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RAPD MOLECULAR MARKERS FOR DIVERSITY ANALYSIS IN WHEAT (TRITICUM SPECIES)

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ABSTRACT
Molecular diversity was assessed among wheat genotypes by employing molecular markers.In total, 25 RAPD markers amplified 23 fragments with 65.22 % polymorphism. Unweighted pair group method with arithmetic mean (UPGMA) analysis generated by RAPD based on genetic distance estimates displayed two major clusters of wheat genotypes; first group includes fifteen genotypes and the second includes ten genotypes.A Jaccard's similarity coefficient of all genotypes derived from RAPD data ranged from 0.54 to 0.95.Marker data subjected to analysis of molecular variances revealed more diversity within population.These molecular markers can be put to use for characterization and selection of wheat genotypes

INTRODUCTION

Assessment of genetic diversity and identification of crop genotypes are essential for efficient conservation and utilization of germplasm resources. Morphological characters have been traditionally used for germplasm characterization. Such characters are, however limited in number and show growth stage and environment dependent expression. In contrast, molecular markers based on difference in the DNA sequence are large in number. The stage of plant growth and the environment do not influence the differences in DNA sequence. Therefore, molecular markers are currently being used for an accurate estimation of genetic diversity and determination of unique identity of crop genotypes (Smith and Helentjaris, 1996). RAPD was the first polymerase chain reaction (PCR) based marker characterized by dominant nature and requiring no sequence information (Asif *et al.,* 2005). There is a plethora of reports on genetic diversity studies employing RAPD markers in wheat (Rahman *et al*., 2004; Asif et al., 2005; Iqbal *et al.,* 2007; Rashed *et al*., 2008; Abd-El-Haleem *et al*., 2009; Patil *et al*., 2011; ElSayed and Rafudeen, 2012). Application of the RAPD markers (Williams *et al.,* 1990; Welsh and McClelland, 1991), which are among the widely used molecular markers in plants (Harris, 1999; Stojalowski *et al.,* 2004) does not need any prior information about the target sequences on the genome and the assay is simple and fast (Varshney *et al.,* 2005).

Wheat occupies a place of prominence among other cultivated cereal crops in India. In view of possible implementation of plant varietal protection in India in near future, increasing attention is being paid towards comprehensive characterization of elite Indian cereal germplasm, supplementing the existing morphological descriptors with reliable and repeatable DNA based molecular profiles (Smith *et al.,* 1991). The total number of accessions of wheat in international and local gene bank around the world is estimated to be in excess of 400000, although many accessions may be duplicated in different collections (Poelham and Sleper, 1995). So, the study of genetic diversity is important in a crop breeding programme for selection of suitable diverse parent to obtain heterotic hybrids as well as for the conservation and characterization of wheat germplasm.

Thus, molecular characterization of the local genotypes which are released varieties or widely cultivated genotypes would be useful for selection for molecular breeding. In the present study, an attempt was made to characterize the wheat genotypes for assessing genetic diversity between genotype and selection of parent for hybridization employing RAPD markers.

MATERIALS AND METHODS

Plant material

Total 25 cultivated wheat genotypes (Table 1), employed in the present study were procured from Wheat Research Station, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S).

DNA isolation and PCR amplification

Two gram fresh leaf sample from a single seedling was grinded in liquid nitrogen to obtain fine powder. The powder was immediately transferred to 50mL polypropylene centrifuge tubes containing 15mL pre warmed (60ºC) extraction buffer and was mixed by inversion. The mixture was incubated for 60 min at 65°C in hot water bath followed with intermediate shaking after every 10 min. 15 mL of Chloroform ($CHCl_3$): Isoamyl alcohol (24: 1) was added and mixed gently but thoroughly to emulsify both the components. Centrifugation was carried out at 8000 rpm for 10 min at room temperature. The upper aqueous phase was transferred into a new 50 ml polypropylene centrifuge tubes with a wide bore pipette. 0.6 volume of ice-cold isopropanol was added and mixed by inversions. CTAB-DNA complexes formed a fibrous network. Alternately after mixing with isopropanol, the samples were centrifuged at 8000 rpm at room temperature for 15 min. After centrifugation a pellet was formed at the bottom of the polypropylene centrifuge tubes.The supernatant was removed and the pellet was washed with 70% ethanol. The pellet wasair-dried for 30 minutes and then dissolved in 0.5 ml of TE buffer. The pellets were allowed to dissolve completely overnight at 40ºC without agitation.

Amplifications were carried out using a 96 thermal cycler (Bio Metra) programmed for 40 cycles as follows: initial denaturation at 94ºC for 4 min, further denaturation at 94ºC for 1 min, annealing at 30ºC for 1 min, extension at 72ºC for 3 min and final extension for 10 min at 72ºC. The amplification products were stored at 4ºC until loading. The PCR products were resolved at 80 Volts for 4 hours on 1.2% agarose gel prepared in 1xTBE buffer. Gel was photographed using Gel-Documentation system (Bio-Rad).

