



Assessment of genetic diversity in bread wheat (*Triticum aestivum* L.) using RAPD markers

Amit Kumar¹, R. S. Sengar², Vivekanand Pratap Rao, ¹Gyanika Shukla, Rekha Dixit¹ Raj Singh³

¹Department of Biotechnology, Faculty of Science, Swami Vivekanand Subharti University, Meerut-250005 (UP), INDIA

²Department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110 (U.P.), INDIA

³Department of Botany, Faculty of science, Swami Vivekanand Subharti University, Meerut-250005 (UP), INDIA

*Corresponding author. E-mail: amit.agbiotech1581@gmail.com

Received: November 11, 2016; Revised received: March 30, 2017; Accepted: August 20, 2017

Abstract: The present study aimed to evaluate the genetic diversity of 10 wheat cultivars by Random Amplified Polymorphic DNA (RAPD) marker. The genomic DNA of 10 wheat genotypes were amplified with 10 RAPD primers that produced 53 amplified band, out of which 23 band were polymorphic (43.39%). The number of fragment amplified per primer ranged from 4 to 9. Primer A01 generated maximum number of amplified band, out of which 5 band were polymorphic. Cluster analysis of wheat genotypes were based on UPGMA method. Cluster analysis of 10 wheat genotypes were classified in to two main group; single variety AKW 1071 was placed in first group and rest 9 variety were placed in second group. The pair wise similarity values ranged from 0.58% to 100% and showed that cultivars Raj-3765 and K-7903 were the closest with highest similarity value (100%), while genotypes AKW 1071 and K9006 showed minimum similarity value (62%). The present study indicated the presence of high genetic diversity among wheat cultivars, which could be used for the developing core collection of wheat germplasm for breeding purpose.

Keywords: Cluster analysis, RAPD marker, UPGMA method, Wheat genotypes

INTRODUCTION

Wheat is a major staple food in all over the world. Wheat production in India has a crucial role in Agriculture. The overall production of wheat in India in 2013-14 was 95.6 mt. In the past few decade morphological characters were using for the characterization of germplasm. Such characters are limited in number and show growth stage and environment dependent expression. From the last few decade isozymes were using for the study of genetic diversity (Hemrick and Godt., 1990). In Recent year molecular marker are being used for the assessment of genetic diversity and identification of crop genotype (Tar'an *et al.*, 2005, Sunil kumar *et al.*, 2016). Most of the molecular markers such as RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter Simple Sequence Repeat) have been used for the assessment of genetic diversity. Among several molecular marker RAPD is mostly use for the assessment of genetic diversity in several important agronomic crops (Mahpara *et al.*, 2012). RAPD could be easily used for the screening of breeding population at large scale (Demekeet *et al.*, 1996). It has been successfully used

for the assessment of genetic diversity of diploid, tetraploid and hexaploid wheat (Sivolap *et al.*, 1999). The wheat genotypes were grown at the pots of the Swami Vivekanand Subharti University, Meerut. The investigation of wheat cultivars by RAPD primers were used to evaluate individual genotype (Ali Ammar *et al.*, 2013, Kumar *et al.*, 2015). The aim of this study was to assess genetic diversity of ten Indian wheat cultivars using RAPD marker.

MATERIALS AND METHODS

Plant material: Seeds of ten selected wheat genotypes (Table-1) were collected from Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut.

DNA extraction and RAPD genotyping: CTAB (Cetyltrimethylammonium bromide) method was used for the extraction of DNA from the leaves of the plant, which is modified method of Doyle and Doyle (1987). The leaves of wheat plants were collected and crushed in liquid nitrogen. After collecting the crushed powder of leaves in eppendorf tubes, 500 micro liter of CTAB buffer was added in to each eppendorf tubes. Composition of CTAB buffer was 1.4 M NaCl, 100 mM Tris, 20 mM EDTA and 2% CTAB. The mixture of leaf

