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Genetic diversity among bread wheat (*Triticum aestivum* L.) genotypes as assessed by SSRs

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Drought is one among the several climatic factors impeding crop productivity and poses a challenge to global food security. The intensity and frequency of droughts are predicted to increase by 50% to 200% during the 21st century in various geographical regions (Trenberth *et al.,* 2014; Zhao *et al.,* 2017). Raising drought tolerant wheat genotypes is the eventual means of safeguarding the crop against water stress. However, drought tolerance is a complex trait governed by various genes, each with minor effects (Bernardo 2008). Knowledge about germplasm diversity significantly impacts the crop improvement programs by supplying novel sources of gene combinations (Ayana and Beleke, 1998). Prior knowledge of genetic diversity and relationships between the elite lines and cultivars are useful for development of new cultivars. It is highly desirable to characterize genetic diversity among wheat germplasm collections to broaden genetic diversity in future wheat breeding programmes (Haung *et al.*, 2002). Molecular markers have proven their role in crop improvement programs by providing selection precision and accelerating the efforts. Assessing genetic diversity within a narrow genetic pool of novel breeding germplasm could make crop improvement more efficient by the directed accumulation of desired alleles. This is likely to speed up the breeding process and decrease the amount of plant material that needs to be screened in such experiments. Genetic variation in common wheat have been studied using different molecular markers such as RAPDs RFLP, AFLPs, SSR, STS, ISSRs, gene based and MIR based SSRs (Siedler *et al.,* 1994, Gupta and Varshney, 2000, Sharma *et al.,* 2021, Mehta *et al.,* 2021). Since SSRs are multi-allelic nature, co-dominant inheritance, reproducibility, abundance and high polymorphic information content (PIC). A small number of SSR markers are adequate to differentiate the closely related wheat and barley species (Plaschke *et al.,* 1995; Russel *et al.,* 1997; Singroha *et al.,* 2020).

In present investigation, we determined genetic diversity and relationships at the molecular level among the fourteen wheat genotypes using microsatellite markers. The phylogenetic relationships and genetic diversity thus analyzed will assist in parental selection in wheat breeding programmes.

We procured fourteen wheat genotypes from the Germplasm Unit, Indian Institute of Wheat and Barley Research, Karnal and were used for cluster analysis at molecular lene. Fresh and young leaves were used to extract genomic DNA according to the method devised by Saghai-Maroof *et al.* (1984). A total number of 44 GWM were selected, representing each wheat chromosome for genotyping (Table 1). Polymerase chain reaction (PCR) was carried out as described earlier (Sharma *et al.,* 2016). The amplification products were resolved in 2% agarose in 1× Tris-borate EDTA buffer (45 mM Tris-borate and 1 mM EDTA) and were visualized under UV light using Gel Documentation System (Alpha Innotech, USA).

Table 1. SSR primers and their chromosome location used for determining the genetic diversity of wheat genotypes.

S.N.	SSR primers	Chromosome location
1.	Xgwm357,Xgwm666,Xgwm497	1A
2.	Xgwm011,Xgwm131,Xgwm140	1B
3.	Xgwm033,Xgwm106	1D
4.	Xgwm296,Xgwm312	2A
5.	Xgwm120,Xgwm148	2B
6.	Xgwm102,Xgwm349	2D
7.	Xgwm030,Xgwm369,Xgwm155	3A
8.	Xgwm77,Xgwm340	3B
9.	Xgwm71,Xgwm161	3D
10.	Xgwm165,Xgwm397	4A
11.	Xgwm107,Xgwm251	4B
12.	Xgwm624	4D
13.	Xgwm205,Xgwm304	5A
14.	Xgwm67,Xgwm68	5B
15.	Xgwm119,Xgwm292	5D
16.	Xgwm459,Xgwm494	6A
17.	Xgwm219,Xgwm132	6B
18.	Xgwm55,Xgwm469	6D
19.	Xgwm260,Xgwm282	7A
20.	Xgwm146,Xgwm344	7B
21	Xgwm44,Xgwm37	7D

The presence of band was scored as 1 and absence of band was scored as 0 in the binary data matrix. Using the *SAHN* module of the *NTSYS-pc* Jaccard coefficients were used to construct unweighted pair-group method of arithmetic average (UPGMA) dendogram.

In PCR amplification, ninety alleles were identified with different size fragments. The average number of alleles per SSR marker was 3.2, ranging from two alleles for *Xwgm*292 to five for *Xwgm*264. A wide range of alleles of expected fragment sizes was obtained by different primer pairs with strong amplifications. The primesr *Xwgm292* and *Xwgm264* yielded five alleles as shown in Fig. 1a and 1b. However, the higher number of alleles per locus has been reported in wheat (4.6 to 18.1), barley (8.6), and several other crops like tomato (3.1), sorghum (2.3), cucumber (2.6), melon (2.9), and watermelons (2.0) (Fahima *et al.,* 1998; Prasad *et al.,* 2000; Huang *et al.,* 2002; Salem *et al.,* 2008; Mohammadi *et al.,* 2009). The average number of alleles per locus (3.2) in this study was lower in comparison to those reported earlier. We identified a total of 23 alleles at 7 loci with an average of 2.67 alleles per locus in A genome, while 15 loci with an average of 3.0 alleles per locus were detected in B genome. In the D genome 30 alleles were detected with 10 microsatellite loci with an average of 2.87 alleles per locus, suggesting diversity at various levels in three genomes.

