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Assessment of Genetic Diversity among Twenty Indian Wheat (*Triticum aestivum* L.) Cultivars using Simple Sequence Repeat (SSR) Markers

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A B S T R A C T

Bread wheat is most cultivated cereal used as food over 95% of the population. Twenty wheat genotypes were assayed to study the genetic diversity using molecular markers. The seventy-five alleles were identified with a mean of 2.34 alleles per locus using 32 SSR markers. Majority of SSR markers showed a high level of polymorphism. PIC values ranged from 0.05 (WMS-169) to 0.75 (CWM-107), with an average of 0.38 per primer. The RP value of primer ranges from 0.92 (WMC-177) to 1.94 (WMS-169) with an average value of 1.51, which explains the ability of primers to resolve the studied germplasm. According to similarity matrix, genetic similarity value ranged from 0.51 to 0.91. The lowest genetic similarity was observed between the WH711 and HD2733 genotypes and the maximum similarity was shown by genotype HD2864 with DBW71. Cluster analysis grouped the twenty wheat genotypes into two main clusters with one separate member. Results indicated that wheat cultivars had high genetic diversity that can be used in wheat breeding programs.

Introduction

Bread wheat (*Triticum aestivum* L.) belongs to the family Poaceae, is the most commonly cultivated cereal, currently grown in most of parts of world (Abdellatif and Abouzeid, 2011). In term of production it is having second place after rice (Trnka *et al.*, 2014). Along with maize it is major part of food for 95% population in developing countries. It is used in form of flour provides one fifth of the global required calories and become most preferred over the rice (Wrigley, 2009; Mwale *et al.*, 2016 and Friedrich *et al.*, 2014). In order to feed the world's growing population, the global demand for wheat yields increase by 50% by 2050 as estimated by Allen *et al.*, (2017). Around the world breeders are working toward the improved grain yield with better quality along with important agronomic traits, therefore the knowledge of the genetic diversity within a germplasm collection has a significant impact for the improvement of crops and useful for production of more efficient crops adapted to diverse conditions (Desheva and Kyosev, 2015). Genetic

diversity is a kind of fundamental study for crop improvement and plays an important role in generating new plant ideotypes with desired traits, which offers prospects for improving the plant characteristics (Manjarrez-Sandoval *et al.,* 1997; Singh, 1991 and Khan *et al.,* 2015). The estimated genetic diversity has great importance for optimal utilization and conservation of germplasm for plant breeding and other activities (Uddin and Boerner, 2008).

Its assessment helps to tackle the threats of environmental fluctuations and for the effective exploitation of genetic resources in breeding programmes. Wheat is one of the most thoroughly studied crops in terms of genetic polymorphism studies but phylogenetic affinities of *Triticum* species have not been assessed to date (Khan *et al.,* 2015). So, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation for future breeding and genetic resource conservation programme.

During the last few decades SSR (microsatellite) markers have been playing an increasing part in genetic studies (Akfirat and Uncuoglu, 2013) permit the fast and high throughput fingerprinting of large numbers of accessions from a germplasm collection in order to assess genetic diversity (Cifci and Yagdi, 2012 and Malik *et al.,* 2013).

They provide new dimension, perfection and accuracy in screening of germplasm (Tar'an *et al.,* 2005). The status of genetic diversity in wheat genotypes assessed by Arora *et al.,* (2014) can be used effectively for future breeding practices. The aim of the present study was done to utilize SSR markers in order to assess the genetic diversity of twenty Indian wheat genotypes. This study was conducted to understand the genetic diversity of Indian wheat genotypes.

Materials and Methods

This investigation was carried out during the crop seasons 2016 and 2017 at the Field research laboratory and experimental station with proper agronomic practices. The molecular works conducted at Department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India (28.99° N Latitude and 77.7° E Longitude with an altitude of 220m above the mean sea level).

Plant material, DNA extraction and SSR analysis

In present study 20 wheat genotypes (Table 1) were used for the assessment of genetic diversity. Total genomic DNA from fresh leaf of plants was extracted using CTAB method (Murray and Thompson, 1980).

DNA was quantified by spectrophotometer, at 260/280nm absorbance. For PCR reaction, the DNA was diluted in the range between 50-100ng/µl. Total thirty-five SSR markers were used for estimation of genetic diversity were selected randomly from prior reported SSR markers (Gao *et al.,* 2003 and Al-naggara *et al.,* 2013). DNA amplification reaction for SSR was performed in a total volume of 25µl.

