

Genetic Diversity and Yield Evaluation of Advanced Wheat Lines Using Agro Morphological Traits and SSR Markers

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Table 1 to 12 are available in the Supplementary Files section.

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Abstract

Crop improvement is the primary goal of plant breeding, and the availability of wide genetic diversity offers breeders the opportunity to enhance targeted traits. This experiment evaluated the ten wheat advanced lines (ALs) with a check variety (BARI Gom 32) to find out their morphological and genetical diversity. The analysis of variance showed significant variation among the studied traits and the ALs. Mean performances recorded the highest grain weight plant⁻¹ in HSTUW 1 (2.81 kg), followed by HSTUW 8 (2.68 kg) and HSTUW 4 (2.67 kg). The correlation analysis revealed a strong positive association between grain yield plant⁻¹ and thousand-grain weight, as well as between spike length, grain weight plant⁻¹, plant height, and days to maturity. Path analyses showed that the grain weight spike⁻¹ and the thousand-grain weight had higher direct positive effects on the yield plot⁻¹. The days to maturity, plant height, and number of spikes showed high heritability and genetic advance. The GCV and PCV recorded medium variation. The SSR markers used were found to be polymorphic. A total of 31 alleles were recorded with an average of 7.75 alleles locus⁻¹. The highest genetic similarity was observed between HSTUW 2 and HSTUW 3 (97%). These ALs can be used for further multi-location yield trials to validate the results for farmers' cultivation.

Keywords: Wheat, Advanced generations, Morphological Variation, Genetic Assessment, SSR Markers

1. Introduction

Maize, rice, and wheat dominate the global cereal production. Among them, wheat (*Triticum aestivum*) is the prominent staple food, accounting for 26% of the world's cereal yield [1]. As a “King of cereals” it not only provides essential nutrients [2] but also contributes to the global economy [3]. Although global wheat production reached a record 778.2 million tons in 2021-22, with a forecasted increase to 781.1 million tons in 2022-23 [4], the growing population necessitates further food by developing high-yielding wheat genotypes to meet the increasing demand of the upsurging human civilization [5].

Over the past decade, Bangladesh has experienced a substantial increase in wheat consumption, due to changes in dietary preferences, enhanced affordability, and a burgeoning baked goods export sector. Although Bangladesh cultivated only 0.32 million hectares of wheat in 2021-22, advancements in modern agricultural practices enabled the country to achieve a remarkable yield of 1.17 million tons, equating to a significant 3.65-ton-per-hectare productivity [6]. Nevertheless, this production remains insufficient to satisfy the nation's escalating wheat demand, which exhibits a consistent annual growth rate of 13%. Imports from other countries now fulfill this ever-increasing demand. Besides the low production rate per unit area, wheat cultivation in Bangladesh faces significant constraints by the synergistic influence of both biotic and abiotic factors [7]. Natural disasters like floods, soil and water salinity, cyclones, droughts, and storm surges significantly impede crop production [8].

Sustainable food crop production hinges on robust germplasm development and the preservation of genetic diversity [5]. To effectively leverage the germplasm, accurately assessing the extent of available genetic variation is crucial. By integrating novel germplasm and rigorously evaluating their desirable traits within existing breeding programs, plant breeders can effectively achieve critical breeding goals [9, 10]. Successful breeding programs necessitate substantial genetic diversity within source populations [11]. This diversity serves as a reservoir for novel alleles, facilitating the incorporation of traits conferring resilience against biotic and abiotic stresses. Rigorous germplasm characterization and identification of a diverse array of statistical methodologies [12, 13].

Due to the polygenic nature of grain yield, directly selecting this trait may result in limited breeding success [14]. To enhance breeding efficiency, wheat breeders must prioritize the identification of targeted traits contributing to yield [15, 16]. Crosses between genetically diverse parents are likely to show higher heterosis, promote beneficial genetic recombination, and increase phenotypic variability in progeny [17, 18]. This broad genetic diversity offers a valuable resource for selecting and characterizing superior genotypes with desirable trait combinations [19-22].

Effective plant breeding programs necessitate a thorough understanding of genetic variability, heritability, and the potential for genetic gain [23]. Heritability quantifies the extent to which phenotypic variation within a population is attributable to genetic factors [24, 25]. This crucial parameter provides valuable insights into the efficacy of selection strategies and the anticipated magnitude of genetic improvement achievable through breeding interventions. The accuracy of heritability estimates is contingent upon a confluence of factors, encompassing the nature of the genetic material, sample size, sampling methodology, experimental design, and the analytical approach employed [26]. Moreover, heritability along with estimates of genetic gain, serve as crucial determinants for optimizing selection efficiency and predicting the outcomes of selection interventions [27, 28]. The presence of both high heritability and substantial genetic advance for various yield-related traits signifies a favorable scenario for the selection of highly productive genotypes [29]. A comprehensive evaluation of heritability and genetic advancement provides a more robust and insightful assessment compared to considering these parameters independently [30, 31]. Genetic advance, a crucial parameter in contemporary breeding initiatives across diverse crops [32], provides superior selection efficacy. Conversely, genotypic and

phenotypic coefficients of variation serve as valuable indicators of the inherent variability within germplasm [33, 34]. Among several statistical techniques for estimating genetic variability among genotypes, multivariate analysis stands out as the most effective and widely adopted approach due to its ability to simultaneously analyze multiple traits and their interactions [35, 36]. It deals with intricate datasets, enabling nuanced exploration of relationships, groupings, and trait selection. These methods offer superior inferential power compared to simpler approaches [35]. Cluster analysis effectively categorizes genotypes based on genetic similarity, minimizing errors [37]. Ultimately, identifying superior genotypes with high genetic gain and desirable traits is paramount for successful breeding programs. So, this study was conducted to assess the performance of advanced wheat lines based on genetic and molecular analyses.

2. Materials and Methods

2.1 Plant materials and description of experimental site

The experiment was conducted at the research field of the Department of Genetics and Plant Breeding (GPB), Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. Ten advanced wheat lines developed by the Department of GPB, HSTU, Bangladesh along with a check variety (BARI Gom 32), a widely cultivated, high-yielding mega-variety in Bangladesh used as the standard benchmark, were used as experimental materials (Table 1). The experimental site was located in the Dinajpur Sadar upazila at 25°13' N latitude and 88°23' E longitude, situated at an elevation of 37.5 meters above mean sea level (Figure 1). This location falls within the “Old Himalayan Piedmont Plain” (Agro-Ecological Zone, AEZ-1). The seeds were sown at the standard sowing time of wheat production (November 15, 2022). The soil, characterized by a silty loam texture, is moderately fertile, rich in organic matter, and has a pH of 6.8. The experimental area was located in a tropical climatic zone, with high temperatures and rainfall during the Kharif season, followed by cooler and drier conditions in the Rabi season. The temperature varied between 15°C and 30°C, with no rainfall during the current experiment growing season. A detailed overview of the prevailing weather conditions throughout the growing season is provided in Figure 2.

2.2 Crop husbandry

The land was plowed using rotary using a plow tiller, followed by repeated laddering to achieve a fine tilth and level the soil. Large clods were broken into smaller pieces, weeds, and crop residues

were removed, and final land preparation was completed before mid-November. The experiment was conducted using a randomized complete block design (RCBD) with three replications. A total of 33 plots were prepared where 11 wheat ALs randomly assigned to five rows per plot. The unit plot size was 5 m × 1 m for each treatment. The total experimental area was 17.5 m × 18 m (315 m²). A spacing of 0.5 m was maintained between adjacent blocks, while the row-to-row distance was maintained at 0.25 m. Seeds were sown in lines at a rate of 120 kg ha⁻¹ and seeds were placed at a depth of 2-2.5 cm, following final land preparation. To prevent soilborne fungal diseases, seeds were treated with the fungicide Provax 200 WP at a rate of 3 g kg⁻¹ of seed before sowing. The manure and fertilizer applied in the experiment was detailed in Table 2. The procedure and methods of fertilizer application described by Ahmmed et al. [38] were followed. Weed and irrigation management were carried out as required to ensure optimal crop growth. Randomly selected ten wheat plants from each plot were used to record data for each AL across three replications. Field data were collected on nine morpho-physiological traits, like DH (days to heading), DM (days to maturity), PH (plant height in cm), GWPS (grain weight spike⁻¹ in g), TGW (1000 grain weigh in g), NOS (number of spikes), SL (spike length in cm), GPS (grains per spike⁻¹), YPP (yield plot⁻¹ in g). For molecular analysis, 2-4 leaf segments, each approximately 2 cm long, were collected from 15-day-old wheat seedlings and placed in Eppendorf tubes. The samples were dried using silica gel for seven days to ensure proper preservation for subsequent molecular studies.