tissue and CTAB buffer were incubated for 1 hour at 60°C. After one hour of incubation equal volume (500 µl) of chloroform and Isoamyl alcohol ratio (24.1) was added and shaken until a homogenous mixture was obtained. Samples were centrifuged at 10000 rpm for 10 minute in bench centrifuge. The supernatant were transferred into fresh eppendorf and cold isopropanol (250 µl) were added in eppendorf and mixed gently to precipitate the DNA at -20°C for overnight. Thereafter samples were centrifuged at 10000 rpm for 10 minute and pellet was deposited at the bottom of eppendorf was recovered after supernatant removal from the samples. After removing the supernatant, the pellet was washed with 70% ethanol and dried at room temperature for 30 minute. Thereafter pellet was dissolved in 50 µl of TE buffer and stored at 4°C until used. Ten RAPD primers are used for the amplification of DNA. The sequence and code of RAPD primer were given in Table-1. The reaction mixture for RAPD genotyping

was carried out as the follows, in 25 µl reaction mixture contained .75ng DNA, 0.2mM of each dNTP, 20pM primer and one unit Taq polymerase were used per amplification. Thermal PCR was used for the amplification of DNA. The PCR program for the amplification was: initial denaturation at 94°C for 5 minute, 40 cycles of 94°C for 1 minute, 35°C of annealing for 2 minute and finally extension at 72°C for 5 minute. After PCR amplification products were separated at 1.5% agarose gel in 1 X TAE buffer at 50 V constant voltage for one hour. Gels were stained with ethidium bromide and images were obtained by molecular analyst program of Bio-Rad. A 100 bp ladder used for analysis of amplified band. Data were recorded as presence (1) or absence (0) of each amplification band, to construct the band matrix.

The Similarity coefficient of 10 wheat cultivars was calculated according to Nei and Lis (1979). The similarity matrix (Table-3) calculated using jaccard's coefficient (Jaccard, 1908) and dendrogram (Fig-1) obtained by clustering according to UPGMA (Unweighted Pair Group Method with Arithmetic average).

RESULTS AND DISCUSSION

In the study 10 RAPD primers (Table-2) were used for the genetic diversity of 10 wheat cultivars. In the wheat genotypes 23 bands showed polymorphism out of 53 amplified bands. Primer A01 and A16 displayed (Fig. 4) high number of Amplified bands while minimum numbers of amplified bands were detected in B-

Table 1. List of Ten Wheat cultivars with their pedigree.

S. N.	Variety	Pedigree
1	AKW 1071	VEE'S/ 3FLN/ACC// ANA
2	K 9006	CPAN 1687 /HD 2204
3	K 0307	K 8321/UP 2003
4	K 7903	HD 1982/K816
5	RAJ 3765	HD 2402/VL639
6	LOK 1	S308 / S331
7	V1	BL 1724/BL1887//BL2031
8	V2	BL 1724/BL1887//BL2031
9	V3	BL 1724/BL1887//BL2031
10	K 65	C591/NP773

Table 2. Primer Sequence, percent of GC content and % of polymorphism of selected RAPD Primer.

RAPD Primer	Sequence	GC%	Amplified Band	Polymorphic Band	% of Polymorphic Band
A01	5' CAG GCC CTT C 3'	70%	9	5	55%
A16	5' AGC CAG CGA A 3'	50%	7	1	14.28%
A20	5' GTT GCG ATC C 3'	60%	5	3	60%
B18	5' CCA CAG CAG T 3'	60%	5	3	60%
B19	5' ACC CCC GAA G 3'	70%	4	1	25%
C13	5' AAG CCT CGT C 3'	60%	5	2	40%
OPA03	5`-CAG GCC TGA C-3	70%	4	2	50%
OPA04	5`AAT CGG GCT G3`	60%	5	2	40%
OPA07	5`GAA ACG GGT G3	60%	5	2	40%
OPA08	5`GTG ACG TAG G3`	60%	4	2	50%
		Total	53	23	

Table 3. Similarity matrix showing the relationship among the 10 wheat genotypes based on RAPD data.

