

EVALUATION OF WHEAT (*TRITICUM AESTIVUM* L.) GERMPLASM USING TOLERANCE INDICES AND SSR MARKERS GROWN UNDER DROUGHT STRESS CONDITIONS

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Abstract

Drought stress impedes wheat production worldwide. For this, an investigation was carried out with 30 wheat genotypes to identify drought tolerant genotypes at seedling and grain filling stages using tolerance indices and SSR genotyping. These experiments were arranged in Split plot design and RCBD Design with three replications, respectively. From the analysis of variance of two factor experiments, highly significant variations were exhibited among the genotypes for traits shoot fresh weight, root fresh weight, and grain yield per plant. The high heritability was recorded for shoot fresh weight (63.83%), root fresh weight (63.94%) and grain yield per plant (63.26%). To reveal drought tolerance amongst the 30 genotypes, drought tolerance indices viz. Stress tolerance index, Stress susceptibility index, Tolerance index, Yield stability index, Mean productivity and Geometric mean productivity were computed. The genotype BARI Gom 30, BARI Gom 22, BARI Gom 32, BARI Gom 27, BARI Gom 26, BWMRI-1 showed drought tolerance because superior rank (<200) recorded for these genotypes. 11 Simple sequence repeat markers were evaluated in 30 wheat varieties for molecular characterization. A total of 54 alleles were detected and the number of alleles per locus ranged from 4 to 9 with an average of 5.4 alleles per locus. Finally, considering the morpho-physiological and molecular study, the genotypes BARI Gom 30, BARI Gom 32, BARI Gom 26 and BARI Gom 22 are identified as drought tolerant genotype and could be utilized in drought tolerant breeding program.

Keywords: Drought Stress, Drought Tolerance Index, SSR Marker, Wheat, Genetic Diversity, Proline Content.

I. Introduction

Wheat (*Triticum aestivum* L.) is a leading cereal diet for millions of the world people. By 2050, global wheat production must be aggravated by 60%, from 3.5 tonnes per hectare to 5 tonnes per hectare, to nourish about 9 billion people (Borisjuk 2019). With a spread of 5.35 million hectares of land, worldwide wheat output was increased from 762.20 million tons (2019/20) to 775.87 million tons (2020/21), but the yield dropped from 3.54 to 3.51 metric tons per hectare (USDA, 2020). Wheat yield is significantly impacted negatively and decreased by 29% by abiotic conditions, notably drought stress (Daryanto et al. 2016).

One of the most restricting elements that affect plant growth and development in dry, warm climates where crops are produced is drought (Qadir 2018). Recently, Bangladesh has been suffering from the effects of high climate variability (Proadhan et al. 2020), where the country's northwestern region being one of the most severely affected by drought (Islam et al. 2019). According to research data, 20–25% of the world's wheat-growing region experiences drought, and depending on the plant growth stage and the stress level, the grain output reduction ranges from 10% to 76% (Grzesiak et al. 2019). The germination stage of a plant's life cycle is the most vulnerable, claimed by Ashraf and Mehmood (1990). To examine the impact on the germination and growth of wheat as well

as the plant's capacity for adaptation, numerous osmotic solutions were employed (Chaniago et al. 2017). Among these, mannitol, a polyhydric alcohol, is employed as the main cause of the plants' osmotic stress brought on by dryness and various crop species employed mannitol-induced drought stress. To evaluate the physiological reactions of wheat to drought stress, however, wheat plants were cultivated under varying mannitol and soil moisture levels in pot and field trials in this study.

The correlations between plant yields obtained during drought and under perfect soil moistening were rendered beneath the drought tolerance indicators in a field (Anwaar et al. 2020; Grzesiak et al. 2019). It was discovered that the stress susceptibility index (SSI), yield stability index (YSI), stress tolerance index (STI), tolerance index (TOL), mean productivity (MP), and geometric mean productivity index (GMP) are useful for identifying genotypes that perform well beneath both non- stress (control) and stress (drought) conditions (Solangi et al. 2021; Kumar et al. 2020).

To increase wheat's ability to withstand drought, marker-assisted selection is helpful. Among the markers that have been exploited often in plants as molecular markers for particular phenotypic features are microsatellites or simple sequence repeats (SSR) (Haque et al. 2021). SSRs, agronomic features, and grain quality criteria are used to analyze the genetic links among selected drought-tolerant wheat genotypes, allowing for the selection of breeding parents (Khan et al. 2020; Nahas et al. 2020; Thungo et al. 2020).

The precise information of drought tolerant study at juvenile stage and molecular characterization of wheat genotypes in Bangladesh is very limited. So, the research hypothesis might be the identification of wheat genotypes based on drought tolerance indices at juvenile stages and molecular characterization of wheat genotypes that might be collected from the Bangladesh Wheat and Maize Research Institute (BWMRI). Therefore, the study was aimed to- a) identify the drought tolerant and susceptible genotypes through different drought tolerance selection indices, and b) study the molecular genetic diversity in wheat using SSR molecular markers.

II. Materials and Methods

Plant Materials, Duration and Location

Thirty (30) wheat germplasms collected from the Germplasm Centre of Bangladesh Wheat and Maize Research Institute in Dinajpur, Bangladesh were used as plant materials. The morphological and molecular screening experiment was conducted between November 2019 and April 2020 at the experimental farm and the laboratory of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Experimental Design, Set Up and Data Collection

At seedling stage, the experiment was conducted using randomized complete block design (RCBD) with model two using factors and three replications. Wheat germplasms acted as factor 1 while the amounts of mannitol (0, 0.2, and 0.4M) utilized as a factor 2. Initially, the wheat seeds underwent grading and 70% alcohol sterilization. The 270 (30×3×3) petridishes were additionally autoclaved. Mannitol stock solution was made by mixing 36.434g and 72.868g of mannitol with 1000 ml of water to create 0.2M and 0.4M mannitol solutions, respectively. In every petridish, (90mm diameter×15mm depth), 5 seeds of each kind were sowed. Over the course of three weeks, the petridishes had received 2 ml of solutions on alternate days of watering. Here, all the experimental data were collected after harvesting of seedlings 21 days aged.

At grain filling stage, a split plot design with three replications and two treatments was used to set up the experiment. Here, two treatments viz. control and drought were assigned to the sub-plots in which genotypes were assigned randomly. For this, five seeds of each genotype were sown in plastic pots measuring 20cm by 20cm by 22cm. 180 plastic

containers included a mixture of topsoil, compost, silica sand, and other materials. Sand that had been thoroughly blended filled each pot to a height of 4.5 cm. Water came in regularly and at the same rate. The drought block will stop receiving regular water delivery after one month of planting. On the other hand, water will be consistently sent to the control block.

Statistical Analyses

Data from the seedling and grain filling stages for various characters were first placed onto an MS Excel sheet. Following that, the data analysis was done by using R software, version 3.4.2 (R Core Team 2017). The drought tolerance indices were estimated according to the equations given in Table 1. Here, at seedling stage, shoot fresh weight and root fresh weight were used as yield, and at grain filling stage grain yield was used as yield.

Genomic DNA Isolation, PCR Amplification and Electrophoresis Separation

Using a modified CTAB technique, genomic DNA was extracted from green leaves that were 21 days old. Prior to PCR amplification, the quality of the extracted DNA samples was examined through quantification using a Thermo Scientific NanoDrop™1000 Spectrophotometer (Thermo Fisher Scientific, USA). In order to amplify SSR markers, diluted DNA (50 ng/l) was utilized. The PCR amplification was done in a 13 µL volume where 5 µL LG2 green PCR master mix, 2 µL nuclease free water, 0.5 µL forward and reverse primer of each, 3 µL mineral oil were mixed with 2 µL DNA sample. For PCR amplification, the conditions were: one cycle at 94°C for 5 min; 35 cycles at 95°C for 0.5 min; 53 to 58°C (based on the individual primers) for 0.5 min; 0.5 min for extension; and a final extension for 5 min at 72°C. Reaction products were mixed with one fifth volume of loading buffer (100 mM/L EDTA pH 8.0, 10 mM/L Tris-HCl pH 7.5, 5% Ficoll 400; 0.05% bromophenol, 0.05% xylene cyanol) and 8 µL were loaded vertically, 8% denaturing polyacrylamide gels in 1 × TBE (90 mM/L Tris borate pH 8.3, 2 mM/L EDTA) at 50 mA for 2 to 3 h for electrophoresis (Wang et al. 2007). Following silver staining, gels were imaged and photograph was taken using digital X-ray film viewer.

