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Genetic finger printing of different cultivars of wheat by using RAPD marker

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Abstract

Genetic diversity analysis of nine wheat (*Triticum astivum*) genotypes was carried out using Random Amplified Polymorphic DNA (RAPD) markers. The 15 RAPD primers amplified a total of 109 bands, out of which 73 were polymorphic. The size of amplified DNA fragment varied from 938.46 to 988.88 bp. The polymorphic bands varied from 45percent in OPA-09 to 100 percent in OPA-12. Dendrogram based Jaccard's similarity coefficient grouped allnine genotypes except one i.e PBW343into one single cluster encompassing threesubclusters. The first subcluster comprised two genotypes *viz;* NIAW1415 and NIAW301while second subcluster grouped another four genotypes *viz;* MACS6222, DBW17, NI5439 and NIAW1885. The third subcluster was comprised remaining two genotypes *viz;* NIAW2030 and NIAW917. The similarity index values ranged from 0.22 to 0.92 indicating the presence of enormous genetic diversity at molecular level. Therefore, RAPD analysis could be used as tool for detecting genetic diversity and can be precisely used for grouping and selection of diverse wheat genotypes.

Key words : *Triticum astivum*, Genetic diversity, RAPD markers.

Introduction

W heat (*Triticum aestivum*L.) is the world's largest cereal crop. It has been described as the 'King of cereals' because of the acreage it occupies, high productivity and the prominent position it holds in the international food grain trade (Shashikala, 2006). W heat is one of the most important and widely cultivated crops in the world, used mainly for human consumption and support nearly 35 per cent of the world population. Nearly 95 per cent of wheat grown today is hexaploid, used for the preparation of bread and other baked products (Debasis and Khurana, 2001). Many wild species of *Triticum* are found in Lebanon, Syria, Northern Israel, Iraq and Eastern Turkey. W heat is cultivated in ancient Greece and Egypt in pre-historic times. Hindukush area is the centre of diversity of hexaploid wheat. The majority of the cultivated wheat varieties belong to three main species of the genus *Triticum*. These are the hexaploid, *T. aestivumL*. (breadwheat), the tetraploid, *T. durum* and the diploid, *T. dicoccum*. Globally, aestivum wheat is most important species which covers 90 per cent of the area. India is the second largest producer of the wheat with 29.9 million ha area and 94.9 million tones production. W heat is grown during winter in the states of Punjab, Haryana, Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh and Maharashtra. W hile in northern states it is grown as irrigated crop, in parts of Madhya Pradesh and Maharashtra it is grown largely under rain fed conditions with little or no irrigation support (FAO, 2013).

Genetic diversity plays an important role in plant breeding either to exploit heterosis or to generate productive recombinants. The choice of parents is of paramount importance in breeding programme. So, the knowledge of genetic diversity and relatedness in the germplasm is a pre-requisite for crop improvement programmers. Reduction in the genetic variability makes the crops increasingly vulnerable to diseases and adverse climatic changes. The precise information on the nature and

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degree of genetic diversity present in wheat collections from its principal areas of cultivation can help to select parents for evolving superior varieties. For the genetic amelioration of this crop, diverse genotypes from the existing germplasm should be selected and used in further breeding programme.

Molecular markers provide new dimension, accuracy and perfection in the screening of germplasm. Accordingly, molecular markers emerged as a tool for many applications of value to crop improvement and proved to be an important way to increase selection efficiency (Bernardo, 2008). Many kinds of molecular markers based on various DNA analysis methods are being used in present day breeding programs in what is known as Marker Assisted Selection (MAS) (Karp, 1997). These include: Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length polymorphism (AFLP), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms (SNPs). RAPD analysis had become popular tool in genetic research for differentiation and determination of phylogenetic relationships among cultivars and detection of genetic differences among species. RAPD assay has been extensively applied to assess genetic diversity in the genus Triticum (Cao *et al*., 1998) showing the narrow genetic base. RAPD provides virtually limitless set of descriptors to compare individual plants and the population. With this innovative tool, genetic diversity can be estimated and equally it is possible to carry out large scale screening of genetic resources held in gene banks, natural populations, ecosystems, and natural reserves with this quick and rapid technique.

Materials and Methods

The present study under titled “Genetic finger printing of different cultivars of wheat by using RAPD marker” was carried out at Department of Plant Biotechnology, Lokmangal College of Agril Biotechnology Wadala.

