Plant Tissue Cult. & Biotech. **34**(2): 173-191, 2024 (December) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v34i2.78900

PTC&B

Screening of Wheat Varieties and Advanced Lines for Salinity Tolerance at the Seedling Stage through Morpho-Molecular Approaches

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Key words: Bread wheat, Hydroponic culture, Salt stress, Selection, SSR marker

Abstract

The selection of potential salinity tolerant varieties is important for the cultivation of wheat in saline prone areas. A screening was performed to assess the salt tolerance capacity of 15 wheat varieties of Bangladesh and 10 advanced lines (exotic) in a hydroponic culture system at four distinct salt concentrations (0, 12, 16 and 20 dS/m). The results revealed that different salinity levels significantly affect the growth attributes by reducing the shoot length and fresh as well as dry weight of roots and shoots, with a few exceptions in some genotypes at 12 dS/m salinity. The highest STI (Salt tolerance index) was observed in nine genotypes, namely BINA Gom-1, ESWYT P-44, ESWYT P-28, BARI Gom-23, ESWYT P-19, BARI Gom-27, BARI Gom-29, Pavon-76 and BARI Gom-32 which are regarded as tolerant varieties and advanced lines. The wheat genotypes were subjected to molecular assessment using 21 Single Sequence Repeat (SSR) markers associated with salinity tolerance. SSR marker assisted assessment identified 116 alleles in 25 wheat genotypes, with an average of 5.52 alleles per locus. In this experiment, the marker Xwmc-24 generated the highest (0.825) polymorphism information content (PIC) and Nei's (1973) gene diversity (0.845). Twenty-five genotypes were categorized into six distinct clusters using similarity indices-based cluster analysis. Advanced lines, namely ESWYT P-44, ESWYT P-28, ESWYT P-19 and HYVs, namely BARI Gom-27 and BARI Gom-29, were grouped in cluster 5, while BINA Gom-1 was identified in cluster 1. Considering all the facts, it can be concluded that these just mentioned varieties and

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inbred advanced lines may be the potential candidates for breeding programs towards salinity tolerance in wheat.

Introduction

The acreage of saline soils is increasing due to the abiotic stress of salinity, which is a result of global warming (Munns and Tester 2008) and it severely affects plant growth and development, including reduction of yield (Mbarki et al. 2018). It was reported that 20% of the total cultivable area in the world is affected by salinity (Oproi and Madosa 2014) and salinity affects more than 20% of present-day agriculture in the world (Mickelbart et al. 2015). The littoral region of Bangladesh spans 2.5 million hectares. Different salinity levels affect approximately 20% of the net arable land in the littoral region of Bangladesh (Khanom and Salehin 2012, Harun et al. 2020). The saline-affected area in Bangladesh is expanding because of sea-level rise, coastal subsidence and enhanced tidal effects, as well as a continuous reduction in river flow, particularly during drought periods. Bangladesh contributes approximately 30% of global wheat production and cultivates wheat on 17% of the country's cultivatable lands (FAO 2016). Bangladesh produces only about 1.5 MMT against the national demand of 7.3 MMT and the country needs to import about 5.15 MMT of wheat grain to compensate for our demand yearly (USDA 2019). The world is in dire need of the development of salinity-tolerant cultivars that can sustain optimal yield levels and satiate the insatiable hunger for food (Al-Ashkar et al. 2020). Much research is going on around the globe to develop salinity tolerant HYV of wheat. Screening lines is a difficult task, as using an incorrect procedure can result in the complete failure of the experiment. The physiological processes may be influenced by soil heterogeneity, climatic factors, and other environmental factors, which made screening at the field level challenging. Therefore, it is deemed more advantageous to screen under laboratory conditions, such as the hydroponic system, than to screen in the field (Munns et al. 2006). Numerous researchers conducted extensive investigations of wheat genotypes for salinity tolerance during the seedling stage in a hydroponic medium (Shazad et al. 2012, Ahmed et al. 2013, Hussain et al. 2015, Haque et al. 2020). Morphological and physiological attributes of salinity stressed plants indicate the level of salinity tolerance and can be used in identifying tolerant and sensitive genotypes. Such experiments are conducted by researchers to filter out salinity tolerant genotypes (Uzair et al. 2022, Rafiq et al. 2006). Plant biomass means the weight of the whole plant that consists of root and shoot weight. Researchers discovered plant biomass may be used for the selection of salt-tolerant genotypes (Munns and James 2003, Oyiga et al. 2016, Genc et al. 2019). Salinity stress has a significant impact on plant growth and development. Salinity reduces plant growth in wheat through osmotic effects and high concentrations of Na^+ and Cl^- and assimilates become less available to growing tissues and organs (Munns 2007). Salinity significantly reduces seedling fresh weight, dry weight of shoots and roots, root number, and root length (Seleiman et al. 2022, Rani et al. 2019). While numerous morpho-physiological characteristics have been recognized as efficacious

screening criteria, their functionality is frequently influenced by environmental factors and is contingent upon the developmental phases of growth. This affects salt tolerance evaluation even more, as environmental fluctuations can influence the salt tolerance of different genotypes (Moraes et al. 2005). Hence, morpho-physiological parameters used alone for screening impose certain limitations on the ability to evaluate genetic diversity in salt tolerance. Fortunately, molecular markers have facilitated the identification of the genes governing the intricate morpho-physiological characteristics that endow numerous field crops with salt tolerance. By doing so, the evaluation procedure has become more streamlined and economical (Al-Ashkar et al. 2020, Abbasi et al. 2015, Abulela et al. 2022). Therefore, to enhance the precision of salt tolerance evaluation in genotypes, it is imperative to integrate phenotypic assessment based on morpho-physiological traits with molecular markers to identify the candidate genes. Several molecular markers are available to compare different genotypes in different environmental circumstances. Furthermore, their versatility allows for the incorporation of various tolerance traits into a single efficient genotype, unconstrained by crop growth stages. Among the markers known to date, Simple sequence repeat (SSR) or microsatellite markers are widely employed in genetic characterization for tasks such as germplasm characterization, cultivar identification, molecular mapping, co-dominant locus specificity, informativeness and absence of biases. These markers are cost-effective, ability to detect multiple alleles, high levels of polymorphism, high throughput capabilities, abundance, and co-dominance (Al-Ashkar et al. 2020, Singh et al. 2018, Devi et al. 2019, Irshad et al. 2022). Therefore, SSR markers, along with morpho-physiological traits, can be a good option for screening salinity tolerant genotypes. The objectives of this study were to screen twenty-five wheat genotypes for salinity tolerance at seedling stages based on morpho-physiological traits and screen wheat genotypes by salt link SSR markers to find out the best candidate for breeding programs.