2.3 Molecular Analysis

The study utilized four SSR markers for PCR-based DNA assay (Table 3). These specific markers were selected due to their previously reported high polymorphism information content (PIC) in South Asian wheat germplasm and their known linkage to functional genomic regions governing yield and stress-tolerance traits. The molecular analysis was conducted at the Molecular Breeding Laboratory of BWMRI and at the Biotechnology Laboratory of the Department of Genetics and Plant Breeding at HSTU, Dinajpur. These specific markers were selected to detect the presence or absence of genes or translocations associated with traits of interest among the studied ALs.

2.3.1 Extraction of DNA by Modified CTAB Method

The DNA samples was evaluated prior to PCR amplification using a NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific, USA) for quantification. PCR amplification was

conducted in 10 μ L reaction volume using a thermal cycler. The reaction mixture comprised 0.01 μ L of 10 mM Tris-HCl, 0.050 μ L of 50 mM KCl, 0.02 μ L of 25 mM MgCl₂, 200 μ L of each dNTP, 250 nM of each primer, 20-40 ng of genomic DNA, and 0.25 U of Taq DNA polymerase. The thermal cycling protocol included an initial denaturation step at 95°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 50-60°C for 1.5 minutes, and extension at 72°C. Post-amplification, the PCR products were mixed with a loading buffer equivalent to one-fifth of the reaction volume. The loading buffer contained 1.0 μ L of EDTA (pH 0.8), 0.01 μ L of Tris-HCl (pH 7.5), 5% Ficoll 400, 0.5% bromophenol blue, and 0.05% xylene cyanol FF. A total of 8 μ L of the TBE buffer. Electrophoresis was performed at 50 mA for 2-3 hours, followed by silver staining to visualize the PCR products. This study employed 4 specific SSR markers for analysis.

2.3.2 Gel Electrophoresis and silver nitrate staining

The reaction products were separated using electrophoresis on polyacrylamide gels in 1 $\times$ TBE buffer (0.90 μ L Tris-borate, pH 8.3, and 2 μ L EDTA) at a constant current of 50 mA for 2-3 hours [39]. The polyacrylamide gel was prepared by mixing 40 mL of 8% non-denaturing polyacrylamide with an acrylamide-to-bisacrylamide ratio of 37.5:1, 400 μ L of 10% ammonium persulfate (APS), and 40 μ L of TEMED. Distinct banding patterns were visualized using silver nitrate staining. The gel was carefully removed from the glass plates and fixed in a stop solution containing 10% ethanol and 0.5% acetic acid for 10 minutes. It was then stained with 0.2% AgNO₃ solution for 15 minutes. After a brief rinse with distilled water for 10 seconds, the gel was immersed in a 0.002% (w/v) sodium thiosulfate solution for 1 minute and subsequently developed in a chilled solution containing 15% NaOH and 0.4% formaldehyde (HCHO) for 5-10 minutes. To terminate the reaction, the gel was agitated in distilled water for 5 minutes using a shaker. Band identification was performed using Gene Tools software.

2.4 Statistical analyses

Data were recorded in a Microsoft Excel spreadsheet and analyzed using R-Studio 4.2.2 [40]. Analysis of variance (ANOVA) was performed using STAR software [41], while correlation analysis was conducted with R software utilizing the ‘Agricolae’ package [42]. Mean performance and genetic parameters were estimated using the “Variability” package. Path coefficient analysis was performed with OPISTAT [43], and combining ability and heterosis analysis were calculated

in Microsoft Excel. UPGMA dendrograms and heatmap clusters were generated using Metabo Analyst 6.0. Population structure analysis was performed with Structure 2.3.4 and Structure Harvester concurrently.

Polymorphism information content (PIC) was calculated using the following formula:

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

Here, P_i depicts the proportion of samples carrying the i^{th} allele. Population structure analysis was conducted using the Bayesian clustering method implemented in STRUCTURE software version 2.3.4 [44]. The analysis was performed with a model-based approach, incorporating a burn-in period of 100,000 iterations followed by 500,000 Markov Chain Monte Carlo (MCMC) replications. The population range was evaluated from $K=2$ to $K=14$. The optimal K -value was determined using the Evanno ΔK method, and the natural logarithm of probability data ($\ln P(D)$) was employed to identify genetically distinct populations using a graphical approach [45]. Each band was treated as a single locus, and all scorable loci were used to generate a bivariate (1-0) data matrix. Genetic distances (GD) among genotypes were estimated using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) as described by Lin and Nei [46]. To assess genetic diversity, a dendrogram was constructed using Metabo Analyst (Online version) as outlined by Chong et al. [47].

3. Result

The analyses of variance (ANOVA) revealed the presence of highly significant differences among the ALs ($p \leq 0.01$) for all the traits under study (Table 4). The substantial variability in the studied materials indicates ample scope for improving the traits investigated. HSTUW 1 required the minimum number of DH (51.67), whereas all other advanced lines (ALs) required more than 60 days, except for HSTUW 2. In terms of DM, all ALs required more than 105 days, except HSTUW 1, which matured in 102.33 days. HSTUW 1 exhibited the desired traits of early heading and maturity, making it well-suited to escape terminal heat stress. The tallest plant was recorded for HSTUW 4 (116.33 cm), while the shortest was observed for HSTUW 2 (92.35 cm). The highest grain weight plant⁻¹ was obtained from HSTUW 5 (1.88 g). Most of the ALs demonstrated statistically similar performances, except for HSTUW 1, HSTUW 2, and HSTUW 3, which were comparable to the check variety. HSTUW 5 exhibited the longest SL (10.73 cm), whereas HSTUW

1 recorded the shortest (8.58 cm). The highest GPS was recorded in HSTUW 9 (45.20), while the lowest was observed in check variety i.e., BARI Gom 32 (30.93). Regarding the NOS, HSTUW 7 produced the lowest (351.67), whereas HSTUW 1 produced the highest (505.67) number of spikes. For TGW, all ALs displayed statistically similar performances, except for HSTUW 3. In terms of yield plot⁻¹, the highest value was recorded in HSTUW 1 (2.81 Kg), followed by HSTUW 8 (2.67 Kg) and HSTUW 4 (2.66 Kg). The lowest yield was observed in HSTUW 2 (2.08 Kg), followed by HSTUW 3 (2.12 Kg) and HSTUW 9 (2.24 Kg) (Table 5).

The correlation analysis revealed that GYPP showed positive associations with PH (0.39), GWPS (0.25), and SL (0.00), but was negatively correlated with GPS (-0.46) and DM (-0.12). TGW exhibited a significant positive association with PH (0.68*) and GWPS (0.63). It also showed positive associations with DM (0.42), DH (0.36), and SL (0.42), but was negatively correlated with GPS (-0.29) and the NOS (-0.45). The NOS recorded a significant negative association with all the studied traits. SL showed highly significant correlations with DM (0.85***), PH (0.85***), GWPS (0.88***), GPS (0.61*), and DH (0.71*). GWPS demonstrated strong positive associations with DM (0.83***), PH (0.88***), and DH (0.79**), as well as a positive association with GPS (0.55). PH displayed strong correlations with DH (0.79**) and DM (0.68*), while showing a positive association with GPS (0.34). DH recorded significant associations with GPS (0.64*) and DM (0.65*). Meanwhile, DM exhibited an insignificant positive correlation with GPS (0.58) (Figure 3). The genotypic correlation analysis revealed a strong positive and significant correlation between YPP and TGW (0.829) at the 5% probability level, while it had non-significant positive correlations with PH (0.421) and GWPS (0.355). YPP was negatively correlated with DH (-0.039), DM (-0.118), NS (-0.1795), SL (-0.058), and GPS (-0.487). GPS showed significant positive correlations with DH (-0.894), DM (-0.859), and PH (-0.855). TGW had a significant positive correlation with PH (0.747) and a moderate positive correlation with GWPS (0.615). GWPS was strongly correlated with PH (0.961), DM (0.903), and DH (0.9034). Finally, DM showed moderate positive correlations with DM (0.669) and PH (0.699) (Table 6).

