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Evaluation of wheat cultivars for salinity tolerance at seedling stage based on morphological and molecular markers

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ABSTRACT

Abiotic stresses are the major constraints to wheat cultivation in Bangladesh. The existence of genetic diversity for salt tolerance is a prerequisite for developing salt-tolerant wheat varieties. Evaluation of wheat cultivars under salt stress at the seedling stage was carried out in the present study using morphological and molecular markers. Twenty four cultivars were tested at 0, 6, 8, 10, and 12 dS/m salt stress in a hydroponic system. Based on morphological traits eight wheat cultivars namely Sourav, Pavon, Prodip, BARI Gom-25, BARI Gom-28, Gourav, Shatabdi and Aghrani were identified as salt tolerant because they showed a lower mean value of root length, shoot length, fresh weight and dry weight reduction at 12 dS/m of salt stress ultimately indicating a higher tolerance to salinity. According to Nei's 1973, the highest value of gene diversity (0.9063) was observed in locus Xgwm577, and the lowest gene diversity value (0.8281) was observed in locus Xbarc84 with a mean value 0.4618. The PIC values ranged from moderate 0.4247 to high 0.8989. The highest PIC value was found in Xgwm577 and the lowest value was inXbarc84. Pair-wise comparison value of genetic distance (D) (Nei's, 1973) between varieties was computed from combined data of 6 markers and ranged from 0.1667 to 1. Molecular marker based grouping indicates the Sub sub-cluster IIA contained ten cultivars; six tolerant cultivars (BARI Gom-25, BARI Gom-28, Prodip, Pavon, Shotabdi, Gourav), two moderately tolerant (Sonalika, BARI Gom-23) and two susceptible cultivars. We, therefore, identified six cultivars as saline tolerant at their seedling stage that clustered together in the same group when analysed by SSR markers linked to salinity. The findings of the present study have the potential for utilization in future wheat breeding for salinity tolerance.

Introduction

By 2050 the world population will be about 9.10 billion, which will be 34% higher than that today, and we need to feed another 2.30 billion people with limited resources. Food production must need to be increased by 70%, and to meet this huge demand, cereal production should be increased to about 3 billion metric tons from 2.10 billion metric tons today (FAO, 2009). But in a dilemma, the world agriculture in the 21st century faces versatile challenges. Soil salinity directly affects plant physiology and causes a drastic reduction in crop production. The world's 25% cultivable lands are salinity affected, and the salt intrusion scenario is alarmingly increasing. Bangladesh is also not beyond this threat. In Bangladesh, the salinity affected area was 83.3 million ha in 1973, 102 million ha in 2000, and in 2009 it has reached up to 105.5 million ha and the area is being expanded with times (SRDI, 2010).

Wheat (*Triticum aestivum* L.) is a temperate cereal crop. It occupies a central place in human nutrition providing 20% of the daily protein and food calories and in terms of food security, it is the second most important food crop in the developing world after rice (Giraldo *et al*., 2019). It is mainly cultivated in the north and north-west region of Bangladesh. In spite of scope of wheat cultivation, a large area of cultivable lands in the coastal belt remains fallow due to high salinity and the sole cropping pattern in that area is single T. Aman (fallow-Aman-fallow) (Haque, 2006; Shahidullah *et al*., 2006). Wheat cultivation in the saline belt seems promising means for optimizing land utilization to supplement the food production and nutritional deficit of the ever-growing population of Bangladesh. Reclamation of saline soil is very much expensive, where salt-tolerant wheat genotype selection could be a feasible and cost-effective mean for the saline belt (Uddin *et al*., 2017).

The soil salinity may be responsible for many detrimental effects on plant growth and development at physiological and biochemical levels (Munns, 2002). In saline soils, seeds with lower osmotic potential fail to absorb water; increase the accumulation of toxic ions (Na^+ and Cl^-) and finally, there is a delay, decrease and disruption of seed germination (Ashraf and Foolad, 2005). Metabolism, physiological act and morphological features of the plant are changed by soil salinity and

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drastically reduce the growth and yield (Ashraf and Harris, 2004). Ramadan *et al*. (1986) reported that higher salinity levels in the germination media build up high osmotic pressure in the solution, which restricted the uptake of soil water required for proper germination of seed. The embryo is severely affected by higher salt concentration, which results in a delay and reduction of germination percentage of seed. Percentage of germination, length of coleoptiles, length of root and seedling growth are reduced by the detrimental effect of salinity (Lallu and Dixit, 2005; Ghannadha *et al*., 2005; Bera *et al*., 2006; Agnihotri *et al*., 2006). The varietal difference in salinity tolerance existing in crop plants has potential use through screening by exposing target traits for salt tolerance (Kingsbury *et al*., 1984). Physiological tolerance along with some agronomic traits and their relationship with salt tolerance indices could be a feasible means are considered strong enough to be a selection tool in the breeding of salt tolerant cultivars (Allakhverdiev *et al*., 2000).

Morpho-physiological traits, as described above, to evaluate the genetic diversity for salt tolerance in crop species are not sufficient to discriminate the genotypes. However, with development of molecular biology, microsatellites or SSR markers have been extensively used for genetic diversity study, genome mapping, varietal identification, etc. There are some reports of the utilization of these markers to investigate the genetic variations and QTL mapping for salt tolerance in different cultivars of wheat elsewhere in the world (Munns, 2002; Huang *et al.,* 2002; Somers *et al*., 2004; Ren *et al*., 2012; Ahmad *et al*., 2013; Batool *et al*., 2018). So far, there are no reports of the potential use of these markers to investigate genetic diversity for salinity tolerance in Bangladeshi wheat genotypes/cultivars. Therefore, with a view to expand the cultivation and to sustain the yield of wheat in the coastal belt, the present piece of work was implemented to evaluate some agronomical, physiological, and molecular markers of wheat as screening criteria against salinity condition.

Materials and Methods

Plant materials

A total of 24 wheat cultivars were used in this experiment. Twentyone cultivars were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur; two cultivars from Regional Wheat Research Center, Rajshahi and one cultivar was received from Bangladesh Institute of Nuclear Agriculture (BINA).

Seed placement for germination

The morphological study was conducted from November 2017 to May 2018 at the glasshouse and the laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The experiment was conducted in a single factor, Completely Randomize Design (CRD), with five replications. The wheat cultivars were tested under four different salinity levels (0, 6, 8, 10 and 12 dSm^{-1}) in the present research. The hydroponic system was constructed according to the IRRI standard protocol (Gregorio *et al.,* 1997) at the glasshouse to screen out the salinity tolerance seedling under salt stress.

