Recent Research in Science and Technology 2013, 5(2): 29-32
ISSN: 2076-5061
Available Online: http://recent-science.com/

Molecular characterization of wheat (*Triticum* species) using random amplified polymorphic DNA marker

Sarika Shende and Sanjay Walunj

Department of Plant Biotechnology, College of Agril. Biotechnology, M.A.U., Latur, M.S, India

Abstract
Thirty five random primers were used to generate the RAPD profiles of 17 wheat accessions. Six markers that showed reproducibility were used to detect polymorphism. Based on the percentage polymorphism and unique banding profiles, five primers OPX-02, OPX-06, OPX-07, OPX-08 and OPX-09 were found to be highly discriminative. Genetic variability between wheat accessions based on Dice's coefficient ranged from 23 to 57% across all genotypes with an average of 46%. Cluster analysis based on UPGMA method separated all the 17 wheat accessions in two distinct clusters. This low polymorphic result shows low genetic base in wheat accessions.

Keywords: Wheat, genetic diversity, genotype, RAPD markers.

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). 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.

In the present study PCR based molecular marker *viz*., Random Amplified Polymorphic DNA (RAPD) was carried out for their potential application in diversity analysis and fingerprinting of wheat genotypes.

MATERIALS AND METHODS

Plant materials

Seventeen cultivated accessions of *Triticum* species, collected from Wheat Research Station, Marathwada Agricultural University, Parbhani (M.S) were used in the present investigation (Table 1).

DNA isolation and PCR amplification

Total genomic DNA was isolated from coleoptiles of each genotype, by following the modified CTAB method (Seghai-Maroof *et al*., 1984; Bhat *et al*., 1999). Purified genomic DNA was subjected to PCR amplification using random primers (RAPD). A 25µl mixture contained 50ng of genomic DNA, 1.5U *Taq* DNA polymerase (Genetix), 1XPCR buffer containing 4mM $MgCl_2$, 0.2 mM of each dNTPs (Genetix), 15 pmol of primers. Amplifications were carried out using a 96 thermal cycler (Bio metra) programmed for 40 cycles as follows: initial denaturation at 94°C for 4 min, further denaturation at 94°C for 1 min, annealing at 30°C for 1 min, extension at 72°C for 3 min and final extension for 10 min at 72°C. The amplification products were stored at 4°C until loading. The PCR products were resolved at 100Volts for 4 hours on 1.2% agarose gel prepared in 1xTBE buffer. Gel was photographed using Gel-Documentation system (ALPHAIMAGER TM 2200).

Data Analysis

Only clear and reproducible DNA fragments were scored as 1-0 matrix for the presence and absence of band, respectively.

*Corresponding Author

Sarika Shende
Department of Plant Biotechnology, College of Agril. Biotechnology, M.A.U., Latur, M.S, India

Email: sarikasinde@gmail.com

Dendrogram was constructed based on UPGMA clustering of a similarity matrix generated by Dice's coefficient. All the above analysis were done using NTSYS-PC (version 2.0) program (Rohlf, 1990).

RESULTS

Molecular marker data in conjunction with the morphological data could be highly useful in precise differentiation of the inbred lines and consequently, their planned utilization in hybrid breeding programme. Thirty five random 25 decamer *viz*., OPA, OPB, OPG, OPH and OPX series (MWG Biotech, Banglore) primers were employed for RAPD analysis of 17 wheat accessions (Table 2). Of these, six primers were selected on the basis of reproducible and scorable amplification products for studying the relationships among 17 wheat genotypes. These six random primers generated 54 amplicons of which 10 were polymorphic, at an average of 1.66 polymorphic amplicons per primer. The number of amplified fragments revealed by each primer ranged from 01 (OPX-07 and OPX-08) to 04 (OPX-09). The amplification profile of one of the primer is presented in fig. 1. The amplified product size ranged from 250bp (OPX-02) to 2000bp (OPX-12). Among these six primers used five primers OPX-02, OPX-06, OPX-07, OPX-08 and OPX-09 were found to represent polymorphism. The primer OPX-09 exhibited highest polymorphism (27.50%) amongst all the primers.

