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Estimation of genetic relationship of wheat germplasm against cereal cyst nematodes using RAPD technique

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Abstract

Investigations were conducted at molecular level, to estimate genetic diversity among wheat germplasm (20 cultivars/lines) in relation to their response against cereal cyst nematodes *Heterodera avenae*, through Random Amplified Polymorphic DNA (RAPD) technique. A total of 589 bands were generated using 14 primers with an average of 28.8 bands/genotype. Maximum percentage of polymorphic loci was 92.86% for genotype TJ-83. Inferences have been made regarding bioassay and molecular characterization that the most diverse and resistant genotype against CCN was Moomal-2002 as compared to the rest of the genotypes studied and the most effective loci to screen diversity was OPA-09.

Key words: Wheat, *Heterodera avenae*, RAPD, genetic diversity.

Cyst nematodes represent a large group of parasites of economically important crops as wheat is one of the most targeted hosts, comprising of 100 or more species. All are specialized parasites to plants including temperate, sub-tropical and tropical crops (Sharma, 1998; Cook & Noel, 2002). The *Heterodera* Schmidt, 1871 is one of the most widely distributed and economically important plant parasitic nematode genus containing over 65 valid species according to the latest classification which have been described from soil and roots of a wide variety of agricultural crops all over the world (Siddiqi, 2000). Among them the most commonly recorded species of economic importance on cereals especially wheat and barley is the cereal cyst nematode *H. avenea* Wollenweber, 1924. It has been reported as the most important pathogen of wheat in South Australia where more than 2 million hectares were found infested causing annual losses up to 70 million US $ (Brown, 1984). It is also a serious pathogen of wheat and barley in India causing "Molya disease" (Dhawan & Pankaj, 1998). From India the Molya disease possibly spread to adjoining wheat growing countries. Maqbool (1981) reported this species on wheat and maize from the then NWFP (Khyber Pakhtunkhwa province) region of Pakistan. Later on, *H. avenae* has been reported from Mirpurkhas, Sindh (Shahina & Erum, 2007) and Bahawalpur, Punjab (Erum, 2011).

Wheat is widely cultivated crop that stands out as the most important in sense of human consumption and covers 35% requirement of the world population. Approximately 95% of the cultivated wheat is hexaploid (Patnaik & Khurana, 2001). Identification of high yielding lines at morphological level is time consuming and needs extensive field trials for evaluation (Astarini *et al*., 2004). Whereas the development of molecular markers for screening of germplasm expedites the process and provides accuracy and perfection (Tar'an *et al*., 2005). RAPD is one of the PCR based technique that used for DNA fingerprinting. This technique is simplest to get product as knowledge of DNA sequences of any particular site is not required (Williams *et al*., 1990). A single arbitrary primer is used in a PCR reaction that results in several discrete DNA products after amplification. Primers are complimentary to the opposite strands that are inverted in their orientation lying on any region of the genome and produce fragments randomly

after amplification (Welsh & Mc Clelland, 1990; Devos *et al*., 1992; Yu & Paul, 1992). This technique is considered one of the techniques for assessment of genetic diversity among cultivars and their wild relatives (Pestsova *et al*., 2000).

Materials and Methods

Biological material

Germplasm: A total of 20 wheat genotypes namely: Sarsabz (1), Pak-81 (2), TD-1 (3), SD-8006 (4), Kiran-95 (5), Mehran-89 (6), Bakhtawar-92 (7), Imdad-2005 (8), Anmol-91 (9), SD-1200/19/1 (10), SD-8012 (11), M-H-97 (12), Inqilab-91 (13), Mommal-2002 (14), Sassui (15), Abadgar-98 (16), SD-4047 (17), TJ-83 (18), Marvi-2000 (19) and Bhittai (20) were utilized in the study.

Cyst culture: Two populations of cereal cyst nematodes *Heterodera avenae* pathotype Ha 41 located in the vicinity of the Northern hilly mountainous region of Pakistan (pathotype identification from National Nematological Research Centre, University of Karachi) and pathotype Ha 12 provided by Dr. Roger Rivoal, France were cultured in screen house located at National Nematological Research Centre, University of Karachi.