Genotypes	AKW1071	K9006	LOK1	V3	RAJ3765	K0307	V1	V2	K7903	K65
AKW1071	1.00									
K9006	0.43	1.00								
LOK1	0.56	0.87	1.00							
V3	0.60	0.81	0.93	1.00						
RAJ3765	0.60	0.70	0.82	0.76	1.00					
K0307	0.58	0.60	0.52	0.56	0.66	1.00				
V1	0.53	0.56	0.58	0.62	0.73	0.75	1.00			
V2	0.61	0.62	0.75	0.68	0.80	0.57	0.64	1.00		
K7903	0.60	0.70	0.82	0.76	1.00	0.66	0.73	0.80	1.00	
K65	0.69	0.58	0.70	0.64	0.86	0.76	0.71	0.78	0.86	1.00

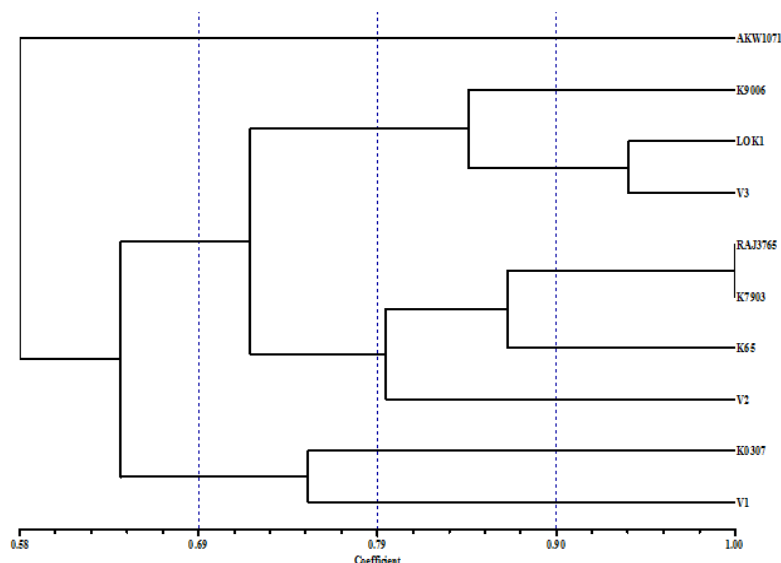


Fig. 1. Dendrogram Illustrating genetic relationships among 10 wheat cultivars, Generated by using UPGMA based on jaccard's coefficient.

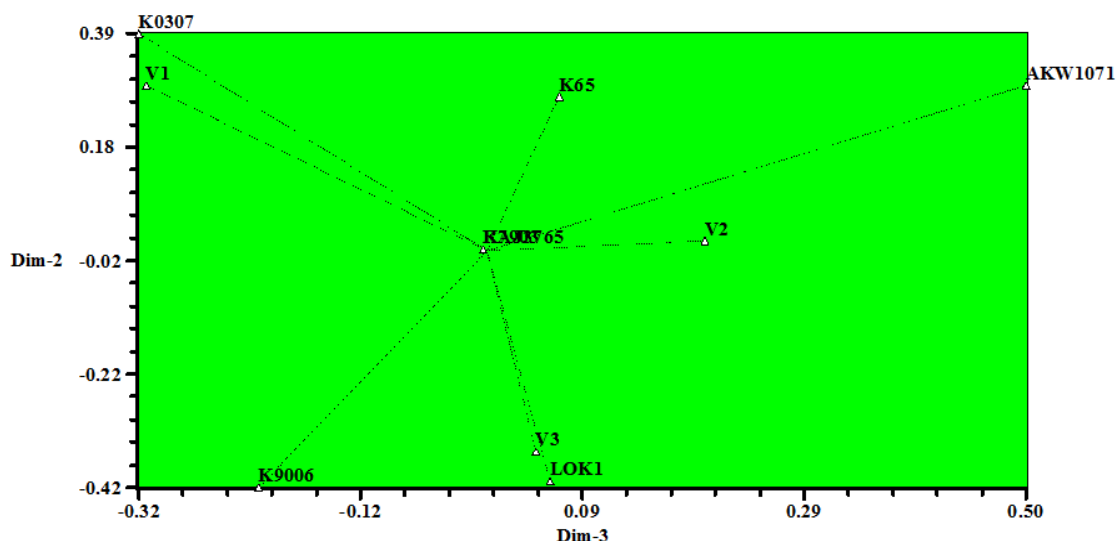


Fig. 2. Two-Dimensional structure of cultivars examined based on RAPD data.

