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Assessing genetic diversity in wheat genotypes by using molecular markers (RAPD and SSR)

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Abstract
Two types of molecular markers, random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR), were assayed to determine the genetic diversity of 10 wheat cultivars. A high level of polymorphism was found with both RAPD and ISSR markers. In RAPD analyses, 200 out of260 bands (73.71%) were polymorphic. The RAPD primer S133 and S138 showed 100% polymorphism while S32 primer showed only 31.03% polymorphism. The cluster analysis reveals that the two clusters contain two major three minor clusters and one out group. In SSR analyses, a total of 118 alleles were detected, among which 68 alleles (56.88%) were polymorphic. PIC value of 0.97 was WMC-156 while the least informative marker was found to be GWM-140 with PIC value of 0.15 amplified alleles ranged from 1 to 3 in all the wheat genotypes. The cluster analysis reveals that the two clusters contain two major three minor clusters and four out group.

Keywords: Assessing, wheat, molecular, RAPD, SSR

Introduction
Wheat belongs to the genus *Triticum*, for which there are 10 species, six which are cultivated and four which are not. The most economically important species, *T. aestivum*. Wheat is one of the main cereal crop which is widely grown and consumed food all over the world. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation. It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay. The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellite), sequence tagged sites (STS), inter-simple sequence repeat polymorphic DNA (ISSR) and so on. These molecular markers had been used in wheat for detecting genetic diversity, genotype identification, genetic mapping (Motawei, M.I. *et al.*, 2007) [4]. Simple sequence repeats (SSRs) are common, informative molecular markers used for genetic diversity studies because of their simplicity, high levels of polymorphism high reproducibility, and co-dominant inheritance patterns (Röder *et al.*, 1998) [6].

Material and Method
Ten genetically diverse genotypes were used in this study (Table 1). Cultivars selected from the wheat breeding program at the Plant biotechnology Department, Vasantrao Naik Marathwada Krushi Vidyapeeth Parbhani, Genomic DNA was isolated from wheat leaves modified CTAB extraction method described by Doyle & Doyle (1987) [1].

RAPD and SSR analysis
A total of ten 10-mer oligonucleotides were used in RAPD analysis, and 10 SSR primers were used in analysis. The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1x PCR buffer, 2.0 mmol/L MgCl2, 100 µmol/L of each dNTP, 0.1 µmol/L primer and 3U Taq polymerase in a 25 µL volume. The amplification protocol was 94 ºC for 5 min to predenature, followed by 45 cycles of 94 ºC for 1 min, 36 ºC (for RAPD analysis) or 56 ºC (for SSR analysis) for 1 min and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. Amplification products were fractionated on 1.5% (for RAPD analysis) or 2% (for SSR analysis) agarose gel.

Table 1: The 10 wheat cultivars used in the study

Sr. No.	Genotypes	Sr. No.	Genotypes
1	PBN-4449	6	PBN-3958
2	PBN1666-1	7	PBW-4905
3	PBN-4876-2	8	PBN-2189
4	PBN-4357	9	HI-1633
5	PBN-4881	10	AKDW-2997-16

Data analysis

RAPD and SSR data were scored (1) for presence and (0) for absence, each band was regarded as a locus. Two matrices, one for each marker, were generated. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes, was constructed using the unweighted pair group method with arithmetic average (UPGMA) 24 through the software NTSYs-pc version 2.11.

Results and Discussion

Identification and evaluation of RAPD and SSR markers for diversity estimates: In RAPD analysis, twenty primers of arbitrary nucleotide sequence were used to amplify DNA segments from 10 wheat genotypes. The RAPD primers generated total number of 260 amplicons in 10 genotypes. Amongst these, 200 amplicons were found to be polymorphic with an average polymorphism 73.71 percent. The RAPD primer S133 and S138 showed 100% polymorphism while S32 primer showed only 31.03% polymorphism. Highest informative marker with the PIC value of 0.93 was S138 with 100% polymorphism followed by S133 (0.31), S98 (0.26), S22 (0.25) while the least informative primer was found to be S32 with PIC value of 0.12. Motawei, M. I. *et al.*, (2007) [4] found that the number of amplification bands per primer varied between 0 and 8.