Plant Material

The seeds of all nine wheat varieties *viz;* NIAW 1815, NIAW 301, PBW 343, NIAW 2030, NIAW 917, MACS 6222, DBW 17, NI 5439 and NIAW 1415 were procured from Agriculture Research Station, Pandharpur.

DNA Isolation

The genotypes were grown in pots. Genomic DNA was isolated from the young leaves as per modified CetylTrimethyl Ammonium Bromide (CTAB) method of Murray and Thompson (1980). The quality and quantity of DNA was determined by nano spectrophotometer.

PCR and RAPD Analysis

PCR amplification was performed with random decamer primers obtained from Operon Technologies (Almeda, Calif., USA). Amplification was performed in a 25 µl reaction volume containing Taq Buffer B (10X Tris without $MgCl_2$), 25 mM MgCl2, 10 pmol RAPD primer, 30 ng genomic DNA, and 1 U Taq DNA polymerase (Bangalore Genei, Bangalore, India). Amplification was performed in an Eppendorf Master Cycler. Amplification conditions comprised initial denaturation at 94°C for 4 min, denaturation94°C for 1 min, annealing 1.5 min, extension 72°C for 2 min and followed by final extension of 4 min at 72°C and total 40 cycles were set. Amplified products were separated on 1.5% agarose gel in 1 × TBE buffer (100 mMTris-HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) at 50 V. The gels were stained with 0.5 µg/ ml ethidium bromide solution and visualized by illumination under UV light.

Data scoring and analysis

RAPD data were scored as present (1) or absent (0) for the estimation of similarity among all varieties. The matrix of similarity was calculated using the Jaccards’ coefficient and the dendrogram obtained by clustering according to the Unweighted Pair-Group Method with arithmetic averages (UPGMA) using NTSYS-pc ver 2.2*i*.

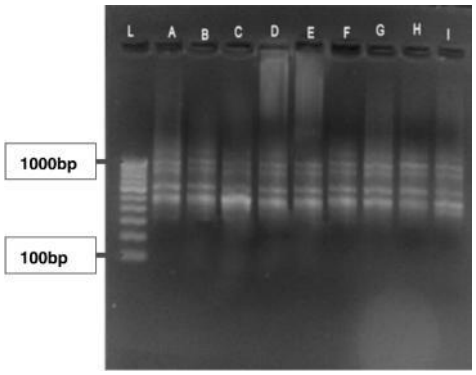
Result and Discussion

Genomic DNA from wheat plants was extracted by CTAB method of Doyle and Doyle (1990) with some minor modification.The quantity and absorbance ratio of isolated DNA was ranged 158 ng/µl to 425 ng/µl and 1.6 to 1.9 respectively. The genomic DNA extracted from each genotype was subjected to polymerase chain reaction using 15random decamer primers. Off 15, four RAPD primers were showed positive results. Hence out of 15, four primers were taken for analysis. The PCR analysis of 9 wheat varieties taken in this study, with 4 polymorphic random markers generated 109 scorable bands (plate no.1 and 2). Among RAPD markers, OPA-9 and OPA-15 produced maximum number of bands (33 in all varieties) followed by OPB-02 (30) and OPA-12 (30). Polymorphic bands in screened markers were ranged from 13 to 24.The percent polymorphism in banding pattern was calculated and it was highest in OPA-12 (100%) followed by OPA-15 (72%), OPB-02 (70%) and OPA-09 (45%).The total percent polymorphism showed by RAPD marker given in table

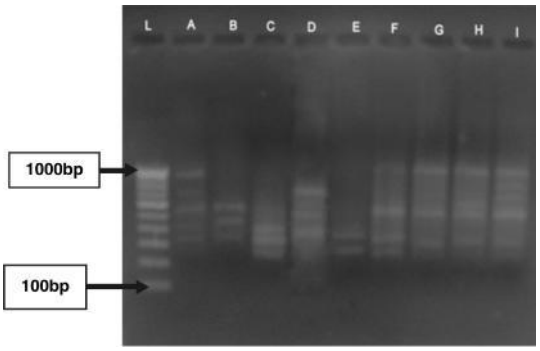
no.1. In case of percent polymorphism near about same result was found byRashed, M.A *et al*.,(2008)was usedRAPD markers for fingerprintingof thirty hexaploid wheat varieties *(Triticum aestivum*L.*)* from different genetic background. A total of 76 DNA fragments were amplified, using five primers, ranging in size from 1884 to 200 base pairs. Out of the 76 amplified products, 19.7% were monomorphic and 80.3% were polymorphic, averaging 12.2 polymorphisms per primer. Bibi*et al*., (2010) assessed twenty four wheat varieties/lines through RAPD for genetic diversity. Of forty primers, thirteen were able to amplify the genomic DNA and yielded 269 polymorphic bands. The percentage of the polymorphic loci was 86.22%. The highest PIC value found in OPB-02 while lowest PIC value found in OPA-12 (Table No : 01)

