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Genetic Analysis and RAPD Polymorphism in Wheat (*Triticum aestivum* L.) Genotypes

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ABSTRACT

Assessment of genotype diversity was studied using released and in pipeline genotypes of wheat, of these 22 were released and 14 were in pipeline. Genetic diversity among 36 wheat genotypes was studied using Random amplified polymorphic DNA (RAPD) analysis. 21 RAPD primers (RPI primers) were used for screening 36 wheat genotypes from which 2,868 fragments were amplified. It was observed that 63.3% bands were polymorphic and 36.4% were monomorphic. The percent of polymorphic bands in banding pattern was calculated and it was highest in RPI-22 (81.3%) while lowest was recorded in RPI-2 (33.8%) and highest PIC value was observed in RPI-22 and RPI-25 (0.88) while lowest in RPI-7 (0.70). Maximum fragments were produced in RPI-1 (200) and minimum in RPI-7 (82). In banding pattern some unique bands were seen, total 7 unique bands were observed. Genetic relationship between wheat genotypes was determined on the basis of Jackard IJ pair wise similarity coefficient values (Similarity coefficient values ranged from 0.09 to 0.99) and dendrogram was generated by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis using dice's similarity matrix through NT-SYS pc software. From dendrogram 5 solitary and 8 clusters were revealed. From above analysis average coefficient values were revealed and highest (0.99) was observed between WSM-175.1 and WSM-163 genotypes while HD-2781 and NIAW-34 represent lowest average similarity coefficient value (0.09). The maximum similarity percentage i.e. 99% was found between WSM-175.1 and WSM-163 and the minimum similarity i.e. 09% was found between HD-2781 and NIAW-34.

Introduction

Wheat (*Triticuma estivum* L.) is an important cereal crop widely cultivated in India and world providing food calories and protein to the human population. Wheat is the annual plant belong to family *Poaeceae* (Singh *et al.,* 2004). It is a good source of protein, minerals, vitamins (Thiamin, Riboflavin), sugar and fats. A protein in wheat varies from 7 to 24 per cent (Cauvain and Stanley, 2003). *Triticum aestivum* commonly contains three different but genetically related genomes (A, B and D) with total genomic size of 1.7 x 10^{10} base pairs (Moore *et al.,* 1995). 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 the 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 marker 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 4,00,000 although many accessions may be duplicated in different collections (Poelham and Sleper, 1995). Thus, 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 germplasm. Molecular marker provides information that helps to define the distinctiveness of species and their ranking according to the number of close relative and phytogenetic position. RAPD analysis has significant level of DNA polymorphism in different plant species. Factors such as speed and efficiency make RAPD a useful method for effective germplasm management to estimate genetic diversity (Sharma, 2006).

RAPD is well established genetic tool which provides a simple and fast approach to detect DNA Polymorphism for cultivars identification and diversity analysis (Welsh and McClelland, 1990) (Williams *et al.,* 1990). RAPD is a dominant marker, RAPD-PCR requires very small quantity of genomic DNA i.e. 10-15µg/ml for assessment. Standard oligonucleotide (10 bp) long random sequence primer can be used to amplify the nanogram amount of total genomic DNA under low annealing temperature by PCR. Amplified product is generally separated on agarose gel electrophoresis (Bardakes *et al.*, 2001). Because of simplicity and low cost of the RAPD technique it has wide range applications in many areas of biology. RAPD markers have been used to examine both interspecific and intraspecific variations in number of plant species (Nawroz, 2008), for Linkage studies (Williams *et al.,* 1990), Gene tagging (Ranade *et al.,* 2010), Plant and animal breeding, Population and evolutionary genetices,Genetic mapping (Bardakes *et al.,* 2001), DNA fingerprinting (Govardhanan *et al.,* 2011) and high polymorphism that enables to generate many genetic markers within short time (Semagh *et al.,* 2006).

Materials and Methods

The plant material

Representative plant samples of 36 accessions of *Triticum aestivum* were collected from NARP, Agriculture Research Station, Washim. Total 36 accessions were included in the study for analyzing genetic diversity of wheat (Table 1). All genotypes were planted in pots.

