, SFW, RFW, SDW and RDW growth parameters of wheat plants were studied under control, 12, 15 and 18 dSm^{-1} salt-stress conditions (Table 4). SL and RL were significantly higher at non-stressed (control), as compared to 12, 15 and 18 dSm^{-1} salt-stressed conditions. Under 15 and 18 dSm^{-1} salinity stress, SL was reduced significantly for all wheat genotypes, ranging from 34.78 cm (*BARI Gom-27*) and 28.36 cm (*BARI Gom-31*) to 27.23 cm (*BARI Gom-23*) and 23.47 cm (*BARI Gom-26*) with an average SL of 30.66 cm and 26.37 cm, respectively (Table 4). The maximum RL was found in *BARI Gom-32* at 15 and 18 dSm^{-1} salt stress, whereas the least was observed in *BARI Gom-26* under 12 and 18 dSm^{-1}. However, the average SL was gradually reduced at 12 dSm^{-1} (18.65 cm), 15 dSm^{-1} (17.52 cm), and 18 dSm^{-1} (17.52 cm) salinity conditions (Table 4). Wheat seedlings had the greatest SFW, RFW, SDW and RDW values in the control condition, and these features declined as saline levels increased. SFW ranged from 1.24 g (*BWMRI-2*) to .0.43 g (*BINA Gom-1*), with an average of 0.89 g 12 dSm^{-1} salinity stress. Under 15 dSm^{-1} salt stress, SFW and RFW ranged from ranging from 1.19 g (*BWMRI-1*) and 0.42 g (*BWMRI-2*) to 0.33 g (*BARI Gom-25*) and 0.08 g (*BINA Gom-1*), with an average SFW of 0.75 g and 0.25 g respectively (Table 4). SDW reduced from 0.31 g (*BARI Gom-32*) to 0.12 g (*BARI Gom-22*) with an average of 0.19 g at 18 dSm^{-1} salinity condition (Table 4). The highest RDW was determined in *BWMRI-2* at 12 and 15 dSm^{-1} stress conditions whereas the lowest was found in *BINA Gom-1* under 15 and 18 dSm^{-1} salt stress (Table 4). Photographic representation of *BARI Gom-33* (A) and *BARI Gom-25* (B) under different salt stress is shown in Fig. 1.

In our study, salt stress affected shoot growth, root elongation and dry mass production in seedlings, with salt -susceptible genotypes suffering more than salt-tolerant genotypes (Table 4). An excess of Na^+ in the soil around the roots may cause an imbalance in nutrient absorption, resulting in reduced plant development. In all wheat genotypes, salt stresses were linked to a significant decrease in shoot and root length as well as shoot and root biomass, implying a decrease in photosynthesis and an increase in respiration rate in growing plants (Table 4). At 12 dSm^{-1} salinity stress, some cultivars e.g. *BARI Gom-33*, *BARI Gom-32*, *BARI Gom-30*, *BARI Gom-22*, *BARI Gom-28* and *BWMRI-2* showed less reduction in their morphological traits. Moreover, several varieties for example *BARI Gom-28*, *BARI Gom-26*, *BARI Gom-33*, *BARI Gom-32* and *BARI Gom-2* exhibited less reduction based on morphological characteristics at 15 dSm^{-1} salt stress conditions. Moreover, few wheat varieties such as *BARI Gom-32*, *BARI Gom-33*, *BARI Gom-31*, *BARI Gom-30* and *BARI Gom-28* displayed the least decrement of shoot and root related parameters at 18 dSm^{-1} conditions. Saline stress lowered the fresh weights of roots and shoots in rice, wheat and other plants, according to a previous study (28, 41-46).

Table 4. Growth parameters of 17 wheat varieties measured 21 days after salinization (EC 12, 15 and 18 dSm^{-1}) at seedling stage.