Materials and Methods

Twenty-five wheat genotypes (Table 1), including one check variety (BINA gom-1) (positive control) and one negative control (BARI Gom-23), were used in this experiment at the glass house of Bangladesh Institute of Nuclear Agriculture (BINA), BAU, Mymensingh, Bangladesh. Wheat seedlings were grown in a hydroponic setup following IRRI standard protocol (Gregoria et al. 1997) with slight modifications. Completely Random Design (CRD) was applied in this experiment. The seed dormancy was broken by subjecting the seeds to thermal treatment in a convection oven at 50°C for five days. Then, the seeds were kept in tap water for 24 hrs for soaking. After that, the seeds were rinsed and cleansed with tap water and subsequently placed on Petri dishes. They were incubated at 30°C for 48 hrs to facilitate germination. Germinated plants were placed on Styrofoam floating in a hydroponic system for 3 days on normal tap water. On 4th day, a nutrient solution (Peter's soluble salt) was added. Four treatments (Control, 12 dS/m, 16 dS/m and 20 dS/m) were imposed on 7-day-old seedlings (2-3 leaf stage) and salinization

was continued for 14 days more. Salinity and pH (5.1) were checked daily and maintained. The nutrient solution's EC was determined utilizing an EC meter (Hanna HI 4321, Weilheim, Germany).

Table 1. List of germplasms used in this experiment.

Germplasm	Type	Germplasm	Type
Akbar	Landrace	BAW-1274	Advanced line
Borkot	Landrace	BAW-1284	Advanced line
Agroni	Landrace	ESWYT P-2	Advanced line
Triticale	Hybrid cereal	ESWYT P-3	Advanced line
Pavon-76	Released variety	ESWYT P-8	Advanced line
BARI Gom-20	High yielding variety	ESWYT P-11	Advanced line
BARI Gom-21	High yielding variety	ESWYT P-12	Advanced line
BARI Gom-23	High yielding variety	ESWYT P-19	Advanced line
BARI Gom-27	High yielding variety	ESWYT P-28	Advanced line
BARI Gom-29	High yielding variety	ESWYT P-30	Advanced line
BARI Gom-32	High yielding variety	ESWYT P-37	Advanced line
BINA Gom-1	High yielding variety	ESWYT P-44	Advanced line
BAW-1262	Advanced line		

After 14 days of salinity treatment, three samples from 3 replications of each treatment and variety were randomly sampled for genomic DNA extraction. In order to eliminate the presence of microorganism spores and any other foreign DNA sources, the leaf tip was cut apart with sterilized scissors, rinsed in distilled water and ethanol, and dried on fresh tissue paper, which was approximately 7-8 cm in length. The collected fresh leaf samples were then put in 50 ml centrifuge tubes and preserved in a -80°C freezer. Data were recorded on standard evaluation system (SES), shoot length (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g) and root dry weight (g). Shoot and root length were recorded with a meter scale. The fresh weight of the seedlings was taken using an electric balance. After recording the fresh weight of each seedling, they were oven-dried at 50°C for a week and then the dry weight was recorded using electric balance.

The salt tolerance index was measured following Tao et al. (2021). The salt tolerance index was measured for all the traits at each salinity level, then all the Salt tolerant index (STI) values of all the traits for a certain variety were summoned to get the Individual salt tolerant index (ISTI), then all the three ISTI values for one particular variety was summoned to get Total salt tolerant index (TSTI). Total DNA was extracted from the leaves of 21-day-old salinized wheat seedlings by cetyltrimethylammonium bromide (CTAB) method (Zidani et al. 2005) with some minor modifications. The concentration of DNA samples was qualitatively assessed by Agarose gel electrophoresis at a 1% concentration.

Table 2. List of the selected SSR loci used for salt tolerance screening in wheat genotypes.

Sl. No.	Locus name	Sequence (5′-3′)	Annealing tem. (°C)
1	Xbarc-45	F CCCAGATGCAATGAAACCACAAT R GCGTAGAACTGAAGCGTAAATTA	57
2	Xcfd-1	F ACCAAAGAACTTGCCTGGTG R AAGCCTGACCTAGCCCAAAT	56
3	Xcfd-13	F CATCCAACAGCACCAAGAGA R GCTACTACTATTTCATTGCGACCA	60
4	Xcfd-49	F TCGTTCCAAAATGCATGAAA R AAGGGCCAGAAATCTGTGTG	60
5	Xcfd-54	F TTCCCATAACTAAAACCGCG R GGAACATCATTTCTGGACTTTG	60
6	Xgwm-160	F TTCCCATAACTAAAACCGCG R GGAACATCATTTCTGGACTTTG	60
7	Xgwm-249	F CAAATGGATCGAGAAAGGGA R CTGCCATTTTTCTGGATCTACC	52
8	Xgwm-296	F AATTCAACCTACCAATCTCTG R GCCTAATAAACTGAAAACGAG	52
9	Xgwm-314	F AGGAGCTCCTCTGTGCCAC R TTCGGGACTCTCTTCCCTG	57
10	Xgwm-455	F ATTCGGTTCGCTAGCTACCA R ACGGAGAGCAACCTGCC	60
11	Xtxp-12	F ATAT GGAAGGAAGAAGCCGG R AACACAACATGCACGCATG	51
12	Xwmc-17	F ACCTGCAAGAAATTAGGAACTC R CTAGTGTTTCAAATATGTCGGA	51
13	Xwmc-18	F CTGGGGCTTGGATCACGTCATT R AGCCATGGACATGGTGTCCTTC	61
14	Xwmc-24	F GTGAGCAATTTTGATTATACTG R TACCCTGATGCTGTAATATGTG	51
15	Xwmc-44	F GGTCTTCTGGGCTTTGATCCTG R TGTTGCTAGGGACCCGTAGTGG	60
16	Xwmc-110	F GCAGATGAGTTGAGTTGGATTG R GTACTTGGAAACTGTGTTTGGG	56
17	Xwmc-154	F ATGCTCGTCAGTGTCATGTTTG R AAACGGAACCTACCTCACTCTT	50
18	Xwmc-170	F ACATCCACGTTTATGTTGTTGC R TTGGTTGCTCAACGTTTACTTC	60
19	Xwmc-405	F GTGCGGAAAGAGACGAGGTT R TATGTCCACGTTGGCAGAGG	60
20	Xwmc-432	F ATGACACCAGATCTAGCAC R AATATTGGCATGATTACACA	51
21	Xwmc-661	F CCACCATGGTGCTAATAGTGTC R AGCTCGTAACGTAATGCAACTG	60