DNA extraction

Total genomic DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) protocol given by (Doyle and Doyle, 1990) with some modifications. DNA was isolated from 0.5 g of fresh leaves of the 10-15 days seedlings. Tissue was crushed to a fine powder using liquid nitrogen and dispersed in 1 ml pre warmed (60^oC) extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 100 mM Tris-HCl, pH 8.0 and 2% β-mercaptoethanol). After incubation for 1 hr at 60°C with intermittent swirling, the mixture was emulsified with an equal volume of chloroform: isoamyl alcohol (24:1). Following centrifugation, the supernatant was collected and mixed with 0.6 volume of isopropanol. The precipitated nucleic acid was spooled out, washed twice in 70% ethanol, dried under vacuum, dissolved in TE buffer (10 mMTris-Cl, pH 8.0) and treated with RNase and proteinase K to remove RNA and protein respectively. DNA was tested for its quality and integrity on 0.8% agarose gel, quantified spectrophotometrically, diluted in TE buffer to a concentration of 25ng/µl and utilized for PCR analysis.

RAPD procedure

The PCR (Eppendorf, India) for RAPD analysis was performed according to (Williams *et al.,* 1990) with certain modifications. Optimum conditions for DNA amplification were as follows. The reaction mixture was mixed well 25µl was distributed in each tube. 1µl of DNA (25ng/µl) sample was added to each tube, mixed well and briefly centrifuged to collect drops from wall of tube, Master mix was prepared and divided into 36 equal parts (each of 12.5µl) into 36 different PCR tubes. After that 10.5µl nuclease free water was added in each tube then 1µl primer and 1µl of 36 different genomic DNA samples of wheat were added to each tube that leads to final quantity of 25µl. PCR tubes were then placed in thermal cycler for amplification of the genomic DNA. The PCR protocol for RAPD markers was standardized for analyzing the samples of wheat as pre heat 101^0C, 94^0C for 5 min(1 cycle), 94^0C for 45 sec, 35^0C for 1 min, 72^0C for 1.5 min (10 cycles), 94^0C for 45 sec, 38^0C for 1 min, 72^0C for 1 min (30 cycles), and 72^0C for 10 min(1 cycle) and 4^0C for 10 min. PCR Amplification was performed in a 25µl reaction mixture volume containing 25ng of DNA, 10x buffer, 10mMdNTPs, 100ng/µl of primer, 2mM of magnesium chloride and 3 U/ µl of Taq (*Thermus aquaticus*) DNA polymerase enzyme (GENEI, Bangalore). Single RPI primer (Table 2) was included in each PCR reaction.

Data analysis

Submerged gel electrophoresis unit was used for fractionating RAPD markers on 2% agarose gel. 4µl loading dye was added to the amplified products in each tube and mixed well. 20µl of amplified products of each sample were loaded on 2% agarose gel containing 1x TAE buffer to separate the amplified fragments. The Gene ruler 100bp DNA ladder plus was used as the standard to determine the size of the polymorphic fragments. The gel was visualized under UV transilluminator (JASCO) and photographed using Gel-Doc system (UV-Tech Ltd).The amplified fragment profiles were visually scored for the presence (1) or absence (0) of bands and entered in a scoring matrix. Jaccard's similarity coefficients were calculated and used to construct dendrograms based on UPGMA and SAHN clustering. The computer package NT SYS-pc version 2.1 was used to carry out cluster analysis. Polymorphic and Monomorphic amplified fragments were counted from PCR amplification image and percentage of the same was calculated by the given formula:

Polymorphic percent (%) =

$$\frac{\text{Polymorphic Bands}}{\text{Total Bands}} \times 100$$

Results and Discussion

Genetic Analysis and RAPD Polymorphism in Wheat Genotypes was carried out using RAPD primers. The results obtained are presented under following points:

Selection of suitable RAPD primers

Universal primers of RPI series were used to evaluate polymorphism of 36wheat genotypes. PCR amplified products of each primer were resolved on 2% agarose gel and the size of the amplified products was compared with marker DNA. 21 primers were screened.

