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RAPD analysis for genetic polymorphism in bread wheat (Triticum aestivum L.em Thell) genotypes varying for grain protein content

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Abstract

Genetic polymorphism was investigated among nine spring wheat genotypes, differing in grain protein content, including C-306M10 (mutant of drought tolerant variety C306), DI 8, DI 9, DI 16, DI 20, DI 716, DI 717, DI 728 (near isogenic lines) and HGPC (from Wheat x Rye crosses) using 55 RAPD primers. Out of 55 primers used, only 36 amplified and generated 2(OPG08, OPD05) and 12 (OPD02) bands. A total of 342 amplified products were observed, of which 168 were polymorphic (49.12%) while 174 were monomorphic. The primer OPC-05 and OPC-07 revealed 92.86% and 80.00% polymorphism, respectively and these primers were most useful in characterization of nine wheat genotypes included in this study. The primer OPG-08 showed no polymorphism. It is concluded that the primers OPC-05 and OPC-07 were very effective in distinguishing wheat genotypes in the present study. Twenty six RAPD primers produced a total of 48 unique bands for high protein content that were either present or absent in HGPC a-high grain protein genotype and thus can be used in wheat improvement through marker-assisted selection (for the bands which are unique by their presence). Data (RAPD analysis) were used to generate the similarity coefficients using 'siMqual' subprogram of software NTSYS-PC. The similarity coefficient values ranged from 0.97(DI8 and DI9) to 0.68 (DI9 and HGPC), indicating high genetic variability among the selected wheat genotypes. The cluster analysis and principle component analysis broadly divided the wheat genotypes into two groups and showed that DI 9 and HGPC were most divergent genotypes.

1. Introduction

Wheat (*Triticum aestivum* L.em Thell) is the world's most widely cultivated cereal crop. India is the second largest wheat growing country of the world. Wheat is cultivated under different conditions due to its versatile genotype, which has wide adaptation to diverse agro-ecological conditions ranging from tropical and sub-tropical to temperate zones and the cold tracts of far North. Wheat protein only accounts for about 10-15 per cent of the mature wheat grain. Nevertheless, they are major determinants of end-use quality, whether the nutritional quality for food and feed or the functional properties for processing. The major storage proteins of wheat; Gliadin and Glutenin, form a Viscoelastic complex called Gluten. Gluten is directly responsible for properties of dough including its gas retaining capability, the oven-spring during baking and the characteristics of the baked bread. Understanding the molecular basis for visco-elasticity of wheat gluten protein is an important pre-requisite for manipulating their properties in order to improve the quality for traditional uses and to develop new properties for novel uses (Shewry *et al*., 2001). The study of genetic diversity is important for characterization and conservation of germplasm as well as for the selection of genetically diverse parents for breeding programme. Assessment of genetic diversity in crop germplasm based on phenotypic and morphological characters and biochemical markers *viz*., isozyme analysis is quite often biased. Molecular markers such as RFLP, RAPD, SCAR, AFLP, SSRs etc. are useful tools for characterization of germplasm /cultivars, to assess genetic variations at DNA level without the influence of environmental variations and developmental stages for various plant traits and to supplement the existing morphological descriptors with reliable and repeatable DNA based marker profiles (Gupta *et al.*, 2000). Random Amplified Polymorphic DNA (RAPD) markers have been shown to be an effective method for detecting polymorphism in wheat (Joshi and Nguyen, 1993a,b, Sun *et al*., 1998, Teshale *et al.*, 2003). The present study was therefore undertaken with the objective to evaluate genetic diversity in 9 spring wheat genotypes, including C-306M10 (mutant of well known drought tolerant traditional tall variety C306), DI 8, DI 9, DI 16, DI 20, DI 716, DI 717, DI 728 (near isogenic lines) and HGPC (obtained from Wheat x Rye crosses) differing for grain protein content (Nimbal *et al.*, 2009) using 55 RAPD primers to search for any co-segreating DNA marker for high grain protein content.

