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Molecular characterization of bread wheat (*Triticum aestivum*) genotypes using SSR markers

SUMAN DEVI[1], VIKRAM SINGH[1], SHIKHA YASHVEER[1], MOHINDER SINGH DALAL[1], PARAS[1], RUKOO CHAWLA[1]*, DARYA KHAN AKBARZAI[1] and HARSH CHAURASIA[1]

Chaudhary Charan Singh Haryana Agricultural University Hisar, Haryana 125 004, India

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ABSTRACT

An experiment was conducted during winter (*rabi*) seasons of 2019–20 and 2020–21 at the research farm of CCS Haryana Agricultural University to study the genetic diversity of 80 bread wheat (*Triticum aestivum* L.) genotypes, using 43 polymorphic SSR markers. A total of 84 alleles were discovered, with an average of 3 alleles amplified per locus. The average value of the allelic PIC varied from 0.26 to 0.82. Primers, viz. Xgwm 129, Xgwm 131, TaGST, CFA2147, Xwmc48, Xbarc 1165 and Xwmc169 may be deemed particularly informative given their high PIC values. Indices of dissimilarity varied from 0.14 to 0.42. Eighty wheat genotypes were clustered into two main groups with 35 and 45 genotypes each using the dendrogram constructed on the basis of molecular data of polymorphic markers. Using STRUCTURE, genotypes were classified into 4 major sub-populations having F_{st} values 0.351, 0.363, 0.508 and 0.313, respectively. Future breeding operations in wheat cultivars for tolerance to abiotic stress should consider genotypes clustering into different groups. Assessing the molecular genetic diversity is a reliable approach to identify cultivars by analyzing of specific regions of the cultivars DNA based on their unique genetic profiles.

Keywords: Cluster, Genetic diversity, PIC, Structure, SSR, Wheat

Wheat (*Triticum aestivum* L.) holds immense significance in ensuring global food security (FAO 2011). It is a nutritionally valuable food for human and animal consumption as it contains 67 g carbohydrate, 15.4 g protein, 612.2 g dietary fiber, 1.8 g total fat and 3.6 mg iron per 100 g seed. According to the latest estimate, India's total wheat production is 109.52 million tonnes from 30.55 mha cultivated area with a 3464 kg/ha on average productivity (ICAR-IIWBR 2021). World food security is in jeopardy due to multiple challenges arising due to rapid growing population and climate change. Wheat constitutes approximately 30% of the global food basket which makes it imperative to increase both wheat productivity and the area under cultivation, including in warmer and drier climates. These goals require significant breeding efforts to withstand biotic and abiotic stresses in wheat.

To achieve this goal, novel genetic resources that possess sufficient genetic variability must be exploited. Molecular markers are considered best for identification of genotypes as they are indifferent to developmental stages and environment. It has been difficult to improve stress tolerance by traditional methods due to complex inheritance and substantial effect of G × E interactions. Microsatellite markers have been discovered to be an effective tool among the various genetic markers available for varietal identification, genotyping, marker assisted selection (MAS), QTL mapping and gene pyramiding etc. These are independent of environment and can be utilized in marker-assisted detection to effectively find variation across genotypes. In numerous crop species, including wheat, investigations have been conducted on markers related to heat stress (Mittler 2006, Wahid *et al.* 2007, Bhusal *et al.* 2017, Prasad *et al.* 2019). These markers have a key significance in genetic diversity research and in the cultivar authentication (Hao *et al.* 2011). The insertion of independently transmitted elite genes into breeding lines can be improved by marker-assisted selection for attributes associated to stress tolerance. A core and reflective collection of wheat germplasm is required to guarantee a high degree of genetic variation, which is critical for the success of the selection procedure (Mathew *et al.* 2019). Even with less markers, a higher incidence of polymorphism and the multi-allelic nature of SSR markers make it easier to establish genotype-to-genotype association (Vieira *et al.* 2016). Numerous studies illustrate the efficacy of SSR markers in polymorphism detection, which aids in genotyping and the evaluation of genetic diversity in wheat (Sheoran *et*

[1]Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. *Corresponding author email: rukoochawlamanjul@gmail.com

al. 2015, Abbasabad and Mohammadi 2016, Phougat *et al.* 2018, Kumar *et al.* 2022). Therefore, in order to select and cross individuals with diverse genetic backgrounds, we have evaluated 80 wheat genotypes using SSR markers.