Virulence/resistance test: Experiments were conducted in the conditions previously described by Martin *et al*., 2004 However, a slight modification of this method was that, 4 day old seedlings were inoculated with two different pathotypes (Ha12 and Ha 41) of *Heterodera avenae* 2nd stage juveniles ranging from 11-20 juveniles per gram of soil and 3 plants were used for the bioassay. To classify the plants, the number of white female cysts on the root of each plant was counted. Observations were recorded after 20 days for cyst count in plant roots system and 40 days for juveniles in soil. A mean value of white female cysts < 4/plant, the plant was recorded as resistant, < 8/plant = moderately resistant, <12/plant = moderately susceptible and > 12/plant = susceptible; whereas the mean value of the number of juveniles/g of soil, the plant scored as (< 10 = R, < 15 = MR, < 20 = MS and > 20 = S). Two-way ANOVA was conducted and means were compared using computer program INSTAT V.3.36 ($p \leq 0.01$).

DNA extraction: One week old fresh leaves about 5-10 cm from the top of the seedling were cut and placed in eppendorf tubes, frozen in liquid nitrogen, powdered with a mortar pestle. DNA was extracted according to the CTAB protocol after Saghai-Maroof *et al*., (1984).

RAPD analysis: DNA concentration in the working solution of approximately 15 ng/µl in d_3H_2O was confirmed by spectrophotometer. For RAPD analysis, concentration of genomic DNA, 10x PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs, 10 mer random primer and Taq DNA polymerase were optimized. Fourteen 10-base oligonucleotide primers obtained from Integerated DNA Technologies, Inc., (USA) were used for the amplification of the genomic DNA (sequences are mentioned in Table 4). Taq polymerase buffer, $MgCl_2$ and dNTPs were purchased from Fermentas (Italy). DNA amplification reactions were performed in a thermal cycler (Eppendorf). The PCR profile was: one cycle of 93°C for 5 min, 40 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min, and a final extension for 10 min at 72°C.

Analysis of RAPD data: To analyze the RAPD fragments through electrophoresis, 1.2% agarose gel was prepared in 10ng/100ml of solution in Tris borate EDTA buffer). The bands were scored from top to the bottom of each lane. Under the label of scoreable fragments, all visible and unambiguous fragments that worked with primers were counted. The PCR resultant profiles of the twenty genotypes were compared with each other, and on the basis of presence or absence of DNA fragments bands, were marked. By utilizing Nei and Li's genetic similarity and distance matrix, detailed analyses were performed (Nei & Li, 1979). The equation used was: No. of shared amplification products = 2 x (No. of common bands between any two lanes)/(Total No. of bands in the same two lanes). For the sake of evaluating genetic relationship among genotypes, dendrograms were constructed using unweighed pair group of arithmetic means

(Sneath & Sokal, 1973) by using software PHYLIP version 3.5; effective number of alleles (Hartl & Clark, 1989); gene diversity between loci Nei (1973); Shannon's information index (Shannon & Weaver, 1949); estimate of gene flow (Slatkin & Barton, 1989) and gene frequency was calculated. Population structure has been developed using Chi-square and likelihood ratio Hardy-Weinberg equilibrium at each locus Levene (1949) using POPGEN 32 version 1.31.

Results

A total of 589 DNA fragments were generated from 20 wheat genotypes using 14 random decamers with an average of 28.8 bands/genotype. The polymorphic number of 14 loci was 225 averaging 11.25 / genotype. Polymorphic loci have been expressed in percentages of all loci that are polymorphic regardless of allele frequency. Maximum percentage of polymorphic loci was 92.86% for TJ-83 (Fig.1 and Table 1). Linear regression was found between the total number of bands and number of polymorphic loci for each wheat genotype.