The morphological screening was done in glasshouse conditions with day/night temperatures of 20-25ºC and at least 50% RH during the day. The volume of each tray was 12 L. Water-soluble fertilizer Peters (Urea: TSP: MP=20: 20: 20) and ferrous sulphate ($FeSO_4.7H_2O$) were used as a nutrient solution. The concentration of nutrient solution was 1.0 g peter fertilizer and 200 mg/L ferrous sulphate were mixed carefully per liter of tap water. The pH was maintained at 5.1-5.2. Salt treatment was applied at the 2-3 leaf stage. Salinization of the nutrient solution was done by adding dry NaCl. The electrical conductivity was measured using an EC meter and adjust the solution at 12dS/m, 10dS/m, 8dS/m and 6dS/m (120 mM, 100 mM, 80 mM and 60 mM). The old solution was replaced with the new one in every weak.

After 21 days of salinization, three samples of each cultivar were collected from each replication. The following data were recorded from the screening in both normal and salinized conditions following shoot length, root length, total fresh weight and total dry weight.

Total dry weight = Dry weight of (Leaves+Shoot+Root)

The % reduction of plant traits =
[(Traits in normal – Traits in saline)/Traits in normal] × 100

DNA extraction

Fresh leaves from 21-day old seedlings were used for DNA extraction following CTAB mini-prep method (IRRI, 1997). Concentrations of DNA samples were analyzed both qualitatively and quantitatively using a nanodrop spectrophotometer.

SSR marker genotyping

Six SSR markers (Huang *et al.,* 2002; Batool *et al*., 2018; Ren *et al*., 2012; Somers *et al*., 2004) were used for genotyping assays. Primer name, sequences and corresponding annealing temperatures are listed (Table 1). The total of 13 µl PCR cocktail composed of 2.0 µl genomic DNA (conc. 50 ng/ml), 1.5 µl 10X PCR buffer (Tris with 15 mM $MgCl_2$, conc. 10X), 0.75 µl dNTPs (Contains dCTP, dGTP, dTTP and dATP all in the conc. of 10 mM)), 1.0 µl forward primer (10 pM), 1.0 µl reverse primer (10 pM), 0.5 µlTaq DNA polymerase (conc. 5 U/µl) and 8.25 µl sterile deionized water was prepared.

Table 1. SSR loci, their sequences and annealing temperature

Locus name	Sequence		Annealing temperature (^{0}C)	Alleles size (bp)
Xbarc121-7A	F	ACTGATCAGCAATGTCAACTGAA	55	68-221
	R	CCGGTGTCTTTCCTAACGCTATG		
Xbarc84-3B	F	CGCATAACCGTTGGGAAGACATCTG	60	123
	R	GGTGCAACTAGAACGTACTTCCAGTC		
Xgwm132-6B	F	TACCAAATCGAAACACATCAGG	64	116-118
	R	CATATCAAGGTCTCCTTCCCC		
Xgwm577-7B	F	ATGGCATAATTTGGTGAAATTG	55	136-222
	R	TGTTTCAAGCCCAACTTCTATT		
Xbarc217-4D	F	GCG TTGTGTTGAAGGCTGAGCATCCA	55	95
	R	GCGGAGTAGCCTAACGGCGGTGGAGGAAAC		
Xgwm350-4A	F	ACCTCATCCACATGTTCTACG	55	145-197
	R	GCATGGATAGGACGCCC		

The reaction mix was preheated at 94ºC for 3 min followed by 35 cycles of 30 sec. denaturation at 94ºC, 45 sec annealing at 55ºC for Xbarc121, Xgwm577, Xbarc217 and Xgwm350 primer and 60 ºC for Xbarc84 and Xgwm132 primer and elongation at 72ºC for 2 min. After the last cycle, a final step was maintained at 72ºC for 7 min to allow complete extension of all amplified fragments followed by holding at 4ºC until electrophoresis. Visualization of amplification products was accomplished on a 8% Polyacrylamide gel in 1X TAE buffer. The Polyacrylamidegel was stained with ethidium bromide solution for 20-25 min. The stained Polyacrylamide gel was illuminated by UV-trans-illuminator and photographed for assessing the DNA profiles. Only three representative gel pictures have been given in this paper to represent allelic variation at DNA level.

Morphological and molecular data analysis

The collected data were managed in MS Excel® and analysed by MINITAB® 14, a computer-based statistical package. Molecular weights of microsatellite products were estimated with AlphaEaseFC 4 software. The major allele frequency, the number of alleles per locus, genetic diversity and polymorphism information content (PIC) values were determined using POWER MARKER version 3.23 Liu and Muse (2005). The data were export in binary format for analysis with NTSYS-PC version 2.1 using the allele frequency data from POWER MARKER (Rohlf, 2000). The genetic similarity was calculated using 0/1 matrix in SIMQUAL subprogram (Nei and Li, 1979). The resultant similarity matrix was employed to construct a dendrogram based Unweighted Pair Group Method of Arithmetic Means (UPGMA) as implemented in NTSYS-PC (version 2.1) to infer genetic relationships and phylogeny (Rohlf, 2000).

Results

Morphological screening at seedling stage

Root length

Plants were grown in a hydroponic tray for 30 days. Root length was estimated in control plants and grown under various treatments. Root length reduction was calculated from the data collected, and it was found that root length reduced due to salinity in all cultivars. However, a great variation in the root length reduction among the treatment and cultivars was found. At 6dS/m salinized condition, BARI Gom-29(8.48%), Durum (7.35%) and Kanchon (12.71%) showed the lower root length reduction while Protiva, BARI Gom-28 and Shotabdi showed the higher root length reduction percentages which were 60.61%, 43.59% and 40.12%, respectively. At 8dS/m salinized condition, Gourav, BARI Gom-25 and BARI Gom-22 showed the lower root length reduction which were 13.96%, 25.47% and 26.39%, respectively but Protiva (61.36%), BARI Gom-30(54.92%) and BINA Gom-1(54.60%) exhibited the highest root length reduction. Again at 10 dS/m salinized condition, Gourav, BARI Gom-22 and Aghrani showed the lower root length reduction which were37.75%, 34.33% and 31.60%, respectively and Kanchon (63.48%), Protiva (62.12%) and Kheri (65.01%) exhibited the highest root length reduction. However, at 12 dS/m salinized condition, Aghrani (34.17%), BARI Gom-22(34.63%) and Akbar (45.45%) showed the lower root length reduction while Kheri (69.66%), Protiva (66.67%) and Kanchon (65.48%) showed the higher root length reduction (Table 2).