Also, Nanodrop spectrophotometer was employed to evaluate the quantity and purity of isolated genomic DNA, as well as the presence of protein and RNA contamination (ND 1000, Thermo Scientific, Madison, USA).

Samples of concentrated DNA were diluted to an approximate volume of 50 ng µl using sterile double-distilled water (ddH_2O). The amplification of DNA (PCR) was performed following the method described by Bala et al. (2017) with some modifications. A set of fifty-one microsatellite primers (Table 2) developed by several investigators was used in this study (Shahzad et al. 2016, Moghaieb et al. 2011, Gajera et al. 2016). To assess the primers' efficacy in amplifying DNA sequences, a subset of four randomly selected individuals from each of the twenty-five wheat genotypes were evaluated to ensure that they could be accurately scored. Twenty-one (21) of the 51 primers exhibited a distinct polymorphism, and they were subsequently employed for further analysis.

PCR consisting of 5 µl of master mix (Promega) and 2 µl of nuclease-free water was used for the final amplification, including 1 µl of forward DNA, 1 µl of primer reverse DNA, and 1 µl of extracted genomic DNA. The PCR reaction was conducted using the following protocol: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at the respective temperature of each primer for 1 min, polymerization at 72°C for 2 min, and incubation at 72°C for 7 min. Electrophoresis was performed in a vertical electrophoresis container with 10X TBE buffer and 2.0 µl of PCR products mixed with loading dye (2X) in each well of the gel following PCR. Finally, the gel was immersed in ethidium bromide (10 mg/l) for 12-15 min. A 50 bp and a 100 bp DNA ladder were employed to determine the size of the DNA.

The data was subjected to statistical analysis using the analysis of variance (ANOVA) and the least significant difference (LSD) at the 5% and 1% probability levels in Duncan's Multiple Range Test (Gomez and Gomez 1984) using MSTAT-C software. The salt tolerance index (STI) at the seedling stage was calculated following Tao et al. (2021).

The POWER MARKER version 3.23 was employed to ascertain the summary statistics, which included the number of alleles per locus, major allele frequency, gene diversity, and Polymorphism Information Content (PIC) values. The cluster analysis and dendrogram construction were conducted using NTSYS-PC (version 2.1), and the Unweighted Pair Group Method with arithmetic mean (UPGMA) was employed.

Results and Discussion

Salinity tolerance screening of twenty five wheat germplasms (HYV, landraces and advanced lines) was conducted in a saline hydroponic system at the seedling stage (Fig. 1). Standard evaluation score (SES) (Gregoria et al. 1997) was taken for salinity tolerance evaluation of wheat germplasm under 12 dS/m salinized condition. Out of 25 wheat germplasms, nine germplasm were categorized as tolerant, 10 were moderately tolerant, and 6 were susceptible. No germplasm was found to be highly susceptible (Table 3).

In this experiment, five morphological attributes were taken into account, viz. shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. These attributes contribute to plant biomass production. Measurement of plant biomass in stress conditions is an effective way of screening tolerant genotypes.

Interactions of variety and treatment had a statistically significant influence on all the parameters mentioned (Table 4).

Table 3. Performance of wheat germplasm under salinized condition (EC 12 dS/m) grown in a hydroponic system at the seedling stage.

Name of wheat germplasm	SES scoring	Tolerance
Akbar, Triticale, Pavon-76, ESWYT P-11, ESWYT P-12, ESWYT P-19, BARI Gom-20, BARI Gom-21, BAW-1284	3	Tolerant
BINA Gom-1, Agroni, ESWYT P-2, ESWYT P-3, ESWYT P-8, ESWYT P-28, ESWYT P-44, BARI Gom-27, BARI Gom-29, BARI Gom-32	5	Moderately Tolerant
Borkot, BARI Gom-23, BAW-1262, ESWYT P-30, ESWYT P-37, BAW-1274	7	Susceptible
None	9	Highly Susceptible

Parameters were analyzed using ANOVA and also compared with control plants to assess change over control. In the case of shoot length, at 12 dS/m, three varieties, namely BAW-1262 (4.27%), ESWYT P-44 (3.86%), BARI Gom-27 (0.73%) showed increment (negative reduction in Table 4) while the rest of the varieties showed decrement. ESWYT P-28 (3.61%) showed the lowest reduction percentage, followed by ESWYT P-11 and ESWYT P-30. At 16 dS/m salinity, ESWYT P-28, ESWYT P-30, and ESWYT P-44 showed less reduction in shoot length. At 20 dS/m salinity, ESWYT P-30 showed the lowest reduction, followed by ESWYT P-37 and ESWYT P-28. BINA Gom-1 showed the highest reduction at 20 dS/m (Table 3b). In the case of root length at 12 dS/m salinity, many varieties showed increment while some showed reduction. Triticale showed the highest