Gel images of 20 wheat genotypes are also furnished for ready reference (Fig. 2). Summary of the genetic similarity among 20 wheat genotypes, calculated for each ten-mer is shown in Nei's genetic distance (Table 2) based on the 225 polymorphic loci. Bioassay results (Table 3) more or less correspond to the RAPD analysis as the highly resistant genotypes-Inqilab-91 and Moomal-2002 had minimum genetic distance. The entire similarity matrix ranged from 0.1% for Inqilab-91 and Moomal-2002 to 2% for Bakhtawar-92 and Sassui. Genetic distance (GD) based on unweighed pair group method with arithmetic average has been made among all genotypes; the average diversity values were calculated based on RAPD derived data.

Dendrogram Interpretation: The dendrogram discriminates the entire genotypes tested (Fig. 3). Four clusters are generated on the basis of genetic distance. First cluster represented seven wheat genotypes viz., TD-1, Bhittai, SD-1200/19/1, Abadgar-98, Bakhtawar-92, Sarsabz and SD-4047. TD-1 has maximum genetic distance value in this cluster i.e., 0.55 and it distinctly separated from the rest of the genotypes of cluster 1. Bhittai and SD-1200/19/1 are the closest genotypes and present at 0.26 GD. Abadgar-98 and Bakhtawar-92, the susceptible genotypes were found at 0.3 GD. Cluster 2 is comprised of 11 genotypes; SD-8006, Marvi-2000, SD-8012, Imdad-2005, Sassui, Pak-81, Kiran-95, Mehran-89, Anmol-91, MH-97 and Inqilab-91. Among these SD-8006, Marvi-2000 and Inqilab-91 (resistant genotypes) existed at less than 0.45 GD in cluster-2, Imdad-2005 and Sassui are the closest genotypes with 0.1 GD value among all, that have been studied. A genotype MH-97 that behaves moderately resistant is erected out from the cluster at GD value= 0.6. The remaining 2 clusters consist of single genotype each that diversifies genetically from the rest of the genotypes and from each other with genetic distance 0.9 and 1.0, respectively. The most diversified genotype is Moomal-2002 with maximum GD value- 1.0.

14 different loci, for effective number of alleles "ne", i.e., estimates the reciprocal of homozygosity was ranged between 1.02-1.43 with an average of 1.26. Genetic diversity for loci "h" ranged between 0.02 for OPA-02 to 0.30 for OPA-09. Shannon Index estimates information index as a measure of gene diversity. OPA-09 has maximum information index as compared to the rest of the loci with an average of 0.34 for all loci (Table 4). It explains that primer pair OPA-09 was most polymorphic in nature and can be used efficiently for screening diversity. Diversity in subdivided population for different examined loci estimates Nm = estimate of gene flow. Highest gene flow value was of OPA-09 whereas the least gained was for OPA-10; average gene flow value of all 14 loci was 11.09 (Table 4) and gene frequency for all loci considering heterozygous individual for the particular loci furnished in Fig. 4. Population structure was estimated using expected and observed values for each loci by calculating Chi- square and likelihood ratio, maximum value was of primer pair OPA-10 and least of OPA-09 for both parameters (Table 5). Highest probability 1.00 was of OPA-09 where df = 19.