Shoot length

Shoot length reduction was calculated from the collected data. It was found that shoot length reduced in all cultivars due to salinity treatment. However, like root length, there was a variation in the shoot length reduction among the cultivars under treatments. Shoot length reduction due to salinity varied significantly in all cultivars. At 6dS/m salinized condition, Kanchon (3.56%), BARI Gom-22(5.83%) and Aghrani (5.66%) showed the lower shoot length reduction percent but Akbar, Kheri and Borkot showed the higher shoot length reduction percent which were 29.15%.26.91% and 27.36%, respectively. Then at 8dS/m salinized condition, Sonalika, BARI Gom-28 and Aghrani showed the lower shoot length reduction which were15.87%,12.50 and 13.09%, respectively but Kanchon (36.29%), BARI Gom-23 (32.45%) and Kolyansona (32.17%) exhibited the highest shoot length reduction. Again at 10 dS/m salinized condition, Prodip, BARI Gom-28 and Aghrani showed the lower shoot length reduction which were 10.09%, 13.53% and 14.02%, respectively but Kanchon (40.27%), Kheri (38.49%) and Triticale (45.83%) exhibited the highest shoot length reduction. However, at 12 dS/m salinized

Table 2. Reduction % root length and shoot length of 24 wheat cultivars at different levels of salinity

Variety name	Root length Reduction (%)				Mean	Shoot length Reduction (%)				Mean
	6 ds/m	8 ds/m	10 ds/m	12 ds/m		6 ds/m	8 ds/m	10 ds/m	12 ds/m	
Sourav	31.87	43.48	54.35	57.96	46.91	13.16	21.05	30.26	43.87	27.09
Sonalika	31.43	36.17	42.86	48.23	39.67	10.07	15.87	34.66	41.83	25.60
Durum	7.35	47.06	48.53	60.29	40.81	14.90	18.00	26.69	27.54	21.78
Pavon	29.61	37.50	50.00	51.83	42.24	6.15	16.15	17.69	25.63	16.41
Prodip	30.14	46.43	57.14	58.71	48.11	18.18	24.99	10.09	33.47	21.68
BINA Gom-1	21.28	54.60	55.32	61.70	48.22	20.39	27.98	24.39	45.27	29.51
BARI Gom-25	22.81	25.47	49.97	56.59	38.71	21.36	26.00	29.40	40.39	29.29
BARI Gom-28	43.59	48.72	49.13	56.41	49.46	9.38	12.50	13.53	33.59	17.25
Gourav	12.77	13.96	37.75	49.41	28.47	5.86	24.32	25.22	31.97	21.84
Kanchon	12.71	42.86	63.48	65.48	46.13	3.56	36.29	40.27	43.38	30.87
Shotabdi	40.12	41.54	43.41	49.07	43.53	21.91	25.71	30.94	36.43	28.75
BARI Gom-23	28.74	38.71	41.38	55.17	41.00	17.43	32.45	38.12	45.70	33.42
BARI Gom-26	36.08	43.04	47.92	54.83	45.47	22.18	26.95	29.56	39.99	29.67
BARI Gom-29	8.48	28.79	55.52	49.09	35.47	5.97	23.82	27.67	31.07	22.13
BARI Gom-30	25.45	54.92	60.12	61.86	50.59	21.69	23.02	26.33	43.83	28.72
BARI Gom-22	20.42	26.39	34.33	34.63	28.94	5.83	25.00	35.00	40.00	26.46
Akbar	36.36	40.91	43.18	45.45	41.48	29.15	31.68	33.75	40.00	33.64
Protiva	60.61	61.36	62.12	66.67	62.69	19.51	29.88	36.59	51.22	34.30
Kheri	21.27	52.76	65.01	69.66	52.18	26.91	30.41	38.49	56.96	38.19
Triticale	28.95	39.47	51.32	50.00	42.43	14.58	29.38	45.83	50.00	34.94
Aghrani	25.47	29.58	31.60	34.17	30.21	5.66	13.09	14.02	27.11	14.97
Borkot	22.86	35.14	58.57	62.40	44.74	27.36	30.13	32.49	43.59	33.39
Kolyansona	35.00	41.65	50.00	53.75	45.10	18.88	32.17	34.77	38.60	31.10
BARI Gom-27	16.04	40.74	53.07	59.85	42.43	19.19	22.58	31.21	40.41	28.35
Mean	27.06	40.47	50.25	54.72	43.12	15.80	24.98	29.46	39.66	27.47
SD	2.44	2.17	1.82	1.81	1.55	1.57	1.30	1.80	1.59	1.26
SE	11.94	10.65	8.91	8.87	7.62	7.71	6.35	8.80	7.80	6.19

condition, Durum (27.54%), Pavon (25.63%) and Aghrani (27.11%) showed the lower shoot length reduction, but BARI Gom-23(45.70%), Protiva (51.22%) and Kheri (56.96%) showed the higher shoot length reduction (Table 2).

Fresh weight of the whole plant

Fresh weight was estimated and fresh weight reduction due to salinity was calculated. The data showed that Fresh weight reduction due to salinity also varied significantly in all cultivars as was found in root and shoot weight. At 6dS/m salinized condition, Kanchon (8.94%), BARI Gom-22 (5.36%) and Sourav (15.15%) showed the lower fresh weight reduction percent but Akbar, Kolyansona and BARI Gom-26 showed the higher Fresh weight reduction percent which were 70.05%, 60.16%and 56.33% respectively. Then at 8dS/m salinized condition, BARI Gom-25, Pavon and Sourav showed the lower fresh weight reduction which were 31.44%, 32.85%and 34.52% respectively but BARI Gom-26 (63.07%), Akbar (72.94%) and Kheri (65.45%) exhibited the highest fresh weight reduction. Again at 10 dS/m salinized condition, Sourav, Pavon and BARI Gom-25 showed the lower fresh weights reduction which was 41.17%, 52.09% and 57.47% respectively but Akbar (77.36%), Kheri (77.35%) and Triticale (73.02%) exhibited the highest fresh weight reduction. However, at 12 dS/m salinized condition, Sourav (56.97%), Pavon (56.74%) and Prodip (51.67%) showed the lower fresh weight reduction but BARI Gom-26 (83.34%), Kheri (82.30%) and Kolyansona (81.67%) showed the higher fresh weight reduction (Table 3).