MATERIALS AND METHODS

DNA extraction and PCR amplification: An experiment was conducted during winter (*rabi*) seasons of 2019–20 and 2020–21 at the research farm of CCS Haryana Agricultural University. The plant material utilized in the present study consisted of 80 bread wheat genotypes screened for genetic variation by using 43 SSR markers (Supplementary Table 1). Fresh young leaves were collected from each genotype of 2–3 week old plants. The CTAB extraction technique described by Murray and Thompson (1980) was used to isolate genomic DNA, which had been adjusted by (Saghai-Maroof *et al.* 1984). Based on prior research and the Grain Genes database, 188 SSR markers were employed to explore molecular variation across the 80 different genotypes to offer coverage of the whole wheat genome. In wheat, 43 of the 128 markers were shown to be linked to drought or heat stress. The thermocycling protocol was employed, which included an initial denaturation for 4 min at 95°C, 35 cycles at 94°C for 1 min, 50 sec at annealing temperature (47–69°C), 1 min at 72°C and a final cycle of 72°C for 15 min. Amersham Biosciences' method was used to resolve amplified products on 4% polyacrylamide gels. After electrophoresis, DNA bands were visualized using ethidium bromide.

Genetic distance and cluster analysis: The genetic diversity among 80 genotypes was identified. Genetic parameters were calculated such as amplification range, total amplified bands (TAB) and polymorphism information content (PIC) (Botstein *et al.* 1980). Using the Jaccard index, the binary data has been used to construct a dissimilarity matrix. The matrix was further used to generate dendrogram based on neighbor-joining algorithm using the unweighted pair group method (UPGMA) subprogram of PAST (Paleontological Statistics) software (Hammer *et al.* 2001).

Population structure analysis: Bayesian model-based clustering algorism (STRUCTURE software) (Pritchard *et al.* 2000) was used to identify the population structure of hexaploid wheat. Population structure analysis was performed 10 times for each K value (K = 1 to 10) assuming an admixture model and uncorrelated allele frequencies, with a burning period of 100,000 and 100,000 Markov Chain Monte Carlo (MCMC) iterations. Delta K (DK) (Evanno *et al.* 2005) used the web-based programme Structure Harvester (Earl and Von 2012) to determine the most likely value of K for each test. Admixture was defined as genotypes with a membership coefficient less than 0.60 at each allocated K.

RESULTS AND DISCUSSION

Polymorphism and allelic diversity: Recent advancements in molecular genetics have strengthened the crop improvement programmes with powerful techniques to identify the plants with desired traits and the selection of complex traits like stress tolerance (Hasan *et al.* 2021). In the current investigation, SSRs were employed to determine the genetic relatedness across 80 wheat genotypes. Out of the 188 SSR primers utilized in this experiment, 144 SSRs produced amplification of which 43 SSRs were discovered as polymorphic (Table 1). The whole wheat genome (AABBDD) was covered equally with the polymorphic SSR primers. The amplified DNA fragments ranged in size from about 100 bp (X gwm 3) to 500 bp (TaGST). The polymorphic SSRs produced a total of 84 alleles. With 77% of markers showing a value below 0.50, PIC values varied from 0.26 (Xwmc552) to 0.82 (X gwm 129). The average PIC value of 0.57 counted a significant degree of a marker's ability to distinguish between the genotypes under study.