The phenotypic correlation coefficients showed YPP had a strong positive significant correlation with TGW (0.519) and a moderate positive significant correlation with PH (0.345). However, it exhibited a moderate negative significant association with GWPS (-0.426), indicating

an inverse relationship between these traits. YPP showed non-significant associations with the NOS (0.086) and SL (0.047). The TGW had strong positive significant associations with GWPS (0.648) and PH (0.585) and a moderate association with DM (0.372). GPS was strongly positively correlated with SL (0.557), DH (0.558), and GWPS (0.555), while it had a strong negative correlation with the NOS (-0.642). SL was positively associated with PH (0.738), DM (0.6544), GWPS (0.624), and DH (0.598) but negatively associated with the NOS (-0.670). DM showed a strong positive correlation with DH (0.627), while PH was positively correlated with DH (0.760) and DM (0.651). GWPS had strong positive correlations with DH (0.617), DM (0.722), and PH (0.741) but strong negative associations with DH (-0.746), DM (-0.687), PH (-0.697), GWPS (-0.825), and TGW (-0.376) (Table 7).

The genotypic path coefficient analysis revealed that GWPS had the highest positive direct effect on YPP (3.573). SL also had a high positive direct effect on YPP (1.198) but showed a negative non-significant association (-0.058). The TGW had a positive direct effect on YPP and positive indirect effects through DH, DM, PH, GWPS, and AL, while It had negative indirect effects with NOS and AGPS. Similarly, NOS exhibited positive indirect effects on YPP but negative effects through DH, DM, PH, GWPS, SL, and GPS. PH had a positive direct effect on YPP, while DH, DM, GWPS, PH, TGW, GPS, and SL contributed positively to yield indirectly, though with high magnitudes. The residual factor was 0.0184, indicating additional unexplained factors influencing YPP (Table 8).

Phenotypic path coefficient analysis revealed that TGW had the highest positive direct effect on YPP (1.108) and a strong positive significant association with grain yield (0.520). GPS also showed a high positive direct effect (0.673) but a moderate negative significant association with YPP (-0.427). While TGW had a positive indirect effect through the NOS, it exhibited negative indirect effects with DH, DM, PH, GWPS, spike length SL, and GPS. PH exhibited a negative direct effect on YPP, and traits such as DH, DM, GWPS, and SL also had negative direct effects though their magnitudes were small. The residual factor value of 0.4134 indicates the presence of other factors influencing grain GYPP (Table 9).

The highest genotypic and phenotypic variances were recorded for YPP (44177.68 and 69323.02), NOS (2631.04 and 3645.52), PH (65.36 and 70.18), and DH (31.93 and 35.17).

Conversely, low genotypic and phenotypic variances were observed for GWPS (0.04 and 0.07) and SL (0.38 and 0.66). The phenotypic coefficient of variation (PCV) exceeded the genotypic coefficient of variation (GCV) for all traits, indicating environmental influence. GCV ranged from 4.53% (DM) to 12.73% (GWPS), with medium GCV observed in GWPS (12.73%), NOS (12.44%), TGW (11.65%), and GPS (10.81%). Similarly, PCV ranged from 4.63% (DM) to 15.62% (GWPS), with medium PCV observed for traits like GWPS (15.62%), NOS (14.65%), and GYPP (10.69%). Heritability (broad sense) ranged from 57.10% (SL) to 95.85% (DM). High heritability (>80%) was observed for DM (95.85%), PH (93.13%), and DH (90.78%), indicating these traits can be effectively improved through selection. Moderately high heritability (60–79%) was recorded for GPS (73.87%), TGW (72.54%), NOS (72.17%), GWPS (66.47%), and GYPP (63.73%). High genetic advance (GA) was observed for GYPP (3345.65), while the lowest was for GWPS (0.35). Genetic advance as a percentage of the mean (GAM) ranged from 9.14% (DM) to 21.77% (NOS). Traits with high GAM (>20%) included the NOS (21.77%), GWPS (21.38%), and TGW (20.44%). Most other traits exhibited moderate GAM values (10–20%): GPS (19.14%), DH (17.46%), PH (15.42%), and GYPP (14.04%). These results indicate varying levels of genetic improvement potential through selection (Table 10).

Four SSR/microsatellite markers namely Gwm495, Barc20, TaBarc101, and Xwmc112 were used to assess variation among the ALs, and all of which were found to be polymorphic. In total, 31 alleles were detected, with the number of alleles per locus ranging from 6 to 9, and an average of 7.75 alleles per locus. The most polymorphic markers were Xwmc112 and Gwm495, each with 9 alleles, followed by Barc20 with 7 alleles and TaBarc101 with 6 alleles (Figures 4a-4d). Polymorphism information content (PIC) values were used to assess marker variability, with markers classified as high, medium, or low polymorphic based on their PIC values: $PIC > 0.5$, $0.5 > PIC > 0.25$, and $PIC < 0.25$, respectively. PIC values ranged from 0.82 (TaBarc101) to 0.87 (Gwm495), with an average of 0.84 per marker. Gwm495 had the highest number of alleles per locus and the highest PIC value of 0.87 (Table 11). All markers had a PIC value greater than 0.5, indicating that they are highly informative and polymorphic. These markers are suitable for use in marker-assisted selection of wheat ALs as they can effectively distinguish between different ALs.

Bayesian clustering analysis was performed to assess the population's genetic structure based on SSR marker data. The clustering model assumes the presence of K underlying clusters,

and the number of populations was determined by plotting the ΔK values derived from the $\ln(PD)$ against K . The ΔK value indicates the highest level of clustering and identifies the number of subpopulations within the main population. The highest log-likelihood value was obtained, resulting in $K=2$. This indicates that the populations are divided into two distinct clusters. To examine the structure based on geographical origin, the analysis was conducted by setting the range of possible subpopulation numbers (K) from 1 to 12 (Figure 5). In the STRUCTURE analysis, accessions were classified as either pure or heterogeneous based on their membership score. Accessions with a score greater than 0.80 were considered pure, while those with a score less than 0.80 were categorized as heterogeneous. Population I, comprising 27.27% of the total ALs, included three pure ALs (HSTUW 1, HSTUW 2, and HSTUW 3), with no admixture observed. Population II, accounting for 72.73% of the total ALs with check, consisted of eight genotypes, of which seven (HSTUW 4, HSTUW 5, HSTUW 6, HSTUW 7, HSTUW 8, HSTUW 10, and BARRI Gom 32) were pure, and one AL (HSTUW 9) was heterogeneous. The phylogenetic dendrogram based on genetic distance showed a similar pattern to the population structure analysis, supporting the presence of two distinct subpopulations, as identified by the model-based STRUCTURE analysis (Figure 6).

Cluster analysis based on genetic similarity values from SSR alleles of wheat ALS was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), resulting in a dendrogram. The analysis revealed two major clusters (Group I and II) with genetic similarity coefficients ranging from 2.5 to 4.5, indicating significant genetic variation among the ALs. Each of these clusters was further divided into three subgroups: subgroup-1 contained 4 ALs, Subgroup-2 included 4 ALs, and Subgroup-3 consisted of 3 ALs (Table 12). The highest genetic similarity was observed between HSTUW 2 and HSTUW 3 (97%), followed by HSTUW 4, HSTUW 5, HSTUW 6 (100%) and HSTUW 7, HSTUW 8, and HSTUW 10 (85%). This suggests that HSTUW 2 and HSTUW 3 are genetically more similar, while HSTUW 7, HSTUW 8, HSTUW 10, and HSTUW 1 exhibit a minimum of 85% genetic divergence (Figure 7). In heatmap clustering, the ALs and their association with SSR microsatellite markers were grouped differently based on their genetic relationships. HSTUW 1, HSTUW 2, and HSTUW 3 fall in similar groups while the rest ALs fall in similar groups (Figure 8).