Genotype	Treatment	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
BARI Gom-20	Control	37.33	14.57	1.03	0.37	0.32	0.12
	12 dSm^{-1}	30.95	17.57	0.74	0.39	0.27	0.11
	15 dSm^{-1}	31.12	18.64	0.82	0.32	0.33	0.09
	18 dSm^{-1}	26.83	13.57	0.30	0.31	0.15	0.06
BARI Gom-21	Control	34.5	17.4	1.37	0.38	0.42	0.11
	12 dSm^{-1}	28.69	19.7	0.83	0.35	0.21	0.10
	15 dSm^{-1}	29.38	16.57	0.82	0.28	0.30	0.08
	18 dSm^{-1}	26.58	14.85	0.43	0.23	0.19	0.07
BARI Gom-22	Control	38.73	15.9	1.29	0.24	0.49	0.07
	12 dSm^{-1}	35.6	21.78	1.04	0.34	0.32	0.09
	15 dSm^{-1}	31.33	19.69	0.66	0.21	0.27	0.06
	18 dSm^{-1}	27.78	16.55	0.22	0.13	0.12	0.05
BARI Gom-23	Control	35.5	16.3	1.34	0.54	0.48	0.13
	12 dSm^{-1}	30.92	20.86	0.85	0.39	0.33	0.10
	15 dSm^{-1}	27.36	17.85	0.67	0.33	0.22	0.08
	18 dSm^{-1}	25.95	13.55	0.28	0.15	0.14	0.05
BARI Gom-24	Control	40.8	24.5	1.44	0.43	0.48	0.16
	12 dSm^{-1}	32.89	21.19	1.03	0.55	0.38	0.15
	15 dSm^{-1}	30.67	19.39	0.80	0.32	0.27	0.10
	18 dSm^{-1}	26.08	16.5	0.33	0.26	0.22	0.08
BARI Gom-25	Control	37.97	15.43	1.72	0.40	0.61	0.14
	12 dSm^{-1}	28.08	19.94	0.53	0.28	0.30	0.09
	15 dSm^{-1}	28.5	16.84	0.33	0.29	0.25	0.09
	18 dSm^{-1}	24.45	12.93	0.30	0.14	0.17	0.05
BARI Gom-26	Control	31.03	11.47	0.93	0.32	0.34	0.09
	12 dSm^{-1}	29.8	14.55	0.81	0.21	0.36	0.07
	15 dSm^{-1}	30.48	18.87	0.59	0.24	0.28	0.07
	18 dSm^{-1}	23.47	12.2	0.19	0.20	0.15	0.05
BARI Gom-27	Control	40.13	12.57	1.26	0.31	0.45	0.09
	12 dSm^{-1}	33.58	17.92	0.80	0.25	0.36	0.08
	15 dSm^{-1}	34.78	14.75	0.80	0.24	0.37	0.08
	18 dSm^{-1}	27.85	12.86	0.34	0.12	0.17	0.05
BARI Gom-28	Control	35.53	13.23	1.30	0.29	0.48	0.06
	12 dSm^{-1}	33.35	18.62	0.99	0.26	0.46	0.09
	15 dSm^{-1}	31.58	16.72	0.82	0.24	0.40	0.08
	18 dSm^{-1}	23.85	15	0.24	0.16	0.17	0.06
BARI Gom-29	Control	34.57	13.27	1.32	0.30	0.50	0.10
	12 dSm^{-1}	36.35	19.39	0.82	0.21	0.32	0.06
	15 dSm^{-1}	32.58	17.28	0.94	0.22	0.35	0.07
	18 dSm^{-1}	28.72	15.22	0.32	0.14	0.17	0.06
BARI Gom-30	Control	37.93	13.03	1.21	0.24	0.50	0.07
	12 dSm^{-1}	36.17	21.11	1.10	0.24	0.47	0.07
	15 dSm^{-1}	33.05	18.45	0.71	0.18	0.38	0.06
	18 dSm^{-1}	27.45	15.22	0.48	0.14	0.26	0.06
BARI Gom-31	Control	36	11.27	1.04	0.22	0.46	0.07
	12 dSm^{-1}	31.05	16.82	0.65	0.18	0.33	0.06
	15 dSm^{-1}	29.4	17.65	0.58	0.18	0.27	0.06
	18 dSm^{-1}	28.36	15.85	0.35	0.14	0.23	0.05
BARI Gom-32	Control	32.77	11.6	1.86	0.24	0.45	0.07
	12 dSm^{-1}	35.67	18.58	1.14	0.25	0.51	0.08
	15 dSm^{-1}	30.24	20.5	0.74	0.17	0.37	0.07
	18 dSm^{-1}	26.88	18.49	0.55	0.19	0.31	0.08