Diversity Analysis

Dendrogram based on genetic similarity values were constructed to reveal similarities between accessions (fig. 2). On the basis of 40% coefficient value the accessions were divided into two groups i.e., A and B, in which cluster A consists of 15 genotypes *viz*., PBN-1666-1, Sehore, Kalyansona, Parbhani-51, Sonalika, PBN-4339, PBNS-4158, PBN-4025, PBNS-3953, PBNS-3933, PBNS-3958, PBNS-3940, Sharbati, PBN-4501, and PBNS-4110 and B consist of two genotypes, PBNS-3905 and HD-2189. The cluster B was found distantly related to cluster A with 29% genetic similarity. The cluster–A is again divided into two subgroups A_1 and A_2. The cluster A_1a_1 in which PBN 1666-1 and Sehore were found closely related and showed 68% genetic similarity as both are MP local genotypes. In cluster A_1a_2 two accessions *viz*., Parbhani-51 and Sonalika were closely related and showed 79% genetic similarity as they were developed through hybridization. The cluster A_1b_2 consists of five genotypes *viz*., PBNS-3958, PBNS-3953, PBNS-3942, PBNS-3933, PBNS-3905 in which all are 50KR mutant line except PBNS-3933 (40KR).

The cluster B comprised of two genotypes PBNS-3905 and HD-2189, was found closely related and showed 41% genetic similarity, as PBN-3905 is derived from Sharbati mutation (30KR) and HD-2189 is developed through hybridization and selection of HD-1931.

Table 1. List of wheat genotypes with their pedigree used in the present study.

S.No.	Genotype	Pedigree
1	PBN 1666-1	MP local
2	Kalyansona	(Fn-K58 x NTHN-103) x (Gobo 55)
3	Sharbati	Local genotype (landrace)
4	Sehore	MP local
5	Parbhani 51	BUC 'S'/FLK'S
6	Sonalika	(II 53-388-An) x (Yt-54xN 10B) L Rojo
7	PBN-4501	PBN 1607 x PBN 3375
8	PBN-4339	PBN 3255 x Kalyansona
9	PBNS-4158	Sharbati M8 50 KR
10	PBNS-4110	Sharbati M8 50 KR
11	PBNS-4025	PBN 51 x Sharbati RM4
12	PBNS-3958	Sharbati M8 50 KR
13	PBNS-3953	Sharbati M8 50 KR
14	PBNS-3942	Sharbati M8 50 KR
15	PBNS-3933	Sharbati M8 50 KR
16	PBNS-3905	Sharbati M8 50 KR
17	HD-2189	HD-1963/HD-1931

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

S. No.	Primer	Sequence (5'-3')	Total number of amplicons	Number of polymorphic amplicons	Percent polymorphism
1	OPX-02	TTCCGCCACC	9	2	22.22
2	OPX-06	ACGCCAGAGG	8	2	25.00
3	OPX-07	GAGCGACGCT	8	1	12.50
4	OPX-08	CAGGGGTGGA	7	1	14.28
5	OPX-09	GGTCTGGTTG	11	4	27.50
6	OPX-12	TCGCCAGCCA	11	0	00.00
		Total	54	10	18.51
		Average	09	1.66	--