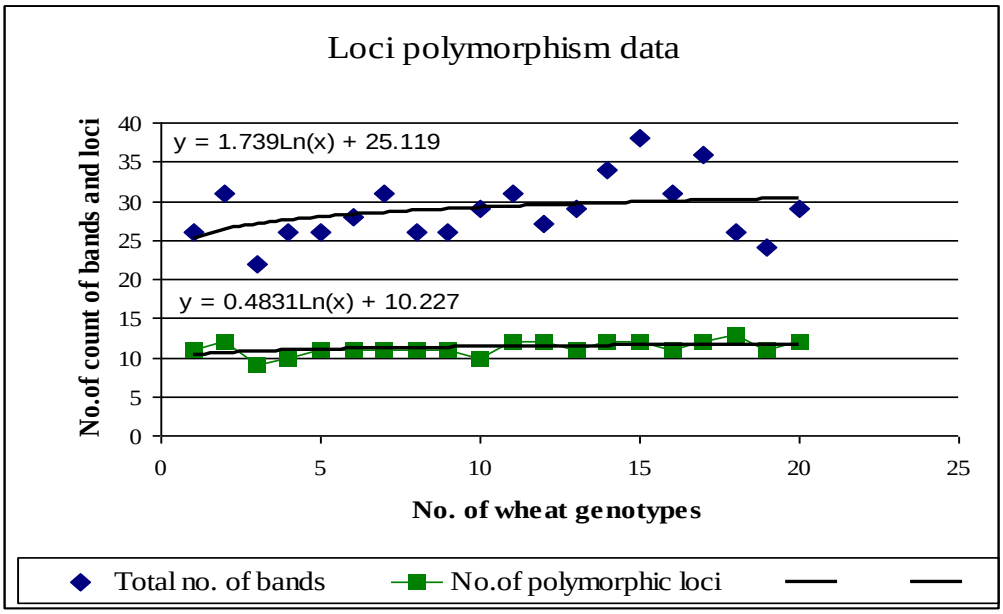

Fig.1. Loci polymorphism data of different wheat genotypes.

Table 1. Description of polymorphic loci originated from different wheat genotypes.

Wheat genotypes	No. of bands	No. of polymorphic loci	Percentage of polymorphic loci
Sarsabz	26	11	78.57
Pak-81	31	12	85.71
TD.1	22	9	64.29
SD-8006	26	10	71.43
Kiran-95	26	11	78.57
Mehran-89	28	11	78.57
Bakhtawar-92	31	11	78.57
Imdad-2005	26	11	78.57
Anmol-91	26	11	78.57
SD-1200/19/1	29	10	71.43
SD-8012	31	12	85.71
MH-97	27	12	85.71
Inqilab-91	29	11	78.57
Mommal-2002	34	12	85.71
Sassui	38	12	85.71
Abadgar-98	31	11	78.57
SD-4047	36	12	85.71
TJ-83	26	13	92.86
Marvi-2000	24	11	78.57
Bhittai	29	12	85.71

Table 2. Nei's original measures of genetic distance

ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	****																			
2.	0.0132	****																		
3.	0.0080	0.0136	****																	
4.	0.0071	0.0133	0.0017	****																
5.	0.0081	0.0149	0.0053	0.0052	****															
6.	0.0170	0.0123	0.0086	0.0085	0.0074	****														
7.	0.0166	0.0116	0.0111	0.0110	0.0123	0.0063	****													
8.	0.0058	0.0159	0.0070	0.0058	0.0020	0.0120	0.0140	****												
9.	0.0191	0.0086	0.0136	0.0111	0.0135	0.0107	0.0137	0.0134	****											
10.	0.0130	0.0083	0.0148	0.0134	0.0158	0.0107	0.0094	0.0145	0.0115	****										
11.	0.0065	0.0063	0.0036	0.0048	0.0076	0.0086	0.0096	0.0087	0.0076	0.0075	****									
12.	0.0117	0.0075	0.0077	0.0070	0.0064	0.0059	0.0068	0.0086	0.0056	0.0067	0.0035	****								
13.	0.0059	0.0086	0.0048	0.0066	0.0093	0.0084	0.0081	0.0110	0.0164	0.0072	0.0031	0.0066	****							
14.	0.0069	0.0082	0.0072	0.0090	0.0087	0.0079	0.0077	0.0104	0.0148	0.0054	0.0031	0.0054	0.0015	****						
15.	0.0168	0.0214	0.0215	0.0191	0.0213	0.0232	0.0292	0.0181	0.0226	0.0180	0.0165	0.0220	0.0168	0.0160	****					
16.	0.0229	0.0087	0.0234	0.0239	0.0245	0.0228	0.0233	0.0249	0.0116	0.0142	0.0117	0.0108	0.0165	0.0167	0.0265	****				
17.	0.0110	0.0066	0.0098	0.0116	0.0138	0.0122	0.0089	0.0144	0.0112	0.0061	0.0031	0.0047	0.0041	0.0043	0.0198	0.0065	****			
18.	0.0086	0.0078	0.0115	0.0103	0.0083	0.0131	0.0128	0.0070	0.0075	0.0077	0.0055	0.0043	0.0096	0.0091	0.0205	0.0080	0.0053	****		
19.	0.0061	0.0111	0.0054	0.0050	0.0080	0.0133	0.0160	0.0069	0.0101	0.0171	0.0055	0.0117	0.0098	0.0115	0.0185	0.0214	0.0133	0.0101	****	
20.	0.0104	0.0149	0.0049	0.0062	0.0067	0.0131	0.0169	0.0092	0.0137	0.0240	0.0065	0.0115	0.0107	0.0122	0.0211	0.0226	0.0137	0.0134	0.0038	****

Nei's genetic distance.