4. Discussion

Genetic diversity plays a vital role in the survival of plants in nature and in enhancing crop productivity. Variation within plant genetic resources provides plant breeders with the opportunity to develop new and improved cultivars possessing desirable traits [35]. Since the dawn of agriculture, the natural genetic adaptability of crop species has been harnessed to meet the demands of subsistence food production [48].

The initial step in improving cereal genetics is the exploitation of natural genetic variation that governs key yield-related traits [49-53]. Phenotypic diversity results from both genetic factors and environmental conditions, and the primary aim of breeding is to decipher the complex interactions that enable the conversion of genotypes into phenotypes [6]. The ANOVA revealed highly significant differences ($p \leq 0.01$) among the ALs for all traits under study, indicating considerable variability and ample potentiality for improving the investigated traits. Therefore, there is a promising opportunity to select a superior advanced line for enhancing grain yield. Several researchers, like Drikvand et al. [54], Bhutto et al. [55], Khan et al. [56], and Phougat et al. [57], have reported significant variability for several important traits in wheat. The experimental materials originated from a genetically diverse population established via hybridization of parents exhibiting advantageous attributes. These desirable traits encompass adaptability, tolerance to environmental stresses, beneficial physiological characteristics, and other traits at both the molecular and phenotypic levels. This variability across ALs arises from differences in the genes present in the genotypes and the interaction of distinct gene combinations with the environment to which the genotypes are exposed. Our research recorded that there are significant opportunities to select early-maturing lines that may escape terminal heat stress. However, the early maturity trait alone is not sufficient, as smallholder farmers also prioritize other important traits, such as spike length, TGW, and grain yield, when selecting lines for field cultivation [1]. Some early-maturing ALs were found to exhibit average to higher yields and yield-contributing traits, such as grain yield, which is noteworthy as it indicates the integration of essential traits, making these ALs desirable. Early maturity, high yield, and good adaptability to various targeted environments have been key focuses in wheat breeding programs [58], which may explain the presence of these desirable traits in the genotypes. We found HSTUW 1 and HSTUW 8 are the best-performing lines due to their high yields of 2812.73 g and 2671.95 g plot⁻¹, respectively. HSTUW 1 is also good in early heading (51.67 days), early maturity (102.33 days), the highest spike count (505.67), and a plant height of 95.23 cm. The HSTUW 8, with a plant height of 108.45 cm, demonstrates strong yield potential

and stability, making both lines suitable candidates for further improvement programs. The cultivation of semi-dwarf wheat varieties has gained significant attention among breeders due to their inherent robustness against lodging and their reduced reliance on external inputs like fertilizers and irrigation. These plants, characterized by their moderate stature, optimize resource allocation by minimizing the energy expenditure required to transport essential nutrients from the leaves to the developing grains (assimilates and photosynthates). This efficient resource management translates into several agronomic advantages, including enhanced resistance to lodging, increased grain number spike, and a favorable harvest index. Consequently, these semi-dwarf cultivars demonstrate superior grain yield and quality [6]. Genetically analyzing these significant traits is essential to understanding their genetic structure and facilitating their incorporation into wheat breeding programs [59, 60].

The correlogram analysis showed that GYPP had a significant positive correlation with TGW and positive associations with PH and GWPS, highlighting these traits as key contributors to yield. Conversely, it was negatively correlated with GPS and DM, indicating that excessive grain number and delayed maturity may negatively impact yield. Abdurezake et al. [61] found that grain yield exhibited a significant positive correlation with DM, grain-filling period, plant height, and thousand-seed weight.

At the genotypic level, GYPP showed a strong positive correlation with thousand-grain weight and moderate positive associations with plant height and GWPS, while it was negatively correlated with DH, DM, NOS, and grains per spike, highlighting the importance of thousand-grain weight for yield improvement. At the phenotypic level, GYPP showed a strong positive correlation with thousand-grain weight and a moderate positive correlation with PH, while exhibiting a moderate negative association with GPS, emphasizing the importance of heavier grains and optimal plant height for higher yield. Hassan et al. [6] found, that wheat yield positively correlates with plant height, effective tillers plant⁻¹, and 100-grain weight, while at the genotypic as well as phenotypic level. Prior studies have consistently demonstrated a significant positive correlation between 100-grain weight and grain yield [62, 63]. Furthermore, several investigations have reported significant associations between yield and other key agronomic traits [64].

Path coefficient analysis enables the partitioning of the correlation coefficient into its constituent direct and indirect effects, providing a more nuanced understanding of causal

relationships within a system. We found that genotypic path coefficient analysis revealed that GWPS had the highest positive direct effect on yield plot⁻¹, while other traits like TGW and SL contributed through a combination of direct and indirect effects. Phenotypic path coefficient analysis showed that TGW had the strongest positive direct effect and significant association with yield. Meena et al. [65] discovered a significant association between grain yield and grain weight at both genotypic and phenotypic levels, emphasizing the importance of grain weight in determining overall yield potential. At the genotypic level, Sabit et al. [66] reported that PH, SL, and YPP positively influenced grain yield in wheat, aligning with our findings. At the phenotypic level, Hassan et al. [6] highlighted that TGW had the greatest influence, supporting our results.

The genetic analysis revealed that the best traits for genetic improvement are YPP, NOS, and TGW, as they exhibit high heritability and significant genetic advance, indicating strong potential for selection. These traits offer a balance of heritability and genetic improvement potential, making them ideal targets for breeding programs focused on enhancing yield. Across all examined traits, phenotypic variance consistently outweighed genotypic variance, suggesting a limited environmental impact on trait expression. This minimal environmental influence is further supported by negligible deviations between the two coefficients. Heritability studies provide valuable insights into the transmission of traits from parents to offspring across generations. These studies are crucial for plant breeders to predict successful crosses and effectively incorporate desired traits into subsequent generations. As emphasized by Johnson et al. [67], heritability estimates, when considered alongside the potential for genetic gain, offer a more robust foundation for selection decisions. Additive genetic effects, which are likely key contributors to heritability, facilitate early selection in segregating populations. This approach can effectively enhance wheat yield by focusing on traits like grain weight. Our findings corroborate previous research [68, 69], which documented substantial heritability for grain yield and TGW. Eid [70] elucidated that non-additive gene action, while potentially associated with high heritability, may limit genetic progress. Conversely, additive gene action typically results in both high heritability and significant genetic advance.

This study used four SSR markers to assess genetic diversity among ALs. All markers exhibited high levels of polymorphism, with a total of 31 alleles detected across loci (range: 6-9 alleles, average: 7.75). All markers exhibited PIC values exceeding 0.5, classifying them as highly

polymorphic. These results surpass those reported in previous studies by Ahmed et al. [71] and Salehi et al. [72]. The high PIC values and allelic diversity observed in this study suggest that these markers are valuable tools for marker-assisted selection in wheat breeding programs. Our findings corroborate previous research by Hassan et al. [6], Haque et al. [73], and Thungo et al. [74]. Using SSR markers and phenotypic data, we assessed the potential application of various wheat genotypes from this study in breeding programs aimed at future sustainable agricultural production [75]. The SSR markers demonstrated notable polymorphism among the wheat varieties analyzed. However, it was hypothesized that genetic similarity might lead to biased selection of breeding material, potentially narrowing the genetic base of wheat germplasm in the region. To mitigate this, additional polymorphic SSR markers could be employed for more effective germplasm screening, covering previously uncharacterized regions of the wheat genome [76]. To assess population genetic structure, we employed a clustering model assuming the presence of K distinct populations. The optimal number of clusters (K) was determined by plotting the natural logarithm of the probability of data (Ln(PD)) against K . The value of K associated with the highest Delta K , a measure of the second-order rate of change in Ln(PD) , was considered to represent the most likely number of clusters and subpopulations within the primary population. The structural analysis based on geographical origin explored the potential number of subpopulations (K) within a range from 1 to 12 [6]. Evanno et al. [45] and Rodriguez-Ramilo et al. [77] applied the maximum log-likelihood method, identifying an optimal K value of 2. This analysis revealed that all populations consisted of two distinct genetic clusters. Accessions were categorized as either pure or heterogeneous based on structure analysis, with a score threshold of 0.80; accessions scoring above 0.80 were classified as pure, while those scoring below this threshold were deemed heterogeneous. We found Population I, representing 27.27% of the total genotypes, consisted of three pure genotypes (HSTUW 1, HSTUW 2, and HSTUW 3), with no evidence of admixture. In contrast, Population II, which accounted for 72.73% of the total genotypes, included eight genotypes. Among these, seven (HSTUW 4, HSTUW 5, HSTUW 6, HSTUW 7, HSTUW 8, HSTUW 10, and BARRI Gom 32) were classified as pure, while one AL (HSTUW 9) was identified as heterogeneous. The population structure analysis, combined with a phylogenetic dendrogram based on genetic distance, identified two potential subpopulations, as suggested by the model-based structure. The dendrogram as constructed using similarity data for SSR alleles from all wheat genotypes, employing the UPGMA. These approaches have been widely adopted by researchers