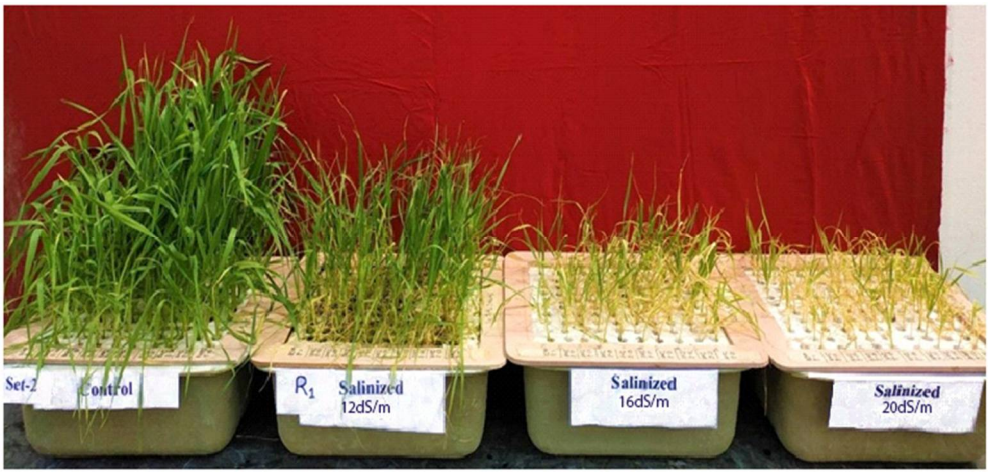

Fig. 1. Wheat germplasm in a saline hydroponic system at control, 12 dS/m, 16 dS/m and 20 dS/m salinity level at the seedling stage.

Table 4. Percentage of reduction of morphological attributes affected by various levels of salinity at seedling stage.

Genotypes	Treatments (dS/m)	Shoot length (% reduction)	Root length (% reduction)	Shoot fresh weight (% reduction)	Root fresh weight (% reduction)	Shoot dry weight (% reduction)	Root dry weight (% reduction)
Akbar	12	19.77	-28.12	59.12	32.26	60.67	28.00
	16	25.66	-52.30	64.86	20.43	64.04	4.00
	20	40.99	-1.55	81.76	69.89	79.78	64.00
Borkot	12	8.78	-34.04	32.63	31.29	38.82	-19.23
	16	26.87	-35.42	60.70	46.26	56.47	15.38
	20	51.60	1.37	85.61	80.95	83.53	65.38
Agroni	12	25.07	-18.13	60.29	51.25	60.49	40.91
	16	33.76	-12.70	74.26	53.75	72.84	45.45
	20	51.93	2.91	87.50	76.25	86.42	72.73
Triticale	12	33.43	-41.71	52.72	9.09	53.77	8.82
	16	27.16	-22.82	64.95	30.91	61.32	29.41
	20	34.46	-3.16	68.48	63.64	63.21	61.76
Pavon-76	12	9.62	-28.07	28.87	-22.73	25.61	-17.86
	16	26.60	-69.49	57.04	11.36	51.22	21.43
	20	41.65	-12.46	79.23	68.18	75.61	67.86
BARI Gom-20	12	17.03	-10.05	53.97	39.36	48.84	41.38
	16	31.89	2.95	63.91	35.11	56.98	34.48
	20	42.11	13.03	78.81	75.53	75.58	72.41
BARI Gom-21	12	23.22	3.48	63.14	-5.88	58.82	-10.53
	16	20.65	-21.70	61.22	10.29	51.76	0.00
	20	34.20	6.25	73.40	60.29	67.06	52.63
BARI Gom-23	12	20.16	-29.81	12.06	-33.82	18.06	-35.00
	16	27.13	-55.91	48.64	-20.59	40.28	-25.00
	20	51.71	11.95	77.82	66.18	73.61	60.00
BAW-1262	12	-4.26	3.62	10.16	58.51	21.52	53.85
	16	9.31	5.16	33.59	12.77	31.65	3.85
	20	29.66	31.97	61.72	68.09	58.23	61.54
BAW-1284	12	5.85	16.47	30.15	44.93	32.11	42.86
	16	17.89	39.36	56.96	33.33	50.46	28.57
	20	22.62	40.41	80.93	65.22	77.06	61.90
ESWYT P-2	12	11.98	-7.24	48.71	46.15	40.70	45.00
	16	28.25	15.65	57.42	23.08	50.00	20.00
	20	34.60	10.62	80.65	56.92	76.74	55.00
ESWYT P-3	12	10.38	-9.26	28.32	38.57	30.86	30.00
	16	14.24	11.60	47.20	25.71	38.27	15.00
	20	27.32	5.28	76.22	67.14	71.60	60.00
ESWYT P-8	12	14.32	15.14	35.19	44.74	39.42	43.48
	16	25.82	-6.66	51.54	32.89	49.04	30.43
	20	30.32	12.21	71.91	63.16	70.19	60.87
ESWYT P-11	12	5.78	-14.29	7.97	23.81	14.29	25.00
	16	20.58	-31.76	50.36	31.75	45.24	30.00
	20	25.99	-40.48	71.38	58.73	67.86	55.00

ESWYT P-12	12	9.90	-8.29	23.34	10.14	29.03	9.52
	16	19.44	8.60	43.21	23.19	43.01	23.81
	20	25.44	-10.20	59.23	44.93	58.06	38.10
ESWYT P-19	12	10.87	-7.72	12.77	-10.34	4.76	0.00
	16	22.46	3.88	40.85	15.52	28.57	16.67
	20	31.87	30.39	65.53	56.90	57.14	50.00
ESWYT P-28	12	3.61	-18.39	-13.09	-26.47	-8.77	-23.81
	16	11.83	38.83	51.31	57.35	45.61	52.38
	20	20.48	4.62	69.11	66.18	64.91	61.90
ESWYT P-30	12	5.84	-15.43	20.42	6.38	9.59	3.57
	16	11.30	29.14	50.52	60.64	34.25	57.14
	20	19.86	8.57	66.44	75.53	54.79	71.43
ESWYT P-37	12	20.53	-10.47	29.34	-3.23	31.08	4.76
	16	21.67	16.98	56.20	41.94	51.35	42.86
	20	20.16	-13.73	69.01	61.29	66.22	61.90
ESWYT P-44	12	-3.86	5.50	-15.61	-52.38	-3.77	-53.85
	16	14.14	2.77	35.84	28.57	30.19	23.08
	20	21.89	1.41	61.27	54.76	58.49	46.15
BARI Gom-27	12	-0.73	-5.63	0.00	7.41	10.94	8.33
	16	17.71	35.96	36.95	70.37	32.81	66.67
	20	26.57	9.00	60.59	71.60	57.81	66.67
BARI Gom-29	12	12.73	-18.25	26.81	0.00	29.41	0.00
	16	16.77	12.32	25.96	50.00	17.65	47.83
	20	31.68	19.41	48.51	71.62	39.71	69.57
BARI Gom-32	12	11.66	-8.52	7.83	10.53	12.00	16.67
	16	24.34	11.59	27.71	12.28	20.00	50.00
	20	36.66	8.56	58.43	70.18	54.00	66.67
BAW-1274	12	18.05	4.82	29.24	47.76	34.94	43.59
	16	17.66	20.68	48.38	62.69	43.37	61.54
	20	24.81	22.12	62.45	78.36	60.24	74.36
BINA Gom-1	12	14.79	-35.93	10.43	26.09	3.64	34.62
	16	19.59	-4.27	43.56	42.03	14.55	42.31
	20	71.92	59.72	45.40	63.77	10.91	65.38