The components used for reaction mixture are 10x Taq Buffer (2.5µl), 10mM dNTP mix 0.5µl, forward primer 0.25µl, reverse primer 0.25µl, Taq polymerase 0.5µl, template 1µl and volume make up to 25µl in 0.2ml thin walled PCR-tubes with the following thermal program; denatured at 94°C for 4 minutes followed by 35 cycles, denature at 94°C for 30 second, annealing at 42-58°C (depending on TM of SSR primer) for 40 second, extension of primer at 72°C for 1minute followed by final extension at 72°C for 10 minutes and hold at 4°C. The amplified products were separated on 1% agarose gels and in 1X TAE

buffer and DNA fragments were visualized under UV trans illuminator using Alpha Imager gel doc.

Data collection and analysis

Thirty-five SSR primers were used for generating the reference data for the usefulness of a primer. Zero-one sheet was prepared by scoring SSR primer on the basis of presence (1) and absence (0) in all wheat genotypes for further analysis. Genetic similarities were calculated using the Jaccard similarity coefficient (Jaccard, 1908) metod and dendrogram acquired by clustering according to the Un-weighted Pair Group Method with Arithmetic average (UPGMA) algorism using the NTSYS-pc software version 2.11s (Rohlf, 2000). The resolving power (RP) for each primer was calculated in order to assess the ability of primers to resolve the different varieties by following Prevost and Wilkinson's (1999) method as RP = Ib (band information) and RP was calculated as 1-[2 x (0.5-p)], where p being the proportion of the 20 varieties containing the bands and Polymorphism information content (PIC) values were obtained using the formula developed by Anderson *et al.,* (1993). PIC = 1- ΣPij2, where Pij is the frequency of jth allele of ith locus, summed across all the alleles for the locus over all genotypes.

Results and Discussion

Loss of genetic diversity has become a problem for agriculturally important species. Decrease in genetic variation affects the productivity and adaptability for improvement of bread wheat (Stoeva *et al.,* 2009). Genetic diversity in wheat is becoming narrowed due to modern breeding, which is a problem for adaptation to biotic and abiotic stresses, like salt or drought tolerance (Nasab *et al.,* 2013). The use of molecular markers for the evaluation of genetic diversity is receiving much attention as they allow calculation of genetic distance based on allele frequencies and useful in studying the relationship of closely related lines (Uddin and Boerner, 2008; Huang *et al.,* 2002). Availability of superior and diverse alleles/genes form the basis of genetic improvement of crop plants including wheat (Abouzied *et al.,* 2013) that can help in identification of new cultivars. Haile *et al.,* (2013) reported that SSR markers are more variable than other molecular markers, which are useful tools for the study of genetic diversity of germplasms.

Polymorphism of SSR markers

Out of 35 tested SSR primers 32 SSR generate clear and reproducible bands have considered for further analysis was polymorphic range from 50.00 to 100% polymorphism. A total of 75 alleles were amplified by 32 SSR primers in 20 genotypes range from minimum 1 to maximum 5 (Figure 1) with an average of 2.34 alleles per primer. Liu *et al.,* (2005) reported on average 1.9 polymorphic loci per reaction. Wang *et al.,* (2007) analyzed on an average 3.3 alleles per locus by using 26 SSR in 60 durum wheat genotypes.

The assessment of efficiency of molecular markers could be assessed with PIC and RP parameters (Phougat *et al.,* 2017). PIC value was calculated for thirty two polymorphic SSR primers and shown in Table 2 and graphically present in figure (Figure 2).

The PIC value ranges from 0.05 for primer WMS-169 to 0.75 was recorded for the primer CWM-107, with an average of 0.38 for all polymorphic SSR primers. These results are comparable with the results reported by Salem *et al.,* (2015), use 17 polymorphic microsatellite markers to explain the genetic diversity of hexaploid wheat and report PIC value ranged of 0.33 and similar results reported by Sharma *et al.,* (2010) also.

Table.1 Names of wheat genotypes used in the study

No	Name/ Identity	Pedigree
1	DBW17	CMH 79A.95/3* CNO 79//Raj 3777
2	DBW71	Prinia/UP 2425
3	HD2733	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ATTILA
4	HD2864	DL 509-2/ DL 377-8
5	HD2888	C 306/T. sphaerococcum//HW 2004
6	HD3086	DBW 14/HD 2733//HUW 468
7	HUW468	CPAN-1962 / TONI // LIRA'S‟ / PRL'S'
8	K-8027	HD1969/K852//K852.
9	K-1256	-
10	K-9107	K 8101/K 68
11	K-9423	HP1633/KAL/UP262
12	PBW226	C591/RN//JN/3/CHR/HD1941
13	PBW343	ND/VG 7944//KAL/BB3YACO S/4/VEE# 5S
14	PBW396	CN067/MFD//M0N 'S73/SERI
15	PBW590	594/RAJ3814//W 485
16	RAJ3765	HD 2402/VL 639
17	RAJ4246	-
18	UP-2425	HD2320/UP2263
19	WH711	ALD ‘S’HUAC//HD 2285/3/HFW-17
20	WH1021	NYOT95/SONAK

