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Assessment of genetic Diversity among nine Bread Wheat (*Triticum aestivum* L.) genotypes using microsatellite markers

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Abstract
Genetic diversity among nine wheat cultivars was evaluated using 23 polymorphic microsatellite (SSR) markers. Analysis of molecular diversity revealed moderate levels of polymorphism in the set of genotypes studied. Fifty six alleles with an average of two alleles per locus were detected. The allelic polymorphism information content (PIC) value ranged from 0.16 to 0.67 with an average of 0.33. The primers like gwm192, gwm111, gwm374, STSS5765, XPSP3000, wmc104 and gwm192 could be considered particularly informative, as they revealed three or more alleles per locus and displayed high PIC values. The most closely related cultivars were 'WH1184' and 'WH1124' displaying the lowest dissimilarity index, whereas highest genetic distance was observed between 'WH711' and 'HD3059'. The dissimilarity indices ranged from 0.62 to 0.85. Three major clusters comprising one, one and seven genotypes were formed. The three clusters and their similarities might assist breeders to set up the appropriate guidelines for successful breeding of wheat cultivars based on the established relationships.

Keywords: Genetic diversity, genotype, microsatellite (SSR) markers

Introduction
Increasing wheat (*Triticum aestivum* L.) yield is an important foundation for ensuring global food security. There is abundant evidence that biodiversity promotes natural ecosystem functions such as productivity, stability, and stress resistance (Cardinale *et al.*, 2013, Oliver *et al.*, 2015, *Pennekamp et al.*, 2018) [6, 20, 21]. It is the principal food crop in most areas of the world and also occupies prominent position in Indian agriculture after rice. The choice of breeding procedure and selection for genetic improvement largely depend on the knowledge of type and relative magnitude of genetic components and the presence or absence of allelic and non-allelic interaction for different characters that are under investigation. In wheat species, it could lead to create new plant populations with a promise to offer segregates of superior agronomic qualities in the process of exploration for variety improvement. Thus, for wheat genetic improvement knowledge of the nature, magnitude of gene effects and their contribution to control trait that is important in formulating an efficient breeding programme. Therefore, the selection for high grain yield and yield contributing components are important focus in wheat breeding programmes. As grain yield is a complex trait and usually controlled by large number of quantitative trait loci (QTL) with their minor effects. It is affected by environmental factors, which make it difficult to be improved and manipulate in breeding programme.

Currently, the methods that reveal polymorphism directly at DNA level are used efficiently for assessing genetic variability. In plant sciences, DNA based molecular markers find widespread application. Khan *et al.*, (2000) [15] used morphological markers that have genetic control for diversity studies and stated that there were certain influence of cultivation practices and environmental factors.

Molecular markers are the tools for improving the efficiency of traditional plant breeding by facilitating the genes of a trait that are linked with molecular markers because they are not influenced by environment and developmental stages and they are scored at any stage of plant growth. To identify the genetic similarity and diversity analysis, microsatellites (SSRs) markers are the most efficient tool. These are the appropriate markers for identifying allelic frequency within population due to co-dominant character, high rate of polymorphism, selective neutrality, cost and labour efficiency and distribution across the genome.

SSRs are the tandem repeats of one to six bases and the loss or gain of repeats resulted in polymorphism. In the DNA of hexaploidy wheat, simple sequence repeats were interspersed ubiquitously (Roder *et al*., 1998) [24]. SSR (Simple sequence repeat) markers the are PCR (Polymerase chain reaction) based markers, that detect a higher level of genetic variation than RFLP (Restriction fragment length polymorphism) and RAPD (Random amplified polymorphic DNA) markers (Plaschke and Roder, 1995 and Korzun *et al*., 1999) [23, 16]. They have chromosome specific features that are valuable for detecting the QTL of interest and localizing linked alleles. Microsatellite markers that are used for genetic analysis in wheat provide more efficient methods of scoring segregating populations and identifying suitable genotype. These markers have a vital role in genetic diversity studies and in the cultivar identification (Hao *et al*., 2011) [11].

The studies of number of workers (Arora *et al*., 2014. Abbasabad, *et al*., 2017 and Phougat *et al*., 2018) [4, 2, 22] clearly demonstrate the effectiveness of SSR markers in polymorphism identification that leads to the estimation of genetic diversity and genotype identification.