Dry weight of whole plant

Dry weight reduction due to salinity was recorded in all cultivars. The data showed that dry weight reduction due to salinity varied significantly with cultivars and salinity levels. At 6dS/m salinized condition, Kanchon (4.63%), Pavon (4.79%) and Sotabdi (5.52%) showed the lower dry weight reduction percentage but Akbar, Kolyansona and Prodip showed the higher dry weight reduction which were 42.39%, 49.32% and 62.58%, respectively. Then at 8dS/m salinized condition, Pavon, BARI Gom-28 and Sotabdi showed the lower dry weight reduction which were 12.33%, 32.52% and 8.97% respectively but, Prodip (63.38%), BARI Gom-22 (60.63%) and Kolyansona (52.19%) exhibited the highest dry weight reduction. Again at 10 dS/m salinized condition, Pavon, BARI Gom-28 and Gourav showed the lower dry weight reduction which were 25.34%, 35.00% and 30.42% respectively but Kanchon (58.30%), Prodip (65.00%) and Triticale(60.44%) exhibited the highest dry weight reduction. However, at 12 dS/m salinized condition, Aghrani (39.43%), Pavon (27.30) and Gourav (41.24%) showed the lower dry weight length reduction but BARI Gom-26(66.26%), Protiva (71.16%) and BARI Gom-30 (68.75%) showed the higher dry weight reduction (Table 3). There were wide variations in the percentage of reduction of root and shoot length and fresh and dry biomass among the cultivars at all the salinity levels.

Table 3. Fresh Weight and reduction % of 24 wheat cultivars under different salinity levels

Variety name	Fresh Weight Reduction %				Mean	Dry weight Reduction %				Mean
	6 ds/m	8 ds/m	10ds/m	12ds/m		6 ds/m	8 ds/m	10ds/m	12ds/m	
Sourav	15.15	34.52	41.17	56.97	36.95	22.09	37.42	40.49	46.63	36.66
Sonalika	48.97	55.88	64.50	76.77	61.53	37.04	38.89	54.63	60.19	47.69
Durum	43.10	63.90	69.66	70.71	61.84	17.19	35.94	42.19	57.03	38.09
Pavon	28.47	32.85	52.09	56.74	42.54	4.79	12.33	25.34	27.40	17.47
Prodip	37.22	44.10	49.19	51.67	45.54	62.58	63.38	65.00	71. 16	65.44
BINA Gom-1	47.97	57.70	73.10	78.65	64.36	27.91	50.70	63.58	66.80	53.84
BARI Gom-25	20.35	31.44	57.47	62.12	42.85	12.32	25.62	36.45	46.80	30.30
BARI Gom-28	32.16	37.80	45.16	66.75	45.47	10.43	14.72	32.52	47.24	26.23
Gourav	36.35	44.02	58.56	62.36	50.32	15.98	24.23	30.41	41.24	27.96
Kanchon	8.94	56.02	72.04	76.28	53.32	4.63	29.17	58.80	61.11	38.43
Shotabdi	30.64	42.76	60.63	61.34	48.84	5.52	8.97	36.55	43.45	23.62
BARI Gom-23	52.25	28.91	62.81	74.13	54.52	14.86	36.49	47.30	60.36	39.75
BARI Gom-26	56.33	63.07	69.94	83.34	68.17	27.98	36.63	41.15	66.26	43.00
BARI Gom-29	27.07	53.11	72.41	73.79	56.60	19.72	30.73	56.42	61.93	42.20
BARI Gom-30	43.84	49.70	65.10	79.86	59.63	23.44	36.33	47.66	68.75	44.04
BARI Gom-22	5.36	52.75	57.44	70.31	46.47	14.98	60.63	62.72	64.81	50.78
Akbar	70.05	72.94	77.35	78.55	74.72	42.39	51.78	57.61	63.11	53.72
Protiva	58.55	60.90	62.39	79.24	65.27	20.26	41.38	49.57	63.36	43.64
Kheri	47.50	65.45	77.36	82.30	68.15	27.24	46.27	50.75	64.55	47.20
Triticale	50.39	56.71	73.02	80.96	65.27	35.16	28.57	60.44	64.47	47.16
Aghrani	38.10	52.82	55.73	61.66	52.08	24.00	27.43	33.71	39.43	31.14
Borkot	53.71	58.43	62.98	79.04	63.54	29.68	34.84	46.45	62.58	43.39
Kolyansona	60.15	62.56	69.00	81.67	68.34	49.32	52.97	54.79	58.45	53.88
BARI Gom-27	46.76	53.68	67.64	76.10	61.04	14.45	32.03	45.70	54.30	36.62
Mean	39.97	51.34	63.20	71.72	56.56	23.50	35.73	47.59	56.72	40.89
SD	3.37	2.42	1.99	1.92	2.05	2.90	2.84	2.34	2.25	2.25
SE	16.52	11.87	9.75	9.41	10.06	14.20	13.93	11.46	11.03	11.03

Table 4. Ranking of 24 wheat cultivars under 12ds/m salinity levels

Cultivars	Root Length Reduction (%)	Shoot Length Reduction (%)	fresh weight reduction %	Dry Weight Reduction %	mean value of four traits reduction	Rank	Tolerance
Sourav	57.96	43.87	56.97	46.63	51.36	7	T
Sonalika	48.23	41.83	76.77	60.19	56.76	12	MT
Durum	60.29	27.54	70.71	57.03	53.89	10	MT
Pavon	51.83	25.63	56.74	27.4	40.40	1	T
Prodip	58.71	33.47	51.67	71. 16	47.95	5	T
BINA Gom-1	61.7	45.27	78.65	66.8	63.11	21	S
BARI Gom-25	56.59	40.39	62.12	46.8	51.48	8	T
BARI Gom-28	56.41	33.59	66.75	47.24	51.00	6	T
Gourav	49.41	31.97	62.36	41.24	46.25	3	T
Kanchon	65.48	43.38	76.28	61.11	61.56	19	S
Shotabdi	49.07	36.43	61.34	43.45	47.57	4	T
BARI Gom-23	55.17	45.7	74.13	60.36	58.84	16	MT
BARI Gom-26	54.83	39.99	83.34	66.26	61.11	17	S
BARI Gom-29	49.09	31.07	73.79	61.93	53.97	11	MT
BARI Gom-30	61.86	43.83	79.86	68.75	63.58	22	S
BARI Gom-22	34.63	40	70.31	64.81	52.44	9	MT
Akbar	45.45	40	78.55	63.11	56.78	13	MT
Protiva	66.67	51.22	79.24	63.36	65.12	23	S
Kheri	69.66	56.96	82.3	64.55	68.37	24	S
Triticale	50	50	80.96	64.47	61.36	18	S
Aghrani	34.17	27.11	61.66	39.43	40.59	2	T
Borkot	62.4	43.59	79.04	62.58	61.90	20	S
Kolyansona	53.75	38.6	81.67	58.45	58.12	15	MT
BARI Gom-27	59.85	40.41	76.1	54.3	57.67	14	MT