2. Materials and Methods

A total of nine wheat genotypes *viz*., C306M10 (mutant of well known drought tolerant variety C306), DI 8, DI 9, DI 16, DI 20, DI 716, DI 717, DI 728 (near isogenic lines derived from crosses between C306M10 and extremely dwarf genotype 'S948A_1' of bread wheat) and HGPC (obtained from Wheat x Rye crosses) having grain protein content (Nimbal *et al*, 2009)ranging from 10.6 %(DI9) to 13.4 % (HGPC)were used for assessing the molecular polymorphism.

2.1 DNA Extraction

Genomic DNA was isolated from young leaves of 2-3 week old plants of all the 4 wheat genotypes using CTAB method (Saghai-Maroof *et al.*, 1984). DNA was checked for its quality and quantity by 0.8% agarose gel electrophoresis using a standard containing 100ng per µl genomic l DNA.

2.2 RAPD Marker Analysis

A total of 55 RAPD primers obtained from Operon Technologies, Almeda USA , were used for PCR amplification which was performed using Eppendorf Master Cycler Gradient thermocycler. The 20µl PCR reaction mixture contained 1x PCR buffer, 2mM $MgCl_2$, 200mM dNTPs, 0.25 mM of primer, 1 unit of *Taq* DNA polymerase (Promega Inc., USA) and 2 µl (50ng) template DNA. PCR amplification was performed with a hot start of 94ºC for 1 minute, annealing at 40ºC for 1 minute and extension at 72 ºC for 2 minutes. Final extension was carried out for 2 minutes at 72 ºC. Amplified DNA fragments were separated by 1.5 % agarose gel electrophoresis in Tris-Borate EDTA buffer. The DNA bands were made visual by staining the gels with ethidium bromide and photographed under UV light using Nikon digital camera attached to gel documentation system (Alpha Innotech, Corp, California, USA).

2.3 Band Scoring and Data Analysis

The frequency of RAPD polymorphisms among 9 wheat genotypes was calculated based on the presence of band taken as '1' or absence of band taken as '0' and the data was entered into NTSYS-PC (numerical taxonomy and multivariate analysis system program) (Rohlf, 1990). The 0/1 matrix was used to calculate the similarity matrices using 'siMqual' subprogram of software NTSYS-PC. Dendrogram was built based on the un-weighted pair group method with arithmetic averages (UPGMA).

3. Results and Discussion

3.1 RAPD Banding Profile and Polymorphism

Out of 55 random primers used, 36 primers (65.4%) produced amplification (Table 1) and generated 2 to 12 RAPD bands while 19 primers showed no amplification and one primer did not show any polymorphism.

Thirty six RAPD primers yielded 342 bands (Table 2), of these 168(49.12 %) were polymorphic while 174 were monomorphic. Average polymorphism across all nine wheat varieties in our study was 49.12 % which is quite consistent with that (45.3 %) reported by Cao *et al.* (2002). Pujar *et al.* (1999) observed 78.6 % polymorphism whereas Teshale *et al.* (2003) observed 79.6 % polymorphism among the selected genotypes, which is also close to our values. Mukhtar, *et al.* (2002) reported 64.3 % polymorphic bands in 29 and 20 wheat genotypes, respectively. The number of bands per RAPD primer ranged from 2 (OPG-08, OPD-05) to 12 (OPD-02). All RAPD primers except OPG-08 showed one or more polymorphic band(s). Lowest number of polymorphic bands was exhibited by OPC-06, OPD-03, OPD-05, OPA-07, OPD-16 (One band each), whereas the highest number of polymorphic bands was exhibited by OPD-02 (12 bands) followed by OPC-11 (10 bands). The maximum percent polymorphism was exhibited by primer OPC-05 (92.86 %) followed by OPC-07 (80%), whereas, the minimum per cent polymorphism was showed by OPG-08 (0.00 %) followed by OPD-03 (9.09%). It showed that the primer OPC-05 was useful to characterize the wheat genotypes, whereas the primer OPG-08 though showed amplification of wheat genomic DNA but was not able to distinguish the wheat genotypes.

For reasons of brevity only selected gel pictures with, RAPD profiles having higher number of bands, obtained from amplification of DNA using most prolific primers in nine wheat genotypes are shown in Figure-plates 1-6.

