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Screening Salt Tolerant Wheat Germplasm by Hydroponic and Pot Culture and Salinity Related Gene Expression Analysis

Muhammad Shahidul Haque[1]*, Md. Shamsul Alam[2], Israt Dolon[3] Sumitra Saha[4] and Fahmida Khatun[5]

[1]*Professor, Department of Biotechnology, Bangladesh Agricultural University, Mymensigh, Bangladesh*
[2&3]*Student, Department of Biotechnology, Bangladesh Agricultural University, Mymensigh, Bangladesh*
[4]*Assistant Professor, Department of Biotechnology, Bangladesh Agricultural University, Mymensigh, Bangladesh*
[5]*Professor, Department of Biotechnology, Bangladesh Agricultural University, Mymensigh, Bangladesh*
E-mail: [1]haquems@bau.edu.bd

Abstract—*Wheat is the staple food grain next to rice. It fulfills global food demands by producing approx. 30% of total cereal crops. Soil salinity is considered as one of the primary abiotic stress factor. It causes significant loss of crop yield. In Bangladesh, about 1.51 Mha of arable lands are affected by varying degrees of salinity. About 53% of coastal soils of Bangladesh are salt-affected. More than 50% of arable land worldwide could be salt-affected by 2050. This study aimed to detect diversity for salt tolerance in wheat Germplasm by morph-physiological attributes; to Identify Salt tolerant wheat Germplasm by hydroponic and pot culture and to analyze Salt tolerant gene expression under saline and non-saline condition. Twenty wheat genotypes were cultured on 0, 8, 12 and 15 dS/m salinity. Wheat seedlings of 12 days old were imposed with salt stress by adding crude salt with the nutrient solution in hydroponic system. Expression of four related genes was analyzed after cultivation of the genotypes in pots with salinity treatments. Morphological characters like shoot and root length, number of roots, shoot and root fresh and dry weight were used to calculate Stress Tolerance Index (STI). Standard Evaluation System (SES) was used visually. Based on the SES and salt tolerance index (STI) using morphological traits at 15 dS/m salt stress BARI Gom (29), BINA Gom 1, BWMRI 2 have been identified as salt-tolerant genotypes. Six genotypes were moderately tolerant and the rest 11 were sensitive. Pot culture with three tolerant, two moderately tolerant and two sensitive genotypes for gene expression analysis of 4 genes related to salinity tolerance has performed and will be discussed. The output of the study will be valuable for developing saline tolerant wheat for sustainable wheat cultivation in Bangladesh.*

INTRODUCTION

Salinity is considered one of the most important abiotic stressors, threatening food security and affecting human life in arid and semi-arid regions [1]. It severely restricts crop production worldwide. More than 6% of the land in the globe is estimated to be saline and sodic soils. The saline prone area is rising gradually [2] and it is further speculated that over 50% of global arable land will be salinized by 2050 [3].

Yield reductions of 50% in durum wheat under dryland salinity [4], 88% in bread wheat under high irrigation salinity [5], and 70% under sodicity have been reported [6]. Due to the genetic and physiological complexities of the salt tolerance trait, and lack of a reliable and rapid screening assay [7, 8], progress in breeding cereal cultivars with salinity or sodicity tolerance has been slow [9]. Moreover, elite germplasm may not have genes able to confer worthwhile salt tolerance, and hence, finding salt tolerance in accessions/landraces/inbreds and wild wheat relatives and introgression by molecular marker aided breeding and/or genetic engineering may be required.