Polymorphic SSRs generated a total of 84 alleles with a mean value of 3.00 alleles amplified per locus. The results were higher than what had previously been reported (Islam *et al.* 2012, Malik *et al.* 2013, Sheoran *et al.* 2015). On the contrary, few studies testified lower mean value than that of 10 (Nasab *et al.* 2013), 3.3 (Sarkar *et al.* 2014), 4.5 (Thungo *et al.* 2020), 5.89 (Abbasabad and Mohammadi 2016), and 8.5 (Choudhary *et al.* 2016). Due to genotypic variations and the use of different samples of the SSR markers, the low number of alleles detected may be explained. There were 2 to 6 alleles per locus, with a mean of 3 alleles per locus (Table 2). According to Thungo *et al.* (2020), who employed 12 polymorphic SSR markers, reported 2–9 (mean = 4.5) alleles per locus in wheat. The PIC value which correlates positively with the number of alleles per locus, makes assessing the discriminating capacity of markers simpler (Hao *et al.* 2011). The mean value of PIC was higher than mean PIC (0.33 and 0.38) reported by Wurschum *et al.* (2013), Naeem *et al.* (2015) and Pervaiz *et al.* (2019). The PIC values for the SSR loci were found to be lower than the PIC value (0.84, 0.60 and 0.58), reported by Choudhary *et al.* (2016), Pankaj *et al.* (2019) and Thungo *et al.* (2020), respectively in wheat. A commonly used measure to quantify genetic diversity is the Heterozygosity index (He), which assesses the proportion of heterozygous individuals in a population. It represents the presence of different alleles at a particular genetic locus within an individual.

Cluster analysis: UPGMA cluster tree (Fig 1) constructed using 43 polymorphic primers discriminated the wheat genotypes into two discrete groups at a dissimilarity coefficient of 0.42. Cluster I was composed of 35 genotypes with a dissimilarity coefficient of 0.27 and was further separated into sub-clusters I-A and I-B. The sub-cluster I-A comprised of 13 genotypes was further delineated into two mini clusters namely mini-cluster-I-A-1(DBW-136, PBW-771, PBW-681, WH-1192, WH-1175, WH-1124, WH-1100) and mini-cluster-I-A-2 (DBW-221, WH-730, WH- 1188, HD-3219, WH-1186, KBRL-79-2) at a dissimilarity coefficient of 0.14. Sub-cluster I-B consisted of 22 genotypes had two mini clusters: mini-cluster I-B-1(UP-2981, GW-477, PBW-769, ESWYT18-121, ESWYT18-122, PBW-752, RWP-2017-21, PBW-766) and mini-cluster-I-B-2 (WH-711, NI-5439,

Table 1 Details of chromosome location, amplification range, total amplified band (TAB) and polymorphism information content (PIC) of 43 polymorphic SSRs

Marker	Amp. Range	TAB	PIC
Tub-Fq	100-450	4	0.695
Qprcp	100-400	4	0.725
Ta18S rRNA-F	100-200	2	0.499
HSP 26	100-500	3	0.563
TaASRP1-RT-F	100-350	2	0.499
X barc 181-1B	150-200	2	0.305
X barc 1152	200-220	2	0.500
Xwmc661-2B	180-200	2	0.375
Xwmc601-2D	100-400	3	0.581
Xgwm374-2B	180-380	3	0.656
Xwmc382-2A	180-200	2	0.486
Xwmc777-3B	100-120	2	0.352
Xwmc552-3D	160-180	2	0.259
Xwmc765-5D	230-260	2	0.497
WMC24-1A	100-200	4	0.708
Xwmc169-3A	150-400	4	0.696
Xwmc48-4B	130-450	5	0.750
Xgwm639-5A	150-170	2	0.498
Xgwm335-5B	180-200	2	0.470
Xwmc283-4A	100-300	3	0.644
Xwmc331-4D	150-170	2	0.499
Xwmc532-3A	180-200	2	0.497
Xwmc364-7B	200-220	2	0.500
Xwmc765-5D	160-210	2	0.641
Xwmc273-7A	180-200	2	0.320
NAC gene	130-280	3	0.663
CFA2147-1A	150-400	5	0.778
Xwmc525-7A	200-280	3	0.593
TaGST	130-500	5	0.755
HSP26.6B	120-350	3	0.618
MADS WP 12-F	190-270	3	0.656
WMC728-1B	200-300	3	0.484
Xgwm174-5D	180-250	4	0.717
TaRCA-L	180-300	3	0.660
X barc 228-2D	180-500	2	0.273
X gwm 165-4B	200-220	2	0.499
Xgwm 155-3A	130-170	3	0.539
Xgwm 249-2D	150-200	3	0.626
X gwm 131-1B	130-300	5	0.766
X gwm 3-3D	100-200	2	0.444
X barc 1165-6A	120-320	4	0.750
Xbarc 188-1B	130-200	3	0.515
X gwm 129-5A	130-400	6	0.816