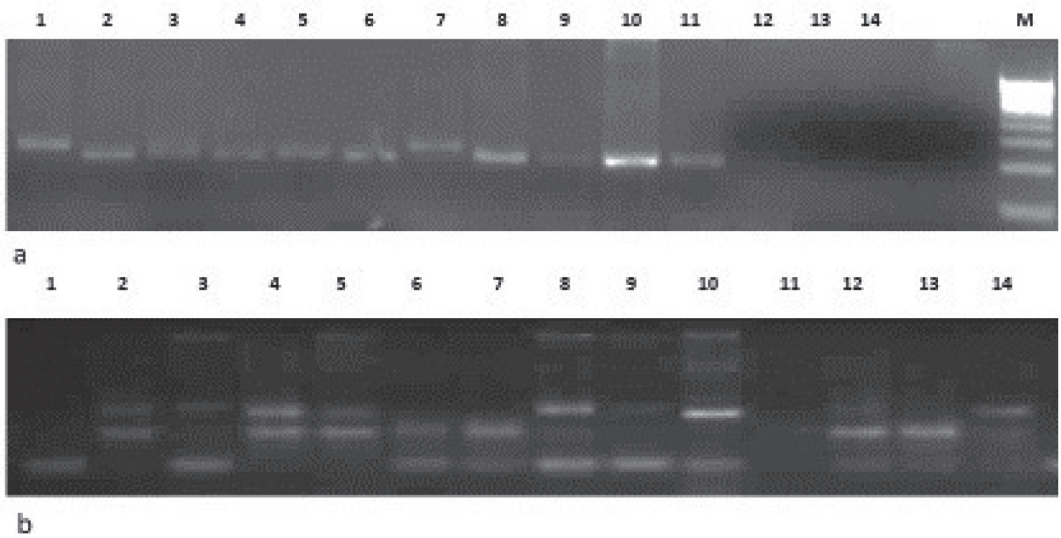

Fig. 1 Gel electrophoresisof amplification productsobtained with microsatelliteprimer pairs Xgwm 292 (a) and Xgwm 264 (b) in 14 wheatgenotypes. M=100 bp standardDNA marker. Lane 1) NI5439, 2) C 306, 3) WH 147, 4) HD 2781, 5) PBW 175, 6) WR 544, 7) HUW468, 8) PBW 343, 9) HD 2733, 10) GW 322, 11) MACS 2496; 12) HD 2932, 13) HUW 234 and 14) Raj 4037.

The lowest allele per locus among the seven homoelogous chromosome groups was obtained in group 6 identifying only one allele for *Xgwm* 148. The group 2 chromosomes were identified to have the highest no. of alleles as presented in Table 2. In a genome-wise comparison, the B genome was found to be highly diverse (0.65) followed by D (0.50) and A (0.44) genomes. For homoeologous chromosome groups, the highest PIC value of 0.53 was observed for group 2 chromosomes markers and the lowest value of 0.27 for group 7 markers. The observations made in this study are contrary to those made by Iqbal *et al.* (2009), where they reported highest PIC value for A genome followed by D and B genomes. Similarly, we report highest value of PIC for homologous group 2 chromosomes in contrast to homoeologous group 7 chromosomes as reported by Roder *et al.,* (2002;) and Haung *et al.,* (2002). This difference can be attributed to the genotypes belonging to different geographical regions and the set of different primer pairs used in this study.

Table 2. Genetic diversity according to genomes and chromosomes across 32 microsatellite loci

Genome	Number of alleles	Gene diversity	Mean no. of alleles/locus
All genomes	90	0.48	3.20
A	23	0.44	2.67
B	36	0.65	3.00
D	31	0.50	2.87
Chromosome			
Group 1	16	0.39	2.50
Group 2	22	0.53	3.20
Group 3	10	0.41	2.67
Group 4	14	0.52	2.95
Group 5	10	0.44	2.87
Group 6	06	0.27	2.00
Group 7	12	0.43	3.00

This analysis therefore identifies the divergence of alleles specific for a particular geographical region. The maximum value of PIC (0.53) in this study is in accordance to the earlier studies where PIC values ranged between 0.23-0.90 (Plaschke *et al.*, 1995, Prasad *et al.*, 2000, Mohammadi *et al.*, 2009). However, the mean PIC value in our results corroborates those reported by Bohn *et al.* (1999).