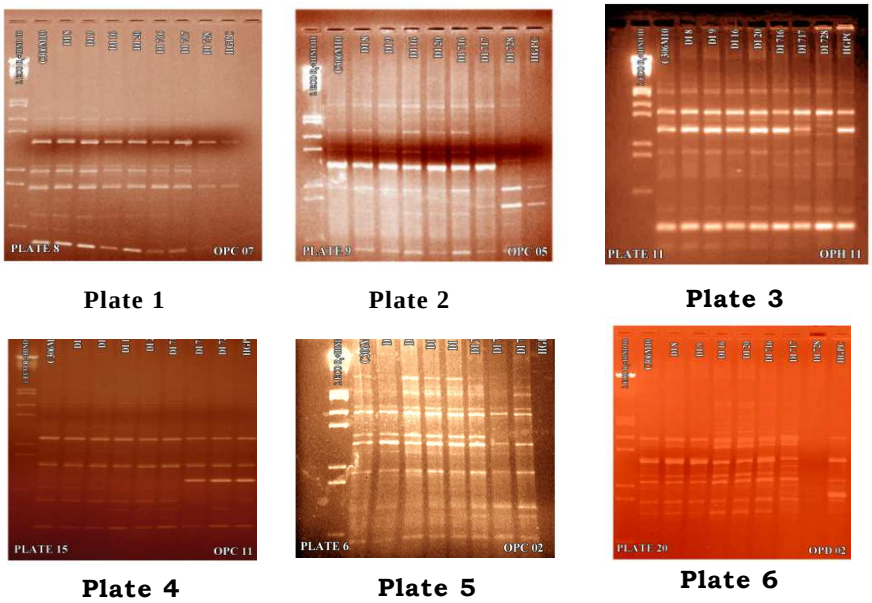

Figure-Plate 1-6: RAPD banding profile of nine wheat genotypes generated by primers OPC-07, OPC-05, OPH-11, OPC-11, OPC-02 and OPD-02.

Table 1. A brief description of RAPD primers (OPERON random primer kit) used for the present investigation**.**

S. No.	Primer	Sequence	S. No.	Primer	Sequence
1	OPA-02	TGCCGAGCTG	19	OPD-02	GGACCCAACC
2	OPA-04	AATCGGGCTG	20	OPD-03	GTCGCCGTCA
3	OPA-09	GGGTAACGCC	21	OPD-05	TGAGCGGACA
4	OPH-20	GGGAGACATC	22	OPD-07	TTGGCACGGG
5	OPB-18	CCACAGCAGT	23	OPD-08	GTGTGCCCCA
6	OPC-02	GTGAGGCGTC	24	OPG-02	GGCACTGAGG
7	OPG-08	TCACGTCCAC	25	OPO-06	CCACGGGAAG
8	OPH-11	CTTCCGCAGT	26	OPQ-07	CCCCGATGGT
9	OPO-18	CTCGCTATCC	27	OPA-07	GAAACGGGTG
10	OPC-05	GATGACCGCC	28	OPD-11	AGCGCCATTG
11	OPC-06	GAACGGACTC	29	OPD-15	CATCCGTGCT
12	OPC-07	GTCCCGACGA	30	OPD-16	AGGGCGTAAG
13	OPC-11	AAAGCTGCGG	31	OPD-18	GAGAGCCAAC
14	OPC-12	TGTCATCCCC	32	OPI-08	TTTGCCCGGT
15	OPC-13	AAGCCTCGTC	33	OPB-05	TGCGCCCTTC
16	OPC-18	TGAGTGGGTG	34	OPO-09	TCCCACGCAA
17	OPC-08	TGGACCGGTG	35	OPD-13	GGGGTGACGA
18	OPC-19	GTTGCCAGCC	36	OPC-01	TTCGAGCCAG