Table 2: RAPD primers for wheat genetic diversity

Primer name	Sequence	Primer name	Sequence
S 22	TGCCGAGCTG	S 132	ACGGTACCAG
S 32	TCGGCGATAG	S 133	GGCTGCAGAA
S 98	GGCTCATGTG	S 135	CCAGTACTCC
S126	GGGAATTCGG	S 138	TTC CCG GGTT
S 127	CCGATATCCC	S 156	GGTGACTGTG

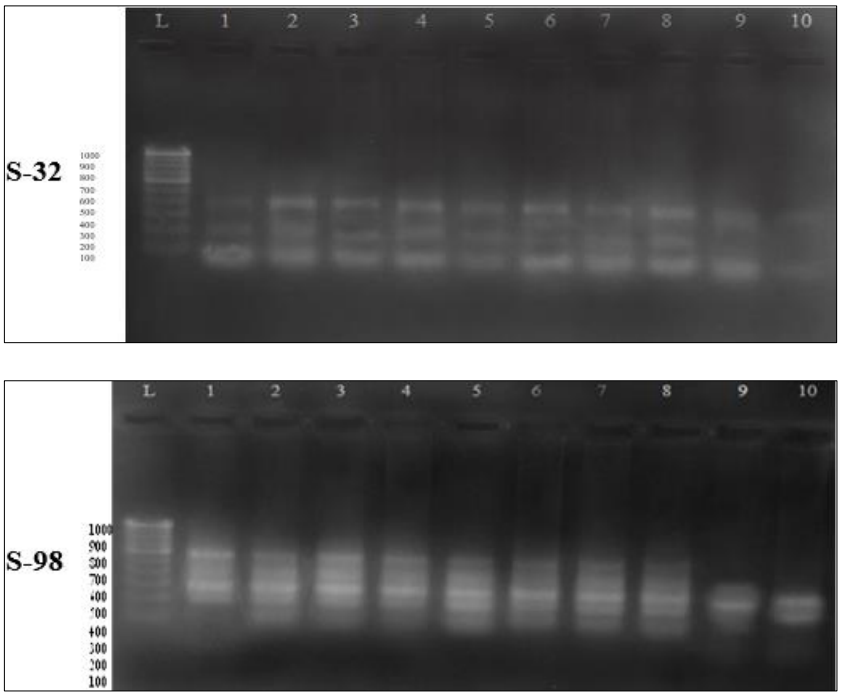

Fig 1: Polymorphism revealed using RAPD primers S-32 and S-98 to amplify genomic DNA purified from wheat genotypes

In SSR analysis

The WMC-154, WMC-245 and WMC-266 primers amplified two alleles whereas as primer WMC-198, WMC-156 GWM-133 and GWM-140 amplified three alleles in wheat genotypes. Primers shows the polymorphic banding patterns in wheat genotypes the polymorphic information content (PIC) value per primer ranged from 0.15 to 0.97with an average of 0.22. Highest informative marker with the PIC value of 0.97 was WMC-156 while the least informative marker was found to be GWM-140 with PIC value of 0.15 amplified alleles ranged from 1 to 3 in all the wheat genotypes 7 polymorphic primers out of 10 amplified a total of 68 loci out of 118 total loci. Primer WMC-154 and WMC-198 respectively showed 100% polymorphism in all genotypes. Primer GWM-133 and GWM-140 showed 33.33 percent polymorphism respectively. P. sharma *et al.*, (2017) [17] found that out of 125 SSR only 41 found polymorphic were distributed across the different wheat chromosome. Maximum no of allele amplified was polymorphic and mean value of PIC 0.39 with a range of 0.12 to 0.75 for the markers Xgwm155 and WMC-156

Primer		Sequence
WMC198	F	CACGCTGCCATCACTTTTAC
	R	TTGAAGTGGTCATTGTTGCT
WMC154	F	ATGCTCGTCAGTGTCATGTTTG

	R	AAACGGAACCTACCTCACTCTT
WMC156	F	GCCTCTAGGGAGAAAACTAACA
	R	TCAAGATCATATCCTCCCCAAC
WMC 266	F	ATGTATTTACGAGCATCGACCG
	R	ATGGTTACTCAGCCCACATTCA
GWM44	F	GTTGAGCTTTTCAGTTCGGC
	R	ACTGGCATCCACTGAGCTG
WMC245	F	GCTCAGATCATCCACCAACTTC
	R	AGATGCTCTGGGAGAGTCCTTA
GWM133	F	ATCTAAACAAGACGGCGGTG
	R	ATCTGTGACAACCGGTGAGA
GWM140	F	ATGGAGATATTTGGCCTACAAC
	R	CTTGACTTCAAGGCGTGACA
GWM156	F	CCAACCGTGCTATTAGTCATTC
	R	CAATGCAGGCCCTCCTAAC
GWM268	F	AGGGGATATGTTGTCACTCCA
	R	TTATGTGATTGCGTACGTACCC