Table.2 Description of SSR markers employed in the study

No	Primer name	No. of allele	PIC	RP	No	Primer name	No. of allele	PIC	RP
1	CWM-101	1	0.51	1.40	17	Xwmc382-2A	1	0.10	**1.90**
2	CWM-103	1	0.44	1.50	18	WMS-06	3	0.25	**1.70**
3	CWM-105	2	0.75	1.00	19	WMS-30	4	0.52	**1.25**
4	CWM-107	2	0.64	1.06	20	WMS-108	3	0.55	**1.12**
5	CWM-110	5	0.51	1.30	21	WMS-118	2	0.40	**1.40**
6	CWM-112	3	0.45	1.40	22	WMS-149	3	0.46	**1.20**
7	CWM-115	2	0.19	1.80	23	WMS-169	2	0.05	**1.94**
8	CWM-118	3	0.30	1.60	24	WMC-177	3	0.74	**0.92**
9	CWM-119	3	0.52	1.30	25	WMC-179	4	0.08	**1.92**
10	CWM-122	3	0.16	1.80	26	WMS-198	1	0.19	**1.80**
11	XGWM-11-1D	1	0.10	1.90	27	WMC-235	2	0.10	**1.90**
12	XGWM-260-7A	4	0.25	1.60	28	WMS-304	1	0.10	**1.90**
13	XGWM276-7A	1	0.44	1.50	29	WMC-307	1	0.27	**1.70**
14	XGWM-350-7A	3	0.50	1.30	30	WMC-322	2	0.47	**1.25**
15	XGWM-573-7B	2	0.60	1.20	31	WMS-375	3	0.44	**1.46**
16	**XGWM-635-7D**	**2**	**0.18**	**1.80**	**32**	**WMC-445**	**2**	**0.45**	**1.45**

Fig.1 SSR primer CWM-103(a) and CWM-107(b), profiling pattern of 20 wheat varieties along with 1Kb (M1) and 100 bp (M2) DNA ladder