DBW-71, ESWYT18-147, ESWYT18-115, ESWYT18-116, WH-1235, WH- 1184, HI-1628, WH-1151, HD-3086, WH-1160, WH-1063, WH-1062) at a dissimilarity coefficient of 0.22. With a dissimilarity coefficient of 0.30, Cluster II, which had 45 genotypes, was further separated into sub-clusters II-A and II-B. The sub-cluster II-A comprised of 21 genotypes further divided two mini clusters namely mini-cluster-II-A-1(MP-3288, C-306, JWS-825, PBW-719, HD-2967, WH- 1164, RAJ-3765, DBW-14, HI-1625, LOK-54, RAJ-4480) and sub-cluster-II-A-2 (WB-2, WH- 1182, DBW-150, WH-1153, HI-1621, WH-1158, WH-542, WH-1132, WH-1152, WH-157) at a dissimilarity coefficient of 0.20. Mini-cluster II-B consisted of 23 genotypes had two sub clusters: mini-cluster II-B-1(WH-1131, WH-1179, HD-3043, HD-2932, WH-1142, GW-463, WH-147, WH- 1021, WH-1129, DBW-233, RW-5, PBW-762, HD-2888, WH-1202, WH-1138) and mini-cluster- II-B-2 (FLW-22, FLW-10, WH-1105, DBW-173, WH-1123, WH-789, FLW-16, PBW-773, PBW- 763) at a dissimilarity coefficient of 0.23.

The identification of diverse genotypes from the gene pool is vital to tailor superior cultivars in breeding programme. The DARWIN UPGMA cluster tree analysis discriminated the genotypes in two major clusters at a dissimilarity coefficient of 0.42 having 35 and 45 genotypes in cluster I and cluster II, respectively. It is interesting to note that there is prominent agreement between the performance of genotypes in field with that of clustering pattern seen in UPGMA based dendrogram. Mini cluster I-A-1 and I-A-2 as well as II-A-1 and II-A-2 perform poor and hence may be designated as sensitive mini clusters based on their stress tolerance indices and mean productivity along with per cent reduction in yield. Cluster II-B-1 includes genotypes HD 2888, RW-5, WH 147 and WH 1021 which are stress tolerant. Particularly RW-5 has high stress tolerance index under drought, heat and combined effect. WH-147 and WH-1021 are heat tolerance checks. PBW-773 placed in Cluster II-B-2 have high stress tolerance index and high mean productivity under all conditions. HD-3086 placed in mini cluster I-B-2 have less tolerance in comparison to RW-5 and PBW-773 to stresses but perform better than sensitive ones. The pattern of concordant clustering reassures the selection procedure and will further help in selection of diverse genotypes for crop hybridization programmes. As a result, all genotypes displayed a high level of genetic heterogeneity. Because of the higher number of alleles per locus and PIC values, and primers such as Xgwm 129, Xgwm 131, TaGST, CFA2147, Xwmc48, Xbarc 1165 and Xwmc169 might be regarded highly informative SSRs. The polymorphism information content (PIC) of each SSR marker ranged from 0.30 to 0.90 (Abbasov *et al.* 2018). Our results underscore the significance of using SSR markers in assessing genetic diversity, allowing for more accurate and comprehensive characterization of wheat genotypes compared to earlier studies that may have relied on less informative markers.