Band statistics

Universal random primers like RPI-1, RPI-2, RPI-3, RPI-4, RPI-5, RPI-6, RPI-7, RPI-9, RPI-10, RPI-11 RPI-12, RPI-14, RPI-15, RPI-16, RPI-17, RPI-18, RPI-19, RPI-22, RPI-23,

RPI-24, RPI-25, were used, 2,868 RAPD amplified fragments were generated. Among RAPD markers, RPI-1 produced maximum number of fragments (200 from all genotypes) followed by RPI-22 (192) and RPI-11 (185) while RPI-7 and RPI-23 generated minimum number of fragments (82) and (87) respectively in the genomic pool. Higher numbers of polymorphic fragments were observed inRPI-22 (156) and lower in RPI-7 (46) and their percentage were calculated which was highest in RPI-22 (81.3%) and lowest in RPI-2 (33.8%). Polymorphism information content (PIC) was calculated and highest PIC value was observed in RPI-22 and RPI-25 (0.88) and lowest was observed in RPI-7 (0.70). Higher number of monomorphic fragments (108) were observed in RPI-3 and RPI-6 and the percent of monomorphic fragments in banding pattern was calculated which was highest in RPI-2 (66.2%) while lowest was recorded from RPI-11 (19.5%). Some unique bands were observed in specific genotypes, total 7 numbers of unique bands were observed in RPI-4 (1), RPI-5 (1), RPI-12 (1), RPI-14 (1) and RPI-15 (1), RPI-10 (2), in genotypes HD-2987, WSM-184, WSM-175.1, WSM-175.1, WSM-175.1, WSM-175.1 and HD-2987 respectively (Table 3).

Table.1 Characteristics of wheat genotypes

Genotype	*aestivum/durum*	Released/ In pipe line	Genotype	*aestivum/durum*	Released/ In pipe line
AKDW 2997.16	*T. durum*	Released	PDKV Washim 1472	*T. aestivum*	Released
MACS 1967	*T. durum*	Released	NIAW 34	*T. aestivum*	Released
N 59	*T. durum*	Released	NIAW 1415	*T. aestivum*	Released
Agra Local	*T. durum*	Released	NI 5439	*T. aestivum*	Released
AKDW 2997	*T. durum*	Released	WSM 55	*T. aestivum*	In Pipe line
FLW 9	*T. aestivum*	Released	WSM 163	*T. aestivum*	In Pipe line
FLW 20	*T. aestivum*	Released	WSM 105	*T. aestivum*	In Pipe line
HD 2987	*T. aestivum*	Released	WSM 42	*T. aestivum*	In Pipe line
KITE	*T. aestivum*	Released	WSM 1472	*T. aestivum*	In Pipe line
PUSA	*T. aestivum*	Released	WSM 175.1	*T. aestivum*	In Pipe line
HD 2781	*T. aestivum*	Released	WSM 175.7	*T. aestivum*	In Pipe line
PKV Washim	*T. aestivum*	Released	WSM 103	*T. aestivum*	In Pipe line
AKW 3722	*T. aestivum*	Released	WSM 135	*T. aestivum*	In Pipe line
AKW 1071	*T. aestivum*	Released	WSM 133	*T. aestivum*	In Pipe line
MACS 6222	*T. aestivum*	Released	WSM 51	*T. aestivum*	In Pipe line
MACS 2496	*T. aestivum*	Released	WSM 184	*T. aestivum*	In Pipe line
LOK 1	*T. aestivum*	Released	WSM 155	*T. aestivum*	In Pipe line
HD 2189	*T. aestivum*	Released	WSM 174.1	*T. aestivum*	In Pipe line

Table.2 Universal RPI primers (GENEI Biotech Pvt. Ltd. Bangalore)