RAPD analysis

Random primers were selected based on the previous studies of Pakniyat and Tavakol, 2007 and Gorji *et al*., 2010 (Table 2). Amplification reaction was performed with 25 μL volume of 2.5 PCR buffer (10 X) with 15 mM MgCl2, primer (10 pmoles/ìL), 0.5 dNTPs mix (10 mM each), 0.3 Taq DNA polymerase (5 U/iL), 1.5 Template DNA (20 ng/iL) (Fermentas, India). Amplification was carried in thermal Cycler (What man Biometra T-Gradient, Germany) with the conditions as follows; initial denaturation at 94ºC for four mins., 39 cycles of denaturation at 94ºC for 30 sec., annealing at 38ºC for 30sec., extension at 72ºC for one min. and final extension at 72ºC for seven mins. The amplified products of RAPD were analysed on 1.8% agarose gel prepared in 1X TBE along with 1 kb standard DNA ladder (Fermentas, India). The electrophoresis was conducted at 60 V current (constant) to separate the amplified bands. The separated bands were visualized under UV transilluminator and photographed using BIORAD Gel Documentation system (BIORAD, USA).

Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function of NTSYS-pc (Rohlf, 1993). Relationships between the wheat cultivars were graphically represented in the form of dendrograms.

RESULTS AND DISCUSSION

RAPD polymorphism

Molecular marker data could be highly useful in precise differentiation of the inbred lines and consequently, their planned utilization in hybrid breeding programme. Twenty five random 25 decamer viz., OPB, OPE and OPG series synthesized from Operon Technologies, USA were used to evaluate 25 wheat parents (Table 2) and RAPD profiling of wheat genotype amplified by two primers OPG-13 and OPG-4 (Fig. 2).

Out of 25 primers screened during present study six primers were polymorphic and five primers were monomorphic and remaining fourteen primers were not amplified for the set of

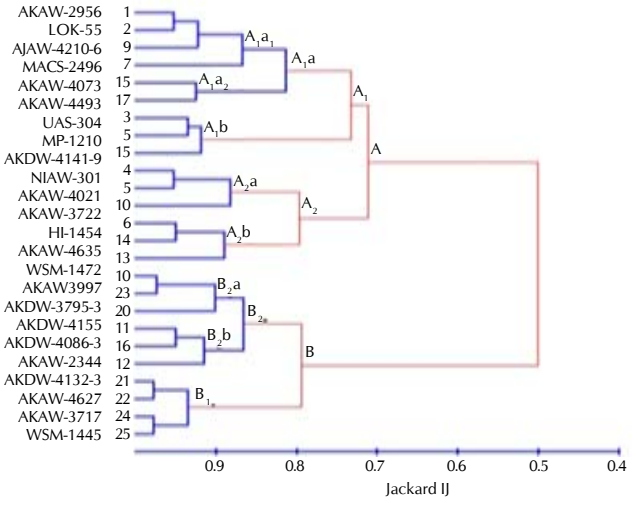

Figure 1: Dendrogram generated through UPGMA analysis showing genetic relationship among the 25 wheat genotypes. Names of the genotypes are given on the termini of branches.

Table 1: List of wheat genotypes used in the present study

Sr no.	Genotype	Sr no.	Genotype	Sr no.	Genotype
1	AKAW- 2956	10	AKAW- 3997	19	AKAW-3722
2	LOK- 58	11	AKDW- 4086-3	20	AKDW- 4155
3	MP- 1210	12	AKAW- 2344	21	AKDW-4132-3
4	NIAW- 301	13	WSM-1472	22	AKAW-4627
5	UAS- 304	14	AKAW- 4635	23	AKDW-3795-3
6	HI- 1454	15	WSM- 4073	24	AKAW-3717
7	MACS- 2496	16	AKAW- 3867	25	WSM-1445
8	AKDW- 4021	17	AKAW- 4493		
9	AKAW- 4210-6	18	AKDW- 4141- 9		

Table 2: RAPD primers used and their characteristics for diversity analysis in wheat.