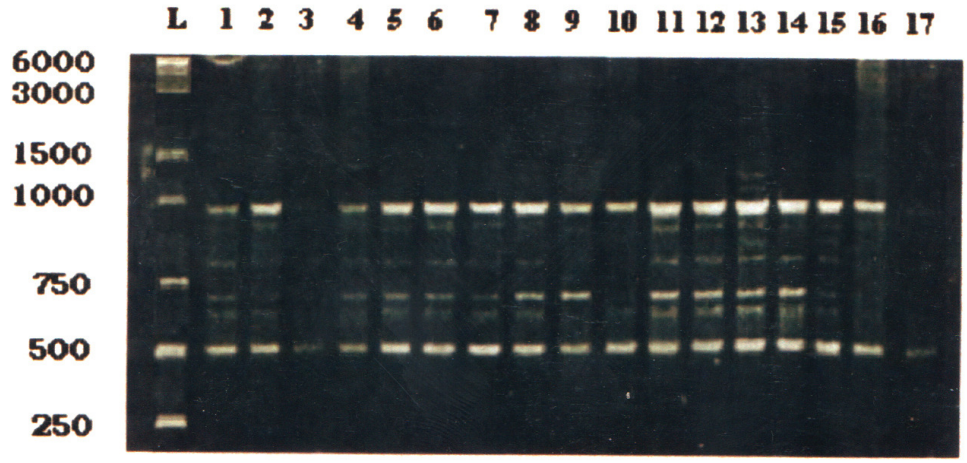

Fig 1. RAPD profile of 17 wheat genotypes generated With primer OPX-06. L: 250bp DNA ladder, Lanes1-17: correspond to wheat genotypes listed in Table 1.

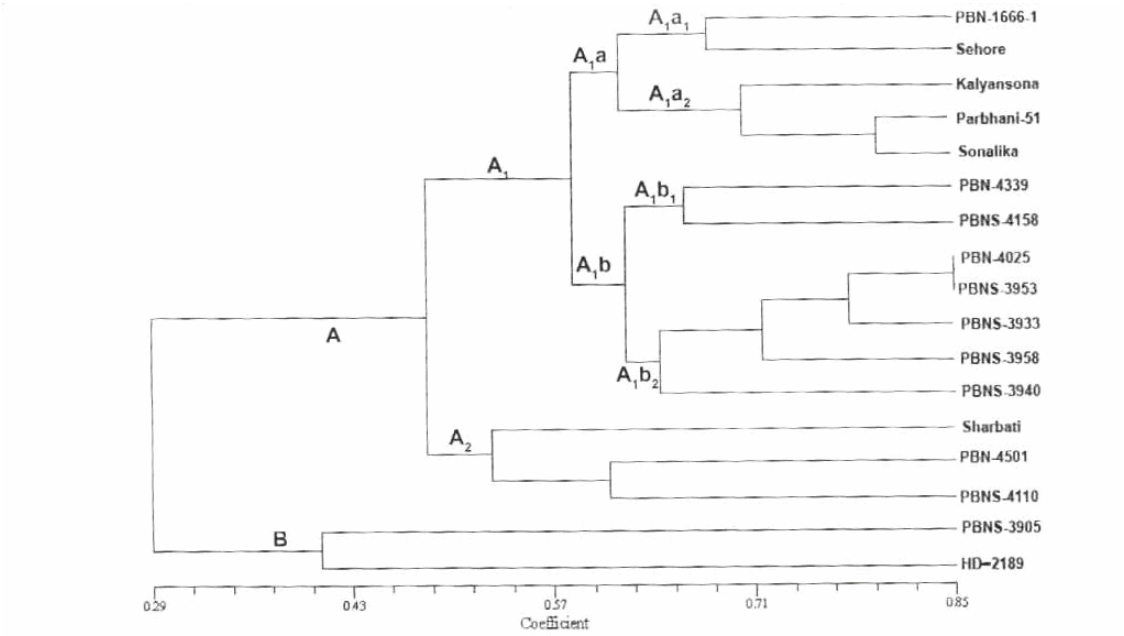

Fig.2. Dendrogram generated through UPGMA analysis showing genetic relationship among the 17 wheat genotypes. Names of the genotypes are given on the termini of branches.