Sarsabz (1), Pak-81 (2), TD-1 (3), 8006 (4), Kiran-95 (5), Mehran-89 (6), Bakhtawar-92 (7), Imdad -2005(8), Anmol-91 (9), 1200/19/1 (10), 8012 (11), M-H-97 (12), Inqilab-91 (13), Mommal 2002 (14), Sassui (15), Abadgar-98 (16), 4047 (17), TJ-83 (18), Marvi 2000 (19) and Bhittai (20).

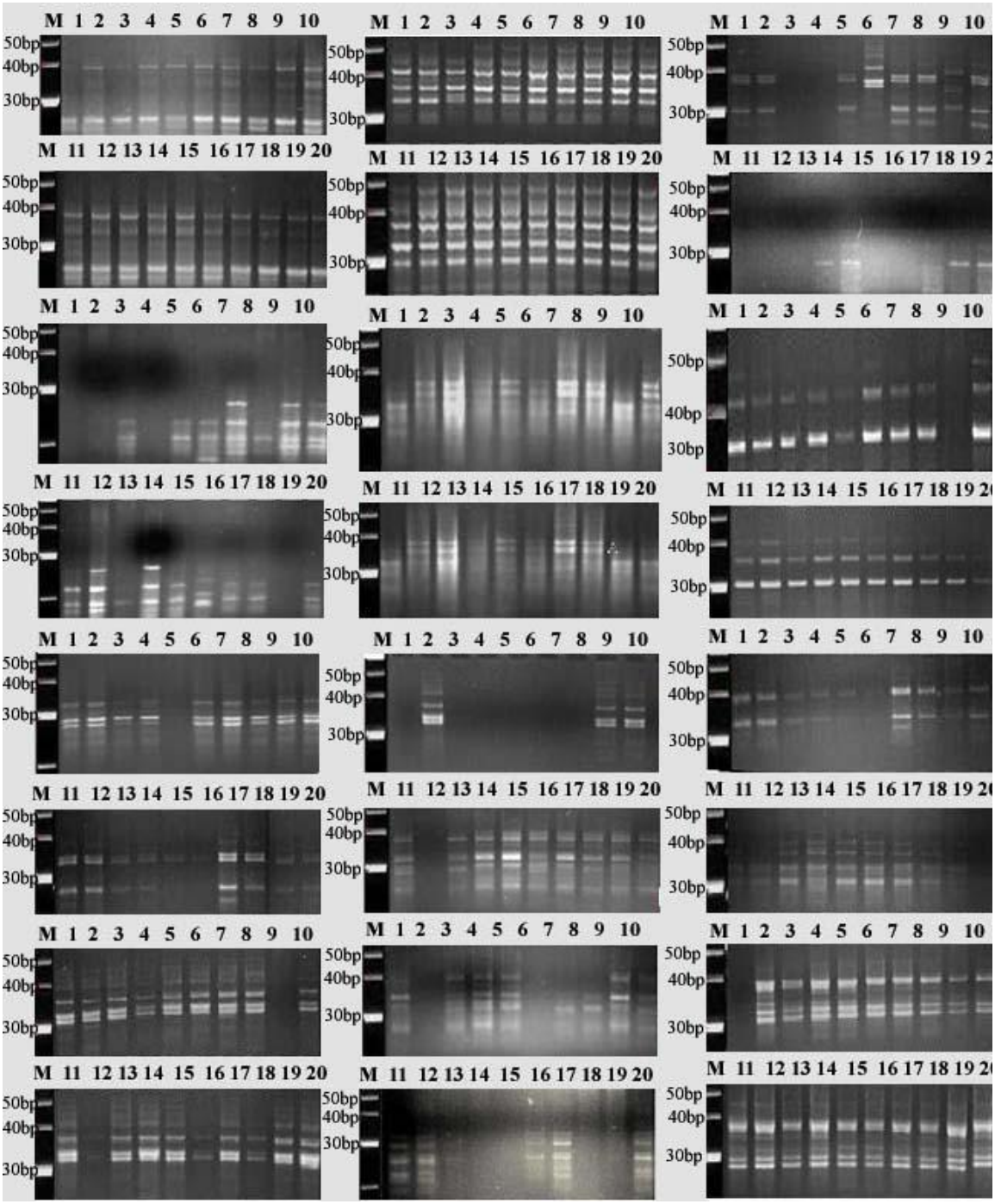

Fig. 2. Transilluminated gel images of 14 RAPD markers applied on 20 wheat genotypes: Sarsabz (1), Pak-81 (2), TD-1 (3), 8006 (4), Kiran-95 (5), Mehran-89 (6), Bakhtawar-92 (7), Imdad-2005(8), Anmol-91 (9), SD-1200/19/1 (10), 8012 (11), M-H-97 (12), Inqilab-91 (13), Mommal-2002 (14), Sassui (15), Abadgar-98 (16), 4047 (17), TJ-83 (18), Marvi-2000 (19) and Bhittai (20).

Table 3. Reproduction rate of *Heterodera avenae* pathotypes (Ha 41 and Ha 12) parasitizing wheat genotypes.

Wheat genotypes	No. of cyst females / plant root system (Mean ± SD)		No. of juveniles / g of soil (Mean ± SD)		Bioassay rating
	Pathotype Ha 41	Pathotype Ha 12	Pathotype Ha 41	Pathotype Ha 12	
Sarsabz	5.33±2.08	3.00±2.00	12.00±6.08	10.33±2.08	MR
