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Assessment of the molecular diversity of a set of wheat genotypes

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Abstract
The genetic diversity of a set of hexaploid and tetraploid wheat genotypes at biotechnology centre Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola was investigated by applying 21 simple sequence repeats (SSR) primers and only six primers gave ploymorphism. The materials consisted of eleven bread wheat and five *durum* wheat. In total 93 alleles were detected with an average of 15.5 alleles per locus. The average PIC values per locus varied from 0.77 for the primer Xgwm-136 to 0.92 for the primer Xgwm-130. Genetic similarity values between genotypes, calculated by Jaccard's similarity coefficient, were used to produce a dendrogram. In the dendrogram the genotypes were clustered in three main clusters and two genotypes remained solitary in dendrogram.

Keywords: Genetic diversity, SSR, alleles etc

Introduction
Estimation of genetic variation among the accessions is prerequisite for germplasm conservation and breeding programs (Fufa *et al*., 2005) [7]. Through selection and breeding, a large number of alleles have been lost, so that more difficulties have egressed for improvement of wheat in modern agriculture system. (Allard, 1996, Hosington *et al*., 1999) [1, 11]. A rich and diverse germplasm collection is the backbone of every successful crop improvement programme. The diversity opens the way for plant breeders to produce convoy of genotypes for climate change and population growth. Progress in wheat breeding also requires broad genetic base with an availability of promising germplasm collection, therefore an analysis of genetic diversity among genotypes can be a useful tool to get information about the genetic variability of genotypes and it possibly changes the direction of breeding program (Kleshtkina *et al*., 2007) [14]. Consequently, such knowledge can also contribute to a purposeful and focused utilization of germplasm. Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. Molecular characterization and genetic manipulation is the best way to raise the wheat production, therefore it is necessary to study and to guesstimate the mode of inheritance and genetic variation in different parameters of plants to start the productive breeding programs in wheat. Molecular markers can provide comprehensive characterization of genetic resources. The use of molecular markers for the evaluation of genetic diversity is receiving much attention. Many wheat scientists have studied genetic diversity in common wheat using different molecular markers such as RAPDs, RFLPs AFLPs, STS and ISSRs However, most of these marker systems showed a low level of polymorphism in wheat, especially among cultivated cultivars. Microsatellites, also termed simple sequence repeats (SSRs), have been proposed as one of the most suitable markers for the assessment of genetic variation and diversity among wheat varieties, because they are multiallelic, chromosome-specific and evenly distributed along chromosomes (Roeder *et al*., 1998). This technology is based on the diagnosis of 2-6 base pair repeated sequence 1-10 base pairs. Genetic studies using microsatellite markers have increased rapidly because they are highly polymorphic, heterozygous conserved sequences, which can be used as co-dominant markers (Gupta *et al*., 2004) [8]. SSR markers assist in classification of the genotypes through the identification of differences in the numbers and locations of the repeated sequences.

Microsatellite markers have been applied widely for tagging resistance genes, (Boerner *et al*., 2000b) [2], identifying QTLs (Parker *et al*., 1998), marker-assisted selection in wheat (Huang *et al*., 2000) and verifying the integrity and genetic stability of gene bank accessions (Boerner *et al*., 2000a). Such markers also revealed a high level of polymorphism among diploid species (Hammer *et al*., 2000) in the accessions of tetraploid wild wheat *Triticum dicoccoides*

(Fahima *et al*., 2002) and of Aegilops tauschii, the D genome donor of bread wheat (Pestsova *et al*., 2000), and as well as in hexaploid wheat varieties (Plaschke *et al*., 1995, Prasad *et al*., 2000). Thus, the most important uses of SSR is to estimate the diversity, genetic mapping and fingerprinting, also it could provide a model for estimation of the variation among isolated generation and assists in improvement of crops efficiently through diagnosis of favorite alleles and collecting them. The objectives of the present study was to use wheat microsatellite markers for assessment of the molecular diversity among a set of *aestivum* and *durum* wheat germplasms.

Materials and Methods

Plant materials and DNA isolation: Eleven hexaploid and five tetraploid wheat genotypes were collected from Wheat Research Unit of Dr. PDKV, Akola, for analysis during 2014 (table 1).