Field evaluation of salt tolerant genotypes requires more cropping seasons for screening and evaluation. Spatial differentiation in soils impacts field evaluation which results in a high coefficient of variation, which adversely affects the reliability of the results [10]. It is also difficult to measure root traits accurately in the field [11]. Testing the salt tolerance of genotypes in a glasshouse/laboratory setting, where plants are under controlled conditions in hydroponic or small-scale pots, can be a useful indicator, as there is a significant correlation between stress resistance observed in the field and stress resistance observed in the laboratory [12]. Important laboratory protocols for the screening of salt tolerance in crop plants include seed germination in saline media, exposure of the plant to water stress, determining control of membrane stability, and measuring leaf water content [13]. Although salinity tolerance is determined by polygenic inheritance, most studies still treat salinity tolerance as a single-gene trait and traditionally use visual scoring [14]. Hence, a combination of morphological, physiological, and genetic characters seems effective for evaluating the salt tolerance. The present study was conducted with the objective to identify salt tolerant wheat germplasm by hydroponic and pot culture and to

analyze Salt tolerance gene expression under saline and no-saline condition.

MATERIALS AND METHODS

Plant Material: Twenty wheat cultivars were used in this study.

Hydroponic Experiment: The salt tolerance of tested genotypes was evaluated in a hydroponic trial. The seeds were germinated on quarter-strength Hoagland's nutrient solution [15]. The pH was adjusted to 6.0. The solution was replaced once a week and aerated continuously. All genotypes were evaluated under 3 salinity levels: 0, 8, 12 and 15 ds/m NaCl. The experiment was carried out in a completely randomized factorial design and replicated 3 times, with 25 seeds for each genotype. After 25 days of salinity treatment, the plants were harvested.

Pot Experiment: Seeds of each genotype were germinated in Petri dishes on filter paper soaked with distilled water and incubated in the dark at 22 ℃. After germination the seedlings were transferred to soil-filled pots and irrigated with deionized water. Four plants per pot were grown in 3 kg capacity pots to enable testing. There were 3 salinity levels: 0, 100 and 200 mMNaCl. The plants were grown until maturity. At heading, penultimate leaves were sampled for gene expression. The pots were kept in net house under natural environment.

RNA Extraction and cDNA Synthesis: Total RNA was isolated using Geneaid total RNA purification mini kit (Taiwan) according to the manufacturer's instructions. First-strand cDNA was synthesized from 500 ng of total RNA using Reverse Transcription system (Bioneer, Korea) with an oligo-dT15 primer. The reaction solution was used as templates for reverse transcriptase polymerase chain reaction (RT-PCR).

Gene Amplification: Target gene and wheat housekeeping B-actin (reference gene) cDNA were amplified using specific primers. Polymerase chain reaction was initiated with hotstart method using the cDNA template on Labnet Thermocycler (USA).

The expression of target gene was examined by SYBR real time RT-PCR using Exicycler real time PCR (Bioneer, Korea). One step RT-PCR was performed using premix RT-PCR qPCR kit (Bioneer, Korea), following the manufacturers protocol. Table: List of primer of Salt related gene of Wheat: TaGSK1: Forward ACGTTTGGTCTGCTGGCTG and reverse GTGCCATGGGTGAGCTTTGATT; TaNIP; Forward: GCATTACGTCCATCTTCGCA and reverse CCTCGAAGCGGATGT GGTG; Actin; Forward TGGCACCCGAGGAGCACCCTG and reverse GCGACGTACATGGCAGGAACA. Expression of four related genes was analyzed after cultivation of the genotypes in pots with salinity treatments. Morphological characters like shoot and root length, number of roots, shoot and root fresh and dry weight were used to calculate Stress Tolerance Index (STI). Standard Evaluation System (SES) was used visually.

RESULTS AND DISCUSSION

The wheat genotypes were cultured in hydroponic system and their relative performance on shoot and root developments are presented in Table 1. It was found the all the characters of root and shoot were affected by the salinity treatments. Shoot length and shoot fresh and dry weight declined with the increasing salt concentrations. On the other hand, there were sharp decrease in root fresh and dry weight and root number, however, root length was less affected by the salinity concentrations.