The percentage of reduction increased with the salinity level. At the highest level of salinity stress, we calculated the average of the reduction of the four important traits, root and shoot length and fresh and dry biomass (Table 5). Our Morphological experiment categorized all the cultivars into three category such as tolerant, moderately tolerant and susceptible. Under 12 ds/m salinity level the variety Sourav, Pavon, Prodip, BARI Gom-25, BARI Gom-28, Gourav, Shatabdi and Aghrani emerged as tolerant to salt by their lower reduction of root and shoot length and fresh and dry biomass over their respective control (Table 5). Sonalika, Durum, BARI Gom-23, BARI Gom-29, BARI Gom-22, Akbar, Kalyansona and BARI Gom-27 were moderately tolerant (MT). The rest of the varieties were found susceptible(S) (Table 4).

Table 5. Summary of Molecular data of 24 wheat cultivars

Marker	Obtained Allele Size	Allele No.	Major. Allele. Frequency	Null Allele	Gene Diversity	PIC
Xbarc121-7A	160-174	4.00	0.6250	-	0.5382	0.4815
Xbarc84-3B	116-123	4.00	0.7083	2	0.4618	0.4247
Xgwm132-6B	93-100	6.00	0.2500	4	0.8021	0.7725
Xgwm577-7B	114-174	14.00	0.1667	-	0.9063	0.8989
Xbarc217-4D	51-58	7.00	0.3333	1	0.7708	0.7365
Xgwm350-4A	131-160	9.00	0.3333	2	0.7951	0.7697
Mean	-	7.33	0.4028	-	0.7124	0.6806

Molecular screening for salt tolerance in wheat cultivars by SSR marker

For molecular characterization of the 24 wheat cultivars, ten SSR Primer pairs Xbarc121, Xbarc84, Xgwm132, Xgwm577, Xgwm130, Xgwm260, Xgwm276, Xgwm332, Xbarc217 and Xgwm350) were used initially for primer selection. Among the primers, six (Xbarc121, Xbarc84, Xgwm132, Xgwm577, Xbarc217 and Xgwm350) were selected for final analysis. These six markers were used to evaluate the wheat for salt tolerance. Amplified microsatellite loci were analysed for identifying polymorphism using polyacrylamide gel electrophoresis. Microsatellite profiles of 24 wheat cultivars at loci Xbarc121, Xbarc84, Xgwm132, Xgwm577, Xbarc217 and Xgwm350 are shown in Fig.1(a), Fig. (b), Fig. 1(c) and Fig. 1(d), respectively.

Among the 24 wheat cultivars, a total of 44 alleles were detected. In this study, the average number of alleles per locus was found to be 7.33 (Table 5). The highest number of alleles was 12 detected for marker Xgwm577 and the lowest number of allele was found for marker Xbarc121 and Xbarc84. A total of nine null alleles were found in SSR primers, Xbarc84 for kanchon and triticale, Xgwm132 for kanchon, aghoroni, borkot and kollayanson, Xbarc217 for triticale and Xgwm350 for prodip and triticale (Table 5).

In this study, the highest level of gene diversity value (0.9063) was observed in locus Xgwm577 and lowest level of gene diversity value (0.8281) was observed in locus Xbarc84 with a mean gene diversity value 0.4618 (Table 6). The PIC values ranged from low 0.4247 to high 0.8989. The highest PIC value was found in Xgwm577 and lowest value for Xbarc84 (Table 5).

Genetic similarity analysis using Neibour Joining Tree

Pair-wise comparison value of genetic distance (D) (Nei's, 1973) between varieties was computed from combined data of 6 markers and ranged from 0.1667 to 1.000. Neibour Joining Tree dendrogram was constructed based on the genetic similarity calculated from the 44 SSR alleles (by 6 markers) generated from 24 wheat cultivars. The cultivars could be easily distinguished. The Neibour Joining Tree cluster tree analysis led to the Grouping of the 24 cultivars into three major clusters (Fig. 2). Cluster I, was formed with Aghroni, BARI Gom-29 and BARI Gom-30. Cluster II, was consisted of two
sub clusters (Sub cluster IIA and IIB). Sub cluster IIA, was formed with Akbar. Sub cluster IIB was consisted of two sub sub clusters (Sub sub cluster II A and II B). In Sub sub cluster IIA Sonalika, BARI Gom-28, BARI Gom-25, BINA Gom-1, Prodip, Pavon, BARI Gom-23, Shotabdi, Gourav and Kanchon were present. Whereas, Sub sub cluster II B was formed with Borkot, Sourav, Protiva, Triticale and Kheri. Cluster III was constructed with BARI Gom-22, BARI Gom-27, Kallyansona, Durum and BARI Gom-26.

Discussion

Morphological screening for salt tolerance in wheat cultivars at seedling stage

Salinity causes a decrease in the root length of wheat. In the experiment, root length was reduced substantially with an increase in salinity stress. The gradual decrease of root length with the increase in salinity might be due to the inhibitory effect of salt. Similar decrease in shoot length was also found with the increase in salinity stress. Reduced vegetative growth of wheat landraces at 200 and 250 mM salt stress was evident in addition to morphological and molecular diversity among the wheat landraces (Shahzad *et al.,* 2012). Akbarimoghaddam *et al.* (2011) reported that shoot and root dry weight of wheat genotypes were also adversely affected due to increase in NaCl concentration. The average root length and shoot length at 12 dS/m were lower than the average root length and shoot length of 10, 8 and 6 dS/m, respectively. The result of the present study revealed that root length was affected at a higher degree than the shoot length. However, as reported previously under imposed stress, shoot growth was inhibited more than root growth (Ma *et al.,* 2007). This contradiction might be due to the use of different genotypes in a different environment.