in wheat breeding programs, yielding significant advancements [72, 78]. The analysis identified two primary clusters, Group I and Group II, with genetic similarity coefficients ranging from 2.5 to 4.5, highlighting substantial genetic variation among the ALs. Each major cluster was further subdivided into three subgroups: Subgroup 1 comprised 4 ALs, subgroup 2 included 4 ALs, and Subgroup 3 contained 3 ALs. Previous research has effectively utilized SSRs to classify wheat cultivars, demonstrating their utility in grouping varieties based on their ancestral lineages and further sub-grouping them according to agronomic characteristics and geographical origins [79]. UPGMA analysis has been shown to yield varying cluster formations, ranging from two to three clusters, depending on the level of genetic diversity observed [80]. Genetic diversity studies have reported a wide range of cluster numbers, with some studies identifying up to nine clusters [80] and others revealing as many as thirteen clusters [81].

The highest genetic similarity was observed between HSTUW 2 and HSTUW 3 (97%), followed by HSTUW 4, HSTUW 5, and HSTUW 6 (100%), and HSTUW 7, HSTUW 8, and HSTUW 10 (85%). These results indicate that HSTUW 2 and HSTUW 3 are the most genetically similar, while HSTUW 7, HSTUW8, HSTUW 10, and HSTUW 1 exhibit a minimum genetic divergence of 85%. Hassan et al. [6] reported that Shatabdi and BARI Gom 25 exhibited the highest genetic similarity at 94%, followed by P3-18-10 and P3-11-3 at 90%, and P3-24-10 and P3-21-10 at 85%.

Molecular characterization employing SSRs has effectively clustered wheat cultivars based on their phenotypic performance, demonstrating a strong correlation between genetic diversity and observed agronomic traits. This marker system has proven particularly valuable in identifying distinct cultivar groups originating from specific parental lineages [82, 83] and in delineating clusters based on agronomic characteristics and geographical origin [79]. Heatmap clustering based on the genetic relationships of ALs and their association with SSR microsatellite markers revealed distinct groupings. HSTUW 1, HSTUW 2, and HSTUW 3 were clustered together in one group, while the remaining ALs formed a separate group. A critical finding of this study is the alignment between the phenotypic performance and the molecular clustering of the advanced lines. Notably, HSTUW 1, HSTUW 2, and HSTUW 3 which exhibited distinct morphological traits such as early maturity and optimal spike counts clustered tightly together in Population I with no genetic admixture. Conversely, lines like HSTUW 8, which demonstrated superior thousand-grain weight

and overall yield potential, were distinctly grouped in Population II. This convergence of morphological and molecular data suggests that the selected SSR markers successfully partitioned the germplasm based on underlying genetic architectures that drive these key agronomic traits, reinforcing the reliability of these markers for targeted selection.

5. Conclusion

Genetic diversity offers plant breeders to select candidate genotypes with the targeted traits. The mean performance of the advanced lines revealed that HSTUW 1 had the number of spikes and yield plot⁻¹, while HSTUW 8 showed the highest thousand-grain weight and, with high genetic advance and heritability indicating the potential for selection in breeding programs. The genetic analysis showed high heritability and genetic advance for days to maturity, plant height, and number of spikes, with medium GCV and PCV. Genotypic analysis using four SSR markers detected 31 alleles, with an average of 7.75 alleles per locus. Moderate polymorphism is indicated by the PIC values. These alleles should be further evaluated at sequencing and expression levels for their potential incorporation into breeding programs to select high-performing wheat lines, possibly linked to traits like stress resistance, adaptability, and climate resilience.

Limitations

While this preliminary screening successfully identified promising advanced lines and demonstrated significant genetic divergence, the field evaluation was conducted over a single location and growing season. Because quantitative agronomic traits, particularly grain yield, are highly subject to genotype-by-environment (G×E) interactions, these findings serve as an exploratory baseline. Multi-location and multi-year trials across diverse agro-ecological zones are currently recommended to validate the temporal and spatial stability of elite lines like HSTUW 1 and HSTUW 8 prior to their recommendation for commercial cultivation.

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Author contributions

MH led the conceptualization, planning, formal analysis, supervision, and reviewing of the study. AAM, MSI, ACM, MBA, and MAH conducted the field research and data collection. MAKKA provided general supervision, while MA supervised the molecular analysis. LR consulted on the research project. MOK was responsible for reviewing, editing, visualization, and preparing the final manuscript for publication. AAM and MOK equally contributed to this study.

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Data availability statement

The datasets supporting the findings of this study are available from the corresponding author upon reasonable request. However, due to ongoing efforts related to the development and release of a new heat-tolerant wheat variety, access to these data is currently restricted and not publicly available. Requests for data access will require appropriate authorization from the Department of Genetics and Plant Breeding at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Declarations

Source of the plant used in your study

The plant materials used in this study were developed by the Department of Genetics and Plant Breeding at Hajee Mohammad Danesh Science and Technology University through several years of rigorous selection and evaluation. The seeds of the check variety used in this experiment were collected from a recognized agricultural research institute (Bangladesh Wheat and Maize Research Institute-BWMRI) by following national guidelines.

Ethics approval and consent to participate

All authors provided their full consent for the publication of this article. The plant material used in this study was self-developed, and the check variety was obtained from the Bangladesh Wheat and Maize Research Institute (BWMRI) with permission from the appropriate authority, in

accordance with national guidelines of Bangladesh. No specific permits were required for the use of cultivated plant material.

Plant Guidelines Statement

The collection, handling, and experimental use of plant materials were conducted in accordance with national agricultural research guidelines of Bangladesh. All experimental procedures complied with institutional biosafety and research regulations.

Permissions for Plant Collection

The wheat seeds (check variety) used in this study were obtained from an agricultural research institution. This plant material is publicly available for cultivation throughout Bangladesh. No wild species, endangered species, or plant materials from protected areas were involved in this study. Therefore, no specific collection permits or licenses were required. All experimental procedures involving plant materials were conducted in accordance with relevant institutional and national guidelines and regulations of Bangladesh.

Consent to publication

All authors have full consent to publish this article.

Competing interests

The authors declare no competing interests.