3.2 Unique Bands in Nine Wheat Genotypes

Some primers gave unique bands in specific wheat genotypes as shown in Table 3. These primers produced a specific allele/DNA band, which distinguished one or a few genotypes from the rest. Primer OPC-05 produced maximum number of unique alleles (6 Bands). The primer OPC-05 produced four unique alleles for genotype HGPC and one unique allele each for DI 20 and DI 728. Likewise primer OPC-19 produced three unique alleles for genotype HGPC and two unique bands for DI 716. The primers OPA-02, OPA-09, OPC-02, OPH-11, OPC-06, OPC-07, OPC-11, OPC-12, OPC-18, OPC-08, OPD-05, OPO-06, OPA-07, OPD-11, OPD-15 and OPC-01 produced only one unique allele in different genotypes. The primers OPA-04, OPH-20, OPB-18, OPG-08, OPD-03, OPG-02, OPD-16, OPD-18, OPB-05 and OPO-09 did not produce any unique band. Total 48 unique alleles were produced by 36 primers in nine genotypes. Of these the genotype HGPC produced maximum number of unique alleles (16) followed by DI 717 (9) while the genotypes DI 8, DI 9 and DI 16 produced minimum number of unique allele (1).

3.3 Unique Banding Patterns in Nine Wheat Genotypes

Of the 36 primers 33 primers gave unique banding pattern in specific wheat genotypes as shown in Table 4. These primers produced a specific banding pattern which distinguished genotype from the rest. The primer OPC-13 produced seven unique banding patterns for genotypes C 306M10, DI 8, DI 9, DI 16, DI 20, DI 716 and HGPC. The primers OPA-02, OPB-18, OPO-18, OPC-06, OPC-12, OPD-03, OPD-05, OPQ-07, OPA-07 and OPD-18 produced only single unique banding pattern in different genotypes. The primers OPG-08, OPG-02 and OPD-16 did not produce any unique banding pattern. Regarding maximum number of groups produced by different primers for different genotypes , the primer OPC-13 produced eight groups, followed by the primers OPC-02, OPC-05, OPC-11, OPD-02, OPD-07 those produced 7 groups. The primer OPG-08 produced only single group, whereas primers OPC-06, OPD-05, OPA-07, and OPD-16 produced two groups each.

Primers OPC-05, OPC-11, OPC-13, OPC-18, OPD-07 and OPD-15 were found very effective in distinguishing the wheat genotypes. Moderately effective primers were OPB-18, OPO-18, OPD-03, OPD-08, OPO-06, OPQ-07, OPD-16, OPD-18, OPI-08, OPB-05, OPD-13 and OPC-01. Out of 36 primers studied 14 were in the category of effective primers. Only primer OPG-08 was found to be non-effective to characterize wheat genotypes, whereas the primers OPC-06, OPD-05 and OPA-07 were least effective to distinguish among the nine wheat genotypes.

3.4 Genetic Relationship among Nine Wheat Genotypes

The RAPD data was used to generate similarity matrices of nine wheat genotypes using 'simqual' sub-programme of software NTSYS-PC (Table 5). The allelic diversity data was used to produce a dendrogram (cluster tree analysis, NTSYS-PC) revealing the genetic relationship among all wheat genotypes (Figure 1). Similarity matrices of nine wheat genotypes revealed the relationship among them. A maximum similarity value of 0.97 was observed between DI 8 and DI 9, whereas DI 9 and HGPC were found to be genetically most diverse (0.68) among these nine genotypes. Teshale *et al*., (2003) also observed a similarity coefficient value ranging between 0.36 and 0.82 among the hexaploid and tetraploid varieties they selected for their investigation.

The cluster tree analysis showed that the wheat genotypes were broadly divided into 2 groups at a similarity coefficient of 0.73. Group-I consisted of six wheat genotypes (C 306M10, DI 8, DI 9, DI 16, DI 20 and DI 716), of which DI 16 and DI 20 formed a sub-group at similarity coefficient of 0.94 and DI 8 and DI 9 formed subgroup at similarity coefficient of 0.97. Group-II consisted of three genotypes namely, DI 717, DI 728 and HGPC. Genotypes DI 717 and DI 728 formed subgroup at similarity coefficient at 0.84 and DI728-formed the group with HGPC at similarity coefficient of 0.79. The major cluster consisted of many sub-clusters, in which a very

close genetic similarity was observed between DI 8 and DI 9 (97%). Pujar *et al*., (1999) studied genetic diversity among 63 tetraploid wheat genotypes. Grewal (2005) studied 20 wheat genotypes by using 25 RAPD primers, which formed 2 groups at a similarity coefficient value of 0.62.