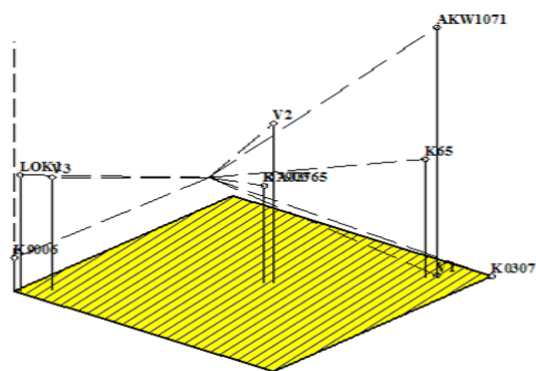


Fig. 3. Three-Dimensional structure of cultivars examined based on RAPD data.

19 followed by OPA 03 and OPA 08. In this experiment highest number of percent of polymorphism were detected in A01 (55%) and minimum number of percent of polymorphism was detected in A16 (14.28%). The Similarity matrix based on possible pair of varieties ranged from 0.43 to 1.00 for hexaploid wheat cultivars. The minimum pair wise similarity (0.43) was recorded between AKW 1071 and K9006 while maximum (1.00) pair wise similarity observed between Raj 3765 and K7903. The present investigation revealed that the high degree of genetic variability within the species.

The dendrogram constructed on the basis of the similarity coefficient showed (Fig-1) that the cultivars of

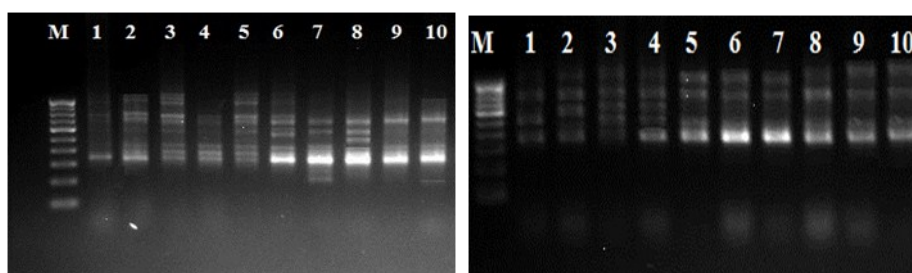


Fig. 4. RAPD amplified Product with 10 wheat cultivars using A01 and A16 Primers; M-Marker, 1- AKW1071, 2-K9006, K0307, K7903, RAJ 3765, Lok-1, V1,V2,V3 and K65.

wheat can be divided into two main groups. AKW1071 was placed in Ist group and rest all nine varieties (K9006, Lok1, V3, Raj 3765, K-7903, K65, V2, K0307 and V1) were placed in IInd group. IInd group can be further divided into two sub groups. II A and II B. In II B K0307 and V1 are placed and rest seven varieties are placed in II A group. II A can be further divided into II A₁ and II A₂. In subgroup II A₁ has three varieties K9006, Lok 1 and V3 and Raj 3765, K7903, K65 and V2 are placed in subgroup II A₂. Two and three dimensional Structures (Fig-2 & Fig-3) of 10 wheat cultivars examined which is based on molecular data. The present research work observed that the RAPD marker is valuable DNA marker for analysis of genetic diversity. Similar research works have been done by Siddiqui *et al.*, (2010), Farrakh *et al.*, (2011) and Shabana Parveen *et al.*, (2013). Grewal *et al.*, (2007) observed that RAPD show maximum polymorphism among 20 Indian wheat cultivars.

Conclusion

It was concluded that the use of molecular marker such as RAPD has been verified for a considerable genetic diversity and genetic relationship between Indian wheat cultivars. The data obtained from this study confirmed that the efficiency of RAPD marker is high for the determination and estimation of genetic similarity among different wheat genotypes. In the present investigation we observed that the high level of diversity seemed in 10 wheat cultivars. We found that the maximum genetic similarity occurs in K7903 and Raj 3765 while minimum genetic similarities were found in AK1071 and K9006. Finding in this study unraveled the efficiency of RAPD marker for assessment of genetic diversity, these marker would prove to be useful resource for wheat breeding. Finding in this study unraveled the efficiency of RAPD marker for assessment of genetic diversity. These RAPD markers would also be helpful in genomic mapping studies and for the development of wheat cultivars with diverse genetic background and to obtain improved crop productivity.