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Figures

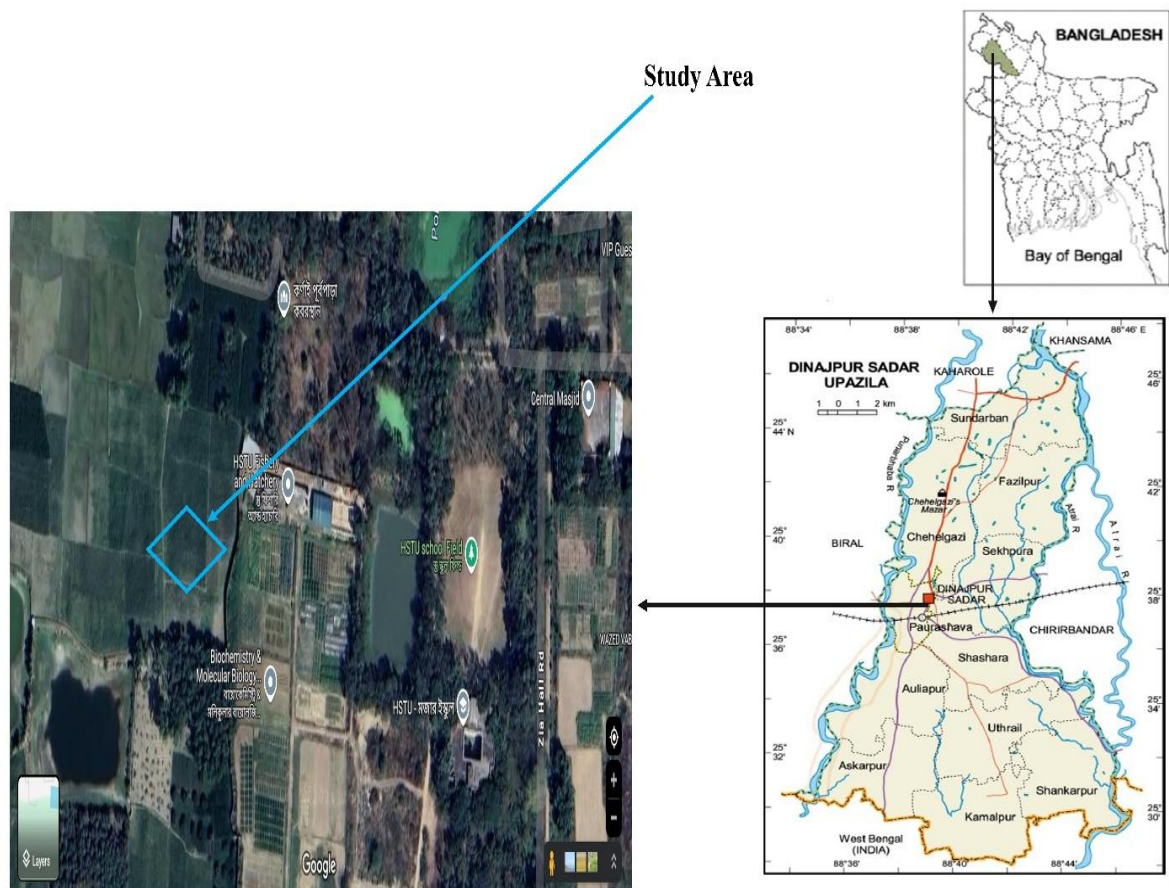


Fig. 1. Map of Dinajpur Sadar Upazila showing study area (HSTU). Source: Google map.

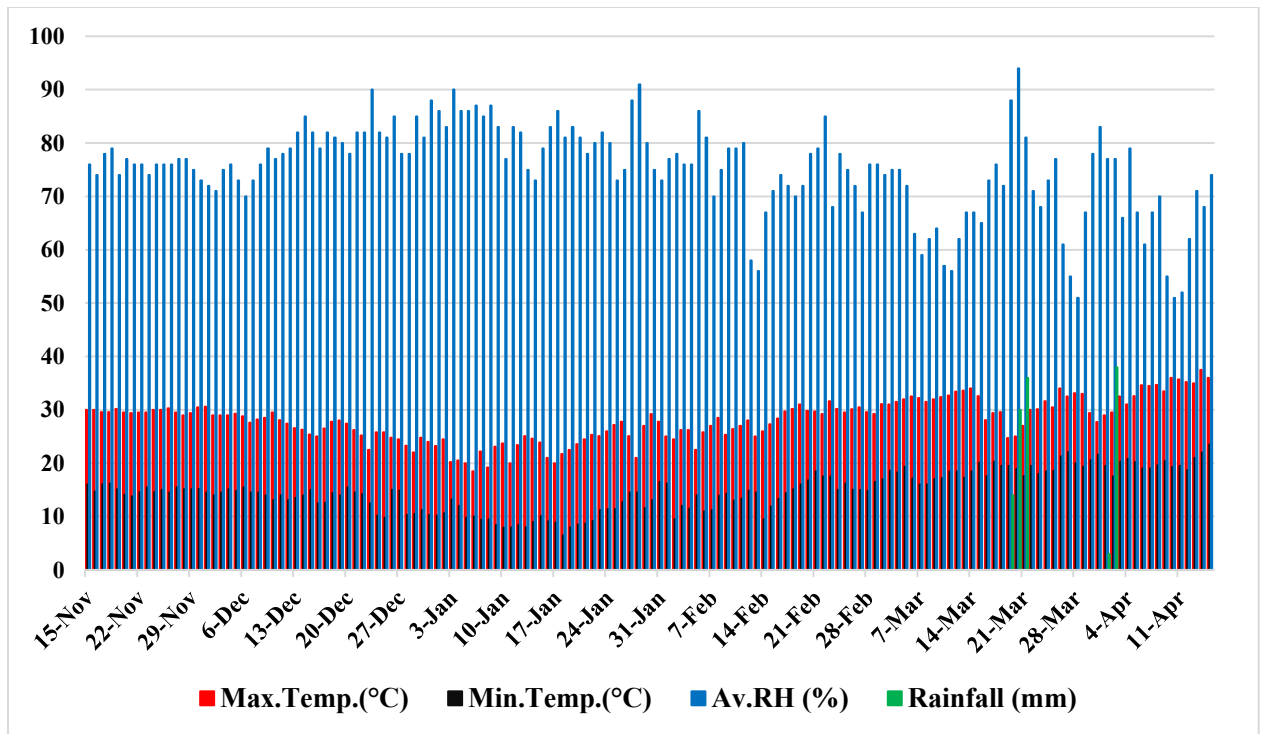
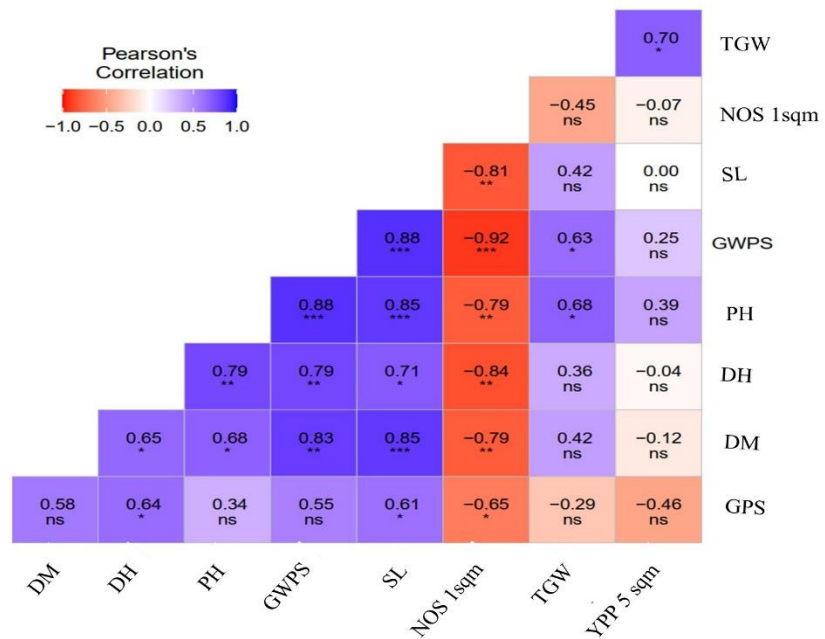


Fig. 2. Average temperature, rainfall and relative humidity from November, 2022- April, 2023



Here, DH= Days to heading, DM= Days to maturity, PH= Average plant height, NOS = Number of spikes, SL= Spike length, GPS= Grains per spike, WPS= Grain weight spike⁻¹, TGW = 1000 grain weight, YPP= Yield plot⁻¹