Table 1. Total percent polymorphism and PIC value of RAPD marker

Sr. No.	Primer code	Low mol. Wt. (bp)	High mol. Wt. (bp)Band	Total No. of band	No. of poly-morphic band	Percent polymorphism	PIC Value
1.	OPA-09	255.21	938.46	33	15	45%	0.75
2.	OPB-02	189.20	965.20	30	21	70%	0.80
3.	OPA-15	211.47	988.88	33	24	72%	0.78
4.	OPA-12	234.31	988.08	13	13	100%	0.72
			Total	109	73	71%	



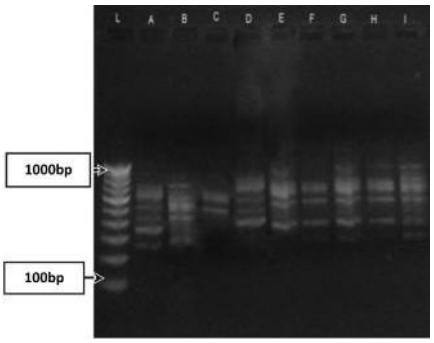
(A) RAPD profile of wheat (*Triticum aestivum*L.) with primer OPA-09



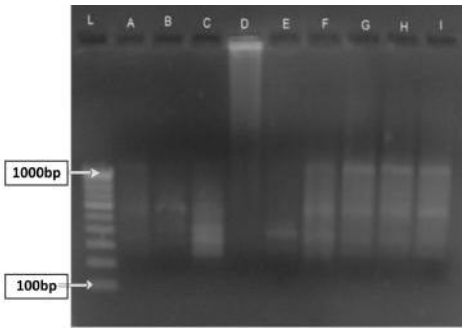
B) RAPD profile of wheat (*Triticum aestivum*L.) with primer OPB-02

Lane L – Ladder 100 bp, Lane A – NIAE-1415, Lane B – NIAW-301, Lane C – NIAW-343, Lane D – NIAW-2030, Lane E – NIAW-917, Lane F – MACS-6222,Lane G – DBW-17, Lane H – NI-5439,Lane I – NIAW-1815

Plate 1. Banding pattern of RAPD marker in wheat varieties with primer OPA-09 and OPB-02.