3.5 PCA Analysis

Principal component analysis (PCA) analysis produced two-dimensional and three-dimensional scaling among the wheat genotypes under study as shown in Fig. 2, respectively. Similar clustering of the nine wheat genotypes was also evident from the two dimensional and three-dimensional scaling based on principal component analysis. Based on two dimensional as well as three-dimensional the nine wheat genotypes were broadly divided into same two groups as showed by cluster tree analysis.

4. Conclusions

End-use quality is one of the priorities of modern wheat (*Triticum aestivum* L.) breeding. Even though quality is a complex trait, high molecular weight (HMW) glutenins play a major role in determining the bread making quality of wheat. DNA markers developed from the sequences of HMW glutenin genes were reported in several previous studies to facilitate marker-assisted selection (MAS). The 16 wheat cultivars with known HMW glutenin subunit composition were genotyped with markers UMN25 and UMN26, and the genotypes perfectly matched their subunit types (Liu *et al.*, 2008) . Wheat quality depends on protein composition and grain protein content (Dumur *et al.*, 2009). High molecular weight glutenin subunits (HMW-GS) play an important role in determining the viscoelastic properties of gluten.

Table 2. DNA profiles and polymorphism generated in wheat genotypes using RAPD primers.

Sr. No.	Primer	Total no. of bands	No. of Monomorphic bands	No. of Polymorphic bands	% Polymorphism
1	OPA-02	7	5	2	28.57
2	OPA-04	9	4	5	55.56
3	OPA-09	14	11	3	21.43
4	OPH-20	14	9	5	35.71
5	OPB-18	11	8	3	27.27
6	OPC-02	13	6	7	53.85
7	OPG-08	2	2	0	0.00
8	OPH-11	14	5	9	64.29
9	OPO-18	4	1	3	75.00
10	OPC-05	14	1	13	92.86
11	OPC-06	5	4	1	20.00
12	OPC-07	10	2	8	80.00
13	OPC-11	14	4	10	71.43
14	OPC-12	4	1	3	75.00
15	OPC-13	17	5	12	70.59
16	OPC-18	9	5	4	44.44
17	OPC-08	7	4	3	42.86
18	OPC-19	19	10	9	47.37
19	OPD-02	21	9	12	57.14
20	OPD-03	11	10	1	9.09
21	OPD-05	2	1	1	50.00
22	OPD-07	7	2	5	71.43
23	OPD-08	12	8	4	33.33
24	OPG-02	8	4	4	50.00
25	OPO-06	10	8	2	20.00
26	OPQ-07	8	3	5	62.50
27	OPA-07	5	4	1	20.00
28	OPD-11	7	4	3	42.86
29	OPD-15	11	5	6	54.55
30	OPD-16	3	2	1	33.33
31	OPD-18	8	6	2	25.00
32	OPI-08	7	4	3	42.86
33	OPB-05	4	2	2	50.00
34	OPO-09	10	6	4	40.00
35	OPD-13	10	5	5	50.00
36	OPC-01	11	4	7	63.64
	Total	342	174	168	49.12

Table 3. Unique band(s) generated by different RAPD primers in wheat genotypes.