DISCUSSION

Genetic variability between 17 wheat accessions based on Dice's coefficient ranged from 23 to 57% across all genotypes with an average of 46%. Similar level of diversity (18-48%) has been reported in *Triticum* species by Grewal *et al*. (2007) whereas Iqbal *et al*. (2007) using RAPD markers observed very low level of polymorphism (7-13.2%). The present findings also supported the previous studies where low level of polymorphism (32%) was detected by the arbitrary primers when compared to other RAPD studies in *Triticum* species (Kumar *et al*., 2006). These low polymorphic results indicates low genetic base in wheat accessions and the genetic diversity may be due to their characters and amplification protocol used for selection of suitable primers.

Cluster analysis revealed that, germplasm lines HD-2189, PBNS-3905, Sharbati, PBNS-4110 and PBN-4501 were found to be quite distant. These accessions can be used as germplasm resource for broadening the genetic base of cultivated wheat in India. Besides, these can be used in generating intra-specific traits like seed boldness and yield. For broadening genetic base of wheat genome, these alone, however, would not be sufficient as we additionally need to screen large number of germplasms and identify the putative diverse germplasms of exotic origin in the background of well adapted Indian cultivars. Inter-specific hybridizations and introgressions are other viable options to diversify the genetic base of wheat.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Biotechnology, Government of India, New Delhi, for providing financial support for above study.

REFERENCES

[1] Bhat K.V., Barekar P.P. and Lakhn Paul S. 1999. Study of genetic diversity in Indian and exotic sesamum (*Sesamum indicum* L) germplasm using RAPD markers. *Euphytica*. 110: 21-33.

[2] Grewal S., Kharb P., Malik R., Jain S. and Jain R. K. 2007. Assessment of genetic diversity among some Indian wheat cultivars using random amplified polymorphic DNA (RAPD) markers. *Indian Journal of Biotechnology*. 6: 18-23.

[3] Harris S.A. 1999. RAPDs in systematics: a useful methodology? In: Hollingsworth PM, Bateman RM, Gornall RJ ed(s). Molecular systematics and plant evolution. London: Taylor and Francis, Pp 211-228.

[4] Iqbal A., Khan A.S., Awan F.S. and Ahmad A. 2007. Study of genetic divergence among wheat genotypes through random amplified polymorphic DNA. *Genet. Mol. Res*. 6(3): 476-481.

[5] Kumar M., Luthra O.P., Chawla S.A. and Chaudhari M.J. 2006. Identification of RAPD markers linked to the karnal bunt resistance genes in wheat. *Biologia Plantarum*. 50 (4): 755-758.

[6] Poelham J.M. and Sleper D.A. 1995. Breeding of field crops, 4th edn. Panima Publishing Corporation, New Delhi. Pp. 207-213.

[7] Rohlf PJ. 1990. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.02. Applied Biostatistics, New York. pp.34-42.

[8] Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A. and Allard R.W. 1984. Ribosomal spacer length polymorphism in Barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci*. USA. 81: 8014-8019.

[9] Smith J.S., Smith O., Bowen S.L., Tenborg R.A. and Walls S.J. 1991. The description and assessment of distance between inbred lines of maize: A revised scheme for testing of distinctness between inbred lines utilizing DNA RFLPs. *Maydica*. 36: 213-226.

[10] Smith S. and Helentjaris, T. 1996. DNA fingerprinting and plant variety protection. In: Paterson AH (ed). Genome mapping in plants, Landes Campany, Texas. Pp 95-110.

[11] Stojalowski A., Lapinski M. and Masojc P. 2004. RAPD markers linked with restorer genes for the C-source of cytoplasmic male sterility in rye (*Secale cereale* L.). *Plant Breeding*. 123: 428-33.

[12] Varshney R.K., Graner A. and Sorrells M.E. 2005. Genic microsatellite markers in plants: features and applications. *Trends Biotech*. 23: 48-55

[13] Welsh J. and McClelland M. 1991. Fingerprinting genomes using PCR with arbitrary primers and a matrix of pair wise combinations of primers. *Nucleic Acid Res*. 19 (19): 5275-5279.

[14] Williams J.K.F., Kubelik A.R., Livak K.G., Rafalki J.A. and Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* 18 (22): 6531-6535.