BARI Gom-33	Control	35.3	17.17	1.18	0.20	0.44	0.06
	12 dSm^{-1}	33.47	19.1	1.13	0.27	0.48	0.10
	15 dSm^{-1}	30.85	14.92	0.87	0.19	0.40	0.08
	18 dSm^{-1}	28.28	18.02	0.60	0.17	0.28	0.07
BINA Gom-1	Control	38.33	14.8	1.37	0.26	0.34	0.06
	12 dSm^{-1}	29.22	14.74	0.43	0.22	0.29	0.06
	15 dSm^{-1}	29.13	14.6	0.46	0.08	0.26	0.06
	18 dSm^{-1}	25.3	13.65	0.30	0.09	0.17	0.04
BWMRI-1	Control	35.00	14.10	1.60	0.30	0.47	0.14
	12 dSm^{-1}	30.64	15.85	1.07	0.35	0.40	0.11
	15 dSm^{-1}	29.32	17.3	1.19	0.35	0.39	0.09
	18 dSm^{-1}	27.03	12.95	0.48	0.22	0.22	0.08
BWMRI-2	Control	38.13	14.1	1.80	0.42	0.50	0.13
	12 dSm^{-1}	32.65	19.35	1.27	0.54	0.51	0.15
	15 dSm^{-1}	31.4	17.78	1.07	0.42	0.34	0.11
	18 dSm^{-1}	23.51	13.33	0.44	0.22	0.18	0.07
$LSD_{0.05}$		4.01	2.04	0.19	0.13	0.03	0.04
Level of sign.		**	**	**	*	**	**
CV%		6.39	6.23	11.08	22.96	4.43	20.28

Estimation of genetic parameters

For all growth contributing parameters, Table 5 showed genotypic and phenotypic variances, heritability, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genetic advance (GA) and genetic advance as a % of mean GA. Among all traits studied in the present investigation, RFW exhibited high estimates of PCV and GCV (28.54% and 25.88%) whereas the lowest was found in SL (5.22% and 3.83%) (Table 5). The heritability of the characteristics evaluated in this study ranged from 45.08% to 82.22%, indicating moderate to extremely high heredity. The RFW (82.22%) had the maximum heritability, whilst RL had the lowest heritability (45.08%) (Table 5). The GA was highest for SL (1.82), followed by RL (1.67), and lowest for RDW among the growth contributing traits (0.03) (Table 5). The RFW (48.34) had the largest genetic progress as a % of mean, followed by RDW (39.19), while SL had the least (5.79) (Table 5).

Screening of salt-tolerant wheat varieties based on TSTI, PCA and Clustering

In the past, salt tolerance indices were employed to identify genotypes and characteristics with substantial salinity tolerance (47, 48). We classified wheat genotypes into salt-tolerant and salt-susceptible using the total salt tolerance index (TSTI) established by (49). The experimented genotypes were divided into three response groups based on TSTI values of measured shoot and root growth parameters (Table 6). Among the tested genotypes, six (35.29%) were recommended as tolerant, six genotypes (35.29%) were recognized as moderate tolerant and the rest of the genotypes (29.41%) were recommended as susceptible (Table 6). TSTI measurements ranged from the value of 10.57 for *BARI Gom-25*, which was recognized as susceptible to the value of 17.49 (*BARI Gom-33*), which was designated as tolerant. The genotypes included in the tolerant class are *BARI Gom-33*, *BARI Gom-32*, *BARI Gom-30*, *BARI Gom-28*, *BARI Gom-31*, and *BARI Gom-26*. The genotypes included in the susceptible class are *BARI Gom*-25, *BARI Gom-23, BINA Gom-1, BARI Gom-24,* and *BARI Gom-21*. The genotypes included in the moderate tolerant class are *BWMRI-2*, *BARI Gom-20*, *BWMRI-1*, *BARI Gom-22*, *BARI Gom-27*, and *BARI Gom-29*. Previously, STI was successfully utilized to differentiate between tolerant and susceptible

Fig. 1. Photographic representation of (A) *BARI Gom-33* and (B) *BARI Gom-25* under different salt stress. Photographs are taken after 21 days of salinization.

Table 5. Variability, heritability (h2b), genetic advance (GA) and GA in percent of mean for six growth and its related characters of wheat.

Characters	Phenotypic variance (d^2p)	Genotypic variance (d^2g)	Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA	GA (%)
Shoot length (cm)	2.70	1.45	31.44	5.22	3.83	53.83	1.82	5.79
Root length (cm)	3.22	1.45	16.42	10.93	7.34	45.08	1.67	10.15
Shoot fresh weight (g)	0.0295	0.0166	0.841	20.43	15.34	56.36	0.20	23.72
Root fresh weight (g)	0.0056	0.0046	0.263	28.54	25.88	82.22	0.13	48.34
Shoot dry weight (g)	0.0031	0.0016	0.333	16.75	11.96	51.00	0.06	17.60
Root dry weight (g)	0.0004	0.0003	0.080	24.57	21.62	77.42	0.03	39.19

Table 6. Screening of 17 wheat varieties based on total salt tolerance index (TSTI).