Under salinity stress, fresh weight, as well as dry weight of all wheat cultivars, was considerably reduced in the present study. El-Hendawy *et al*. (2005) reported that salt stress decreased dry weight of plant at all growth stages. Similarly, the average fresh weight and dry weight at 12 dS/m were also lower than the fresh weight and dry weight of 10, 8 and 6 dS/m, respectively. According to Mass (1986), dry matter production permits direct estimation of economic return under

saline conditions, because it is a useful criterion to evaluate salt tolerance.

The lower mean value of root length, shoot length, fresh weight and dry weight reduction indicate a higher tolerance level of the cultivars to salinity and the cultivars with higher value of root length shoot length, fresh weight and dry weight reduction are salinity susceptible cultivars. In considering root length, shoot length, fresh weight and dry weight reduction; under the highest salinity stress level (12ds/m) eight cultivars namely Sourav, Pavon, Prodip, BARI Gom-25, BARI Gom-28, Gourav, Shatabdi and Aghrani were the most salt-tolerant wheat cultivars. The BARI Gom-25 was released as a moderately salt tolerant variety. Another variety, BINA Gom-1, released by Bangladesh Institute of Nuclear Agriculture as salt tolerant variety could not perform well in our study and it was found to be susceptible at 12ds/m.

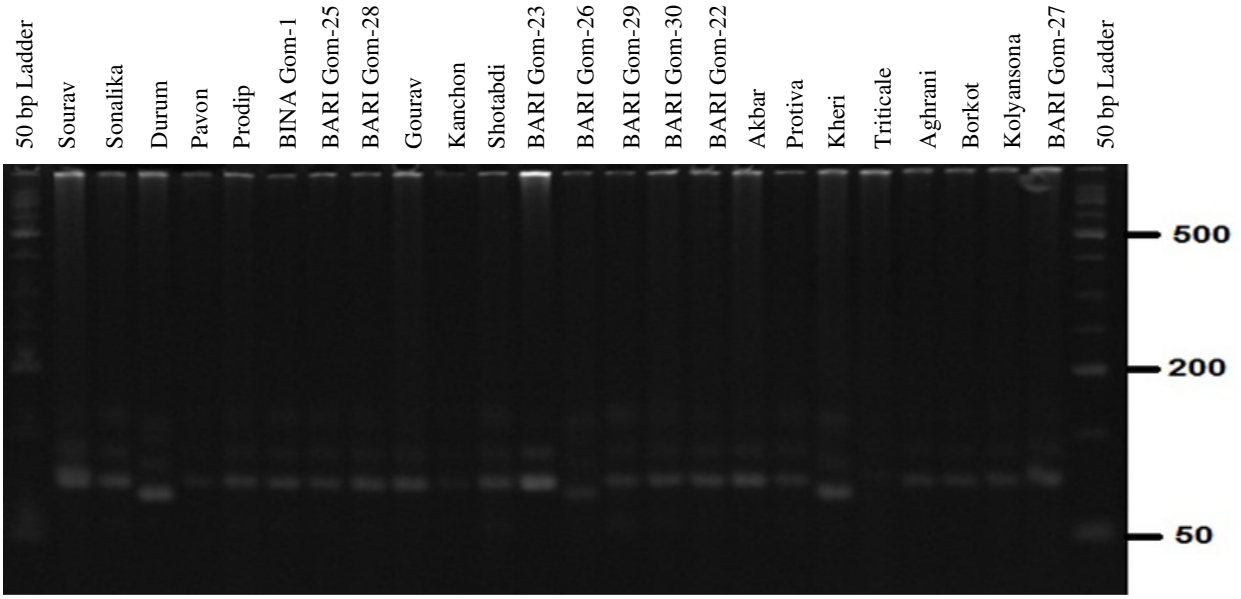

Fig.1(a): Microsatellite profile of 24 wheat cultivars at locus Xbarc84

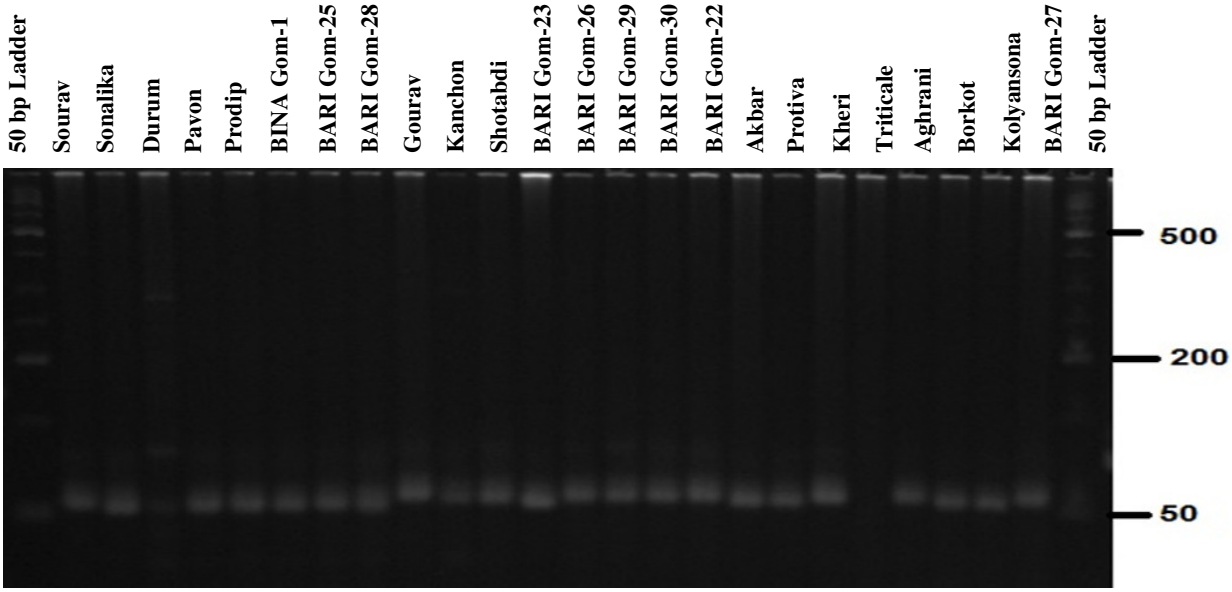

Fig.1(b): Microsatellite profile of 24 wheat cultivars at locus Xbarc217

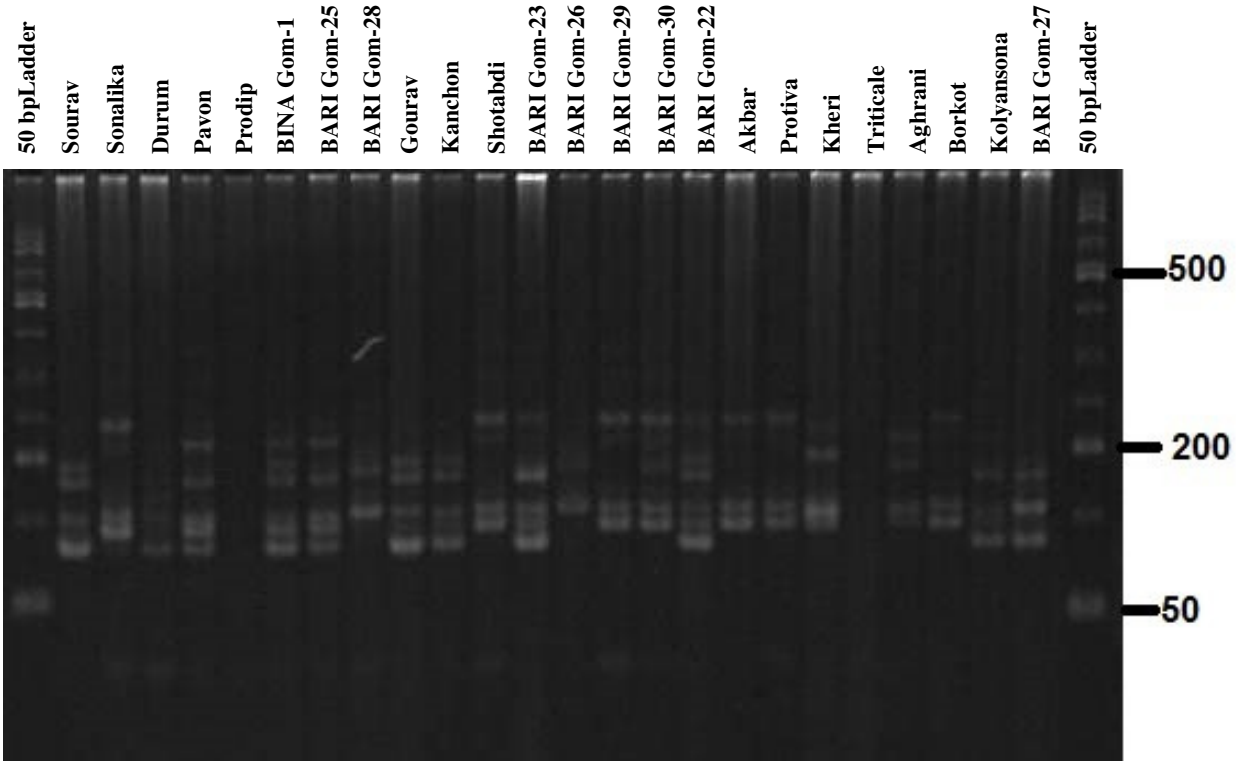

Fig.1(c): Microsatellite profile of 24 wheat cultivars at locus Xgwm350

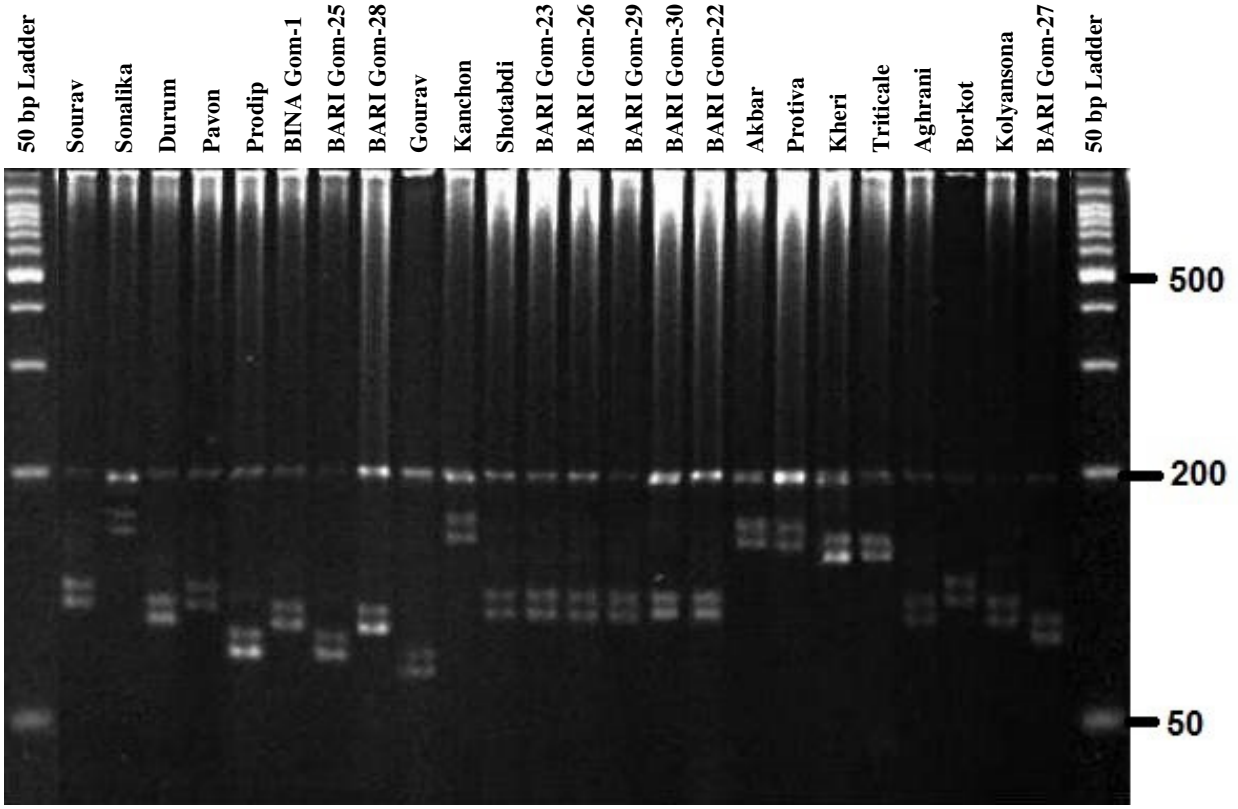

Fig.1(d): Microsatellite profile of 24 wheat cultivars at locus Xbarc577

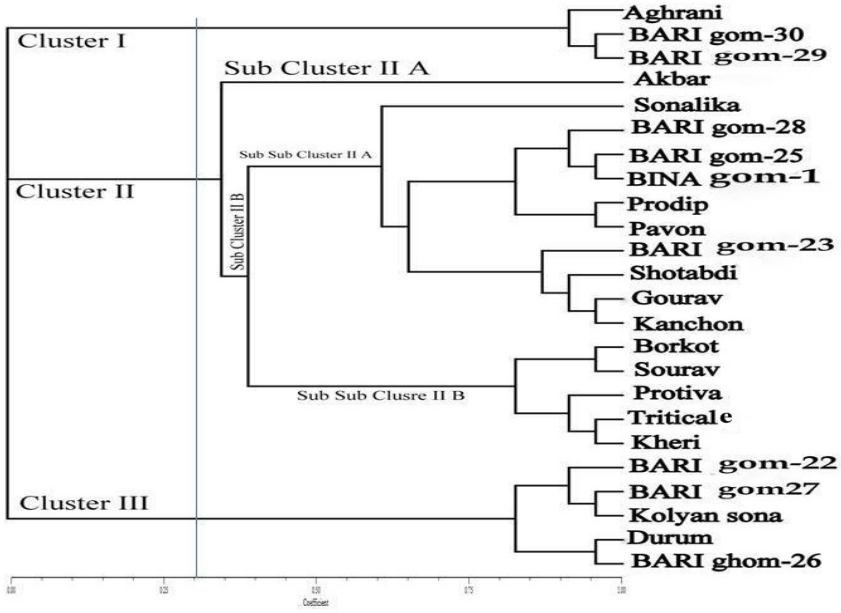

Fig. 2. Neibour Joining Tree Dendrogram for 24 Wheat cultivars showing the genetic similarity and relatedness

Molecular screening for salt tolerance in wheat cultivars by SSR marker

In the present study, the PIC value ranged from 0.4247 to 0.8989 with an average of 0.6806. Similar result was reported by Ciuca and Petcu (2009). Also, PIC values showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluate in this study. This can be used as valuable genomic resources for the identification of genes, alleles and genomic regions for developing number of tolerant varieties. The allele size range and the number of alleles were also highly correlated in consistent with previous works (Ciucă and Petcu, 2009). Ahmad *et al.