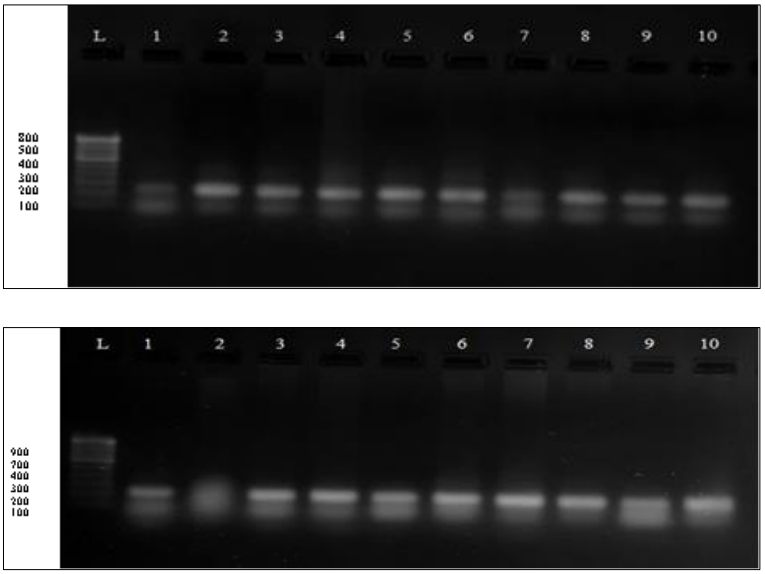

Fig 2: Polymorphism revealed using SSR primers WMC-156 and WMC-154 to amplify genomic DNA purified from the tested wheat genotypes

Genetic diversity

The relationships among wheat cultivars were estimates by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using RAPD markers alone (Fig. 3), SSR markers alone (Fig. 4), has revealed groupings in cases.

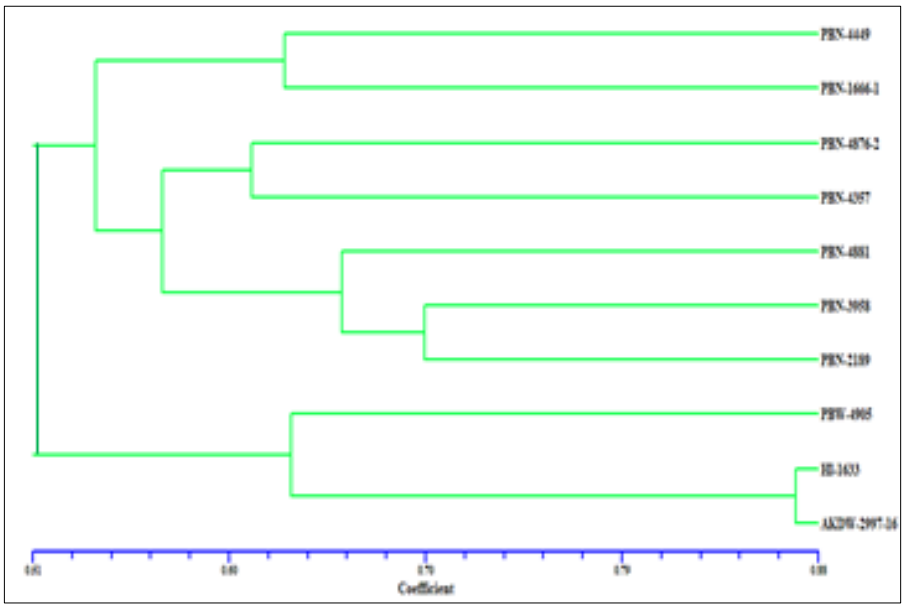

Fig 3: Dendrogram constructed from similarity coefficients and showing the clustering of wheat genotypes using RAPD markers