Primers	Accession No.	Sequence 5'-3'	Primers	Accession No.	Sequence 5'-3'
RPI 1	AM765819	AAAGCTGCGG	RPI 14	AM773774	ACTTCGCCAC
RPI 2	AM750044	AACGCGTCGG	RPI 15	AM773775	AGCCTGAGCC
RPI 3	AM773310	AAGCGACCTC	RPI 16	AM773776	AGGCGGCAAG
RPI 4	AM773769	AATCGCGCTG	RPI 17	AM911710	AGGCGGGAAC
RPI 5	AM773770	AATCGGGCTG	RPI 18	AM765830	AGGCTGTGTC
RPI 6	AM773771	ACACACGCTG	RPI 19	AM773777	AGGTGACCGT
RPI 7	AM773312	ACATCGCCCA	RPI 22	AM911711	CATAGAGCGG
RPI 9	AM773315	ACCGCCTATG	RPI 23	AM911712	CCAGCAGCTA
RPI 10	AM750045	ACGATGAGCG	RPI 24	AM765821	CCAGCCGAAC
RPI 11	AM911709	ACGGAAGTGG	RPI 25	AM750054	GAGCGCCTTC
RPI 12	AM773316	ACGGCAACCT			

Table.3 Characteristics of amplified fragments obtained from 21 primers for RAPD analysis of wheat genotypes

Sr. No.	Primers	Mono-morphic Bands	Mono-morphic percent (%)	Poly-morphic Bands	Poly-morphic percent (%)	Unique Bands	Total Bands	PIC Values
1	RPI- 1	72	36.0%	128	64.0%	-	200	0.87
2	RPI-2	108	66.2%	55	33.8%	-	163	0.81
3	RPI-3	36	32.7%	74	67.3%	-	110	0.79
4	RPI-4	72	55.8%	56	43.4%	01	129	0.80
5	RPI-5	36	24.8%	108	74.5%	01	145	0.85
6	RPI-6	108	65.1%	58	34.9%	-	166	0.83
7	RPI-7	36	43.9%	46	56.1%	-	82	0.70
8	RPI-9	36	29.8%	85	70.2%	-	121	0.84
9	RPI-10	36	32.1%	74	66.1%	02	112	0.82
10	RPI-11	36	19.5%	149	80.5%	-	185	0.87
11	RPI -12	36	31.3%	78	67.8%	01	115	0.78
12	RPI-14	72	42.9%	95	56.5%	01	168	0.85
13	RPI-15	36	31.3%	77	66.9%	01	115	0.81
14	RPI-16	36	33.0%	73	66.9%	-	109	0.78
15	RPI-17	36	34.6%	68	65.4%	-	104	0.78
16	RPI-18	72	55.4%	58	44.6%	-	130	0.80
17	RPI-19	36	27.1%	97	72.9%	-	133	0.81
18	RPI-22	36	18.8%	156	81.3%	-	192	0.88
19	RPI-23	36	41.4%	51	58.6%	-	87	0.75
20	RPI-24	36	30.5%	82	69.5%	-	118	0.81
21	RPI-25	36	19.6%	148	80.4%	-	184	0.88
Total		1044	36.4%	1816	63.3%	07	2868	-