Susceptible (10.57-13.00)	Moderate Tolerant (13.01-14.75)	Tolerant (14.76-17.49)
Bari Gom-25 (10.57)	Bari Gom-29 (13.63)	Bari Gom-26 (14.76)
Bari Gom-23 (11.64)	Bari Gom-27 (13.64)	Bari Gom-31 (14.86)
BINA-1 (11.99)	Bari Gom-22 (14.55)	Bari Gom-28 (15.82)
Bari Gom-24 (12.44)	BWMRI-1 (14.59)	Bari Gom-30 (15.83)
Bari Gom-21 (13.00)	Bari Gom-20 (14.70)	Bari Gom-32 (17.33)
	BWMRI-2 (14.73)	Bari Gom-33 (17.49)
5 (29.41%)	6 (35.29%)	6 (35.39%)

wheat genotypes (49).

Following Ward's Methods Euclidean classification, seventeen wheat genotypes were divided into four groups based on their diversity for salinity tolerance (Fig. 2).

The dendrogram showed that cluster I include *BWMRI-1*, *BWMRI-2* and *BARI Gom-24*; cluster II includes *BARI Gom-20*, *BARI Gom-21*, *BARI Gom-23* and *BARI Gom-25*; cluster III includes *BARI Gom-22*, *BARI Gom-27*, *BARI Gom-28*, *BARI Gom-29*, *BARI Gom-30*, *BARI Gom-32* and *BARI Gom-33*; cluster IV includes *BARI Gom-26*, *BARI Gom-31* and *BINA Gom-1*. Based on the TSTI results, the genotypes of Cluster II, Cluster III are susceptible and tolerant respectively whereas clusters I and IV contain moderately tolerant genotypes. Bio plot model of principal component analysis (PCA) is shown in Fig. 3. According to the PCA it is clear that the genotypes *BARI Gom-30*, *BARI Gom-32* and *BARI Gom-33* lie in the tolerant (PC1 + axis and PC2 - axis) plot so they are positioned in the salt-tolerant group. Likewise, varieties *BARI Gom-22*, *BARI Gom-27*, *BARI Gom-28*, *BARI Gom-29* and *BARI Gom-31* are positioned in the tolerant (PC1 – axis and PC2 – axis) plot so they are moderately salt-tolerant. On the other hand, genotypes *BWMRI-1*, *BWMRI-2*, *BARI Gom-21*, *BARI Gom-23* and *BARI Gom-24* are positioned in the susceptible (PC1 + axis and PC2 + axis) plots and they are susceptible. Besides, *BARI Gom-20*, *BARI Gom-25*, *BARI Gom-26* and *BINA Gom-1* are positioned in the

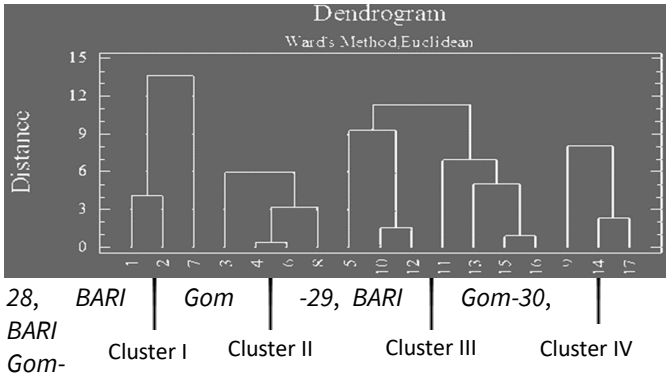

Fig. 2. Dendrogram designed on summary data and differentiation among 17 wheat varieties of landraces following Ward's approach.

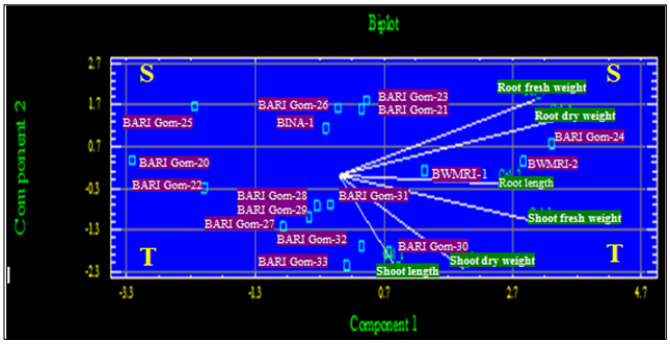

Fig. 3. Bioplot model of PCA component analysis (S= Susceptible T= Tolerant).