Fig. 3. Correlogram coefficient among the morphological characters

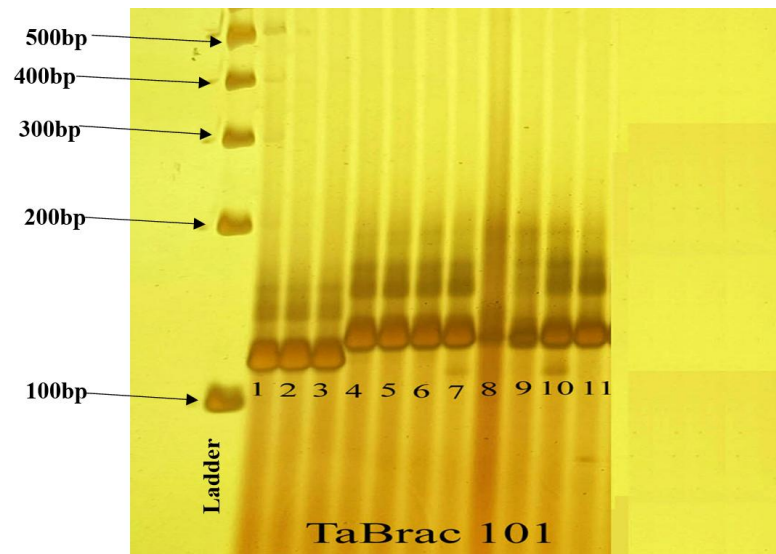


Fig. 4(a). The DNA profile of wheat genotypes using **TaBrac101**

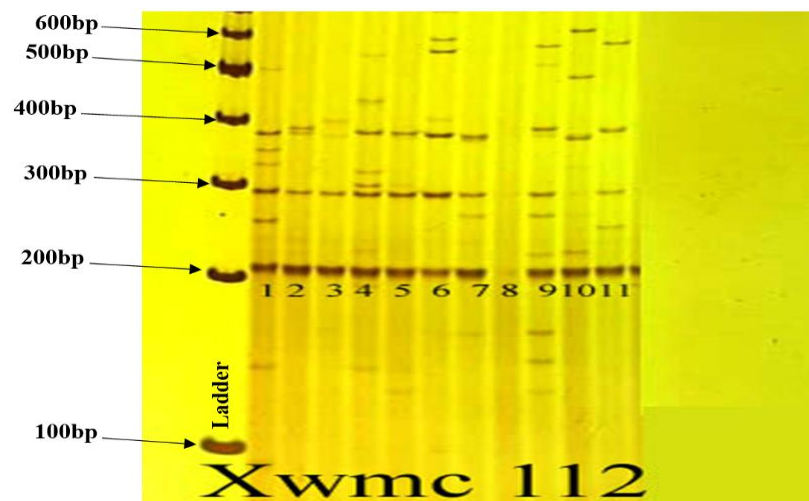


Fig. 4(b). The DNA profile of wheat genotypes using **Xwmc112**

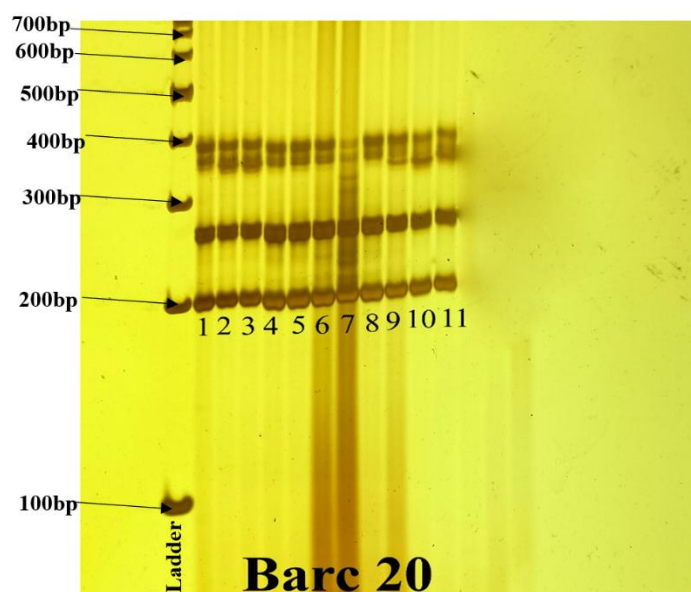


Fig. 4(c). The DNA profile of wheat genotypes using **Barc20**

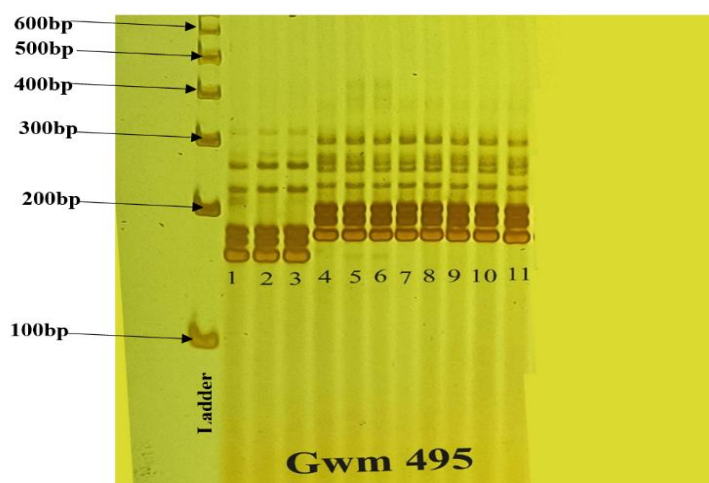


Fig. 4(d). The DNA profile of wheat genotypes using **Gwm**

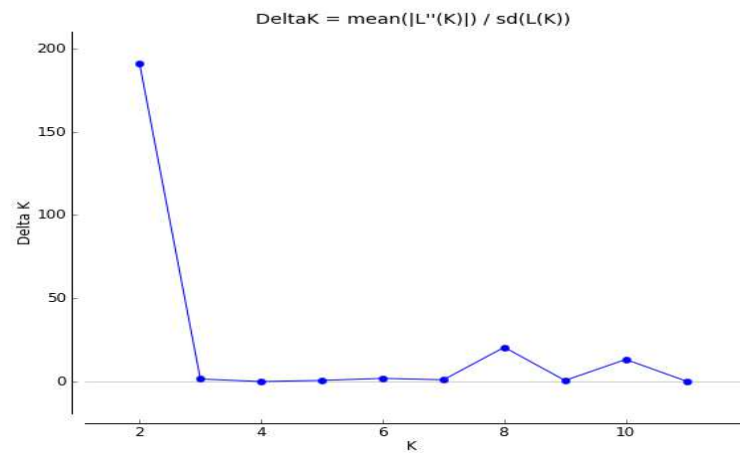


Fig. 5. number of groups among locations estimated by Evanno test methods

The best

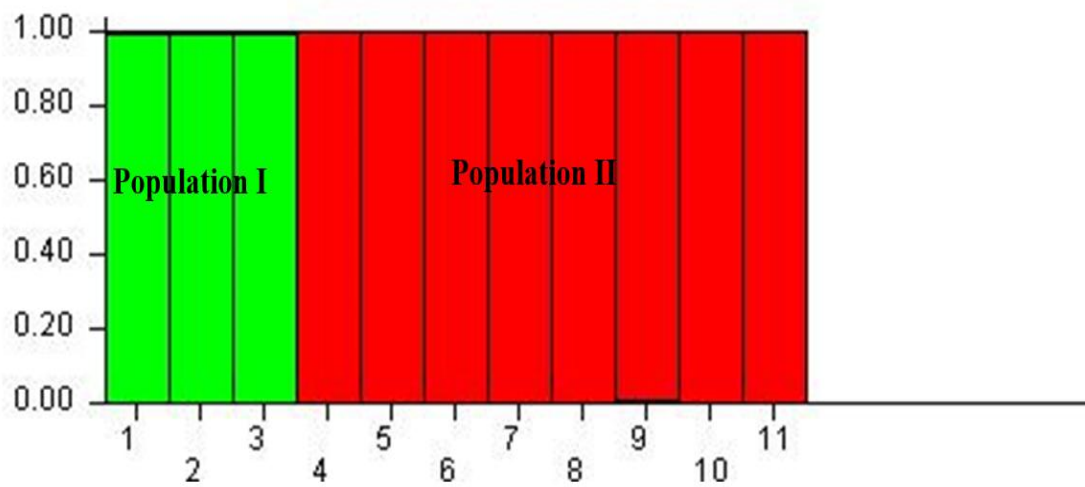


Fig. 6. Model-based population structure plot for each isolate with K=2, using Structure with SSR markers data. Color codes are as follows: Population I red, Population II green