Sr. No.	Primer	Unique Band (s)	Genotype
1	OPA-02	1	DI 717 (-2)
2	OPA-04	Nil	
3	OPA-09	1	DI 8 (-6)
4	OPH-20	Nil	
5	OPB-18	Nil	
6	OPC-02	1	DI 717 (-4)
7	OPG-08	Nil	
8	OPH-11	1	DI 717 (+7)
9	OPO-18	2	HGPC (+2, +3)
10	OPC-05	6	HGPC (-1,-2,-3, +10); DI 20 (+4); DI 728 (+8)
11	OPC-06	1	DI 717 (+1)
12	OPC-07	1	HGPC (+6)
13	OPC-11	1	DI 716 (+1)
14	OPC-12	1	DI 717 (+1)
15	OPC-13	3	C-306M10 (-5); DI 716 (-10,-15)
16	OPC-18	1	DI 728 (-9)
17	OPC-08	1	HGPC (+6)
18	OPC-19	5	DI 716 (-1,-9); HGPC (+3,-6, +7)
19	OPD-02	3	DI 717 (+8); HGPC (-16, +19)
20	OPD-03	Nil	
21	OPD-05	1	DI 716 (-2)
22	OPD-07	2	DI 20 (-3,-7)
23	OPD-08	3	DI 717 (-3,-4); DI 728 (+1)
24	OPG-02	Nil	
25	OPO-06	1	DI 728 (-3)
26	OPQ-07	4	DI 728 (-4,-6,-7,-8)
27	OPA-07	1	C-306M10 (-1)
28	OPD-11	1	DI 9 (-3)
29	OPD-15	1	HGPC (+6)
30	OPD-16	Nil	
31	OPD-18	Nil	
32	OPI-08	2	HGPC (-4,-5)
33	OPB-05	Nil	
34	OPO-09	Nil	
35	OPD-13	2	C-306M10 (-1); DI 717 (-1)
36	OPC-01	1	DI 16 (-1)

+ indicates presence of unique band(s)
-indicates absence of unique band(s)

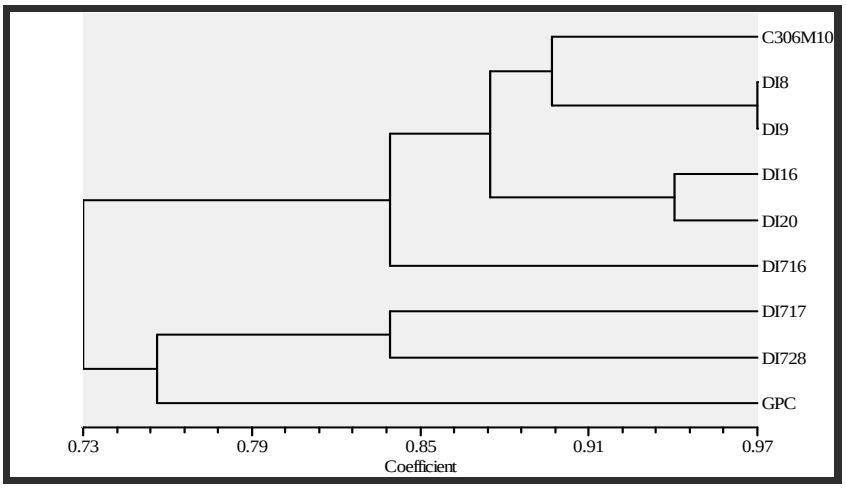

Figure 1. Dendrogram of cluster analysis of RAPD primer of nine wheat genotype

Table 4. Differential DNA banding patterns generated by RAPD primer of nine wheat genotypes.