SSR Genotyping

Thirty genotypes of wheat were examined to see the molecular variation among them using eleven SSR markers. List of primer names, sequences, and associated annealing temperatures are presented in Table 2. The original sources, repeat motifs, primer sequences, anticipated length, chromosomal position, and other pertinent data for these markers are published on Grain Genes website (<http://www.wheat.pw.usda.gov>).

Molecular Statistical Analysis

The allele frequency data were exported in binary format (allele presence = “1” and allele absence = “0”) and input in excel spreadsheet for analysis. Using the following formula, polymorphism information content (PIC) was estimated:

$$PIC = 1 - \sum (P_i)^2$$

Here, P_i denotes the ratio of samples bearing the i th allele.

Through structure software version 2.3.4 (Pritchard et al. 2000), the population structure analysis was performed using the Bayesian clustering method. In order to inferring the population structure, the model-based program structure 2.3.3 was utilized with a burn in period of 10,000 and a run length of 100,000 Markov Chain Monte Carlo number (MCMC) replications for a population range of $K = 1$ to 12 (Pritchard et al. 2000). To identify the presence of genetically separate populations using a graphical technique, the Evanno's K method final K value and \ln probability data were employed (Evanno et al. 2005). Each band was viewed as a distinct locus. Genetic distances (GD) between genotypes were calculated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method as described by Nei & Jin (1989), and a dendrogram was made to estimate genetic

diversity using the MetaboAnalyst program (Online Version). All of the scorable loci were taken into consideration when creating the bivariate 1-0 data matrix.

III. Results

The analysis of variance of morphological characters is presented in Table 3. It revealed highly significant variations among genotypes for the traits shoot fresh weight, root fresh weight, and grain yield per plant. Again, treatment and genotype effects as well as genotype and treatment interaction effects were also highly significant for all studied traits. Here, the coefficient of variation ranged from 20.34% to 29.10%. The high heritability was revealed for shoot fresh weight (63.83%), root fresh weight (63.94%) and grain yield per plant (63.26%). The traits imparted a grand mean as 61.35mg, 49.49mg and 100.01mg for shoot fresh weight, root fresh weight and grain yield per plant, respectively.

Tolerance Indices of 30 Bread Wheat Genotypes

At both the seedling and grain filling stages, values for the yield of genotype in control condition (Y_{pi}), yield of genotype in stress condition (Y_{si}), and six drought tolerance indicators were assessed under both drought stress and control conditions. Drought adversely affects the root length, shoot length, root fresh weight and shoot fresh weights which are clearly shown on Fig. 1. The lowest and highest values of Y_{pi} were recorded for genotypes Ananda (67) and BARI Gom 24 (266.11) respectively (Table 4). The superior Y_{pi} was detected for genotypes BARI Gom 24 (266.11), Kanchan (238.79) and Shotabdi (235.78). Similarly, the lowest and highest values of Y_{si} were found for genotypes BARI Gom 25 (41.21) and BARI Gom 30 (118.02), respectively.

Stress tolerance index (STI) values were ranged between 0.15 and 0.79 for genotypes Ananda and BARI Gom 30 respectively. Genotypes identified with superior STI values were BARI Gom 22 (0.72), BARI Gom 33 (0.75), and BARI Gom 30 (0.79). Conversely, stress susceptibility index (SSI) values were detected with a range from -1.23 to 1.54 to -1.23 for genotypes BARI Gom 24 and Barkat, respectively. Superior SSI values were noted for genotypes Barkat (-1.23), Akbar (-1.32) and BAW 1200 (0.29). Yield stability index (YSI) values were ranged from 0.19 to 1.69 for the genotypes for BARI Gom 24 and Akbar respectively. Again, superior YSI were identified for genotypes Akbar (1.69), Barkat (1.64) and BAW 1200 (0.84). Tolerance index (TOL) showed minimum and maximum values as -33.33 and 214.46 for genotypes Akbar and BARI Gom 24, respectively. The superior genotypes for TOL were recorded as Barkat (-33.33), Akbar (-35.71) and Ananda (10.56). Mean productivity (MP) ranged from 61.72 to 158.88 for genotypes Ananda and BARI Gom 24 in that order. The highest values for MP were detected for genotypes BARI Gom 24 (158.88), Kanchan (153) and BARI Gom 33 (147.98) respectively. The minimum and maximum values of Geometric mean productivity (GMP) were 61.50 and 140.22 for genotype Ananda and BARI Gom 30, respectively. The maximal values of GMP were observed for genotypes BARI Gom 30 (140.22), BARI Gom 33 (136.70) and BARI Gom 22 (133.66).

In table 4, superior Y_{si} was detected for genotypes BARI Gom 27 (1.28), BARI Gom 26 (1.18) and BARI Gom 28 (0.97) at grain filling stage. A wide range of stress tolerance index (STI) values was from 0.17 to 1.05 for genotypes Sonara 64 and BARI Gom 25, respectively. Genotypes identified with superior STI values were BARI Gom 25 (1.05), BARI Gom 27 (0.90) and BARI Gom 28 (0.81). Conversely, superior stress susceptibility index (SSI) was detected for genotypes Akbar (0.45), Agraphani (0.50) and BARI Gom 33 (0.57). The genotypes BARI Gom 24 (1.11), BARI Gom 26 (1.05) and Gourab (0.86) represented superior Yield stability index (YSI). Superior tolerance index (TOL) were recorded for genotypes BARI Gom 26 (-0.06), BARI Gom 24 (-0.07) and Gourab (0.12). Genotypes BARI Gom 25 (1.71), Shotabdi (1.47) and BARI Gom 27 (1.42) showed highest mean productivity

(MP) as well as highest geometric mean productivity (GMP) was recorded for BARI Gom 25 (1.51), BARI Gom 27 (1.41) and BARI Gom 28 (1.34).

Ranks for 30 Wheat Genotypes using 6 Drought Tolerance Indices

Considering the yield and six drought tolerance indices, the ranking of wheat genotypes during the seedling and grain filling stage was presented in table 5. Superior wheat genotypes were those found with low rank values. Based upon indices such as STI, SSI, YSI, TOL, MP and GMP, the superior rank was recorded for the genotypes BARI Gom 30 (45), and it is followed by BARI Gom 22 (59), BARI Gom 33 (77), BARI Gom 32 (79) and BWMRI 1 (82) at seedling stage under mannitol induced drought conditions. In case of grain filling stage, superior drought tolerance indices rank was detected for genotypes BARI Gom 27 (61), BARI Gom 28 (66), BARI Gom 25 (72), BAW 1163 (77) and Shotabdi (81). Considering both stages, the genotypes BARI Gom 30 (141), BARI Gom 22 (167), BARI Gom 32 (186), BARI Gom 27 (192), BARI Gom 26 (195), and BWMRI 1 (198) appeared as suitable lower indices carrying genotypes.

Categorization of 30 Wheat Genotypes using 6 Drought Tolerance Indicators

As per previous ranking value, 30 wheat germplasms were categorized and presented in Table 6. Here, BARI Gom 30, BARI Gom 22, BARI Gom 32, BARI Gom 27, BARI Gom 26 and BWMRI 1 were identified as tolerant genotype as well as BARI Gom 28, BARI Gom 33, BAW 1163, Shotabdi, Sonalika, BARI Gom 29, BAW 1177, Kalyansona and BAW 1200 were recorded as moderately tolerant genotype. On the other hand, BARI Gom 23, BARI Gom 25, Protiva, Balaka, Barkat, Kanchan, Agrahani and Sonara 64 were noted moderately susceptible genotype as well as BAW 1135, Akbar, Ananda, BARI Gom 24, Sourov, BARI Gom 31 and Gourab were identified as susceptible genotype.