Table 7. *In vitro* callus formation of wheat as affected by different salt concentrations.

Genotypes	Treatment	Callus induction (%)	Reduction (%)	Total Reduction (%)
BARI Gom-28	Control	55.67	-	65.87
	9 dSm^{-1}	39.00	29.94	
	12 dSm^{-1}	11.67	79.04	
	15 dSm^{-1}	6.33	88.63	
BARI Gom-30	Control	45.67	-	55.48
	9 dSm^{-1}	35.67	21.90	
	12 dSm^{-1}	20.00	56.21	
	15 dSm^{-1}	7.000	88.33	
BARI Gom-32	Control	48.33	-	56.32
	9 dSm^{-1}	37.00	23.44	
	12 dSm^{-1}	21.00	56.55	
	15 dSm^{-1}	5.33	88.97	
BARI Gom-33	Control	75.00	-	53.78
	9 dSm^{-1}	58.00	22.66	
	12 dSm^{-1}	38.67	48.44	
	15 dSm^{-1}	7.33	90.23	
$LSD_{0.05}$		5.64		
Level of sign.		**		
CV%		9.80		

susceptible (PC1 – axis and PC2 + axis) plots so they are moderately susceptible (Fig. 2). Plant biomass and shoot traits may be better descriptors of salt tolerance whereas root length exhibited no significant link with salinity tolerance (40). While we discovered that specific shoot characteristics and biomass must be critical. These findings are in agreement with those many studies (19, 38, 49, 50).

In vitro response of wheat callogenesis under salinity treatment

In vitro selection utilizing tissue culture methods has been generally acknowledged as a low-cost and effective tool for creating salt-tolerant crops during the last two decades. *In vitro* responses of callus induction in four selected wheat genotypes against salt stress viz., 0, 9, 12 and 15 dSm^{-1} were observed in the present study. Callus induction % was significantly higher at non-stressed (control) conditions as compared to salinity stress (Table 7) Callus induction ranged from 35.67% (*BARI Gom-30*) to 58.00% (*BARI Gom-33*) at 9 dSm^{-1} salinity stress. Moreover, callus induction ranged from 11.67% (*BARI Gom-28*) to 38.67% (*BARI Gom-33*) and from 5.33% (*BARI Gom-32*) to 7.33% (*BARI Gom-33*) at 12 and 15 dSm^{-1} salt treatments respectively. According to the total reduction % of callus induction, the lowest reduction of callus induction was recorded in *BARI Gom-33* (53.78%) whereas the highest reduction was found in *BARI Gom-28* (65.67%) (Table 7). Photographic representation of *in vitro* callus induction callus in *BARI Gom-33* at different salt stress conditions is shown in Fig. 4. This study revealed that the callus induction percentage, in all wheat genotypes was significantly reduced in all 3 salt stress conditions than in control conditions. Similar