Material and Methods

Plant Material

Nine wheat genotypes *viz*, WH1105, HD2967, HD3086, HD3059, Raj3765 WH1124, WH283, WH711 and WH1184 studied for genetic polymorphism using chromosome specific SSR markers. Table 1 shows the parentage and pedigree of the varieties. Genomic DNA was extracted from a small amount of fresh leaf tissue (5.0 g) from each of the parental genotypes using the CTAB method of Saghai-Maroof *et al*. (1984) [26]. Agarose gel electrophoresis (0.8%) was used to check quality and quantity of genomic DNA. The quality and quantity of genomic DNA were checked using a 0.8 percent agarose gel electrophoresis. Visual comparison of band intensity with Lambda (λ) DNA of known concentration was used to estimate DNA concentrations. For molecular characterization, parental genotypes were screened using fifty SSR markers, 41 of which were polymorphic and used for diversity analysis.

The PCR amplification conditions were optimized. The PCR reaction was conducted in a reaction volume of 20 µl containing 2ul of 1X PCR buffer, 100 µM dNTPs, 0.5 µl of each primer, 1.5 unit Taq DNA polymerase and 50 mg template DNA. The thermocycling program consisting of an initial denaturation at 95 °C for 4 minutes followed by 40 cycles of 95°C for 1 minute, 1 minute and 20 second at annealing temperature (55-63 °C), 1 minute at 72°C and a final cycle of 72C for 10 minutes was used. Amplified products were resolved on 4% polyacrylamide gels using Amersham Biosciences system as described by Chen *et al.* (1997). Gels were pre-run until an adequate temperature (50-600C) was reached. DNA bands were visualized by using silver staining protocol (SILVER SEQUENCETM DNA Sequencing System, Promega Inc., Madison, WI, USA) after completion of electrophoresis. The frequency of polymorphism between different varieties of wheat for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of bands (Ghosh *et al*. 1997) [9]. The 0/1 matrix was used to calculate similarity genetic distance using„ simqual‟ sub-program of software NTSYSPC (numerical taxonomy and multivariate analysis system programme) (Rohlf 1993) [25]. The resultant distance matrix was employed to construct dendrograms by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC.

Results

Genetic diversity analysis was done with high-quality genomic DNA that is isolated from nine bread wheat genotypes (Table 1). A total of 50 SSRs were used to screen parental genotypes, out of these 23 primers showed polymorphism (Table 3 and Fig 1 & 2). Database of parental genotypes was generated visually using 23 polymorphic SSRs on the basis of amplified scored products (Fig 1, and 2).

Table 1: Name, pedigree and source of the parents used in the present study:

Parent	Pedigree	Source
WH1105	MILAN/S87230//BABAX	CCS HAU, Hisar
HD3059	KAUZ//ALTAR84//ADS/3/ MILAN/KAUZ/4/HUITES	IARI, New Delhi
WH1124	MUNIA/CHTO/AMSEL	CCS HAU, Hisar
WH711	ALD'S'/HUAC//HD2285/3/HFW17	CCS HAU, Hisar
HD2967	ALD/CUC//URES/HD2160/HD2278	IARI, New Delhi
WH1184	HD2850/WH147	CCS HAU, Hisar
WH283	HD1981/Raj821	CCS HAU, Hisar
Raj3765	HD2402/VL639	RAU, Durgapura
HD3086	DBW14/HD2733//HUW468	IARI, New Delhi