Correlation between Grain Yields and Drought Tolerance Indices

At seedling stage, yield under control condition (Y_{pi}) was significantly correlated with STI (0.75***), SSI (0.83***), YSI (-0.83***), TOL (0.96***), MP (0.95***), and GMP (0.78***) (Table 7). Similar positive and significant correlations had been found between Y_{si} and STI (0.46*), YSI (0.41*), and GMP (0.41**). A significant association of TOL with STI (0.54**), SSI (0.86***), YSI (-0.86***); mean productivity (MP) with STI (0.90***), SSI (0.72***), YSI (-0.72***) and TOL (0.83***) were identified. Here, STI was also identified as significantly positively correlated with all the parameters except YSI.

At grain filling stage, the yield (Y_{pi}) under control conditions had a very weak correlation with stress conditions (Y_{si}) (Table 7). Yield under control conditions (Y_{pi}) exhibited a strong positive association with STI (0.71***), TOL (0.89***), MP (0.90***), and GMP (0.73***), but SSI (-0.74***) and YSI (-0.74***) showed a substantial negative correlation with Y_{pi}. Similar positive and significant correlations had been found between Y_{si} and all tolerance indicators, with the exception of TOL. Further, STI was identified as significantly positively correlated with all the parameters except TOL. A significant correlation also appeared between SSI and all other indices. Significant relationships existed between TOL and STI (0.32*), SSI (-0.94***) and YSI (-0.94***). Mean productivity (MP) showed a substantial negative association with SSI (-0.39*) and YSI (-0.39*), but a significant positive correlation with STI (0.94***) and TOL (0.60***). GMP exhibited significant association with STI (0.99***), TOL (0.34*) and MP (0.95***).

SSR Markers based DNA Fingerprinting Analysis

Total eleven (11) SSR markers were used to identify the variation among 30 wheat genotypes. Among them, ten (10) SSR markers viz. XGWM44, XGWM164, XGWM642, XGWM111, XWMC667, XGWM484, XWMC96, XWMC405, XGWM120 and XGWM33 were found polymorphic whereas marker XCFD183 was found monomorphic. So, ten SSR markers were taken for further analysis. A total of 54 alleles were detected and the number of alleles per locus ranged from 4 to 9 with an average of 5.4 alleles per locus (Table 8). The

polymorphic information content (PIC) was measured to quantify the genetic diversity. With an average PIC value of 0.74 per marker, the studied SSR markers' PIC values varied from 0.63 for XGWM164 to 0.87 for XGWM111. The most polymorphic microsatellite marker is XGWM111 (0.87), which has 9 alleles. Following it, the markers XGWM642, XGWM44, and XWMC96 had 7 and 6 alleles, respectively. (Table 8 and Fig. 2). All eleven SSR markers employed in the current investigation had PIC values better than 0.5.

Population Structure

In the context of structural analysis, accessions were classified as pure or admixture; those with a score greater than 0.80 were deemed pure, while those with a score lower than 0.80 were deemed admixtures. Here, the population I consisted of 50% genotypes; 12 genotypes (Kalyansona, Sonalika, Gourab, BARI Gom 23, BARI Gom 25, BARI Gom 28, BARI Gom 30, BARI Gom 32, Balaka, BAW 1200, BAW 1163, Protiva) were pure, where 3 genotypes (Sourov, BARI Gom 33, Agrahani) were admixed. Population II comprised of total 15 genotypes; 12 genotypes (Sonara 64, Kanchan, Shotabdi, BARI Gom 22, BARI Gom 24, BARI Gom 26, BARI Gom 27, BARI Gom 29, BARI Gom 31, BWMRI 1, Ananda, Akbar, Barkat, BAW 1135, BAW 1177) were found as pure and 3 genotypes (Shotabdi, BWMRI 1, Barkat) were found admixture (Fig. 3). Similar to the population structure study, phylogenetic dendrogram based on genetic distance showed two potential populations utilizing model-based STRUCTURE (Fig. 3).

Pairwise Genetic Dissimilarity

As shown in Table 9, the values for pairwise genetic dissimilarity ranged from -0.003 to 0.884 (-0.3% to 88.4%). BARI Gom 32 and Balaka show the highest dissimilarity of 88.4% followed by Akbar and BAW 1177 (80.8%), BARI Gom 30 and Protiva (77.4%) and BARI Gom 22 and BARI Gom 26 (77.4%). On the other hand, Protiva and Sonara 64 had the lowest genetic dissimilarity of -0.3% followed by Barkat and Sonara 64 (-0.3%), Ananda and BAW 1163 (-0.9%), BARI Gom 30 and BAW 1163 (-0.9%) which means they are intergeneric to each other.

Genetic Diversity

As a consequence of the cluster analysis, the wheat accessions under investigation could be separated into two populations, population I and II, with similarity coefficients ranging from 0.5 to 4 indicating significant genetic variation among the wheat accessions studied. Both groups could be further separated into four clusters (Fig.4). Cluster I comprised 7 genotypes, Cluster II comprised 7 genotypes, Cluster III comprised 9 genotypes and Cluster IV comprised 7 genotypes (Table 10). Balaka and BARI Gom 32 shared the most genetic similarity, 94.44%, followed by Protiva and BARI Gom 30 (90.7%) and BARI Gom 22 (Sufi) and BARI Gom 26 (90.7%). (Fig. 4).

Principal Component Analysis (PCA)

The first two main components of principal coordinates in PCA scatter plots have eigen values of 15.4% and 12.1%, respectively, with a maximum cumulative variance of 27.5%. (Fig. 5). The UPGMA-tree revealed that PC₁ could clearly discriminate across variations and discovered 4 unique clusters. The spatial distribution of the genotypes was displayed using PCA's graphical perspectives. Sonalika, Balaka, BARI Gom 32, Protiva, BARI Gom 25, BARI Gom 23 (Bijoy), Akbar, BAW 1177, and BARI Gom 24 (Prodip) were discovered far from the cluster's center, whereas the other genotypes were distributed roughly around it.

IV. Discussion

The goal and a significant problem for wheat breeders nowadays is the creation of wheat genotypes that are resistant to drought. Selecting resources for advanced breeding

projects can be done effectively by screening germplasm for drought tolerance, especially under controlled drought circumstances.

The genotypes showed very significant variance in ANOVA, indicating that there is a good deal of variability that can be used to choose characters for crop improvement programs. Any effective breeding program for drought tolerance must start with this knowledge. Genotype and treatment interaction also showed incredibly significant changes for each of the study's variables. Similar findings were observed by Iyem et al. (2021). For all of the studied traits, the highly significant treatment influence was observed, all of which had previously been reported by Solangi et al. (2021); Arifuzzaman et al. (2020).

Heritability measures how much of a trait is passed down from one generation to the next which is the proportion of genetic variance to total phenotypic variation. Heritability is a crucial selection characteristic that aids plant breeders in selecting the traits that will be selected. Here, the traits root fresh weight, shoot fresh weight and grain yield per plant showed a substantial amount of heritability. According to Regmi et al. (2021), moderate to high heritability is observed for grain yield.

At the moment, one of the primary obstacles preventing crop plants from expressing their genetic potential is drought. To ensure productivity, it is essential to identify genotypes that are resistant to drought. In the current study, we assessed 30 wheat genotypes with various drought response traits. Several drought tolerance indices were utilized to identify genotypes that were drought tolerant. For drought evaluation and monitoring at the global and regional levels, drought indices are frequently used to show the length, severity, and the extent of a drought (Edossa et al. 2016).

Exuberant quantitative factors have been proposed for the genotypes selection based on their yield performance in stress and non-stress conditions. In this study, six drought tolerance indices namely, stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), geometric mean (GM), mean productivity (MP), and yield stability index (YSI) were assessed for genotypes under drought and control conditions at both the seedling and grain filling stages.