ACKNOWLEDGEMENTS

Authors are highly obliged to Dr. Rekha Dixit, Depart-

ment of Biotechnology in the Keral Verma Faculty of Science, Swami Vivekanand Subharti University, Meerut for the encouragement and constructive suggestions.

REFERENCES

- Ali, Ammar., Ali, Nawab, Ali, Imran., Adnan, Muhammad., Ullah, Nimat., Ahmed Zahoor (2013) Morphological and genetic diversity of Pakistani Wheat germplasm under drought stress. *International Journal of Advancements in Research & Technology*.2(5):186-193.
- Demeke, T., Laroche, A., Gaudet, D.A. (1996). A DNA marker for the Bt-10 common bunt resistance gene in wheat. *Genome J.* 39: 51–55.
- Doyle, J. J. and Doyle, J. L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phyto. Bull.* 19: 11–15.
- Farrakh, S., Ahmad, Mirza, J. I., Hameed, S., Kazi, M., Ashraf, M. (2011) RAPD analysis of stripe rust resistant synthetic hexaploid of wheat. *Int. J. Appl. Sci. Technol.* 1:3.
- Grewal, S., Kharb, P., Malik, R., Jain, S., Jain, R.K. (2007) Assessment of genetic diversity among some Indian wheat cultivars using random amplified polymorphic DNA (RAPD) markers. *Indian J. Biotech.* 6:18-33.
- Hemrick, J.L., and Godt, M.J.W. (1990) Alloenzyme diversity in plant species, In: *Plant Population Genetics, Breeding and Genetic Resources.* (Eds.): A.H.D. Brown, M.T. Clegg, A.L. Kahler, B.S. Weir. *Sinauer Associates Inc.*, Sunderland MA, pp. 43-63.
- Jaccard, P. (1908) Nouvelles recherches sur la distribution florale. *Bulletin Societe Vaudoise Des Sciences Naturelles.* 44: 223-270.
- Kumar, A., Singh, R., Singh, R., and Sengar, S. R. (2015). Molecular Approach for Detection of Plant pathogen. *An International Journal of Biological Sciences Biotech today.* 5(2):14 -19.
- Kumar, S., Deepali, Mishra D. C., Bansal R., Kumari J., Kumar S. (2016) Molecular characterization of Indian wheat germplasm line for stay green & other heat tolerance genes using linked SSR markers. *An International Journal of Biological Sciences Biotech today.* 6(1):90 -94.
- Mahpara, S., Farooq, J., Ali, Z., Petrescu-Mag IV., Hussain, F. (2012) Assessment of genetic distance among wheat genotypes through RAPD markers. *Advances in Agriculture and Botany J.* 4(1):31–35.
- Nei, M., Li, W. H. (1979) Mathematical models for studying genetic variation in terms of restriction endonucleases.

- Proc. Natl. Acad. Sci. USA* 76:5269-5273.
- Parveen, S., Saharan, M.S., Verma, A and Sharma, I. (2013). Comparative analysis of RAPD and ISSR marker assays for detecting genetic polymorphism in *Tilletia indica*. *European Journal of Experimental Biology* 3 (1):380-387.
- Siddiqui, M. F., Iqbal, S., Erum, S., Naz, Khan, S. (2010) DNA landmarks for genetic relatedness and diversity assessment in Pakistani wheat genotypes using RAPD markers. *Pak. J. Bot.* 42(2):1013-1020.
- Sivolap, Y.M., Chebotar, S.V., Topchieva, E.A., Korzun, V. N., and Totskiy, V. N. (1999) RAPD and SSRP analyses of molecular-genetic polymorphism in *Triticum aestivum* L. cultivars. *Russian J. Genet.* 35: 1433—1440.
- Tar'an, B., Zhang C., Warkentin T., Tullu, A., and Vandenberg (2005) A genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. *Genome*, 48: 257-272.