Fig.1 Fragments obtained from RPI-1 over 36 wheat genotypes

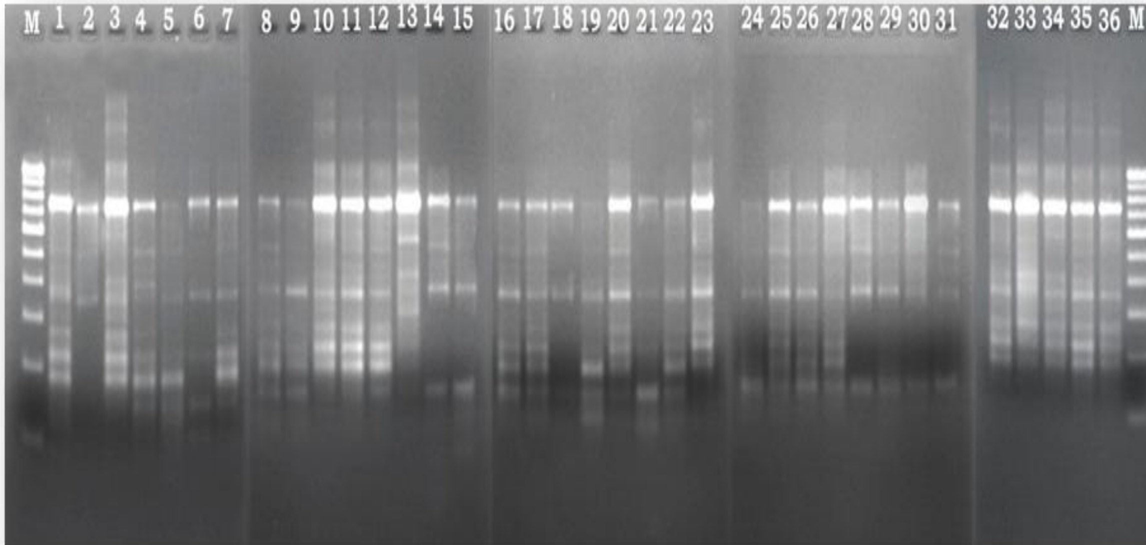


Fig.2 Fragments obtained from RPI-25 over 36 wheat genotypes

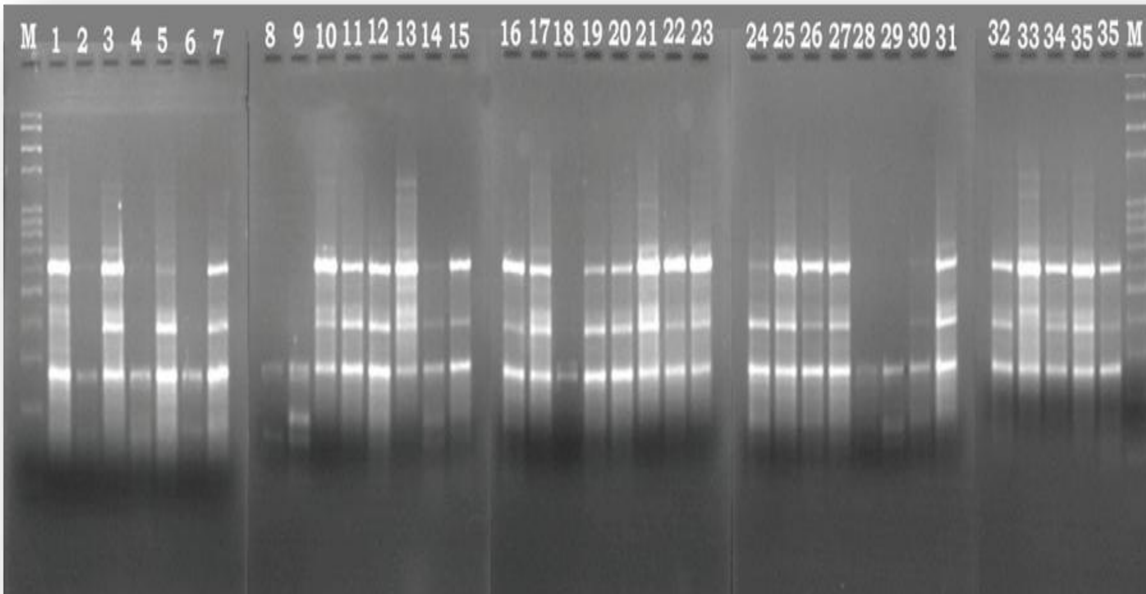
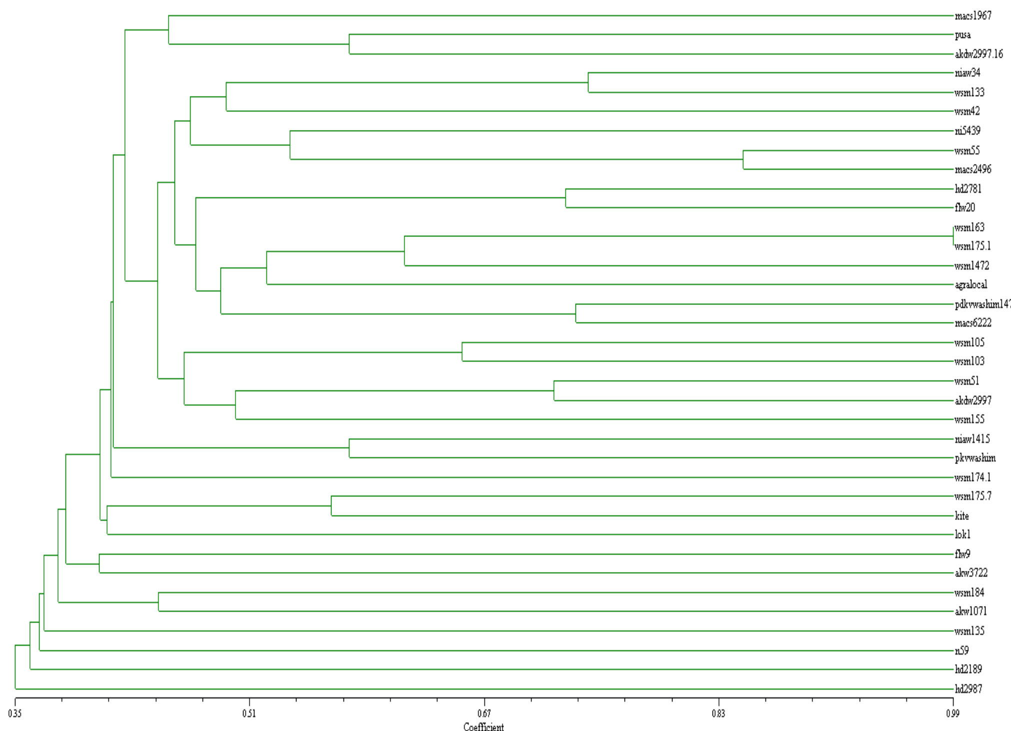