* (2013) reported the PIC value ranged from 0.22 to 0.76 with an average of 0.49 using 38 SSR markers, was highly correlated with this works. Genetic similarity coefficients ranged from 0.45 to 0.95, indicating the presence of considerable genetic variation in the cultivars tested. Using 21 SSR markers, an average PIC value of 0.47 was recorded (Singh *et al.,* 2006). While, Almanza-Pinzon *et al.* (2003) reported PIC value of 0.45. These markers can be considered as useful for marker-assisted selection (MAS). The higher the PIC value of the SSR markers, the higher the informative the markers are. In this study, we found a higher PIC (0.4247 to 0.8989 with an average of 0.6806) value that indicates the SSR markers we used were of considerable information.

A limited research has been done to identify genetic markers associated with salt tolerance in different plants. Thus, understanding of genetic factors affecting salt tolerance as well as to identify new diagnostic markers to be deployed in MAS are necessary. It will ensure faster yield gains under salt stress environments. The morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage has previously been investigated (Ahmad *et al*., 2013). The salt-tolerant genotypes produced more alleles than sensitive once. The genetic dissimilarities among 24 cultivars were determined using Nei's genetic distance-based analysis. The higher genetic distance between them indicates that genetically they are diverse compared to lower genetic distance value. Basically, this value indicates that they are genetically much closer to each other.

In this study, Neibour Joining Tree dendrogram separated the wheat cultivars distinctly. As all the markers in the present study were related to salt tolerance (Huang *et al*., 2002; Batool *et al.,*2018; Ren *et al.,*2012), genetic similarity based clustering might be indicative to the genetic potentiality for salt tolerance. It is clearly evident from the present study that morphological and molecular genetic variation for salt tolerance existed in the wheat genotypes tested. Our results confirmed that salt stress significantly affected dry matter accumulation. The genotypes responded differently due to the different NaCl stress levels.