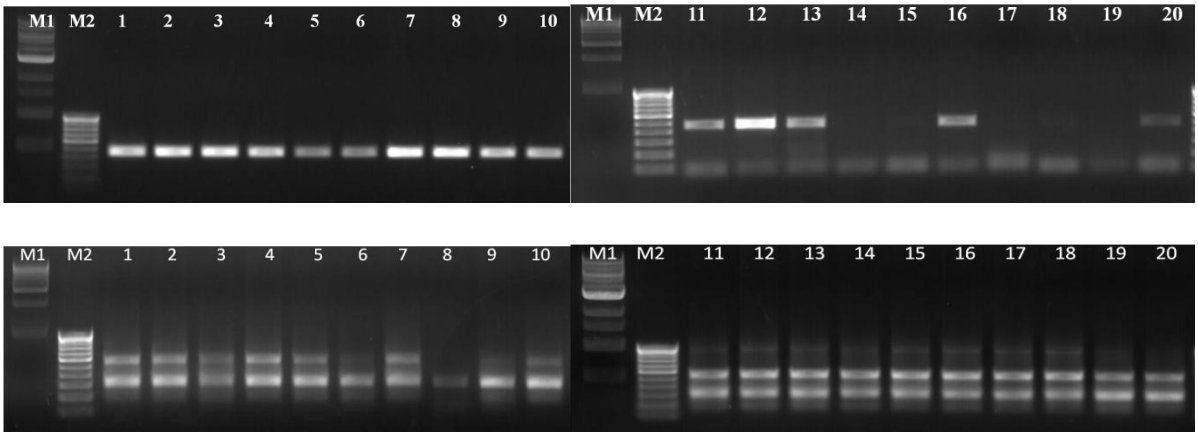


Fig.2 Graphical representation of PIC value of SSR markers

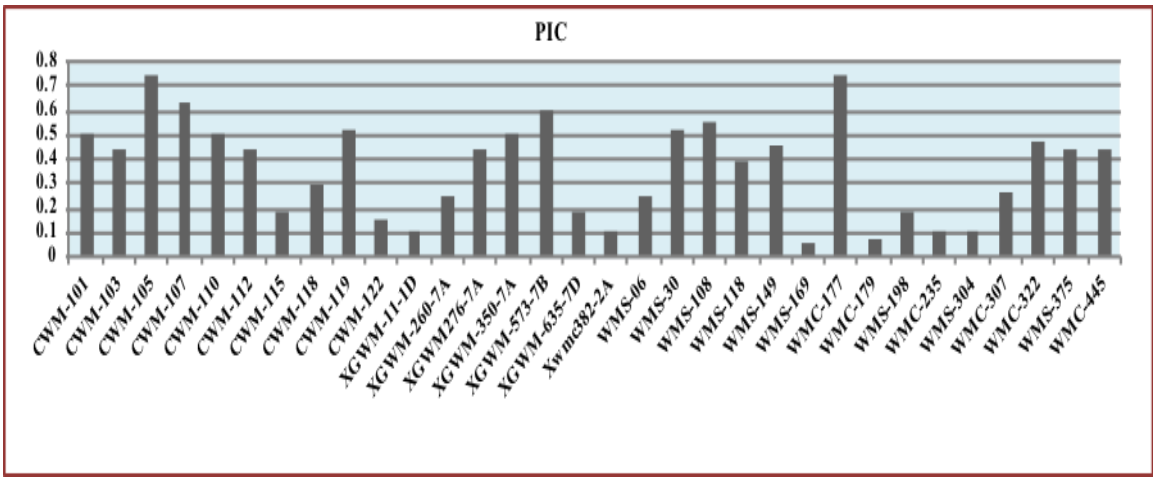


Fig.3 Graphical representation of resolving power (RP) value of SSR markers

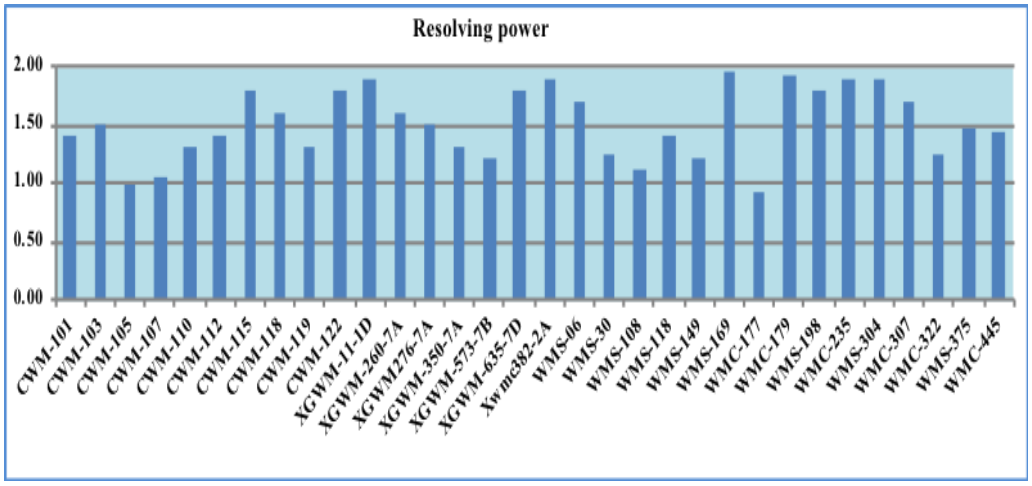
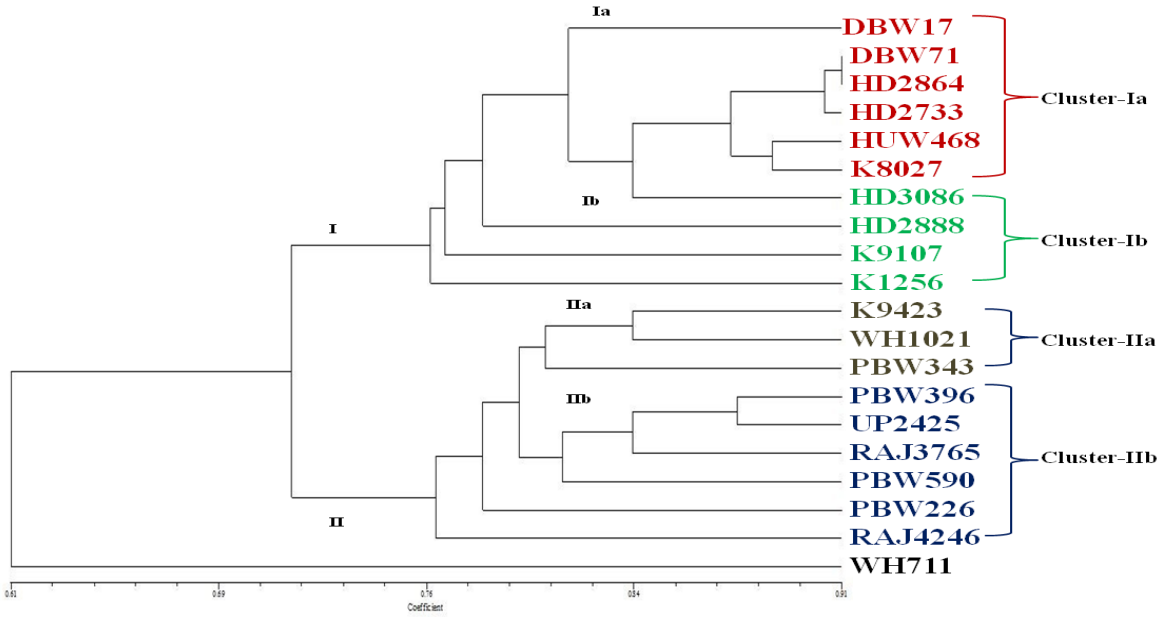