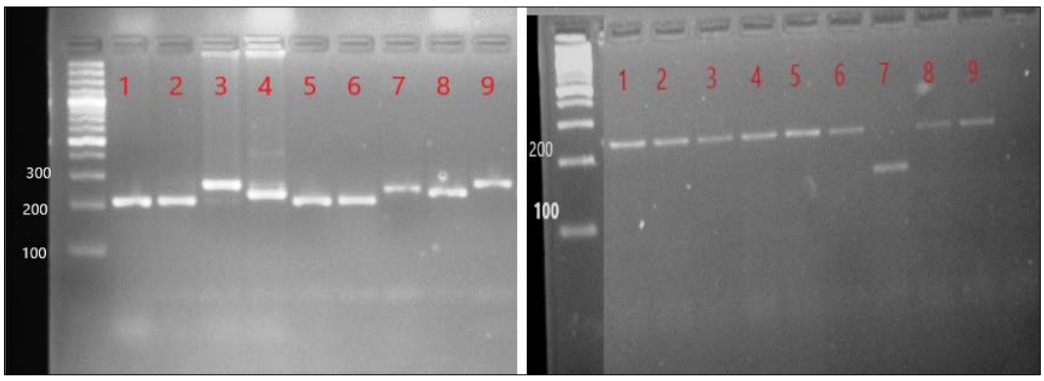

Fig 1 and 2: Agarose gel showing allelic polymorphism among nine bread wheat genotypes using primer xwmc198 and xpsp3000

SSRs amplified products scored visually as the presence of band was taken as 1 and absence of band was taken as zero. A total of 56 alleles were detected and number of alleles per locus ranged from 1-4 with average alleles per locus was 2. The overall size of PCR products amplified ranged from 100-300 bp (Table 2). The maximum number of alleles was

observed at gwm111 and gwm374 marker. The values of PIC were also estimated, and the highest value of 0.67 was recorded for xgwm120. A representative polymorphic banding pattern with two primers (xwmc198 and xpsp3000) is shown in (Fig. 1 & 2). Among the classes of repetitive DNA sequences used for PCR amplification, SSRs remains the unsurpassed choice of markers (Jacob *et al.*, 1991) [14]. SSR markers are valuable genetic markers because they detect high levels of allelic diversity, co-dominant, easy and economically assayed by PCR (Weber and May 1989) [37], easily automated (Smith 1998) [32], abundance and even genomic distribution (Weber and May 1989) [37], high level of polymorphism (Saghai – Maroof *et al.*, 1994) [26], high variability (Brown *et al.*, 1996) [5], highly polymorphic even between closely related lines (Gupta *et al.*, 1999) [10]. The average number of alleles detected (2.00per locus) was found to be higher than those reported by the earlier workers (Malik *et al.*, 2013; Islam *et al.*, 2012, Sheoran *et al.*, 2015) [18, 13, 31] in elite wheat genotypes. Whereas, the average number of alleles detected in the present study was found to be lower than the average of 3.2 (Salem *et al.*, 2008) [27], 3.2 (Schuster *et al.*, 2009) [29], 5.7 (Spanic *et al.*, 2012) [33], 10 (Nasab *et al.*, 2013) [19], 3.3 (Sarkar *et al.*, 2014) [28] and 5.89 (Abbasabad *et al.*, 2016) [1] reported in the genetic diversity studies on bread wheat using microsatellite markers. This discrepancy might be related to the genotypes used and the selection of SSR primers with scorable alleles. Number of alleles per marker depends on the relative distance of the locus from the centromere (high genetic variation occurs in the non-centromeric regions compared to the centromeric regions of chromosomes.) and also it was related to the motif and repeat number of the allele frequencies (Huang *et al.*, 2009) [12].

Table 2: DNA amplification profile of nine bread wheat genotypes

Number of SSRs used	50
Number of markers that did not show amplification	7
Number of polymorphic markers	23
Number of alleles detected using polymorphic markers	56
Range of alleles	1-4
Average number of alleles	2
Size of PCR products	100-300 b p

The mean PIC value of (0.33) observed in the present study was found to be higher than those reported in earlier studies (Malik *et al.*, 2013; Sarkar *et al.*, 2014) [18, 28]. But it was found to be lower than 0.76 reported in wild diploid wheat (Wang *et al.*, 2017) [36], 0.6 in Iranian landraces (Abbasabad *et al.*, 2016) [1], 0.55 among Egyptian wheat varieties (Salem *et al.*, 2008) [27], and 0.58 among the Indian wheat varieties (Arora *et al.*, 2014) [4], respectively. PIC measures the informativeness of the DNA markers over a set of genotypes during gene mapping, molecular breeding, and germplasm evaluation (Varshney *et al.*, 2007; Wang *et al.*, 2007) [34, 35]. Microsatellite markers exhibit high PIC value because of their codominance and multi-allelism (Ferreira and Grattapaglia 1998) [8]. The polymorphism in SSR could be due to a change in the SSR region itself caused by the expansion or contraction of SSR or interruption (Li *et al.*, 2007) [17]. Markers with PIC values 0.5 or higher are considered as highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a specific locus (De Woody *et al.*, 1995; Akkaya and Buyukunal Bal 2004) [7, 3]. Out of 23 polymorphic SSR markers used in the present study, three markers have a PIC value ≥0.5. This indicates that the SSR markers used in the present study were informative and robust indicating their utility to study genetic diversity and molecular mapping in bread wheat.