Sr. No.	Name	Primer sequence (5′-3′)	Sr. No.	Name	Primer sequence (5′-3′)
1	opB-03	CATCCCCCTG	14	opg-04	AGCGTGTCTG
2	opb-13	ttcccccgct	15	opg-05	CTGAGACGGA
3	ope-01	cccaaggtcc	16	opg-06	GTGCCTAACC
4	ope-02	ggtgcgggaa	17	opg-07	GAACCTGCGG
5	ope-03	ccagatgcac	18	opg-08	TCACGTCCAC
6	ope-04	gtgacatgcc	19	opg-09	CTGACGTCAC
7	ope-05	tcagggaggt	20	opg-10	AGGGCCGTCT
8	ope-16	ggtgactgtg	21	opg-11	TGCCCGTCGT
9	ope-17	ctactgccgt	22	opg-12	CAGCTCACGA
10	ope-19	acggcgtatg	23	opg-13	CTCTCCGCCA
11	opg-01	CTACGGAGGA	24	opg-14	GGATGAGACC
12	opg-02	GGCACTGAGG	25	opg-15	ACTGGGACTC
13	opg-03	GAGCCCTCCA			

Table 3: Characteristics of the amplification products obtained with six primers for RAPD

Sr.no.	Primer	Total No. of amplicons	Monomorphic ampliconsamplicons	Polymorphic polymorphism	Per cent
1	OPB-13	4	1	3	75
2	OPG-4	4	1	3	75
3	OPG-5	2	1	1	50
4	OPG-13	4	2	2	50
5	OPE-4	4	1	3	75
6	OPE-16	5	2	3	60
	Total	23	8	15	65.22
	Average	3.83	1.33	2.5	

Table 4: Details of Dendrograms generated by RAPD

Details	Cluster	Sub clusters			Genotypes
	A	A1	A1a	A1a1	AKAW-2956, LOK-58, AKAW-4210-6, MACS-2496
				A1a2	AKAW-4073, AKAW-4493
			A1b		UAS-304, MP-1210, AKDW-4141-9
		A2	A2a		NIAW-301, AKDW-4021, AKAW-3722
			A2b		HI-1454, AKAW-4635, WSM-1472
	B	B1			AKDW-4132-3, AKAW-4627, AKAW-3717, WSM-1472
		B2	B2a		AKAW-3997, AKDW-3795-3, AKDW-4155
			B2b		AKDW-4086-3, AKAW-3867-10, AKAW-2344
Total	2	4	6	2	25

Table 5: Average similarity values of wheat genotypes

Sr no.	Genotype	Average similarly value	Sr no.	Genotype	Average similarly value
1	AKAW- 2956	0.73	14	AKAW- 4635	0.74
2	LOK- 58	0.79	15	WSM- 4073	0.68
3	MP- 1210	0.72	16	AKAW- 3867	0.76
4	NIAW- 301	0.77	17	AKAW- 4493	0.76
5	UAS- 304	0.78	18	AKDW- 4141- 9	0.76
6	HI- 1454	0.70	19	AKAW-3722	0.69
7	MACS- 2496	0.68	20	AKDW- 4155	0.83
8	AKDW- 4021	0.78	21	AKDW-4132-3	0.87
9	AKAW- 4210-6	0.78	22	AKAW-4627	0.88
10	AKAW- 3997	0.81	23	AKDW-3795-3	0.84
11	AKDW- 4086-3	0.80	24	AKAW-3717	0.95
12	AKAW- 2344	0.72	25	WSM-1445	0.88
13	WSM-1472	0.74		Average	0.777

wheat genotypes under study. In total, 25 RAPD markers amplified 23 fragments with 65.22 % polymorphism. Similarly, Harshvardhan *et al*. (2014) reported 66.83% polymorphism and Bahurupe *et al*. (2013) in chilli have also reported

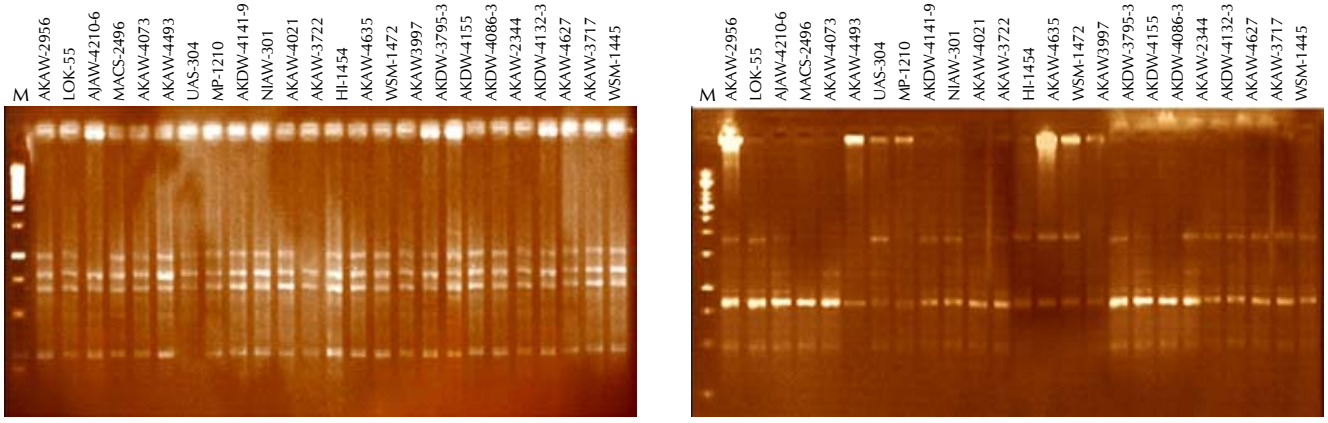

Figure 2: RAPD profile of 25 wheat genotypes generated with primer OPG-13 and OPG-4.

substantial degree of DNA marker-based polymorphism.The details of these six polymorphic markers are given below in (Table 3).