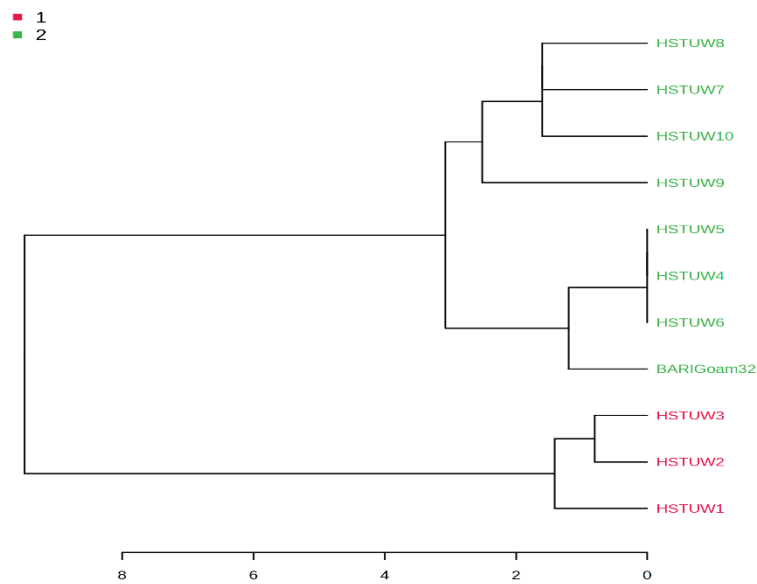


Fig. 7. UPGMA tree displaying the distribution of the wheat genotypes in two groups, and presenting the genetic similarities and dissimilarities within and between the groups. Names of the genotypes are given on the termini of branches

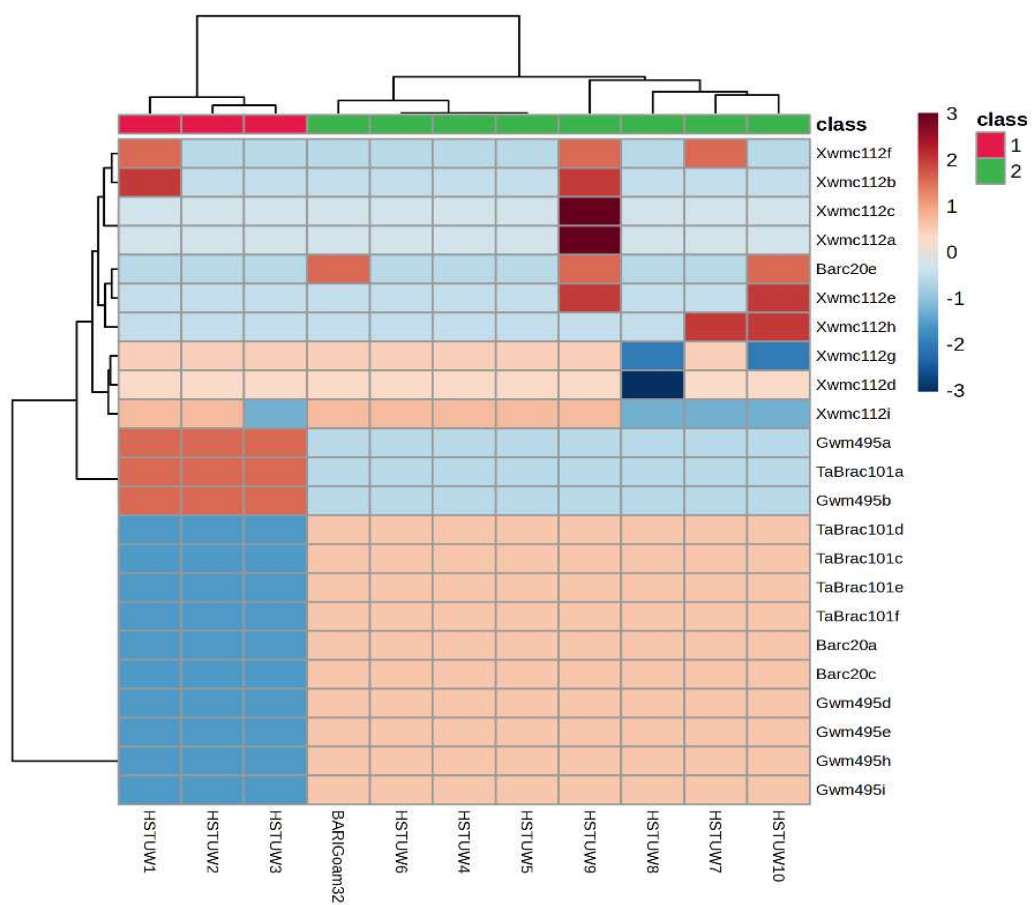
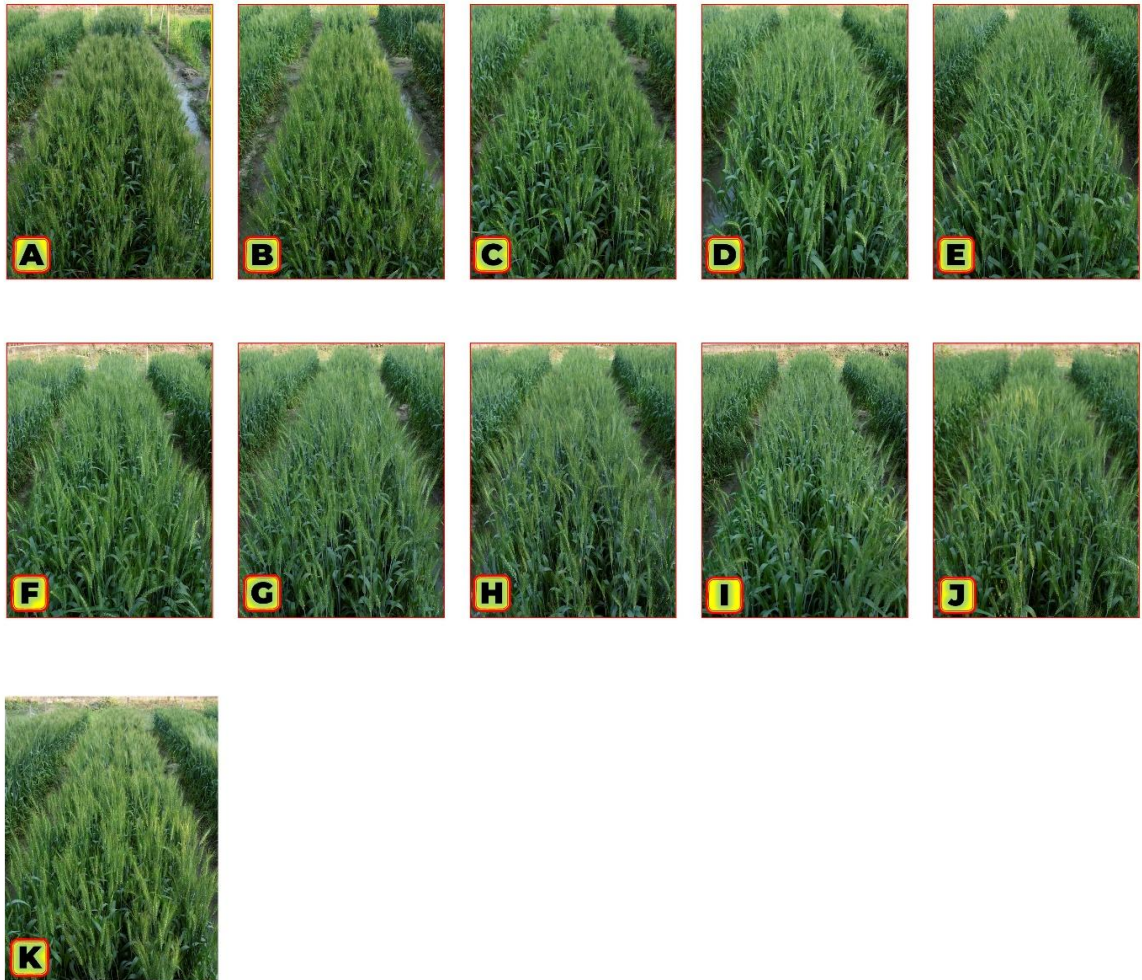


Fig. 8. Heatmap cluster analysis based on genotypical traits



Here, A) HSTUW1, B) HSTUW2, C) HSTUW3, D) HSTUW4, E) HSTUW5, F) HSTUW6, G) HSTUW 7, H) HSTUW 8, I) HSTUW 9, J) HSTUW 10, and K) BARI Gom 32

Fig. 9. Morphological features of the wheat advanced lines and check variety

Supplementary Files

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