Table 3: DNA amplification profile of nine parental genotypes as assessed by 23polymorphic SSRs primer and PIC (Polymorphic information content) value

Sr. No.	SSRs Primer	No. of Alleles	PIC (Polymorphic information content)
1	barc75	Two	0.20
2	gwm192	Three	0.39
3	gwm111	Four	0.38
4	gwm374	Four	0.59
5	p6m12	Two	0.35
6	STSS5765	Four	0.36
7	xgwm389	Two	0.44
8	cslv4	Two	0.20
9	wmc533	Two	0.20
10	xgwm120	Two	0.16
11	xgwm419	Two	0.20
12	xpsp3000	Three	0.67
13	xwmc120	Two	0.35
14	xwmc198	Two	0.20
15	wmc104	Three	0.34
16	xgwm418	Two	0.37
17	xgwm136	Two	0.52
18	wmc18	Two	0.35
19	barc98	Two	0.21
20	barc108	Two	0.35
21	gwm192	Three	0.39
22	wmc245	Two	0.20
23	wmc776	Two	0.44

Data obtained from 23 polymorphic markers was further used for constructing similarity indices among using 'Simqual' subprogram of NTSYS PC- version 2.0 program (Fig. 3). The cluster analysis performed by using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) based on Jacquard's similarity co-efficient values between the nine wheat into main three clusters with 0.68 similarity coefficient (Fig.3). The genetic similarity coefficient between all the nine genotypes ranged from 0.68 to 0.85 with an average of 0.73. Similarity coefficient index showed that most closely related wheat genotypes were WH1124 and WH1184 with highest similarity index 0.85. The most diverse genotypes were WH711and HD3086 with lowest similarity index 0.68.

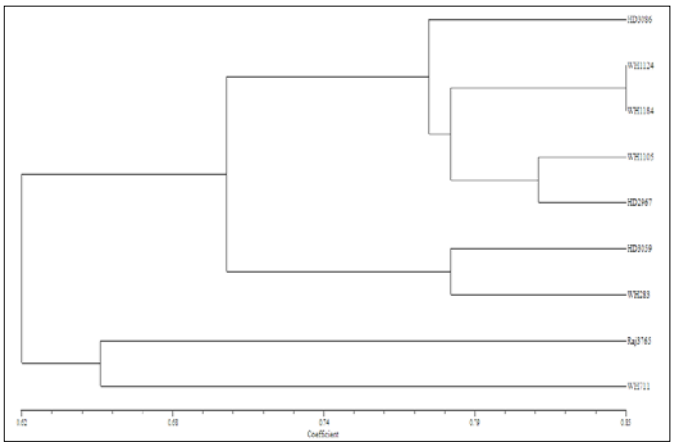

Fig 3: Dendrogram constructed among nine bread wheat genotypes using 23 polymorphic SSRs primers

This analysis grouped the nine bread wheat genotypes in majorly three clusters in such a way that highest similarity present between the clusters and minimum similarity among the clusters. Cluster I include WH711 and cluster II include Raj3765. Third cluster include seven genotypes namely, HD2967, WH283, WH1184, WH1104, WH1124, HD3059 and HD3086. Similar investigations have been carried out by Sharma *et al.,* (2021) [30] using SSR markers. Therefore, a wide genetic variability was present between all the parents from the dendrogram and similarity indices (Fig.3). 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. The primers like gwm192, gwm111, gwm374, STSS5765, XPSP3000, wmc104 and gwm192 could be considered particularly informative, as they revealed three or more alleles per locus and displayed high PIC values. It is further suggested that more polymorphic 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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