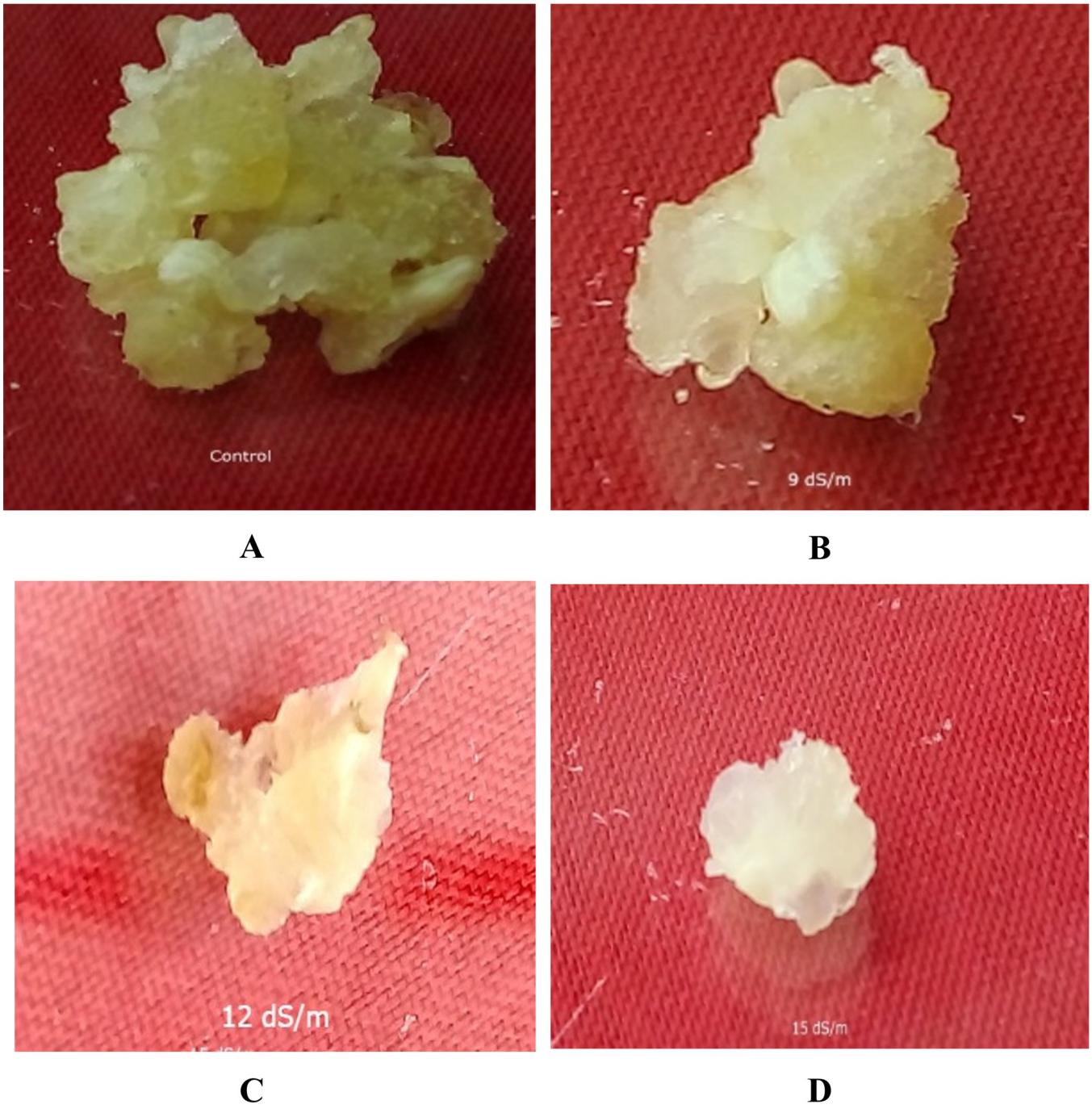

Fig. 4. Photographic representation of *in vitro* callus induction in *BARI Gom-33* at (A) Control (B) 9 dSm^{-1} (C) 12 dSm^{-1} (D) 15 dSm^{-1} salinity stress. Photographs are taken after 14 days of saline stress.

trends were also observed (51). According to total reduction indices, the chronologies of salt tolerance are as follows: *BARI Gom-33>BARI Gom-30>BARI Gom-32>BARI Gom-28*. The genotypes, *BARI Gom-33* showed the greatest tolerance to salinity and may develop salt tolerance mechanisms and improve their defense activities to improve morphological characteristics. The addition of NaCl to the callus culture medium enhanced callus necrosis in 5 rice genotypes (52). The results of the current study were inconsistent with the findings of the other studies on several crop plants (31, 51, 53-56). The *in vitro* results strongly supported the morphological screening and clarified the salt tolerance mechanism. This established combined screening protocol is reliable as well as cost-effective. The most salt-tolerant candidate varieties were separated successfully through morphological screening and strongly supported by the *in vitro* screening results.

Conclusion

Salt stress had a substantial influence on seedling growth and development, according to the findings of morphological research. Based on TSTI values of all recorded shoot and root-associated characteristics, wheat genotypes were divided into 3 response groups (tolerant, moderately tolerant and sensitive). The genotypes *BARI Gom-33* were shown to be the most salt-tolerant genotypes based on TSTI, PCA and Clustering. Moreover, this variety (*BARI Gom-33*) exhibited the best performance of *in vitro* callus induction under salt stress treatment and strongly supported our morphological findings. The newly screened genotypes might be employed in wheat breeding to generate salt-tolerant cultivars and the developed screening process will be effective in identifying candidate genotypes.

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Authors contributions

MSH, SY, and SNB planned and designed the experiment. MTR and MH carried out the experimental work and analyzed the experimental data. MH wrote the first draft of the paper. MSH and SNB revised and edited the manuscript.

Compliance with ethical standards

Conflict of interest: The author did not disclose any potential conflicts of interest (s).

Ethical issues: None.

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