Table 1: The *aestivum* and *durum* wheat genotypes and their sources

S. No	Genotypes	Species	Source
1	AKAW -4739	*T. aestivum*	Dr. PDKV, Akola
2	AKAW- 4798	*T. aestivum*	Dr. PDKV, Akola
3	AKAW- 4800	*T. aestivum*	Dr. PDKV, Akola
4	PBN- 4876	*T. aestivum*	M.K.V. Parbhani
5	PBN -4881	*T. aestivum*	M.K.V. Parbhani
6	NIAW -2495	*T. aestivum*	M.P.K.V., Rahuri
7	NIAW -2539	*T. aestivum*	M.P.K.V., Rahuri
8	NIAW -2595	*T. aestivum*	M.P.K.V., Rahuri
9	AKAW -3722 (C)	*T. aestivum*	Dr. PDKV, Akola
10	MACS- 6478 (C)	*T. aestivum*	A.R.I. Pune
11	NIAW -301 (C)	*T. aestivum*	M.P.K.V., Rahuri
12	AKDW -4525	*T. durum*	Dr. PDKV, Akola
13	PBND- 4825	*T. durum*	M.K.V. Parbhani
14	PBND -5175	*T. durum*	M.K.V. Parbhani
15	NIDW -0950	*T. durum*	M.P.K.V., Rahuri
16	NIDW- 295 (C)	*T. durum*	M.P.K.V., Rahuri

Total genomic DNA was extracted using Cetyltrimethyl ammonium bromide (CTAB) protocol as given by Sharma *et al.* (2002) with some modifications.

21 wheat SSR primers (Table 2) were selected for the investigation on the basis of their polymorphic nature in wheat species.

Microsatellite Markers

Table 2: The list of identified SSR primer used for Amplification

Sr. No.	Primer Name	Sequence (5'-3')	Direction
1	Xgwm-130	AGCTCTGCTTCACGAGGAAG	F
		CTCCTCTTTATATCGCGTCCC	R
2	Xgwm-136	GACAGCACCTTGCCCTTTG	F
		CATCGGCAACATGCTCATC	R
3	Xgwm-193	CTTTGTGCACCTCTCTCTCC	F
		AATTGTGTTGATGATTTGGGG	R
4	Xgwm-493	TTCCCATAACTAAAACCGCG	F
		GGAACATCATTTCTGGACTTTG	R
5	Xgwm-610	CTGCCTTCTCCATGGTTTGT	F
		AATGGCCAAAGGTTATGAAGG	R
6	Xgwm18	GGA GTC ACA CTT GTT TGT GCA	F
		CAC TGC ACA CCT AAC TAC GTG C	R
7	Xgwm-133	ATCTAAACAAGACGGCGGTG	F
		ATCTGTGACAACCGGTGTGA	R
8	Xgwm-120	GATCCACCTTCCTCTCTCTC	F
		GATTATACTGGTGCCGAAAC	R
9	Xgwm-469	CAACTCAGTGCTCACACAACG	F
		CGATAACCACTCATCCACACC	R
10	Xgwm-325	TTTCTTCTGTCGTTCTCTTCCC	F
		TTTTTACGCGTCAACGACG	R
11	Xgmw-106	CTG TTC TTG CGT GGC ATTA AA	F
		AAT AAG GAC ACA ATT GGG ATG G	R
12	Xgwm-533	AAG GCG AAT CAA ACG GAA TA	F
		GTT GCT TTA GGG GAA AAG CC	R
13	Xgwm-389	ATC ATG ATC TCC TTG ACG	F
		TGC CAT GCA CAT TAG CAG AT	R
14	Xgwm-219	GAT GAG CGA CAC CTA GCC TC	F
		GGG GTC CGA GTC CAC AAC	R
15	Xgwm-459	ATG GAG TGG TCA CAC TTT GAA	F
		AGC TTC TCT GAC CAA CTT CTC G	R
16	Xgwm-508	GTT ATA GTA GCA TAT AAT GGC C	F