Fig.4 Dendrogram from UPGMA analysis based on Jaccard similarity coefficient of 20 Indian wheat cultivars



Tekeu *et al.*, (2017) aimed to estimate the levels and genetic structure within 17 bread wheat variety using 11 microsatellite markers revealing 77 alleles. PIC value between 0.16 and 0.91 for SSRs was also reported by Bohn *et al.*, (1999). Ahmad, (2002) evaluated 13 wheat cultivars of diverse origin using 43 SSR markers and report similar results also. Marmar *et al.*, (2013) carried out research to screen 12 wheat cultivars to study genetic diversity with 24 allele specific SSR markers showing polymorphism information content ranging from 0.16 to 0.89. In the present study besides primer CWM-105, the other primer like CWM-107, WMC-177 and XGWM-573-7B also show higher PIC value. The higher mean PIC value indicated the informativeness of the primers pairs in detecting genetic diversity and can be used in future studies in the field of taxonomical and genetic resource management. The resolving power (RP) of primer explains the ability of primers to resolve the studied germplasms and ability of a primer to distinguish between large numbers of genotypes (Provost and Wilkinson, 1999; Ablett *et al.*, 2006). The resolving power of 32 polymorphic SSR primers varies from 0.92 (WMC-177) to 1.94 (WMS-169) with an average value of 1.51 (Table 2) and graphically present in figure 3). Singh *et al.*, (2017) reports resolving power with an average value of 1.79 in wheat genotypes.

Cluster analysis

The cluster analysis based on the UPGMA method, SSR primers allowed the discrimination of cultivars and represent the estimated relations between different genotypes (Singh *et al.*, 2017). To present the genetic relationship a dendrogram was constructed, which generate two major groups i.e. cluster I and cluster II groups (Figure 4). The genotype WH711 did not grouped in any cluster and stays separated at the one end of the cluster. The group I subdivided into two sub clusters viz. cluster Ia and cluster Ib. The sub cluster Ia further divided into small clusters includes 7 genotypes namely

DBW17, DBW 71, HD2864, HD2733, HUW468, K8027, HD3086 and cluster Ib includes 3 genotypes HD2888, K9107 and K1256. The cluster II subdivided into two sub cluster (IIa and IIb). The sub cluster IIa includes 3 genotypes namely K9423, WH1021, PBW343 and subcluster IIb includes PBW396, UP2425, RAJ3765, PBW590, PBW226 and RAJ4246 which are further grouped into small clusters. Grouping of wheat genotypes into different clusters have relevance to the future breeding programs (Sharma *et al.*, 2010). Genetic similarity value for all the 20 genotypes ranged from 0.51 to 0.91. The minimum similarity exhibited by genotype WH711 with HD2733 and the maximum similarity was shown by genotype HD2864 with DBW71. The distribution of similarity coefficient is shown in figure 4. Higher similarity values provide greater confidence for the assessment of genetic diversity and relationships in related genotypes. Islam *et al.*, (2012) report microsatellite markers are helpful to characterize and discriminate the diversity within the wheat genotypes. For evaluating genetic relationships diversity analysis is a key factor, which is use in breeding of improved varieties (Al-Doss *et al.*, 2011).

In this investigation, SSR markers showed a high level of polymorphism and are more informative in hexaploid wheat. The genetic diversity levels observed in bread wheat that cultivated in India would be useful indicators if such an approach is planned for the wheat genome and outcome of this research could provide appropriate guidelines for plant breeders towards the implementation of future crop improvement programs for proper management of the wheat cultivars.

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