Population structure: The STRUCTURE was used to calculate the number of sub-populations among the

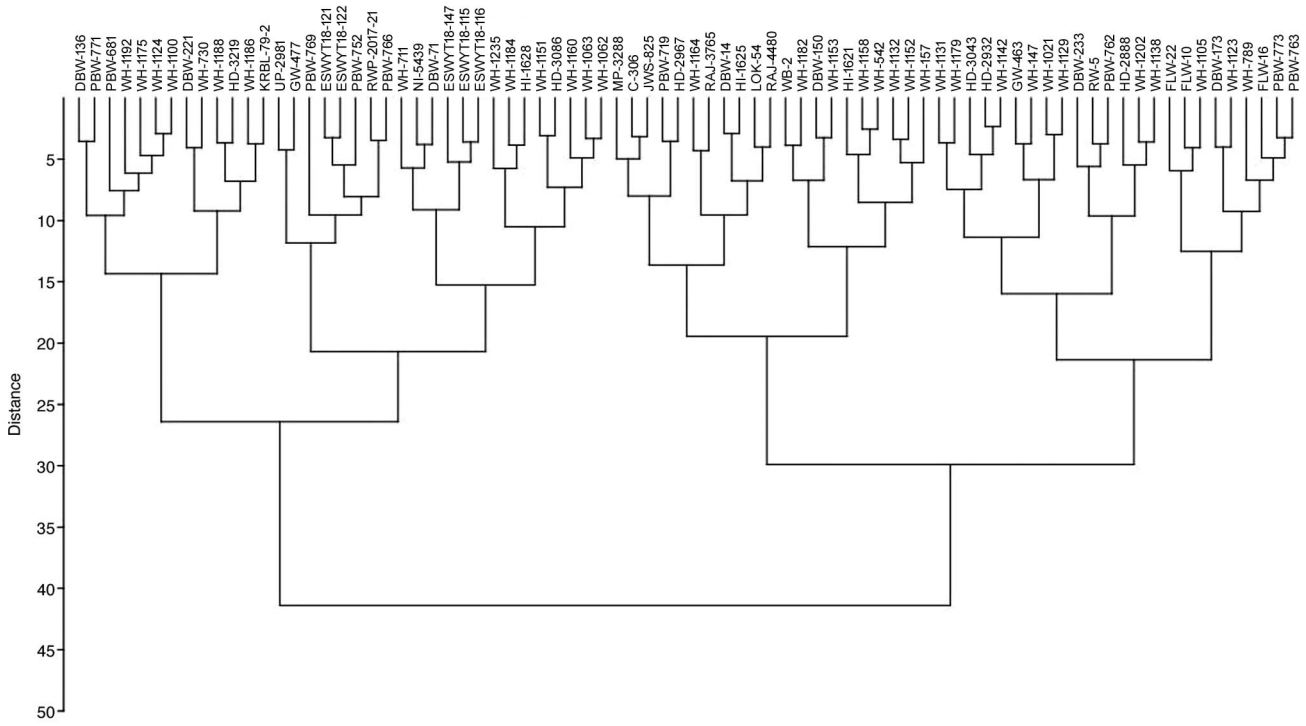

Fig 1 Dendrogram showing relationships among 80 wheat genotypes generated by UPGMA analysis using 43 polymorphic SSR primers.