At the seedling stage, for genotypes Ananda and BARI Gom 24, minimum and maximum values for Ypi were noted. For the BARI Gom 24, Kanchan, and Shotabdi genotypes, superior Ypi was observed. Superior Ysi was found for the genotypes BARI Gom 30, BARI Gom 22, and BARI Gom 32. Here, genotypes identified with superior stress tolerance index (STI) values were BARI Gom 30, BARI Gom 33, BARI Gom 22. Conversely, superior stress susceptibility index (SSI) was detected for genotypes Barkat, Akbar and BAW 1200. Yield stability index was indicated superior for the genotypes Akbar, Barkat and BAW 1200. The superior genotypes indicated for tolerance index (TOL) were recorded as Barkat, Akbar and Ananda. The highest values for MP were obtained for genotypes BARI Gom 24, Kanchan and BARI Gom 33. The highest values of Geometric mean productivity (GMP) were recorded for genotypes BARI Gom 30, BARI Gom 33 and BARI Gom 22. According to Anwaar et al. (2020), lower SSI and TOL values with higher MP and GMP values can be used to select tolerant genotypes. Again, Kumar et al. (2020) also demonstrated that MP, TOL, and HM were recommended if the stress was less intense, whereas when the stress was intense, SSI was implied as a good indicator for wheat breeding.

Comparing the yield reduction under drought circumstances with control conditions, drought tolerance indices are frequently employed to identify drought tolerant genotypes in the majority of field-based research. In this study, yield (Ypi) under control conditions has no significant correlation with drought stress conditions (Ysi), demonstrating that maximal yield potential under control conditions does not foretell greater yield under stress situations. Here, at the grain filling stage, superior Ysi was detected for genotypes BARI Gom 27, BARI Gom 28 and BARI Gom 26. Genotypes

identified with the superior stress tolerance index (STI) values were BARI Gom 25, BARI Gom 28 and BARI Gom 27. Conversely, superior stress susceptibility index (SSI) was detected for genotypes Akbar, Agrahani and BARI Gom 33. Yield stability index (YSI) was indicated as highest for genotypes BARI Gom 24, BARI Gom 26 and Gourab. For drought, Tolerance index (TOL) superior values for genotypes BARI Gom 26, BARI Gom 24 and Gourab. The highest values for MP were recorded for genotypes BARI Gom 25, Shotabdi and BARI Gom 27. The highest values of GMP was seen for genotypes BARI Gom 28, BARI Gom 27 and BARI Gom 25. Amare et al. (2019) used the MP, GMP, YI, and STI indices to assess 256 bread wheat genotypes and demonstrate how breeders can choose the best genotypes under drought stress conditions and contrast their performance under control conditions. Additionally, Sheibanirad et al. (2018); Jahan and Ahmed (2017) found similar results.

At seedling stage, based upon all six indices such as STI, SSI, YSI, TOL, MP and GMP; BARI Gom 30 to be appeared the most drought tolerant cultivar as it recorded superior rank (45) followed by BARI Gom 22 and BARI Gom 33. Similarly, at grain filling stage, BARI Gom-27 to be appeared the most drought tolerant cultivar because it recorded as superior rank (61) genotypes pursued by BARI Gom-25 and BARI Gom-28 using drought indices such as STI, SSI, YSI, TOL, MP and GMP. Considering both seedling and grain filling stage, for tolerance a large range of variation was found amongst the wheat genotypes. The genotype BARI Gom-30 exhibited a minimum rank value (141) which indicated maximum tolerance to drought stress. Again, BARI Gom-22, BARI Gom-32, BARI Gom-27, BARI Gom-26, BWMRI-1 revealed tolerance amongst the 30 genotypes. BARI Gom-28, BARI Gom-33, BAW-1163, Shotabdi, Sonalika, BARI Gom-29, BAW- 1177, Kalyansona, BAW-1200 showed moderate tolerance against drought exhibiting medium rank values. BARI Gom-23, BARI Gom-25, Protiva, Balaka, Barkat, Kanchan, Agrahani, Sonara-64, Gourab were moderately susceptible genotypes. BAW- 1135, Akbar, Ananda, BARI Gom-24, Sourov, BARI Gom-31 showed maximum rank and were predicted as susceptible genotypes against drought. According to Jahan and Ahmed (2017), SATYN-25 BARI Gom-28 and BARI Gom-26 all produced higher yields while having lower SSI values and higher STI, GMP, and YSI values, showing that those genotypes would be acceptable for growing in locations that are prone to drought.

The estimate of stress tolerance features showed that based on a single criterion, identifying drought-tolerant genotypes was contradictory. Therefore, correlation coefficients between drought tolerance indicators and yield under drought stress conditions and control conditions were performed. In order to infer the most appropriate drought tolerance parameters, the genotypic correlation coefficient (r_g) among Y_{pi} , Y_{si} and other quantitative markers of drought tolerance was assessed. In this study, the relationship between yield (Y_{pi}) under control conditions and yield (Y_{si}) under drought stress conditions is very weak, demonstrating that high yield efficiency under ideal conditions does not narrate superior yield under stress conditions. Therefore, as illustrated by indirect selection for stressful situations based on how well irrigated conditions perform, as supposed by Gholipouri et al. (2013), will not be successful.

In this study, a favorable and substantial correlation between yield under drought stress circumstances (Y_{si}) and STI, YSI, and GMP was also found at the seedling stage. Additionally, at the grain filling stage, a positive and substantial correlation between yield under drought stress circumstances (Y_{si}) and all tolerance indices, viz. SSI, STI, YSI, MP, and GMP, had been found. Similar to this, the suitable selection criteria for genotypes that produced large yields and were drought tolerant were STI, MP, and GMP, which had the strongest correlation coefficient with grain production (Ozturk et al. 2018).

SSR markers have been exercised in the past to compute genetic diversity of domesticated and wild wheat species as well as their improved germplasm for the wheat

crop. For the selection and development of promising wheat varieties, the presence of the greatest genetic diversity is helpful (Lopes et al. 2015). In this study, molecular characterization of thirty wheat genotypes through SSR markers XGWM44, XGWM164, XGWM642, XGWM111, XWMC667, XGWM484, XWMC96, XWMC405, XGWM120, XGWM33 showed different level of polymorphism whereas XCFD183 showed monomorphism. The XGWM111 marker showed a good level of polymorphism with the thirty wheat genotypes followed by XGWM642, XGWM44 and XWMC96. The experimental data were analyzed to obtain the genetic diversity of the 30 wheat germplasms and drought tolerance screening in wheat. The 11 SSRs generated a total of 54 alleles, averaging 5.4 alleles per marker and ranging from 4 for XGWM484, XWMC667, XWMC405, and XGWM120 to 9 for XGWM111. Earlier studies by Thungo et al. (2020) provided support for our findings. In this work, the polymorphic bands showed variations between the genotypes and were utilized to evaluate and create systematic correlations among genotypes.

Here, SSR markers were highly polymorphic and informative, as shown by their polymorphic information content (PIC) value. If $PIC > 0.5$, $0.5 > PIC > 0.25$, or $PIC < 0.25$, respectively, the locus polymorphism was categorized as high, medium, or low (Vaiman et al. 1994). With an average of 0.75 for each marker in the going investigation, PIC values ranged from 0.63 for XGWM164 to 0.87 for XGWM111. All 11 SSR markers that were employed in the present investigation had PIC values better than 0.5. These markers appeared to be highly informative and polymorphic, and since they can distinguish between genotypes, they can be practiced in the marker-assisted selection of wheat genotypes. PIC values from this study are significantly higher than those prior examinations from Ahmed et al. (2020) and Salehi et al. (2018). In this study, the average significantly high PIC values of the SSR markers provided further evidence of their high polymorphism. However, a higher PIC value, on the other hand, denotes a larger diversity of materials, which is preferred for the development of novel varieties. A lower PIC value, on the other hand, suggests that the genotypes being researched are closely related kinds. In advanced wheat breeding programs, simple sequence repeats (SSRs) have been utilized successfully to define genetic diversity, report Hammer et al. (2000), and are the ideal marker system for wheat (Dreisigacker et al. 2004).