Table 1: Effects of salinity on root and shoot characters of wheat grown under hydroponic system

Salinity (dS/m)	Shoot Length	Root length	Shoot fresh Weight	Root Fresh Weight	Shoot Dry Weight	Root Dry Weight	Root Number
0	46.03a	14.51ab	2.59a	0.78a	0.40a	0.094 b	20.30a
8	43.04 b	15.17a	1.94 b	0.59 b	0.26 b	0.102a	15.42 b
12	41.67 b	14.81ab	1.76 bc	0.51 b	0.29 b	0.063 c	13.58 c
15	41.66 b	14.10 b	1.53 c	0.33 c	0.28 b	0.067 c	13.62 c
Level of sign.	**	*	*	**	**	**	**

** and * indicate significant at 0.01 and 0.05 probability levels.

Wheat genotypes were grown hydroponically at 12 and 15 ds/m salinity to sort out the 20 genotypes into tolerant, moderately tolerant and susceptible on the basis of salinity tolerance index (STI) and standard evaluation system (SES). The result has been presented in Table 2. It is evident by STI that six genotypes emerged as tolerant, eight as moderately tolerant and six as susceptible. Among the six tolerant genotypes, five were tolerant at 12 ds/m and three were found tolerant at 15 ds/m using SES evaluation method. On the other hand, the eight moderately tolerant genotypes, six were moderately tolerant at 12 ds/m and only two were found moderately tolerant up to 15 ds/m using SES evaluation method.

Table 2: Screening of .wheat genotypes by salinity tolerance index (STI) and standard evaluation system (SES) at 12 and 15 sd/m of salinity

Variety Name	Salinity Tolerance Index, STI	Salinity Tolerance	SES score at 12 ds/m	Salinity Tolerance	SES score at 15ds/m	Salinity Tolerance
BARI Gom-28	0.81	T	3	T	5	MT
BARI Gom-25	0.78	T	7	S	7	S
BARI Gom-29	0.78	T	3	T	3	T
BWMRI-2	0.76	T	3	T	3	T
BINA Gom-1	0.74	T	3	T	3	T
BARI Gom-20	0.71	T	3	T	5	MT

BARI Gom-30	0.66	MT	5	MT	7	S
BARI Gom-33	0.63	MT	5	MT	5	MT
BARI Gom-21	0.62	MT	5	MT	7	S
BARI Gom-27	0.62	MT	5	MT	7	S
BARI Gom-24	0.61	MT	5	MT	7	S
BWMRI-1	0.60	MT	7	S	7	S
TRITICLE	0.59	MT	7	S	7	S
BARI Gom-32	0.58	MT	5	MT	5	MT
BWMRI-3	0.53	S	5	MT	5	MT
BARI Gom-23	0.48	S	3	T	5	MT
GOURAB	0.47	S	7	S	7	S
BARI Gom-22	0.47	S	5	MT	7	S
BARI Gom-31	0.46	S	7	S	7	S
BARI Gom-26	0.43	S	5	MT	5	MT

As presented in Table 3, we could identify three genotypes namely BARI Gom 29, BINA Gom 1, BWMRI 2 as salinity tolerant variety by 0.71 to 0.81 STI (their performance at salinity was 71% to 81% those of the control condition) and SES score 3. While, five BARI released varieties (BARI Gom 23, 26, 28, 32, 33) and BWMRI 3 were found moderately tolerant to salinity. They had a score of 0.58 to 0.66 STI and SES 5. BARI Gom (20, 21, 22, 24, 25, 27, 30, 31), BWMRI 1, Gourab, Triticale were identified as sensitive genotypes. Many studies investigated the tolerance of wheat into the salinity and found that the tolerated genotypes can survive in the level of 150 mMNaCl [16, 17]. In our study, three genotypes survived and performed better in 15 ds/m salinity. However, in the present study there were tremendous reproductive damage in all the genotypes. The damage included wrinkled grains, lower grain weight, less number of grains/spikelet, spikelet/ear size of ear and tiller number. All the yield and yield character were affected by salinity and some the damages are shown in Figure 1. In the gene expression analysis, preliminary result showed upregulation of the TaGSK1 and TaNIP genes (data not shown)