(41.71%) increment, while ESWYT P-44 roots were reduced (5.50%). At 16 dS/m salinity, varieties showed both increment and decrement. But BARI Gom-23 showed the highest increment (55.90%). At 20 dS/m salinity, ESWYT P-11 showed the highest (40.48%) increment of root length, though many other varieties showed increment as well as acceptable percentage of reduction in root length. For shoot fresh weight at 12 dS/m salinity, two varieties, namely ESWYT P-44 (15.61%) and ESWYT P-28 (13.09%) showed increased shoot fresh weight value, while others showed decreased value. Bina Gom-1 showed an acceptable degree of reduction in fresh weight (10.43%) at 12 dS/m. At 16 dS/m salinity, BARI Gom-29 (25.96%) showed the lowest reduction, followed by BARI Gom-32. At 20 dS/m salinity, BINA Gom-1 (45.4%) showed the lowest reduction in shoot fresh weight, followed by BARI Gom-29 (48.51%). In the case of 12 dS/m salinity, ESWYT

P-44 (52.38%) showed a tremendous increment for root fresh weight, followed by BARI Gom-23 (33.82%). At 16 dS/m salinity, only BARI Gom-23 (20.59%) showed an increment for root fresh weight, while other varieties showed a reduction. At 20 dS/m, ESWYT P-12 (44.93%) showed minimum reduction. BINA Gom-1's reduction was at an acceptable level (63.77%). For shoot dry weight at 12 dS/m, ESWYT P-28 (8.77%) showed increased weight, followed by ESWYT P-44 (3.77%), while others showed reduction. Among them, BINA Gom-1 (3.64%) had the lowest decrement of shoot dry weight at 12 dS/m. At 16 dS/m, BINA Gom-1 (14.55%) showed minimum reduction. Also, at 20 dS/m, BINA Gom-1 showed its supremacy, showcasing the lowest reduction (10.91%). At 12 dS/m salinity, root dry weight showed both increased and decreased values. ESWYT P-44 (53.85%) showed maximum increased value followed by BARI Gom-23 (35%). At 16 dS/m, BARI Gom-23 showed an increment (25%) and ESWYT P-44 (23.08%) showed an acceptable reduction. At 20 dS/m, ESWYT P-12 (38.1%) showed the lowest reduction, while ESWYT P-44 (46.15%) showed less reduction than many varieties (Table 4).

At 12 dS/m salinity, the Individual salt tolerant index (ISTI) ranged from 3.80 (Agroni) to 8.28 (BINA Gom-1) followed by ESWYT P-44 (7.24), ESWYT P-28 (6.87) and BARI gom-23 (6.48). At 16 dS/m salinity, Individual salt tolerant index (ISTI) ranged from 2.91 (ESWYT P-2) to 6.87 (BINA Gom-1) followed by ESWYT P-44 (6.11), ESWYT P-28 (5.60) and BARI gom-23 (5.12). At 20 dS/m salinity, the Individual salt tolerant index (ISTI) ranged from 2.22 (Agroni) to 5.05 (ESWYT P-44), followed by BARI gom-23 (4.97), BINA Gom-1 (4.37). Total salt tolerance index (summation of all ISTI) was highest of BINA Gom-1 (19.53) followed by ESWYT P-44 (18.40), ESWYT P-28 (16.78), BARI gom-23 (16.57) ESWYT P-19 (14.88) (Table 5).

For molecular screening of salt tolerance of the twenty-five (25) wheat genotypes, we used twenty-one (21) SSR primer pairs. A total of 116 alleles were detected at 21 loci (Fig. 2). A wide range of allelic variants was observed for each locus. The lowest number of alleles was 2.0 found for marker Xwmc-405 and the highest number of alleles was 9.0 detected for marker Xbarc-45, with an average of 5.52. Observed allele sizes were within the expected allele size ranges in most of the primers. Rare alleles were observed at 11 SSR loci (Xbarc-45, Xcfd-1, Xcfd-13, Xgwm-249, Xgwm-296, Xgwm-455, Xwmc-24, Xwmc-44, Xwmc-432, Xwmc-661) with an average of 0.761 alleles per locus (Table 6). Among 21 SSR loci used in this study, Xgwm-249, Xgwm-296, Xwmc-18, Xwmc-44, Xwmc-110 and Xwmc-661 primer showed one null allele, with an average of 0.285 in 25 genotypes. A major allele is defined as the allele with the highest frequency and is also known as the most common allele at the locus. The size of the different major alleles at different loci ranged from 111 bp for Xgwm-296 to 264 bp for Xcfd-13. The highest genetic diversity (0.845) was observed in loci Xwmc-24 and the lowest (0.477) was observed in loci Xwmc-432 with the mean diversity of 0.694 as estimated following the formula of Nei's, (1973). The PIC value ranged from 0.375 to 0.825 with an average value of 0.650). PIC values also showed a significant, positive correlation with the number of alleles and allele size (Table 6).

Table 5. Ranking of wheat genotypes for salinity tolerance according to STI value.