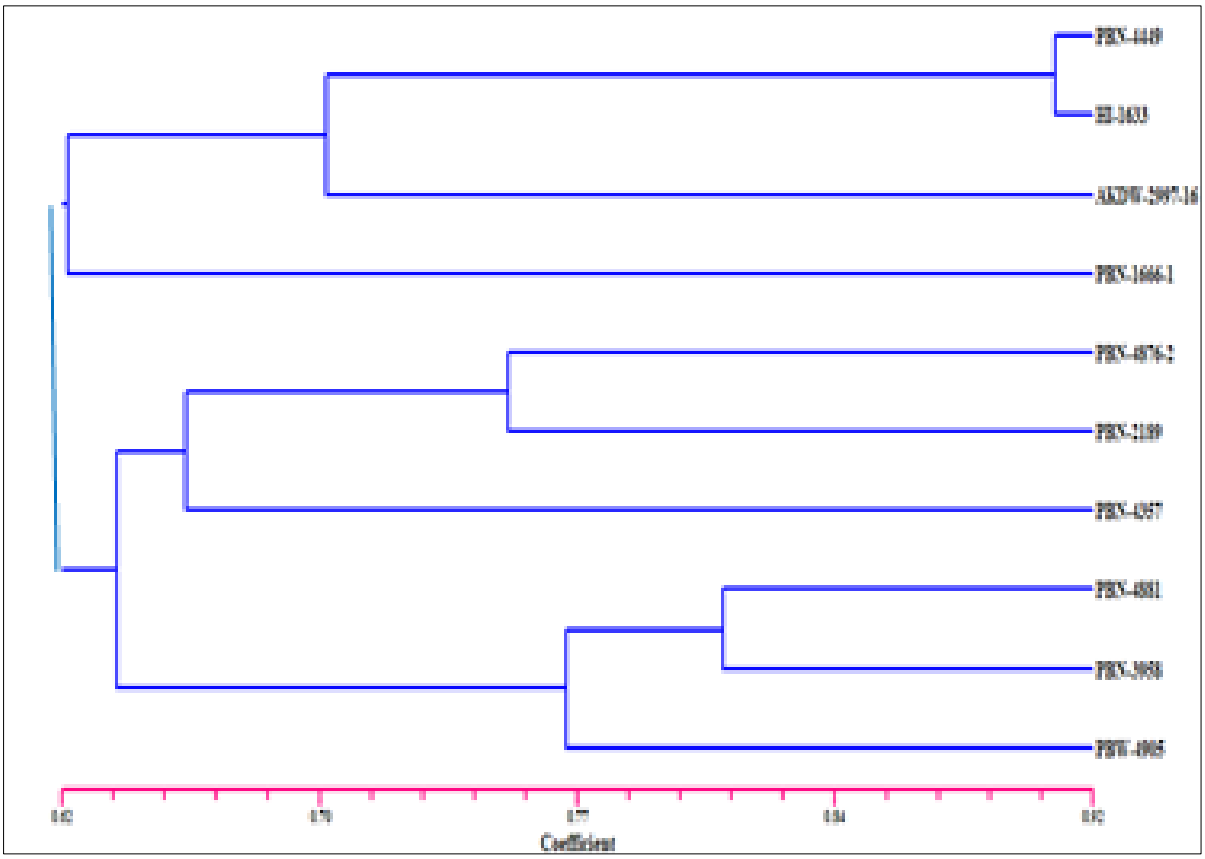

Fig 4: Dendrogram constructed from similarity coefficients and showing the clustering of wheat genotypes using SSR markers

The cluster analysis reveals that the two clusters contain two major three minor clusters and one out group. The cluster-I being the major cluster with 80% similarity amongst the eight genotypes. The cluster-II was minor cluster with two genotypes i.e. HI-1633 and AKDW-2997-16 and one out group with PBW-4905. The dendrogram also generated 1 out group namely AKDW-2997-16. The major cluster-I was further divided in to three sub cluster sharing similarity with each other. Three cluster shares similarity with remaining one out group with PBN 4881. The sub cluster IA contains two genotypes namely PBN-4449 and PBN-1661. The sub cluster I-BI contains two genotypes namely PBN- 4876-2 and PBN-4357, and sub cluster I-B-II three genotypes namely PBN-2189 and PBN-3958 with one out group PBN-4881. The cluster-I contains eight genotypes with PBN-4449, PBN-1666-1, PBN-4876-2, PBN-4357, PBN-4881, PBN-3958, PBN-2189 and cluster- II contains three genotypes with PBW-4905, HI-1633 and AKDW-2997-16. Motawei, M.I. *et al*., (2007) [4] they generated dendrogram from RAPD data clearly indicated two main clusters.

The cluster analysis reveals that the two clusters contain two major three minor clusters and four out group. Cluster I was major cluster with two sub-cluster in which sub-cluster I contain two genotypes i.e. PBN-4449, HI-1633 and one out-group (AKDW-2997-16). Sub-cluster I with PBN-4449 and HI-1633 sharing 0.9% genetic similarity with each other. Sub-cluster II contains out-group with PBN-1666-1 shares 0.58% similarity with AKDW-2997-16.

The major cluster-II was further divided in to two sub cluster sharing similarity with each other. The sub cluster IIA contains two genotypes namely PBN-4876-2 and PBN-2189 with one outgroup PBN-4357 shares 0.62% genetic similarity with PBN-2189. The sub cluster II-B contains two genotypes namely PBN- 4881 and PBN-3958 with one out-group namely PBW-4905 shares 0.73% genetic similarity with PBN-4881. El-Rawy, M. A (2015) [2] Similarity and Cluster analysis Cluster analysis was performed to categorize the 10 RILs selected in both directions for genetic variation in heat tolerance based on the 1000-KW, grain yield per plant, traits. The dendrogram constructed based on these phenotypic traits classified the 10 RILs into two groups or clusters. Hassan, M.I. (2016) [3] did cluster analysis of similarities using Jaccard's coefficients based on SSR markers data classified the ten bread wheat genotypes evenly into two groups. Vasantrao, J. M. *et al*., (2019) [8] found Allelic diversity data was used to produce a dendogram in order to elucidate the relationship among the 19 wheat varieties. The consensus tree showed that it divided the wheat genotypes into 2 main clusters.

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