Nei's (1983) genetic distance between thirty wheat genotypes was measured pairwise and ranged from -0.3% to 88.4%. (Table 9). Akbar and BAW-1177, Sufi and BARI Gom-26, BARI Gom-30 and Protiva, and BARI Gom-32 and Balaka exhibit the greatest dissimilarity. It appears that the cross between BARI Gom-32 and Balaka will produce desirable results for the breeder. A higher genetic distance across genotypes suggests that their genetic makeup is more varied than a smaller genetic distance number. In essence, this value represents how genetically distinct they are. The observed genetic divergence between these genotypes may be due to chromosomal mutation and distinct geographic origin. The observed genetic divergence suggests that the species share a common ancestor or that there may have been extensive interbreeding that shared similar genes across their genomes. These findings were consistent with what Islam et al. (2012) reported, which stated that a larger genetic distance (0.469) was found between the Gourab vs. Akbar and BAW-1064 pair. Elshafei et al. (2019) and Wang et al. (2017) all reported comparable results.

By using the UPGMA cluster tree and the STRUCTURE analysis, 30 bread wheat genotypes were sorted into 4 clusters and 2 populations, respectively. Many scientists used these strategies in their wheat breeding scheme and got the results they were looking for in terms of explanations (Ahmed et al. 2017). Accessions in this study were further classified as pure or admixture using the Structure analysis; those with a score of more than 0.80 were regarded as pure, and those with a score of less than 0.80 as admixture.

In the current experiment, both populations could be further separated into four clusters. Cluster I comprised 7 genotypes, Cluster II comprised 7 genotypes, Cluster III comprised 9 genotypes and Cluster IV comprised 7 genotypes. Balaka and BARI Gom 32 shared the most genetic similarity, followed by Protiva and BARI Gom 30 and BARI Gom 22 and BARI Gom 26. So, Balaka and BARI Gom 32 are genetically more divergent while BAW 1200 and BARI Gom 27, Sonara 64 and BAW 1135 showing minimum 72% similarity are less divergent.

Distances between clusters make it evident how different the 30 bread wheat genotypes are from one another genetically. The maximum genetic difference between clusters existence suggests that they have different genetic make-ups. This is a sign of genetic divergence among the clusters, and as a result, more genetic diversity is present in the analyzed germplasms. Within each cluster, there is a small genetic distance between the genotypes that demonstrates the genetic similarity among the 30 genotypes. Using similar methods, other wheat breeders assessed the genetic diversity of their crops (Ahmed et al. 2017), and they came to similar conclusions.

Once more, the principal coordinate analysis (PCoA) provides a platform to show the relative genetic distances between individuals spatially. Additionally, it evaluates how robustly the groups in the dendrogram are differentiated (Liu et al. 2013). According to the current study, the first two PCA enumerated for 27.5% of the total variation, which was then displayed to demonstrate genotype diversity. Similarly, Khan et al. (2015) explained the first two main components, which each accounted for 17.6 and 10.7% of the overall variation. The classification of wheat subgroups by Narantsetseg et al. (2017) revealed a resemble pattern of eigen values for the first principal components. The data can be utilized along with cluster analysis to determine the linked genotypes if the variation for the principal components is high ($\geq 25\%$). The first three principal components were found to be the most significant in capturing the patterns of variation among accessions, according to the results, which are in agreement with those findings. It is advised to differentiate between population subgroups using the markers involved in genotype classification. PCA and dendrogram groupings were compared, and the results showed largely comparable tendencies with a slight variation in cluster composition.

Through the analysis of seedling stage and grain filling stage data, it can be considered that BARI Gom 30, BARI Gom 32, BARI Gom 22, BARI Gom 26, BARI Gom 27, BWMRI 1 are tolerant genotypes; BARI Gom 28, BARI Gom 33, BAW 1163, Shotabdi, Sonalika, BARI Gom 29, BAW 1177, Kalyansona, BAW 1200 are moderately tolerant. In molecular level, the respected tolerant genotypes obtained from previous data i.e. BARI Gom 30, BARI Gom 22, BARI Gom 26 are located in same cluster as UPGMA cluster II; the respected moderately tolerant genotypes BARI Gom 28, Shotabdi, and Sonalika are located in the UPGMA cluster III. According to Nei's genetic distance or dissimilarity (1972), BARI Gom 32 and Balaka (88.47%); genotypes BARI Gom 30 and Protiva (77.4%) regained genetic distance or dissimilarity whereas they are considered as respected tolerant and moderately susceptible genotypes, respectively. The PCA graphical views displayed the genotypes spatial distribution. The results indicated that the respected tolerant genotype BARI Gom 32, BARI Gom 26; the respected moderately susceptible genotype Balaka and susceptible genotype BARI Gom 31 were placed far away from the centroid that means they were more genetically diverse.

V. Conclusion

BARI Gom 30, BARI Gom 22 (Sufi), BARI Gom 32, BARI Gom 27, BARI Gom 26, BWMRI 1 were identified as most drought tolerant genotypes among 30 genotypes through assessment of drought indicator such as STI, SSI, YSI, TOL, MP and GMP. Based on the molecular diversity Balaka, BARI Gom 32, Protiva, BARI Gom 30, BARI Gom 26, BARI Gom 22 (Sufi) were identified as diverse genotypes and might be useful for the future

hybridization program. Finally, considering the morpho-physiological study and molecular study, the genotypes BARI Gom 30, BARI Gom 32, BARI Gom 26 and BARI Gom 22 (Sufi) might be very effective in drought tolerant breeding strategy.

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List of tables

Table 1 Abbreviations and Formula used for calculation of various drought indices in wheat

Name of index	Abbreviation and Formula	Reference
Stress susceptibility index	SSI= (1-(Ysi/Ypi))/SI SI= 1- (Ys/Yp)	Fischer & Maurer (1978)
Stress tolerance index	STI= (Ypi× Ysi)/Yp ²	Fernandez (1992)
Tolerance index	TOL=Ypi -Ysi	Rosielle & Hamblin (1981)
Yield stability index	YSI=Ysi/Ypi	Bousslama & Schapaugh (1984) and Gavuzziet al.. (1997)
Mean productivity	MP= (Ypi+Ysi)/2	Rosielle & Hamblin (1981)
Geometric mean productivity	GMP= 2 √Ypi× Ysi	Fernandez (1992)

Ypi- yield of genotype in control condition, Ysi- yield of genotype in drought stress condition, SI - stress intensity, Ys- total yield mean in drought stress condition, Yp- total yield mean in control condition

Table 2 SSR Markers used for molecular study in 30 wheat genotypes

SSR Loci	Location	Forward primer (5'–3')	Reverse primer (5'–3')	Annealing temperature (°c)
XGWM-111	2A, 2B, 4A, 6D, 7B, 7D	TCTGTAGGCTCTCTCC GACTG	ACCTGATCAGATCCCAC TCG	58
XGWM-164	1A	ACATTTCTCCCCATC GTC	TTGTAAACAAATCGCAT GCG	53
XGWM-642	1D	ACGGCGAGAAGGTGC TC	CATGAAAGGCAAGTTCG TCA	55
XGWM-44	2A, 4A, 5A, 5B, 7D	GTTGAGCTTTTCAGTT CGGC	ACTGGCATCCACTGAGC TG	56
XWMC-667	2A	GAGGAGAGGAAAAGG CAGGCTA	AACTCTTGCGTGTCTCA AACCG	58
XGWM-484	2D	ACATCGCTTTCACAA ACCC	AGTTCCGGTCATGGCTA GG	55
XCFD-183	5D	ACTTGCACTTGCTATA CTTACGAA	GTGTGTCGGTGTGTGGA AAG	55
XGWM-120	2B	GATCCACCTTCCTCTC TCTC	GATTATACTGGTGCCGA AAC	55
XGWM-33	1A, 1B, 1D	GGAGTCACACTTGTTT GTGCA	CACTGCACACCTAACTA CCTGC	57
XWMC-96	3A, 4A, 5A, 5D, 6A	TAGCAGCCATGCTTA GCATCAA	GTTCAGTCTTTCACGA ACACG	54

XWMC-405	1D, 5D, 7D	5B, 7A,	GTGCGGAAAGAGACG AGGTT	TATGTCCACGTTGGCAG AGG	57
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Table 3 Mean square values of some characters at seedling and grain filling stage
Mean sum of square