		GTG CTG CCA TGA TAT TT	R
17	Xgwm-518	AAT CAC AAC AAG GCG TGA CA	F
		CAG GGT GGT GCA TGC AT	R
18	XPSP-2999	TCCCGCCATGAGTCAATC	F
		TTGGGAGACACATTGGCC	R
19	XPSP-3000	GCA GAC CTG TGT CAT TGG TC	F
		GAT ATA GTG GCA GCA GGA TAC	R
20	gwm18	GTGAGGCAGCAAGAGAGAAA	F
		CAAAGCTTGACTCAGACCAAA	R
21	gwm 133	CAAATGGATCGAGAAAGGGA	F
		CTGCCATTTTTCTGGATCTACC	R

*The primers in bold letters are polymorphic.

Polymerase chain reaction and fragment analysis
The amplification protocol was as follows: the DNA was denatured initial at 94^0C for 2 minutes, followed by 35 cycles each consisting of denaturation at 94^0C for 30 sec. annealing for ; 1 min either at 60 or 62^0C, elongation at 72^0C for 1 min; and a final extension of 10 min at 72^0C.
Amplified products were analyzed on 8 per cent PAGE with 100 and 1kb ladder as molecular size standards.

Data analysis: Results were analyzed on gel doc (Syngene Biorad) and fragments amplified by microsatellite primers were scored for presence (1) or absence (0) and used for a binary data matrix. The binary data were used to compute a pair-wise similarity according to Jaccard (1908). The similarity matrix was subjected to cluster analysis using the UPGMA algorithm on NTSYSpc version 2.0 (Rohlf 1998) Allelic polymorph information content (PIC) was calculated according to formula of Nei (1973). PIC = Σ(Pij)2 ; where pi is the frequency of the jth allele for ith locus summed across all alleles for the locus.

Results and Discussion
Microsatellite polymorphism: Out of 21 SSR primers, only six were polymorphic and produced the clear bands. In total 93 different alleles were detected by these six primers. The average number of alleles per locus was 15.5. Amplified alleles ranged from 13 (Xgwm136) to 20 (Xgwm130). The Xgwm130 primer amplified higher number of alleles i.e. 20 followed by primer XPSP2999 with 17 number of alleles. Several authors reported that microsatellites are more variable than most of other molecular markers that are useful as tools for studying the genetic diversity of germplasm. Plaschke *et al.* (1995) has used wheat microsatellite for the first time for studying the genetic diversity in closely related European bread wheat varieties and obtained on an average 6.2 alleles per locus by using 23 makers. On the other hand, Amer *et al*. (2001) detected genetic diversity in fifteen Libyan wheat genotypes and found the average number of 4.5 alleles per locus. The authors suggested that a relatively small number of primers could be used to distinguish all genotypes and to estimate the genetic diversity. In recent years Ayman and Mohamed (2019) investigated seventeen wheat cultivars obtained from Gene Bank, Egyptian Ministry of Agriculture and reported the alleles ranged from eight to twenty. The PIC values in this study ranged from 2.21(Xgwm136) to 5.69 (XPSP2999) with an average of 3.73. It means the used markers are very informative and the chosen genotypes are more diverse. A higher number of alleles per locus and higher values of PIC for the markers used in these studies may be attributed to the use of more informative markers and relatively more diverse genotypes.

Genetic diversity
A dendrogram derived from unweighted pair group method with arithmetic mean cluster analysis based on the genetic similarity coefficient matrix for sixteen genotypes was constructed (Fig. 1). The genotypes were clustered in clearly defined three main groups at 75 per cent cut off level and subdivided into sub clusters at 80 per cent cut off level (Table 3.). Recently Hermuth *et al*. (2019) evaluated the twenty wheat genotypes and got the clusters and sub clusters.
In this study, it was observed that genotypes AKDW-4525 and PBND-4825 showed 88 per cent similarity. They also grouped in the same sub-cluster and cluster, respectively. Based on similarity index lowest similarity (0.634) between three main clusters across 16 genotypes was found between NIAW-2539 and AKAW-4739 genotypes. The reason may be that these two genotypes developed at different geographical locations and different parental materials used for development. NIAW-2495 and NIDW-0950 did not belong to any cluster and remained single. In cluster 'I' 'II" and 'III' the *aestivum* and *durum* were clustered together that means these genotypes have the common loci and introgressed from related parents. Uddin and Boerner (2008) also reported the grouping of *durum* and *aestivum* in the same cluster where Ragina 24, belonging to *durum* clustered with Riscicola 67 which belongs to *aestivum* type.