The marker assisted study revealed a discrimination of the 24 cultivars into three major clusters, each of which is divided into sub-cluster and further sub sub-clusters. Molecular grouping indicates the genetic potential of Sub sub cluster IIA that contained morpho-physiologically identified five tolerant (BARI Gom-28, Prodip, Pavon, Shotabdi, Gourav), two moderately tolerant (Sonalika, BARI Gom-23) and two susceptible cultivars (BINA Gom-1, Kanchon) with check variety BARI Gom-25.

There were some discrepancies between the morphological and molecular diversity in salt tolerance among the wheat cultivars. Not all the genotypes found tolerant to salinity by morphological study grouped together in the molecular study. BINA Gom-1, salt

tolerant variety, was not found tolerant in morphological diversity but grouped together with the tolerant genotypes in the molecular analysis. Previous reports suggested that molecular diversity provides remarkably higher estimates of genetic diversity than morphological or physiological methods (Karp *et al*., 1996; Beyene *et al*. 2005). Karhu *et al*. (1996) described that the diversity at the molecular level may not reflect in the diversity at the morphological or physiological level. Increasing the number of markers in molecular analysis to several thousands and including all possible morphological or physiological traits as parameters can give similar diversity pattern among genotypes based on molecular and morphological diversity. Morphological and physiological characters are the ultimate expression of molecular constitution of a variety where a number of biochemical processes are involved. Therefore, different types of clustering in different methods are not unusual (Han-yong *et al*., 2004).

Conclusion

The present study aimed to assess genetic variation for salinity tolerance in 24 wheat genotypes based on morphological and molecular marker screening. Data were collected by screening the cultivars under different salt stress. Morphological and molecular variations were observed for salt tolerance in wheat cultivars. Combining morphological findings with that of the molecular assessment, six cultivars, BARI Gom-28, Prodip, Pavon, BARI Gom-25, Shotabdi, Gourav may be considered as true salt tolerant cultivars which may contribute in a greater way in the development of salt tolerant genotypes. The findings will be helpful for both plant breeders and farmers of saline belts in general. The tolerant and moderately tolerant genotypes have been identified as resource base population, which could to be utilized suitably for further improvement programme for salt tolerance in Bangladeshi wheat.

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