80 wheat genotypes based on the peak of delta K at K = 4 (Fig 2). Genotypes have been divided into four major sub-populations and portrayed with different colour codes (Fig 3), with each genotype symbolized by a single straight line whose length is proportional to each of the four sub-populations, and the colour length in the line suggests the proportion of a genotype belonging to that sub-population. Using a probability to sub-population of 60.00% for each genotype in the four sub-populations, a total of 38 genotypes (47.50%) were assigned to one of the four sub-populations. The first sub-population accounted for 5 (6.25%) of total genotypes, the second for 13 (16.25%), the third for 12 (15%), and the fourth for 8 (10%) (Supplementary Table 2). Remaining 42 genotypes were admixture. Fst values

Table 2 Clustering of 80 wheat genotypes based on UPGMA analysis

Major and minor clusters	Genotype	No. of genotypes	Dissimilarity value
Cluster I		35	0.42
Sub-clusters I-A		13	0.27
Sub-clusters I-B		22	0.27
Mini-clusters I-A-1	DBW-136, PBW-771, PBW-681, WH-1192, WH-1175, WH-1124, WH-1100	7	0.14
Mini-clusters I-A-2	DBW-221, WH-730, WH-1188, HD-3219, WH-1186, KBRL-79-2	6	0.14
Mini clusters I-B-1	UP-2981, GW-477, PBW-769, ESWYT18-121, ESWYT18-122, PBW-752, RWP-2017-21, PBW-766	8	0.22
Mini clusters I-B-2	WH-711, NI-5439, DBW-71, ESWYT18-147, ESWYT18-115, ESWYT18-116, WH-1235, WH-1184, HI-1628, WH-1151, HD-3086, WH-1160, WH-1063, WH-1062	14	0.22
Clusters II		45	0.42
Sub-clusters II-A		21	0.30
Sub-clusters II-B		24	0.30
Mini-clusters II-A-I	MP-3288, C-306, JWS-825, PBW-719, HD-2967, WH-1164, RAJ-3765, DBW-14, HI-1625, LOK-54, RAJ-4480	11	0.20
Mini-clusters II-A-2	WB-2, WH-1182, DBW-150, WH-1153, HI-1621, WH-1158, WH-542, WH-1132, WH-1152, WH-157	10	0.20
Mini clusters II-B-1	WH-1131, WH-1179, HD-3043, HD-2932, WH-1142, GW-463, WH-147, WH-1021, WH-1129, DBW-233, RW-5, PBW-762, HD-2888, WH-1202, WH-1138	15	0.23
Mini clusters II-B-2	FLW-22, FLW-10, WH-1105, DBW-173, WH-1123, WH-789, FLW-16, PBW-773, PBW-763	9	0.23

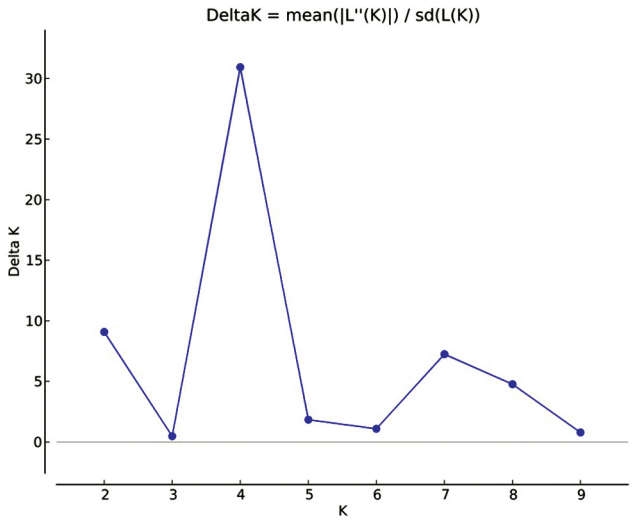

Fig 2 Estimated Delta K values against the number of subpopulations tested (K).