Genetic diversity and relationship

Genetic relationship between wheat accessions was determined on the basis of Jackard IJ pair wise similarity coefficient values.

A dendrogram was generated by UPGMA cluster analysis based on Jackard IJ similarity coefficients (*Fig 1*) and cluster analysis on the basis of coefficient value the accessions, which could be divided into two groups or classes i.e. A and B in which cluster A consist of 15 genotypes and B consist of 10 genotypes.

The cluster A is divided into two sub cluster i.e. A1 and A2. The cluster A1 is again divided into A1a and A1b. The cluster A1a consist of six genotypes and cluster A1b consist of three genotypes. The cluster A1a consist of A1a1 cluster consisting four genotype AKAW-2956 and LOK-58 were found closely related and showed 90.48 per cent genetic similarity. The cluster A1a2 consisting two accessions viz., AKAW-4073 and AKAW-4493 which were closely related and showed 85 per cent genetic similarity while one accession MACS-2496 was distantly related to AKAW-2956 and LOK-58 with 76 per cent genetic similarity. The cluster A1b consists of three genotypes and genotypes UAS-304 and MP-1210 were found closely related and showed 86.04 per cent genetic similarity.

The cluster A2 consist of two clusters A2a and A2b in which each sub cluster consists of three genotype in A2a genotype NIAW-301 and UAS-304 were found closely related and showed 90.48 per cent genetic similarity, however, the third accession AKAW-3722 was found distantly related to NIAW-301 and UAS-304 showing 80 per cent genetic similarity. The cluster A2b consists three genotypes and HI-1454 and AKAW-4635 were found closely related and showed 90 per cent genetic similarity however, the third accession WSM-1472 was found distantly related with HI-1454 and AKAW-4635, showing 78.26 per cent genetic similarity.

The cluster B consist of two sub cluster B1 and B2 in which B1 consist of B1a and B1b cluster in which B1a consists of genotypes AKDW-4132-3 and AKAW-4627 were found closely related and showed 95.45 per cent genetic similarity and B1b consist of AKAW-3717 and WSM-1472 which were found closely related and showed 95.45per cent genetic similarity. The cluster B2 consists of cluster B2a and B2b in which both clusters consists three genotype out of B2a genotype AKAW-3997 and AKDW-3795-3 were found closely related and showed 94.74 per cent genetic similarity and in B2b genotypes AKDW-4086-3 and AKAW-3867-10 were found closely related and showed 80.95 per cent genetic similarity. Rashad (2008) revealed 32 to 97 percent genetic similarity and Mukhtar (2002) 75 to 92 percent similar finding were reported by khaled (2008). The present study was found much similar as compared with previous studies in the different wheat genetic similarity analysis (Table 4).

Similarity based on Jackard IJ coefficient

Genetic relationship between wheat genotypes was determined on the basis of Jackard IJ pair wise similarity coefficient values. The value of similarity coefficient ranged from 0.54 to 0.95.

The genotype AKAW-3717and WSM-1472 represent highest average similarity coefficient value (0.95) similar found in genotype AKAW-4627 and AKAW-3717 the genotype AKAW-2344 and AKAW-4073 represent lowest average similarity coefficient value (0.54). The maximum similarity coefficient of 90.48 was found between AKAW-2956 and LOK-58 and also in AKAW-4021 and NIAW-301 and the lowest similarity coefficient of 56.52 was found AKAW-2956 and AKAW-2344 and AKAW-3867-10. the similarity coefficient of 77.27 was found between HI-1454 with between LOK-58 and UAS - 304,in general the similarity coefficient was ranges from 0.54 to 0.95. Teshale (2003) reported the similarity coefficient between wheat genotype ranges from 0.63 to 0.95 similar result found by Thomas *et al*. (2006), Iqbal *et al*. (2007) and Sajida Bibi *et al*. (2009) the present study was found much similar as compared with previous studies in the different wheat similarity coefficient analysis (Table 5).

Average similarity index

The average similarity coefficient value of each genotype was calculated. The genotype AKAW-3717 a recorded highest average similarity coefficient of 0.95 (Table 5) while, the genotype MACS-2496 and WSM-4073 recorded lowest

average similarity coefficient value of 0.68. The average genetic similarity coefficient value was 0.777.

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