Genotypes	12 dS/m ISTI	16 dS/m ISTI	20 dS/m ISTI	TSTI	Ranking
Akbar	4.28	4.73	2.65	11.67	19
Borkot	5.42	4.30	2.32	12.03	16
Agroni	3.80	3.33	2.22	9.35	25
Triticale	4.84	4.09	3.12	12.05	15
Pavon-76	6.05	5.02	2.80	13.86	8
BARI gom-20	4.09	3.75	2.43	10.27	23
BARI gom-21	4.68	4.78	3.06	12.52	13
BARI gom-23	6.48	5.12	4.97	16.57	4
BAW-1262	4.57	3.47	3.77	11.80	18
BAW-1284	4.28	3.32	3.11	10.71	21
ESWYT P-2	4.15	2.91	3.13	10.19	24
ESWYT P-3	4.71	3.58	3.66	11.95	17
ESWYT P-8	4.08	3.11	3.46	10.65	22
ESWYT P-11	5.37	4.08	4.22	13.67	10
ESWYT P-12	5.26	4.09	3.90	13.25	11
ESWYT P-19	5.90	4.70	4.28	14.88	5
ESWYT P-28	6.87	5.60	4.31	16.78	3
ESWYT P-30	5.70	3.60	3.07	12.37	14
ESWYT P-37	5.28	4.16	3.69	13.14	12
ESWYT P-44	7.24	6.11	5.05	18.40	2
BARI gom-27	5.80	4.56	3.90	14.25	6
BARI gom-29	5.49	4.27	4.16	13.92	7
BARI gom-32	5.50	4.29	4.08	13.86	9
BAW-1274	4.22	3.27	3.35	10.83	20
BINA Gom-1	8.28	6.87	4.37	19.53	1

Genetic similarity analysis using Unweighted Pair Group Method of Arithmetic Mean (UPGMA): A dendrogram was constructed based on Nei's (1973) genetic distance calculated from the 116 SSR alleles (by 21 markers) generated from 25 wheat genotypes. The UPGMA cluster analysis showed significant genetic variation among the wheat genotype studied, with a similarity coefficient varying between 0.14 and 0.86. The UPGMA cluster analysis divided 25 germplasm into six major clusters (Fig. 3).

Hydroponic culture of wheat in collaboration with salinity stress is a method of screening and characterizing wheat germplasms and advanced lines (Hussain et al. 2015). The growth phase "seedling stage" is considered an important factor for a plant to survive abiotic stress conditions later in the cycle (Shah et al. 2021, Khan et al. 2020, Zulfiqar et al. 2014). Pre-selection of accessions before field trial is an effective approach to rule out unfit advanced lines in any breeding program (Ali et al. 2012, Munns and James 2003). Controlled conditions should be prioritized for the screening of wheat for

salt tolerance, as they exhibit minimal ecological variations, such as altering soil pH and deleterious components from one region to another and even from land to land (Munns and James 2003). Standard evaluation score (SES) is a test based on phenotypes only and thus just gives a slight idea about the true nature of any variety. The response to salinity is different among species and even among genotypes within the same species. Previous research indicated that root length appeared to be a critical parameter for salt stress tolerance (Ali et al. 2007, Shahzad et al. 2022). Most of the previous reports revealed that

Table 6. Summary statistics of 21 Simple Sequence Repeat (SSR) markers found among 25 wheat genotypes.

Marker	Chromosome No.	Allele No.	Rare Allele	Null Alleles	Major Allele		Gene Diversity	PIC
					Frequency	Size(bp)		
Xbarc-45	3A	9.0	2	-	0.240	188	0.835	0.815
Xcfd-1	6D	7.0	1	-	0.320	175	0.784	0.754
Xcfd-13	6B	7.0	2	-	0.520	156	0.659	0.620
Xcfd-49	6D	7.0	-	-	0.320	195	0.813	0.790
Xcfd-54	4B/4D	6.0	2	-	0.560	167	0.627	0.588
Xgwm-160	4A	4.0	-	-	0.360	182	0.726	0.677
Xgwm-249	2D	4.0	1		0.520	165	0.630	0.572
Xgwm-296	2D/7D	6.0	1	1	0.240	136	0.810	0.781
Xgwm-314	3D	3.0	-	1	0.560	124	0.589	0.523
Xgwm-455	2D	6.0	1	-	0.280	172	0.781	0.747
Xtxp-12	-	4.0	-	-	0.480	208	0.646	0.585
Xwmc-17	7A	4.0	-	-	0.600	136	0.582	0.539
Xwmc-18	2D	6.0	-	1	0.280	164	0.790	0.758
Xwmc-24	1A	8.0	1	-	0.200	174	0.845	0.825
Xwmc-44	1B	8.0	2	1	0.360	246	0.774	0.744
Xwmc-110	5A	4.0	-	1	0.680	158	0.490	0.445
Xwmc-154	2B	6.0	-	-	0.400	192	0.765	0.736
Xwmc-170	2D	6.0	-	-	0.280	212	0.784	0.751
Xwmc-405	7D	2.0	-	-	0.520	206	0.499	0.375
Xwmc-432	1D	4.0	1	-	0.680	195	0.477	0.420
Xwmc-661	2B	5.0	2	1	0.440	224	0.659	0.598
Mean		5.52	0.761	0.285	0.421		0.694	0.650

salinity causes a reduction in root length (Uzair et al. 2022, Rafiq et al. 2006, Ghonaim et al. 2021, Zafar et al. 2015, Attaullah et al. 2019). In this experiment, we have found that some accessions and varieties increase root length instead of decreasing (Table 4). This is a unique finding as no other varieties have shown this phenomenon before except the rice experiment conducted by Amirjani (2011) and in a wheat experiment by Gholizadeh et al 2021. As salinity causes root length reduction in susceptible varieties, if any variety shows increased root length, then we can assume that this variety may be salinity tolerant. So, we can guess that some varieties of this experiment may be salt tolerant (Table 4), especially ESWYT P-11 and ESWYT P-44. Shoot length decreases as a result of salinity stress in maximum experiments (Bilkis et al. 2016, Saddiq et al. 2021, Seleiman et