Sr. No.	Primer	Unique Pattern(s)	Genotypes	Max. no. of groups.
1	OPA-02	1	DI 717	3
2	OPA-04	3	C-306M10, DI 16, DI 717	5
3	OPA-09	2	DI 8, DI 9	4
4	OPH-20	4	DI 8, DI 20, DI 716, HGPC	6
5	OPB-18	1	C-306M10	4
6	OPC-02	6	C-306M10, DI 8, DI 9, DI 716, DI 717, DI 728	7
7	OPG-08	nil	-	1
8	OPH-11	5	DI 20, DI 716, DI 717, DI 728, HGPC	6
9	OPO-18	1	HGPC	3
10	OPC-05	6	C-306M10, DI 20, DI 716, DI 717, DI 728, HGPC	7
11	OPC-06	1	DI 717	2
12	OPC-07	3	DI 717, DI 728, HGPC	5
13	OPC-11	6	DI 16, DI 20, DI 717, DI 728, HGPC	7
14	OPC-12	1	DI 717	4
15	OPC-13	7	C-306M10, DI 8, DI 9, DI 16, DI 20, DI 716, HGPC	8
16	OPC-18	2	C-306M10, DI 728	5
17	OPC-08	2	DI 728, HGPC	4
18	OPC-19	4	DI 716, DI 717, DI 728, HGPC	5
19	OPD-02	6	C-306M10, DI 8, DI 9, DI 716, DI 717, HGPC	7
20	OPD-03	1	HGPC	3
21	OPD-05	1	DI 716	2
22	OPD-07	5	DI 9, DI 16, DI 20, DI 716, HGPC	7
23	OPD-08	3	DI 716, DI 717, DI 728	4
24	OPG-02	nil	-	3
25	OPO-06	2	C-306M10, DI 728	3
26	OPQ-07	1	DI 728	3
27	OPA-07	1	C-306M10	2
28	OPD-11	2	DI 9, DI 728	4
29	OPD-15	3	C-306M10, DI 716, HGPC	6
30	OPD-16	nil	-	2
31	OPD-18	1	DI 716	3
32	OPI-08	2	DI 728, HGPC	3
33	OPB-05	3	DI 717, DI 728, HGPC	4
34	OPO-09	3	DI 717, DI 728, HGPC	5
35	OPD-13	3	C-306M10, DI 716, DI 717	5
36	OPC-01	5	C-306M10, DI 16, DI 20, DI 716, DI 717	6

Table 5. Similarity indices of nine wheat genotypes based on RAPD primer.

	C306M10	DI 8	DI 9	DI 16	DI 20	DI 716	DI 717	DI 728	HGPC
C306M10	1.00								
DI 8	0.90	1.00							
DI 9	0.90	0.97	1.00						
DI 16	0.86	0.88	0.89	1.00					
DI 20	0.84	0.89	0.88	0.94	1.00				
DI 716	0.80	0.82	0.83	0.86	0.87	1.00			
DI 717	0.74	0.79	0.78	0.77	0.76	0.79	1.00		
DI 728	0.70	0.71	0.71	0.71	0.71	0.76	0.84	1.00	
HGPC	0.72	0.69	0.68	0.69	0.70	0.70	0.72	0.79	1.00

The wheat fingerprint data thus generated is useful in identifying wheat genotypes and random primers, which have the potential to be used further in varietal identification and classification. The diversity and cluster analysis has helped identifying most diverse genotypes DI9 and HGPC which is further validated due diverse pedigree involved in their development. These results as generated by 36 RAPD primers can be further used to manipulate genetic determinants (Xu *et al.*, 2008) of agriculturally important wheat grain quality traits particularly grain protein content based on DNA markers through genetic enhancement followed by selection for potent genotypes.

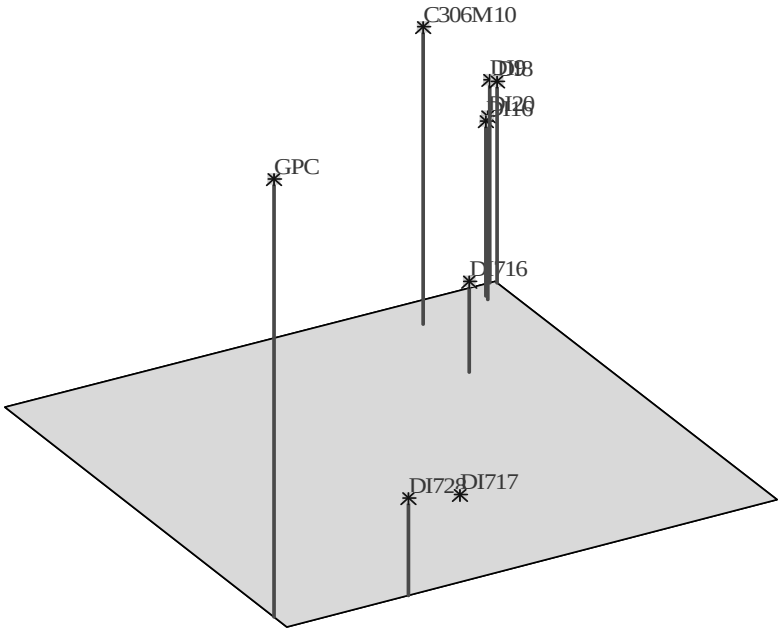

Figure 2. Three dimensional scaling by principal component analysis (PCA) of nine wheat genotypes using genetic diversity data from 36 RAPD primers.