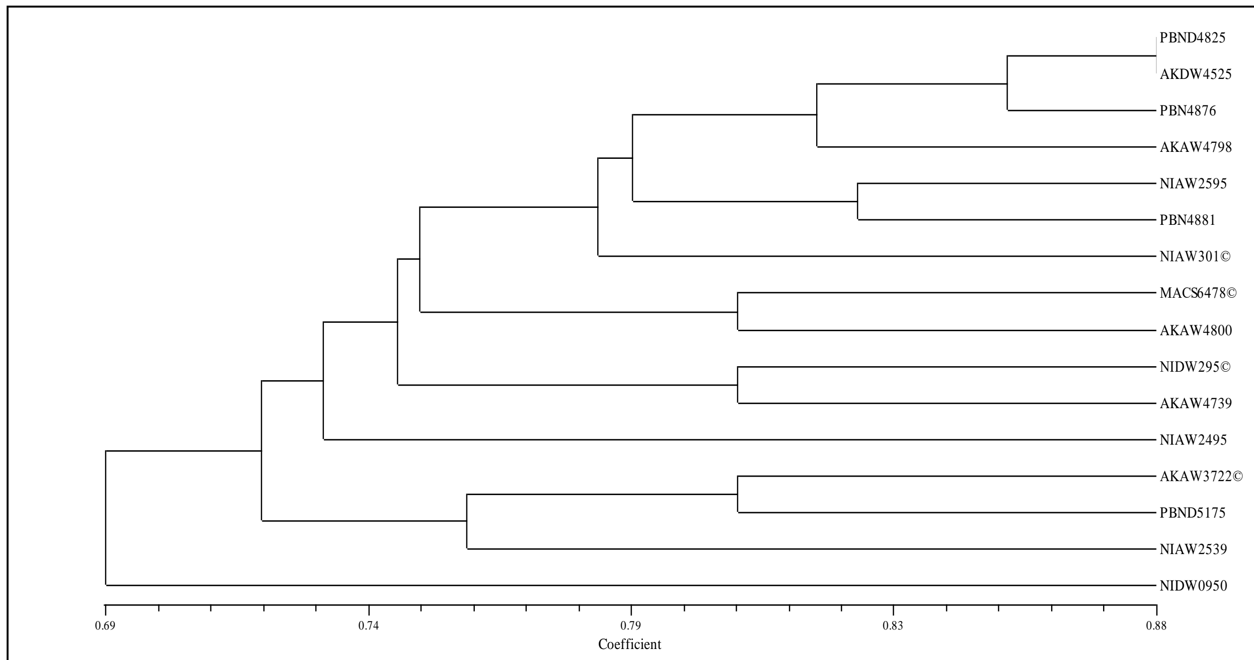

Fig 1: Dendrogram of the sixteen genotypes of wheat based on genetic similarity.

Table 3: Grouping of genotypes at 75 and 80 per cent cut off level based on similarity coefficient matrix

S. No.	Cluster at cut of 75%	Sub cluster at cut of 80%	Genotype
1	I	A	PBND-4825, AKDW-4525, PBN-4876, AKAW-4798
		B	NIAW-2595, PBN-4881
		C	NIAW-301
		D	MACS-6478, AKAW-4800
2	II	A	NIDW-295, AKAW-4739
3	III	A	AKAW-3722, PBND-5175
		B	NIAW-2539
4	Single		NIAW-2495
5	Single		NIDW-0950

The genotypes of AKAW types developed at Dr. PDKV, Akola did not clustered in the same group, similarly the other genotypes also. The reason for this might be that genetic variation occurred independently in these genotypes in their geographic regions. The results presented here confirm the utility of SSR marker for the characterization of genotypes of wheat. The marker data can be used in selecting diverse parents in breeding improved cultivars and in appropriately maintaining genetic variation in the germplasm.

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