al. 2022, Zeeshan et al. 2020). Our experiment revealed some varieties with higher plant height, even in salinity stress (Table 4). This indicates the salinity resistance capability of the germplasms (ESWYT P-28, ESWYT P-30, and ESWYT P-44). Shoot fresh weight decreased as salinity increased in our experiment except for ESWYT P-28 and ESWYT P-44 at 12 dS/m salinity. ESWYT P-44 and BINA Gom-1 showed relatively lower reduction rates at every salinity level. Root fresh weight showed a similar pattern as shoot fresh weight. Shoot dry weight followed the shoot fresh weight's pattern but the lowest decrement was of BINA Gom-1, maybe it's because of genetics. Root dry weight revealed a similar result to shoot dry weight. In short, BINA Gom-1, ESWYT P-28, and ESWYT P-44 showed better performance with respect to plant biomass. The salt tolerance index gives a clear idea about the tolerance capacity of any given variety (Hasan et al. 2015, Al-Ashkar et al. 2020, Tao et al. 2021). BINA Gom-1 ranked first at STI because it showed the overall best performance in the morphological aspect, followed by advanced line ESWYT P-44 and ESWYT P-28.

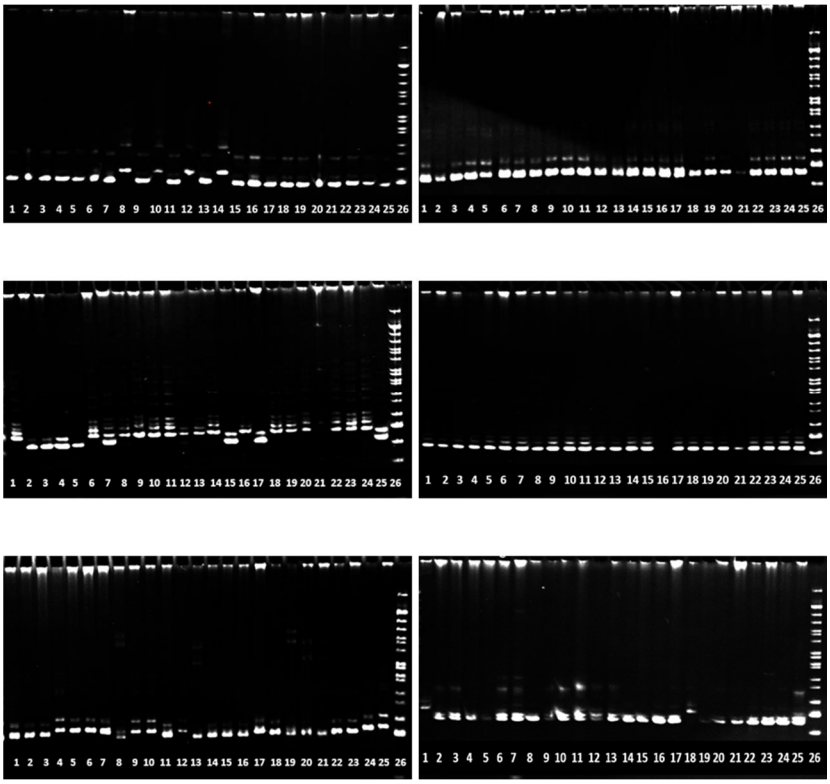

Fig. 2. SSR profiles of 25 wheat genotypes using primer Xcfd-1(a), Xwmc-17(b), Xcfd-49 (c), Xwmc-110(d), Xwmc-170(e) and Xwmc-432(f). [(1) BINA Gom-1, (2) Akbar, (3) Borkot, (4) Agroni (5)Triticale, (6) Pavon-76, (7) BARI Gom- 20, (8) BARI Gom- 21, (9) BARI Gom- 23, (10) BAW- 1262, (11) BAW- 1284 (12) ESWY TP-2 (13)ESWY TP-3 (14) ESWY TP-8 (15)ESWY TP-11 (16)ESWY TP-12, (17) ESWY TP-19, (18)ESWY TP-28, (19)ESWY TP-30, (20) ESWY TP-37, (21) ESWY TP-44, (22)BARI Gom- 27, (23)BARI Gom-29, (24) BARI Gom-32, (25) BAW- 1274, (26) 50bp ladder.

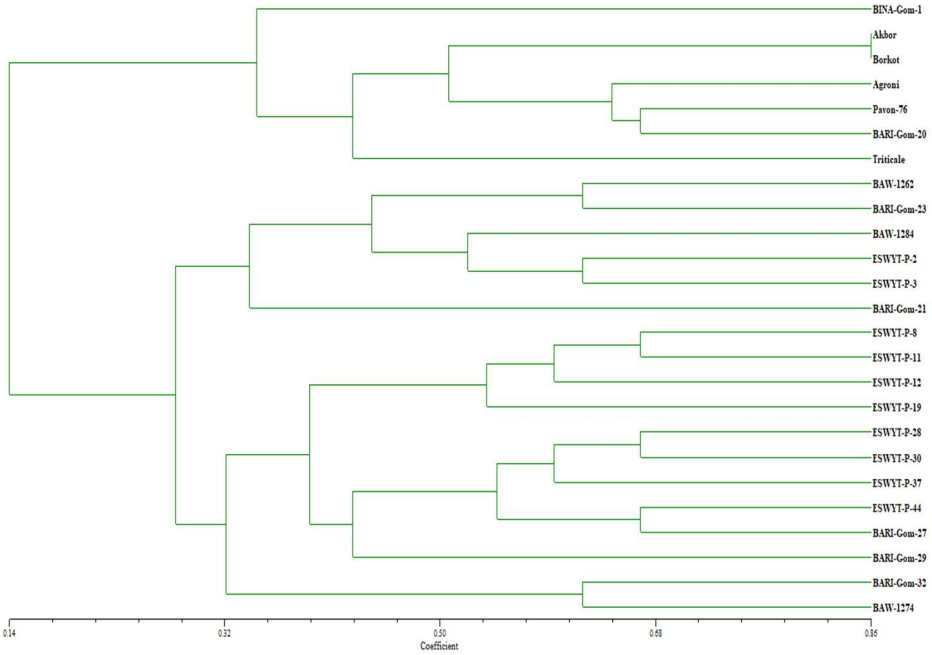

Fig. 3. SSR-based genetic relationship between 25 wheat genotypes shown by UPGMA cluster analysis based on Nei's (1973) genetic distance.