We observed highest genetic diversity confirming that there is highest polymorphism in B genome and A genome is based on polymorphism studies least genetic diversity. The highest polymorphism among B genome is also reported by Eujayl *et al.* (2002) and Wang *et al.* (2007) in wheat as well as by Cho *et al.* (2000) in rice.

It might be correlated to evolution of each of the three wheat genomes. The B genome has originated from species closely related to the *A. speltoides*, a cross-pollinated species, whereas A and D genomes are traced to have originated from *T. urartu* and *Ae. tauschii*, respectively and are self-pollinating species. In general, a cross pollinating species exhibit higher genetic diversity in comparison to a self-pollinating species. This might be the reason why, B genome is highly diverse in primitive hexaploid wheat as comparison to genomes A and D. During the course of evolution of the hexaploid wheat, tetraploid wheat crossed with *Aegilops tauschii*, and produced the hexaploid wheat. Consequently, the opportunity of the gene exchange of the D genome with A or B genome was lower than that between B and A genome. Evidences also suggest that B genome chromosomes are rich in repetitive DNA sequences and the length of B genome is longer than A and D genomes.

For all possible pairs of varieties, the genetic similarity (GS) coefficient ranged from 0.50 to 0.92. The similarity coefficient generated a tree for cluster analysis using UPGMA as shown in Fig. 2. The varieties C306/NI5439 (drought tolerant) had highest similarity of~ 0.92. Apart from this more pairs *viz.* HD 2781/C306, HD2733/HD 2781 and HD2932/MACS 2496 also showed high degree of commonness. The dendrogram based on UPGMA algorithm grouped the fourteen wheat varieties into two major clusters, I (10 varieties), and II (04 varieties). However, two varieties in cluster I (PBW 175) and II (GW 322) showed considerable diversity with other varieties in their respective clusters. The clusters I, and II were further divided into two sub-clusters (Ia: five varieties, Ib: three varieties; and IIa: four varieties) as shown in Fig. 2. Similar investigations have been carried out by Ram *et al.,* (2007) using SSR markers.

It was postulated that biased selection of material in the previous breeding program might have resulted into high level of similarity and narrowed the genetic base of wheat germplasm. It is further suggested that more polymorphic

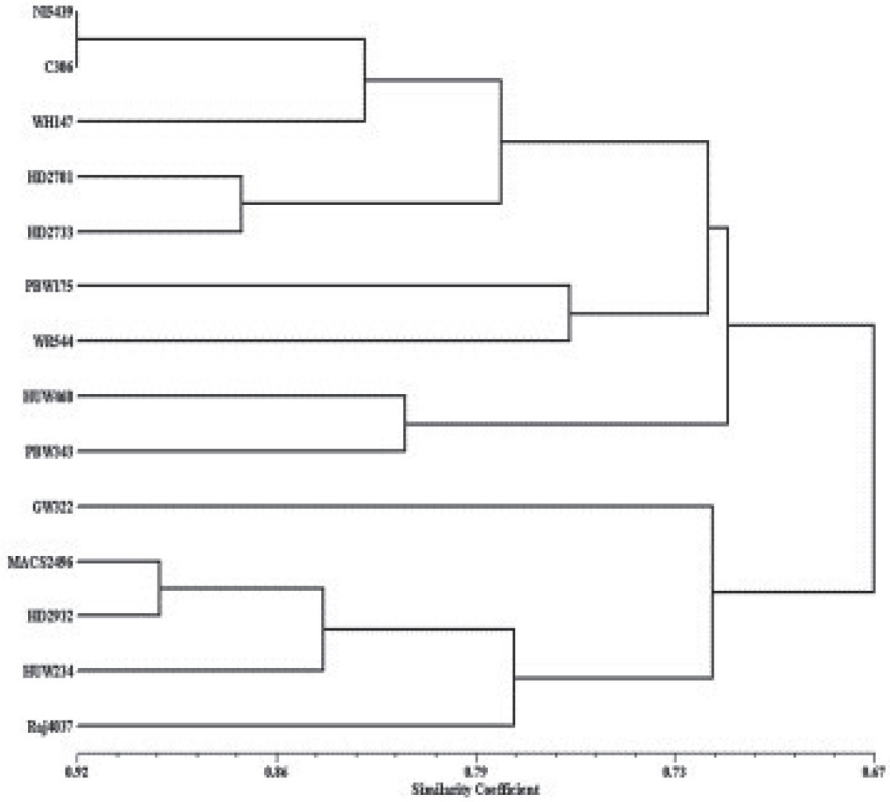

Fig. 2 UPGMA dendrogram of 14 wheat genotypes based on 32 SSR markers.

microsatellite markers could be used for efficient screening of the wheat germplasm by saturating more regions of the wheat genome and these microsatellite marker data will be useful in identifying diverse parents and for maintaining genetic variation in germplasm for trait improvement.

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Compliance with ethical standards

Conflict of interest: No

Author contributions:

RS, PS worked for the wet lab experiments. PS, SK, SKS edited the manuscript. All Authors read the manuscript.

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