(C) RAPD profile of wheat (*Triticum aestivum*L.) with primerOPA-15



(D) RAPD profile of wheat (*Triticum aestivum*L.) with primer OPA-12

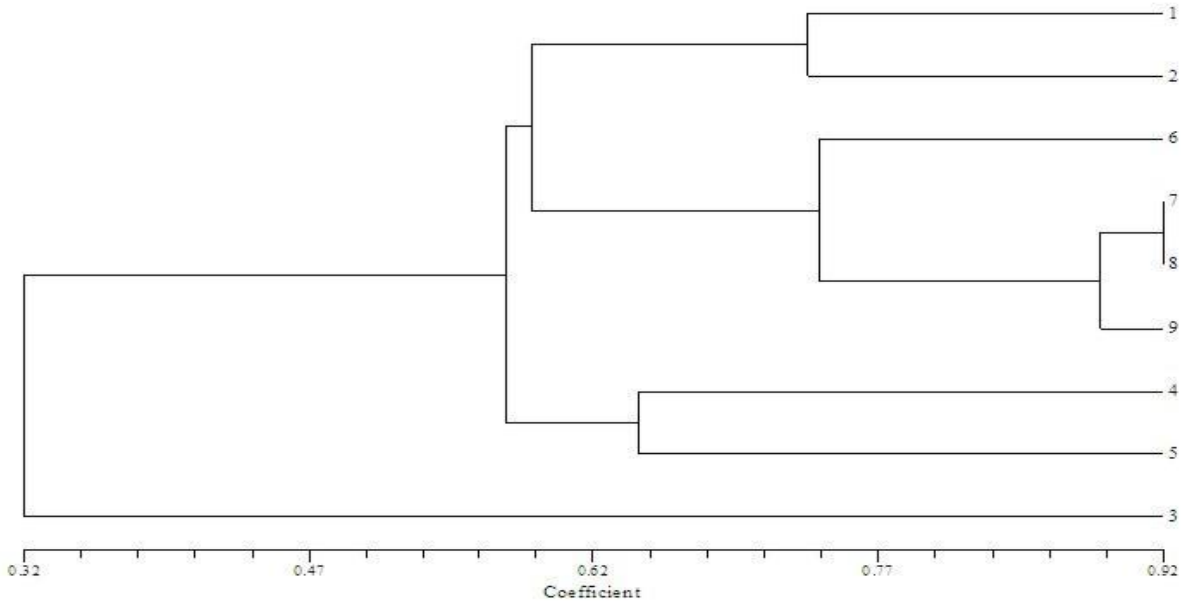
Lane L – Ladder 100 bp, Lane A – NIAE-1415, Lane B – NIAW-301, Lane C – NIAW-343, Lane D – NIAW-2030, Lane E – NIAW-917, Lane F – MACS-6222, Lane G – DBW-17, Lane H – NI-5439,Lane I – NIAW-1815

Plate 2. Banding pattern of RAPD marker in wheat varieties with primer OPA-15 and OPB-12.

A dendrogram was generated using UPGMA cluster analysis based on Jaaccard's similarity coefficient revealed that average similarity was sixty six. Diversity analysis of wheat using RAPD markers through clustering (Fig. no.1) showed that 9 wheat varieties were grouped in one major cluster except PBW 343.In major cluster covered total 8 verities namely NIAW 1415, NIAW 301, NIAW 2030,NIAW 917,MACS 6222, DBW 17, NI 5439, NIAE 1815.

In similarity index, NI-5439 and NIAW-1815shows highest similarity (0.92)while NIAW-1415 and PBW-343 shows lowest similarity coefficient (0.22) (Table 02). The findings are similar to Nawroz A. T. *et al.*, (2008) reported genetic diversity among 11 durum and bread wheat genotypes was studied using random amplified polymorphic DNA (RAPD) analysis. A total of 70-75 DNA fragments were amplified with 10 random decamer primers 40 per cent (bread) and 35.7per cent (durum wheat) of which were polymorphic. Genetic similarity matrix based on Jaaccard'S index detected coefficients ranging from 0.5 to 0.952 bread wheat and 0.102 to 0.917 durum wheat.

Fig 1 : Dendrogram showing genetic diversity for RAPD marker in wheat



1. NIAW1415 2.NIAW301 3.PBW343 4.NIAW2030 5.NIAW917 6.MACS6222, 7.DBW17 8.NI5439 9. NIAW1885.

Table 2. Similarity matrix of RAPD marker in wheat varieties

Varieties	NIAW1415	NIAW301	PBW343	NIAW2030	NIAW917	MACS6222	DBW 17	NI5439	NIAW 1815
NIAW1415	1.0000000								
NIAW301	0.7333333	1.0000000							
PBW343	**0.2222222**	0.2857143	1.0000000						
NIAW2030	0.5294118	0.4666667	0.5000000	1.0000000					
NIAW917	0.5882353	0.6428571	0.3571429	0.6428571	1.0000000				
MACS6222	0.7058824	0.6666667	0.3125000	0.5625000	0.6250000	1.0000000			
DBW17	0.5555556	0.5000000	0.3333333	0.6000000	0.5625000	0.8000000	1.0000000		
NI 5439	0.5882353	0.5333333	0.2666667	0.5333333	0.6000000	0.7333333	0.9230769	1.0000000	
NIAW 1815	0.6470588	0.5000000	0.2500000	0.6000000	0.5625000	0.6875000	0.8571429	**0.9230769**	1.0000000

Conclusion

The primer OPA-12 shown 100% percent polymorphism followed by OPA-15 (72 %), OPA-02 (70%), hence all these three primers are very informative for the analysis of diversity in wheat.Average percent polymorphism is found to be 71% across the nine wheat varieties so here it can be conclude that the RAPD primers are very feasible to reveal different molecular insights in wheat.

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