Fig.3 Dendrogram generated by UPGMA cluster analysis showing genetic diversity among the different wheat genotypes



Cluster analysis

A Dendrogram was generated by UPGMA cluster analysis based on Jackard IJ similarity coefficients and cluster analysis on the basis of coefficient value, the accessions further divided into 5 solitary and 8 clusters which are divided further into different genotypes. First cluster contains 3 genotypes(MACS-1967, Pusa, AKDW-2997.16) second, 6 genotypes (NIAW-34, WSM-133, WSM-42, NI-5439, WSM-55, MACS-2496) third, 8 genotypes (HD-2781, FLW-20, WSM-163, WSM-175.1, WSM1472, Agra Local, PDKV washim-1472, MACS-6222) fourth, 5 genotypes (WSM-105, WSM-103, WSM-51, AKDW-2997, WSM-155) fifth, 2 genotypes (NIAW-1415, PDKV washim) sixth, 3 genotypes, (WSM-175.7, Kite, Lok-1) seventh, 2 genotypes, (FLW-9, AKW-3722) and eighth contain 2 genotypes (WSM-184, AKW-1071).

Similarity based on Jackard IJ coefficient

Genetic relationship between wheat genotypes were determined on the basis of Jackard IJ pair wise similarity coefficient values. The value of similarity coefficient ranged from 0.09 to 0.99. GenotypesWSM-175.1 and WSM-163 represent highest average similarity coefficient value (0.99) genotypes HD-2781 and NIAW-34 represent lowest average similarity coefficient value (0.09). The maximum similarity coefficient percentage i.e. 99% was found between WSM-175.1 and WSM-163 and the lowest i.e. 9% was found between HD-2781 and NIAW-34. Thus, RAPD plays an important role in securing plant variety right by virtue of its unique efficiency in distinguishing closely related germplasm. Future thrust will be directed towards the holistic use of RAPD primes for DNA fingerprinting, genetic analysis and linkage mapping in wheat.

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