Sources of variation	At seedling stage			At grain filling stage		
	df	Shoot fresh weight	Root fresh weight	df	Grain yield per plant	
Genotype	29	1139.00***	1334.10***	29	0.33***	
Replication	2	58.00	102.40	2	0.04	
Treatment	2	67143.00***	19845.10***	1	29.25***	
Genotype × Treatment	58	1675.00***	593.10***	29	0.26***	
Error	178	156.00	207.50	58	0.05	
Coefficient of variation (%)	-	20.34	29.10	-	21.62	
Heritability (%)	-	63.83	63.94	-	63.26	
Grand mean ± SE	-	61.35 ± 7.2	49.49 ± 8.32	-	1.01 ± 0.135	

***- significant at 0.1% level of probability

df - Degrees of freedom, SE- Standard error

Table 4 Tolerance indices of 30 bread wheat genotypes under stress and non-stress environments at seedling and grain filling stages

Genotype	Ypi		Ysi		STI		SSI		YSI		TOL		MP		GMP	
	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G
Kalyansona	99.32	1.18	77.11	0.96	0.31	0.51	0.43	1.59	0.78	0.81	22.21	0.22	88.22	1.07	87.51	1.06
Sonara 64	192.56	0.71	79.23	0.52	0.61	0.17	1.12	1.43	0.41	0.73	113.30	0.20	135.90	0.62	123.53	0.61
Sonalika	173.56	1.87	73.22	0.74	0.51	0.63	1.11	0.77	0.42	0.39	100.33	1.14	123.39	1.31	112.73	1.17
Kanchan	238.79	1.69	67.22	0.51	0.64	0.39	1.38	0.59	0.28	0.30	171.56	1.19	153	1.10	126.70	0.93
Sourov	205.56	1.34	55.52	0.59	0.46	0.36	1.40	0.86	0.27	0.44	150.03	0.75	130.54	0.96	106.83	0.89
Gourab	184.56	0.92	61.82	0.79	0.46	0.33	1.27	1.70	0.33	0.86	122.74	0.12	123.19	0.85	106.81	0.85
Shotabdi	235.78	2.16	55.78	0.79	0.53	0.77	1.46	0.72	0.24	0.37	180	1.37	145.78	1.47	114.68	1.30
BARI Gom 22	172.67	1.69	103.46	0.70	0.72	0.54	0.77	0.81	0.60	0.41	69.20	0.99	138.07	1.19	133.66	1.09
BARI Gom 23	197.67	1.36	76.07	0.69	0.60	0.43	1.18	1.00	0.38	0.51	121.60	0.67	136.87	1.02	122.62	0.97
BARI Gom 24	266.11	0.65	51.66	0.72	0.56	0.21	1.54	2.18	0.19	1.11	214.46	0.07	158.88	0.69	117.24	0.69
BARI Gom 25	199.79	2.51	41.21	0.91	0.33	1.04	1.52	0.72	0.21	0.36	158.56	1.59	120.50	1.71	90.74	1.51
BARI Gom 26	201.56	1.12	80.11	1.18	0.65	0.60	1.15	2.07	0.40	1.05	121.44	0.06	140.83	1.15	127.07	1.15
BARI Gom 27	205.11	1.55	67.33	1.28	0.56	0.90	1.29	1.63	0.33	0.83	137.78	0.26	136.22	1.42	117.52	1.41
BARI Gom 28	126.94	1.85	72.74	0.97	0.37	0.81	0.82	1.03	0.58	0.52	54.20	0.88	99.84	1.41	96.10	1.34
BARI Gom 29	126.33	1.45	81.36	0.71	0.41	0.47	0.68	0.97	0.64	0.49	44.98	0.73	103.84	1.08	101.38	1.01

BARI 30	Gom 8	166.5	1.56	118.0	0.79	0.79	0.56	0.56	1.00	0.71	0.51	48.56	0.76	142.30	1.18	140.22	1.11
BARI 31	Gom 4	120.4	1.39	66.63	0.50	0.32	0.31	0.86	0.70	0.55	0.36	53.82	0.90	93.54	0.95	89.58	0.83
BARI 32	Gom 32	173	1.39	97	0.80	0.67	0.51	0.84	1.14	0.56	0.58	76	0.58	135	1.09	129.54	1.06
BARI 33	Gom 7	204.6	1.76	91.29	0.51	0.75	0.41	1.06	0.57	0.45	0.29	113.38	1.25	147.98	1.14	136.70	0.95
BWMRI 1		166.5	1.22	96.33	0.85	0.64	0.47	0.81	1.37	0.58	0.70	70.22	0.37	131.44	1.04	126.67	1.02
Balaka		113.67	1.26	73.33	0.76	0.33	0.43	0.68	1.19	0.65	0.61	40.33	0.50	93.5	1.00	91.30	0.98
Ananda		67	1.53	56.44	0.57	0.15	0.39	0.30	0.73	0.84	0.37	10.56	0.96	61.72	1.05	61.50	0.93
Akbar		48.22	1.65	81.56	0.38	0.16	0.28	-1.32	0.45	1.70	0.22	-33.33	1.28	64.89	1.02	62.71	0.79
Barkat		55.56	1.35	91.27	0.67	0.20	0.41	-1.23	0.98	1.64	0.50	-35.71	0.67	73.41	1.01	71.21	0.95
BAW 1200		104.0	1.55	88.44	0.62	0.37	0.43	0.29	0.78	0.85	0.40	15.63	0.93	96.26	1.08	95.94	0.98
BAW 1135		123.11	0.79	71.78	0.62	0.35	0.22	0.80	1.53	0.58	0.78	51.33	0.18	97.44	0.71	94.00	0.70
BAW 1163		141.8	1.61	69.96	0.92	0.40	0.67	0.97	1.12	0.49	0.57	71.93	0.69	105.92	1.26	99.63	1.22
BAW 1177		167.0	1.74	84.22	0.59	0.56	0.46	0.95	0.67	0.50	0.34	82.80	1.14	125.62	1.16	118.60	1.01
Agrahani		148.4	1.88	74.07	0.48	0.44	0.41	0.96	0.50	0.50	0.26	74.38	1.40	111.25	1.18	104.85	0.95
Protiva		115.11	1.78	61.37	0.74	0.28	0.60	0.90	0.82	0.53	0.42	53.74	1.04	88.24	1.26	84.05	1.15

Y_{pi}- yield of genotype in control condition, Y_{si}- yield of genotype in drought stress condition, STI - Stress tolerance index, SSI – Stress susceptibility index, YSI - Yield stability index, TOL - Tolerance index, MP - Mean productivity, GMP- Geometric mean productivity, S = Seeding stage and G = Grain filling stage

Table 5 Ranks for 30 wheat genotypes using 6 drought selection indices under seedling stage and grain filling stage

Genotype	Rank in seedling stage	Rank in grain filling stage	Total rank
Kalyansona	134	106	240
Sonara 64	110	180	290
Sonalika	137	88	225
Kanchan	117	152	269
Sourov	154	164	318
Gourab	154	148	302
Shotabdi	144	81	225
BARI Gom 22	59	108	167
BARI Gom 23	117	137	254
BARI Gom 24	145	165	310
BARI Gom 25	187	72	259
BARI Gom 26	107	88	195
BARI Gom 27	131	61	192
BARI Gom 28	135	66	201
BARI Gom 29	110	123	233
BARI Gom 30	45	96	141
BARI Gom 31	159	173	332
BARI Gom 32	79	107	186
BARI Gom 33	77	145	222
BWMRI 1	82	116	198
Balaka	132	133	265
Ananda	155	154	309
Akbar	131	175	306
Barkat	123	145	268
BAW 1200	108	135	243
BAW 1135	136	167	303
BAW 1163	146	77	223
BAW 1177	107	126	233
Agrahani	136	140	276
Protiva	169	92	261

Table 6 Categorization of 30 wheat genotypes using 6 drought tolerance indicators