Table 3: Separation of wheat genotypes based on the performance (STI) and SES under salinity stress

Varieties	(STI)	Varieties (12 DS/m)	SES	Varieties (15 DS/m)	Tolerance
BARI Gom (20, 25, 28, 29), BWMRI 2, BINA Gom 1	0.71 to 0.81	BARI Gom (20, 23, 28, 29), BINA Gom 1, BWMRI 2	3	BARI Gom 29, BINA Gom 1, BWMRI 2	Tolerant
BARI Gom (21, 24, 27, 30, 32, 33), BWMRI 1, Triticale	0.58 to 0.66	BARI Gom (21, 22, 24, 26, 27, 30, 32, 33), BWMRI 3	5	BARI Gom (23, 26, 28, 32, 33), BWMRI 3	Moderately Tolerant
BARI Gom (22, 23, 26, 31), Gourab, BWMRI 3	0.43 to 0.53	BARI Gom (25, 31) BWMRI 1, Gourab, Triticale,	7	BARI Gom (20, 21, 22, 24, 25, 27, 30, 31), BWMRI 1, Gourab, Triticale	Sensitive

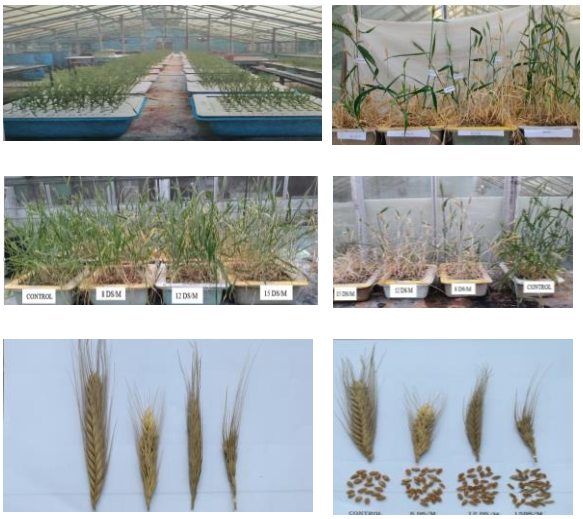

Figure 1: Hydroponic culture of wheat genotypes at various stages of growth

High salinity lowers agricultural productivity. It is well-known that improving the salt-tolerance of crop genotypes is a much more effective strategy for alleviating the negative effects of salinity stress on crop production than other agronomic practices. Thus, it is necessary to improve wheat germplasm, by introducing new genes or alleles from the rich allelic repertoire found in landraces and some cultivated wheat varieties, to enable higher tolerance for salt-stress [18].

There has been little specific research on salinity and mechanisms of tolerance [19], as screening for salinity tolerance has been difficult in laboratory or glasshouse environments [20]. The aims of our study were to characterize the genetic variability of different traits as screening criteria for evaluating the salt tolerance of wheat genotypes under

different salinity conditions using hydroponic and pot culture. In addition, attention will be paid to investigate the expression of target gene were examined. Screening for salinity tolerance based on morphological characters is time consuming and requires extensive field trials and evaluation. Therefore very few reports are available, especially in Bangladesh [21]. Molecular markers play pivotal role in efficient and quick screening of genotypes and speed up the process of varietal evaluation. The morphological and microsatellite based molecular markers have been used for wheat genetic diversity analysis by ourgroup [22-26].

CONCLUSION

Given the limited studies in Bangladesh, there is clear importance of the present study on detecting salt tolerance in Bangladeshi wheat germplasm/accession and salinity related gene expression analysis. The study enabled detection of saline tolerant lines and genes associated with salinity tolerance, which can be deployed in breeding programs.

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