Table 3 Mean value of F_{st} within the population

Population	Mean F_{st} value
A	0.351
B	0.363
C	0.508
D	0.313

This enhanced resolution of genetic diversity is crucial for understanding the population structure and designing effective breeding strategies to enhance crop improvement (Crossa *et al.* 2007, Wurschum *et al.* 2013).

The current work sought to investigate the genetic relationships among 80 wheat genotypes, which is critical for breeding programmes. The primers used in this study exhibited PIC values from 0.26 to 0.82, indicating their efficiency in distinguishing between the studied genotypes.

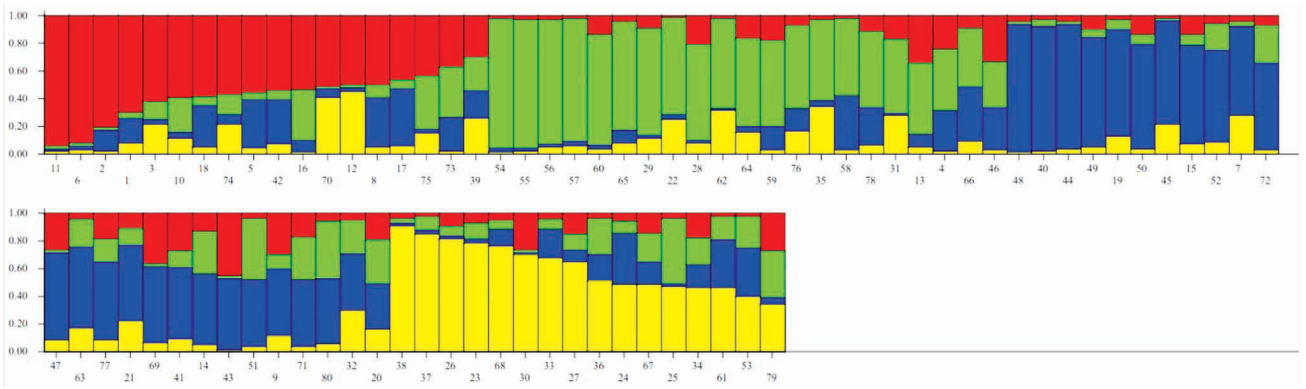

Fig 3 Dendrogram showing diversity among 80 wheat genotypes generated by STRUCTURE software.

for sub-populations were 0.351, 0.363, 0.508, and 0.313, respectively. Furthermore, sub-population D had the lowest Fst (0.313). The total Fst value obtained within the sub-populations was likewise moderate (0.383, Table 3). The occurrence of sub-populations might be attributed due to the genetic differentiation of the genotypes based on their geographical origin, ecological adaptation and breeding history (Rufo *et al.* 2019). The fraction of an individual's membership in a sub-population determines their placement in Structure. The Fst values of the sub-populations ranged from 0.313 to 0.508, indicating that they were reasonably uniform. All pairwise Fst values in the current research were substantially different from one another, indicating considerable genetic diversity in the population. The sub-populations D and A exhibited lower F_{st} value of 0.313 and 0.351, respectively, indicating that individuals of these two subpopulations shared their alleles. In comparison to previous studies on population structure in wheat, our research provides novel insights into the extent of genetic heterogeneity within the genotypes. The higher number of alleles per locus and elevated PIC values observed using SSR markers suggest a more diverse gene pool than reported in some earlier studies that utilized different marker systems.

The clustering pattern observed in the UPGMA-based dendrogram showed a good agreement with the phenotypic performance of the genotypes. Furthermore, the genotypes were categorized into sensitive and stress-tolerant groups, demonstrating the existence of significant genetic differences among heat and drought-responsive wheat genotypes. These genotypes could be strategically crossed to elevate their genetic gains for output grain yield under stress conditions. The identified sub-populations could be further analyzed to identify genotypes suitable for specific agro-ecological zones based on their agronomic traits. Moreover, the identified markers could be used for developing high-density genetic maps for marker-assisted selection.

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