Category	Rank	Genotypes
Tolerant	<200	BARI Gom 30, BARI Gom 22, BARI Gom 32, BARI Gom 27, BARI Gom 26, BWMRI 1
Moderately tolerant	201-250	BARI Gom 28, BARI Gom 33, BAW 1163, Shotabdi, Sonalika, BARI Gom 29, BAW 1177, Kalyansona, BAW 1200
Moderately susceptible	251-300	BARI Gom 23, BARI Gom 25, Protiva, Balaka, Barkat, Kanchan, Agrahani, Sonara 64
Susceptible	> 300	BAW 1135, Akbar, Ananda, BARI Gom 24, Sourov, BARI Gom 31, Gourab

Table 7 Correlation coefficients between drought tolerance indices and yield under drought and control conditions at seedling stage

Parameter	Stage	Y _{pi}	Y _{si}	STI	SSI	YSI	TOL	MP
Y _{si}	S	-0.22						
	G	0.02						
STI	S	0.75***	0.46*					
	G	0.71***	0.70** *					
SSI	S	0.83***	-0.41*	0.53**				
	G	- 0.74***	0.60** *	-0.10				
YSI	S	-						
	G	0.83*** - 0.74***	0.41* 0.60** *	-0.53** -0.10	-1*** 1.00***			
TOL	S					-		
	G	0.96*** 0.89***	-0.47** -0.43*	0.54** 0.32*	0.86*** -	0.86*** -		
MP	S			0.90** *		-	0.83** *	
	G	0.95*** 0.90***	0.08 0.46*	0.94** *	0.72*** -0.39*	0.72*** -0.39*	0.60** *	
GMP	S			0.99** *				0.92** *
	G	0.78*** 0.73***	0.41** 0.69** *	0.99** *	0.59 -0.14	-0.59* -0.14	0.59 0.34*	0.95** *

*= significant at 5%, **= significant at 1%, and ***= significant at 0.1% level of probability

Y_{pi}- yield of genotype in control condition, Y_{si}- yield of genotype in drought stress condition, STI - Stress tolerance index, SSI – Stress susceptibility index, YSI - Yield stability index, TOL - Tolerance index, MP - Mean productivity, GMP- Geometric mean productivity

S = Seeding stage and G = Grain filling stage

Table 8 Number of alleles, allele range and PIC values of 10 polymorphic SSR markers in 30 wheat genotypes

SSR loci	Number of alleles	Range of allele size (bp)	PIC
XGWM44	6	170-650	0.78
XGWM164	5	120-375	0.63
XGWM642	7	125-550	0.77
XGWM111	9	130-370	0.87
XWMC667	4	110-270	0.72
XGWM484	4	150-310	0.70
XWMC96	6	230-600	0.75
XWMC405	4	190-450	0.72
XGWM120	4	120-150	0.70
XGWM33	5	90-240	0.76
Average	5.4		0.74

PIC=Polymorphic Information Content, bp = Base Pair

Table 9 Nei's genetic dissimilarity among 30 wheat genotypes obtained from 10 SSR markers

Genotype	Sonara BAW 1135	Kanchan BARIGo m 29	Ananda BARIGo m 31	Gom 22 BARIGo m 26	Agrahani BARIGo m 30	Protiva BARIGo m 32	Balaka BARIGo m 33	Barkat BARIGo m 27	Akbar	Prodip BAW 1177	Sourov BWMKI 1	Shotabdi BARIGo m 28	Sonalika	Gourab BARIGo m 25	Bijoy
BAW 1135	0.37														
Kanchan BARIGo m 29	0.44	0.72													
Ananda BARIGo m 31	0.43	0.57	0.59												
Gom 22 BARIGo m 26	0.42	0.56	0.55	0.78											
Agrahani BARIGo m 30	0.45	0.61	0.60	0.63	0.30										
Protiva BARIGo m 32	0.46	0.62	0.61	0.64	0.31	0.30									
Balaka BARIGo m 33	0.47	0.63	0.62	0.65	0.32	0.31	0.30								
Barkat BARIGo m 27	0.48	0.64	0.63	0.66	0.33	0.32	0.31	0.30							
Akbar	0.49	0.65	0.64	0.67	0.34	0.33	0.32	0.31	0.30						
Prodip BAW 1177	0.50	0.66	0.65	0.68	0.35	0.34	0.33	0.32	0.31	0.30					
Sourov BWMKI 1	0.51	0.67	0.66	0.69	0.36	0.35	0.34	0.33	0.32	0.31	0.30				
Shotabdi BARIGo m 28	0.52	0.68	0.67	0.70	0.37	0.36	0.35	0.34	0.33	0.32	0.31	0.30			
Sonalika	0.53	0.69	0.68	0.71	0.38	0.37	0.36	0.35	0.34	0.33	0.32	0.31	0.30		
Gourab BARIGo m 25	0.54	0.70	0.69	0.72	0.39	0.38	0.37	0.36	0.35	0.34	0.33	0.32	0.31	0.30	
Bijoy	0.55	0.71	0.70	0.73	0.40	0.39	0.38	0.37	0.36	0.35	0.34	0.33	0.32	0.31	0.30

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BAW	0.2	0.	0.1	-	0.	0.	0.	0.1	0.2	0.2	0.	0.2	0.1	0.	0.1	0.1	0.2	0.	-	-	-	-	-	-	0.	0.	0.	0.	0.	0.	0.
1200	9	09	8	02	08	05	4	3	0	08	3	0	07	2	4	9	04	02	1	0	3	09	04	05	04	20	16	48			

Table 10 Cluster groups of 30 wheat genotypes based on 10 SSR marker data

Cluster	Size	Genotypes
I	7	Sonara-64, BAW 1135, BARI Gom 29, Kanchan, BAW 1177, Akbar, BARI Gom 24 (Prodip)
II	7	Protiva, BARI Gom 30, Agrahani, Ananda, BARI Gom 31, BARI Gom 26, BARI Gom 22 (Sufi)
III	9	BWMRI 1, Sourov, BARI Gom 28, Gourob, Kalyansona, Sonalika, Shotabdi, Balaka, BARI Gom 32
IV	7	Sonara 64, BARI Gom 29, Kanchan, Ananda, BARI Gom 31, BWMRI BARI Gom 27, BAW 1200, Barkat, BARI Gom 33, BARI Gom 25, BARI Gom 23 (Bijoy), BAW 1163

List of figures

Fig. 1 Effect of drought on root and shoot of wheat plant at seedling stage. Here, A= control condition, B = drought condition at 0.2M mannitol solution and C = drought condition at 0.4M mannitol solution.



Fig. 2 DNA profile of 30 bread wheat germplasm using 10 polymorphic SSR markers (a) to (j) and 1 monomorphic SSR marker (k)

As seen, the marker showed different polymorphism patterns within wheat genotypes. Legend: 1= Sourov, 2= BARI Gom 32, 3= Balaka, 4= BARI Gom 28, 5= BARI Gom 24, 6= BAW 1163, 7= Ananda, 8= BWMRI 1, 9= Barkat, 10= BARI Gom 33, 11= BAW 1177, 12= Kalyansona, 13= BARI Gom 29, 14= Kanchan, 15= Akbar, 16= BARI Gom 26, 17= BARI Gom 27, 18= BAW 1135, 19= Shotabdi, 20= Agrahani, 21= BAW 1200, 22= BARI

Gom 23, 23= Protiva, 24= BARI Gom 25, 25= BARI Gom 31, 26= BARI Gom 22, 27= Sonara 64, 28= Sonalika, 29= BARI Gom 30, 30= Gourab; Ladder =100bp