This experiment witnessed a high level of genetic variation among the wheat genotypes at the DNA level using SSR markers. The genotypes of the present study included 15 Bangladeshi and 10 exotic landraces and inbred lines. SSR analysis indicated that allele frequency ranged between 2 and 9, with an average of 5.52 alleles per locus. Abbasov et al. (2018) found 111 alleles and 10 alleles per locus, which was similar to the findings of the present study. The highest number of alleles (9) was amplified by the marker Xbarc-45. On the contrary, the Xwmc-405 marker was found to produce a monomorphic allele. Several factors, like the structure of a primer and the number of annealing sites in the genome, are responsible for creating variations in the number of alleles detected by different sets of primers (Kernodle et al. 1993). Polymorphic bands revealing differences among genotypes would be used to examine and establish systematic relationships among genotypes (Hadrys et al. 1992). The null alleles resulted from the locus Xgwm-249, Xgwm-296, Xwmc-18, Xwmc-44, Xwmc-110 and Xwmc-661 primer and the lowest frequency of null allele was detected by six loci with an average of 0.285, in this study. Null alleles are the alleles that fail to amplify during PCR, probably due to polymorphism at the hybridization sites of one or both primers (Dakin et al. 2004). Null alleles can arise from a point mutation(s) in one or both the primer binding sites thereby inhibiting primer annealing. In this study, D genome-based SSR markers (Xcfd-1, Xcfd-49, Xgwm-249, Xgwm-296, Xgwm-314, Xgwm-455, Xwmc-18, Xwmc-17, Xwmc-405

and Xwmc-432) produced maximum alleles, followed by A and B genomes. Therefore, the D genome appeared as the richest in identifying SSR-based polymorphisms. 11 markers were found as rare alleles because they were amplified in less than 5% of genotypes only. Among them, seven rare allele-producing markers are located on the D genome. Gorham et al. (1987) reported in their experiment that the D genome controls salinity tolerance. So, these rare alleles contain potential genetic materials that control the tolerance capacity of the hexaploid wheat. Genetic diversity among the existing genotypes is considered as a raw material in breeding to spur genetic improvement, both in increasing the yield potential and decreasing reliance on production inputs like fertilizers, water, and pesticides, and assures potential progress in plant breeding and insurance against unforeseen threats to agricultural production of biotic or abiotic stresses (Gepts 2006). The genetic diversity was measured by the polymorphic information content (PIC). Vaiman et al. (1994) considered loci polymorphism to be high, medium, or low when PIC >0.5, 0.5> PIC >0.25 and PIC <0.25, respectively. The PIC value ranged from 0.375 in Xwmc-405 to 0.825 in Xwmc-24, with an average value of 0.650 per marker. The PIC values recorded in this study are higher than the PIC values reported in other studies by Bányai et al. (2006). However, Uddin and Boerner (2008) reported similar observations. The simple sequence repeats (SSRs) represent the most suitable marker system in wheat (Hammer et al. 2000) and have been successfully used to characterize genetic diversity in advanced wheat breeding materials. Generally, the salt-tolerant genotypes tend to cluster together, indicating the efficiency of SSR markers in distinguishing between sensitive and tolerant genotypes regarding their similarity matrix. The coefficient of similarity matrix ranged from 0.14 to 0.87, with an average of 0.55, indicating the presence of considerable genetic variation in the genotypes tested. The dendrogram (UPGMA) was constructed based on Nei's (1973) genetic distance, which separates 25 wheat genotypes distinctly. As all the markers in the present study were related to salt tolerance, genetic similarity-based clustering might be indicative of the genetic potentiality for salt tolerance. The marker-assisted study divided 25 genotypes into 6 clusters at 0.40 cut-off similarity coefficient. Most salt-tolerant genotype BINA Gom-1 found in cluster 1 and moderately tolerant varieties such as ESWYT P-8, ESWYT P-11, ESWYT P-12, ESWYT P-19, ESWYT P-28, ESWYT P-30, ESWYT P-37, ESWYT P-44, BARI Gom-27 and BARI Gom-29 in cluster 5A. Cluster 5 consisted of the highest number of genotypes. Among 10 Mexican CIMMYT lines in our experiment, 8 germplasm grouped into this cluster 5 and most of them are categorized as moderate tolerant of salinity. But ESWYT P-44, ESWYT P-28, BARI Gom-23, ESWYT P-19, BARI Gom-27, BARI Gom-29 and BINA Gom-1 showed high to moderate tolerance levels of salinity. So, by both morphological and molecular analysis it is strongly suggested that these varieties and inbred advanced lines may be suitable candidates to incorporate in a high salinity tolerant wheat breeding program.

This experiment explored the salt tolerance ability of 25 wheat genotypes, and it appeared that salinity had an inhibitory effect on plant biomass. Root length and shoot

length characteristics of some genotypes were unique and surprising for some genotypes. The genotypes contrasted in their attitude to salinity stress. This experiment concludes that genetic variability, along with biomass-related parameters due to their genetic constitution, can be utilized as a selection criterion to identify salt tolerant wheat genotypes. Among the genotypes, advanced lines, namely ESWYT P-44, ESWYT P-28, ESWYT P-19 and HYVs, namely BARI Gom-23, BARI Gom-27, BARI Gom-29 and BINA Gom-1 seen to be more tolerant considering all the aspects of morphological and molecular indices. The present investigation is well explained to recognize the best genotypes and selection criteria for the preferred traits. So, genotypes displayed in this instance may be employed in breeding programs, which can prompt work on the financial status of the stakeholders overall and farmers living in salt-impacted regions. Further assessments are relied upon to draw stronger conclusions for the advancement of salt-tolerant wheat genotypes.

Acknowledgment

We cordially acknowledge our funding collaborator from the Ministry of Education (MoE), Peoples Republic of Bangladesh.

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(Manuscript received on 13 November, 20224; revised on 3 December, 2024)