References

Cao, W. G. Hucl. P., Scoles, G., Chibbar R.N., Fox, PN., Skovmand, B. and Cao, W.G. 2002. Cultivar identification and pedigree assessment of common wheat based on RAPD analysis. *Wheat Infor. Service*, **95**, 29 – 35.

Dumur, J., Branlard, G., Tanguy, A. M., Dardevet, M., Coriton, O., Huteau, V., Lemoine J. and Jahier, J. 2009. Development of isohomoeoallelic lines within the wheat cv. Courtot for high molecular weight glutenin subunits: transfer of the Glu-D1 locus to chromosome 1A. Theoretical and Applied Genetics, **119**(3), 471.DOI 10.1007/s00122-009-1053-y.

Grewal, S. 2005. Genetic diversity analysis in wheat using random amplified polymorphic DNA (RAPD) markers, M.Sc. Thesis, CCS HAU, Hisar.

Gupta, P.K., Varshney, R.K., Sharma, P.C., Ramesh, B.2000. Molecular markers and their applications in wheat breeding. Plant Breeding, **118**(5), 369-390.

Joshi, C.P. and Nguyen, H.T. 1993a. Application of random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats. *Genome*., **36**, 602-609.

Joshi, C.P. and Nguyen, H.T. 1993b. RAPD (random amplified polymorphic DNA) analysis based on intervarietal genetic relationships among hexaploid wheats. *Plant Sci*. **93**, 95-103.

Liu, S., Chao, S. and Anderson J. A. 2008. New DNA markers for high molecular weight glutenin subunits in wheat. Theoretical and Applied Genetics, **118 (1),** 177-183

Mukhtar, M.S., Mehboob-ur-Rehman and Zafar, Y.2002. Assessment of genetic diversity among wheat (*T. aestivum* L.) cultivars from a range of localities across Pakistan using random amplified polymorphic DNA (RAPD) analysis. *Euphytica*, **28,** 417-425.

Nimbal,S, Behl, R.K.,Chhabra,A.K., Madan,S and Dethi,S.K. 2009.Characterization of Wheat Genotypes for Grain Quality Traits. Eds R.K.Behl, D.P.Singh, V.S.Tomar, M.S.Bhale, D.Khare and S.D.Upadhyaya. Agrobios International Publishers, Jodhpur, pp.

Pujar, S., Tamhankar, S.A., Rao, V.S., Gupta, V.S., Naik, S. and Ranjekar, P.K. 1999. Arbitrarily primed-PCR based diversity assessment reflects hierarchial groupings of Indian tetraploid wheat genotypes. *Theor. Appl. Genet.*, **99,** 868-876.

Rohlf, F.J. 1990. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System. Version 1.60. Applied Biostatistics, New York.

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

Shewry, P.R., Tatham, A.S., Fido, R., Jones, H., Barcelo, P. and Lazaeri, P.A. 2001. Improving the end use properties of wheat by manipulating the grain protein composition. *Euphytica*, **119**, 45-48.

Sun, Q., Ni, Z., Liu, Z., Gao, J. and Huang, T. 1998. Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. *Euphytica*, **99**, 205-211.

Teshale, E.T., Bansal, S., Mishra, A., Vijaipal, and Khanna, V.K. 2003. DNA fingerprinting of wheat genotypes by RAM markers. *Wheat Infor. Serv.*, **96**, 23-27.

Xu, Q, J. Xu, C.L. Liu, C. Chang, C.P. Wang, M.S. You, Li' B.Y.' and Liu G.T. 2008. PCR-based markers for identification of HMW-GS at *Glu-B1x* loci in common *wheat Journal of Cereal Science*, **47(3)**, 394-398.

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