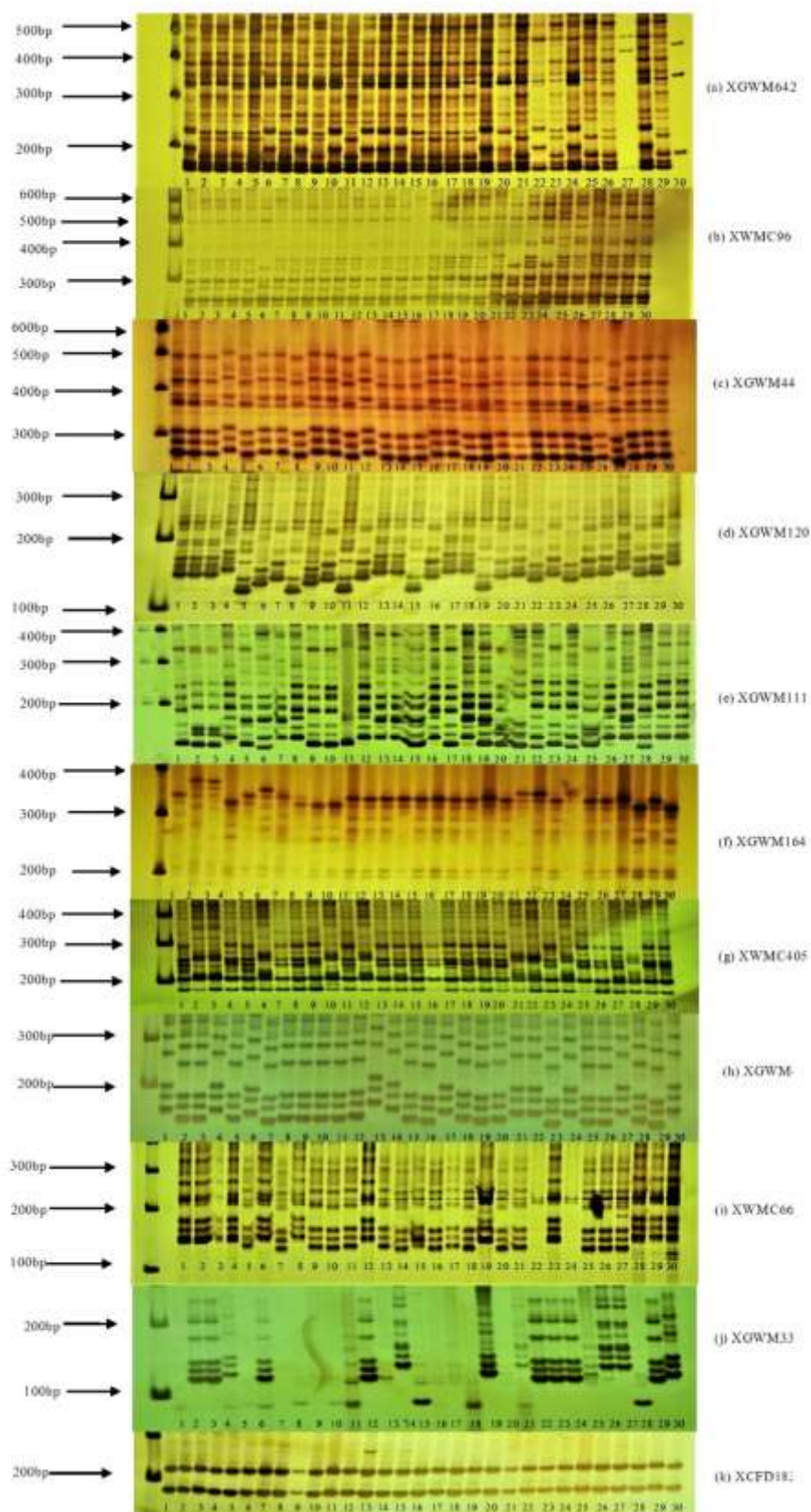


Fig. 3 Population structure of 30 genotypes of wheat. A=The best number of groups among locations estimated by Evano test methods, and B= Model based population structure plot for each isolate with K=2, using Structure with SSR markers data. Color codes are as follows: Population I red, Population II green.

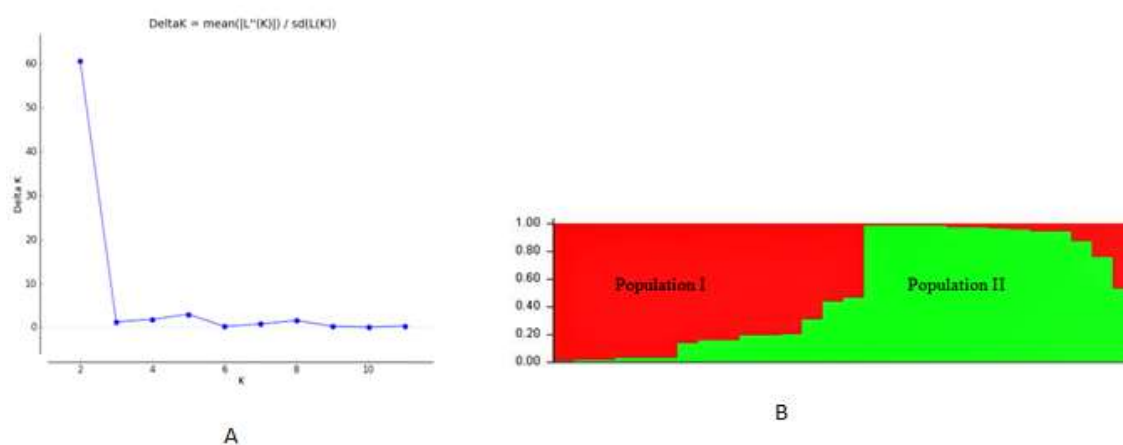


Fig. 4 UPGMA tree displaying the distribution of the 30 bread wheat genotypes in four clusters, and presenting the genetic similarities and dissimilarities within and between the populations. Names of the genotypes are given on the termini of branches.

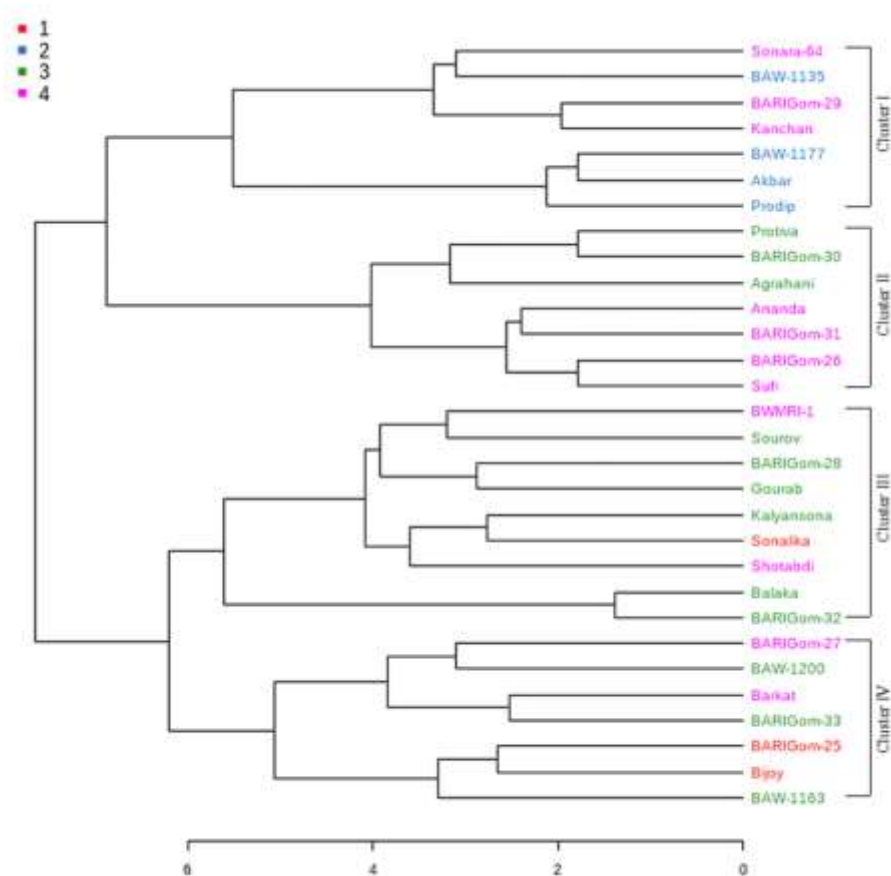


Fig. 5 Principal coordinates analysis of 30 wheat genotypes based on SSR marker data.