Pak-81	9.33±2.51	10.67±1.52	45.00±6.24	35.33±4.50	S
TD.1	3.67±1.52	2.66±1.52	8.60±5.50	11.67±2.08	R
SD-8006	4.33±1.15	3.00±1.00	8.66±2.51	12.00±2.64	R
Kiran-95	3.00±1.73	1.17±0.57	11.67±1.52	13.67±2.88	MR
Mehran-89	11.33±3.21	13.00±3.60	34.00±7.21	30.00±6.00	S
Bakhtawar-92	10.67±1.15	9.33±1.52	25.67±2.08	20.33±5.03	S
Imdad–2005	0.33±0.57	2.33±0.57	14.00±4.58	12.00±3.60	MR
Anmol-91	10.00±2.00	9.33±1.52	35.00±9.64	39.33±2.08	S
SD-1200/19/1	8.67±1.15	7.00±1.00	18.66±2.51	20.00±9.16	MS
SD-8012	3.00±1.73	0.67±1.15	12.67±6.02	12.33±3.51	MR
MH-97	4.00±2.00	3.33±2.08	13.67±4.72	15.00±4.35	MR
Inqilab-91	2.00±1.00	1.67±0.57	13.33±4.16	8.00±4.35	R
Mommal-2002	2.00±1.00	2.67±1.15	1.00±1.00	8.67±4.72	R
Sassui	9.00±1.00	9.00±1.00	38.33±4.04	24.00±6.24	S
Abadgar-98	9.33±2.51	9.00±3.60	28.66±3.21	39.00±5.19	S
SD-4047	10.00±3.46	8.67±3.21	18.33±2.08	15.33±3.51	MS
TJ-83	11.67±2.51	10.67±1.15	19.33±1.52	18.00±2.00	MS
Marvi-2000	1.00±1.00	0.67±1.15	4.66±3.05	7.33±4.04	R
Bhittai	12.33±2.30	2.33±0.57	10.00±1.00	8.33±3.21	R
LSD$p \leq 0.01$	1.95 *		2.64*		

*=Significant values

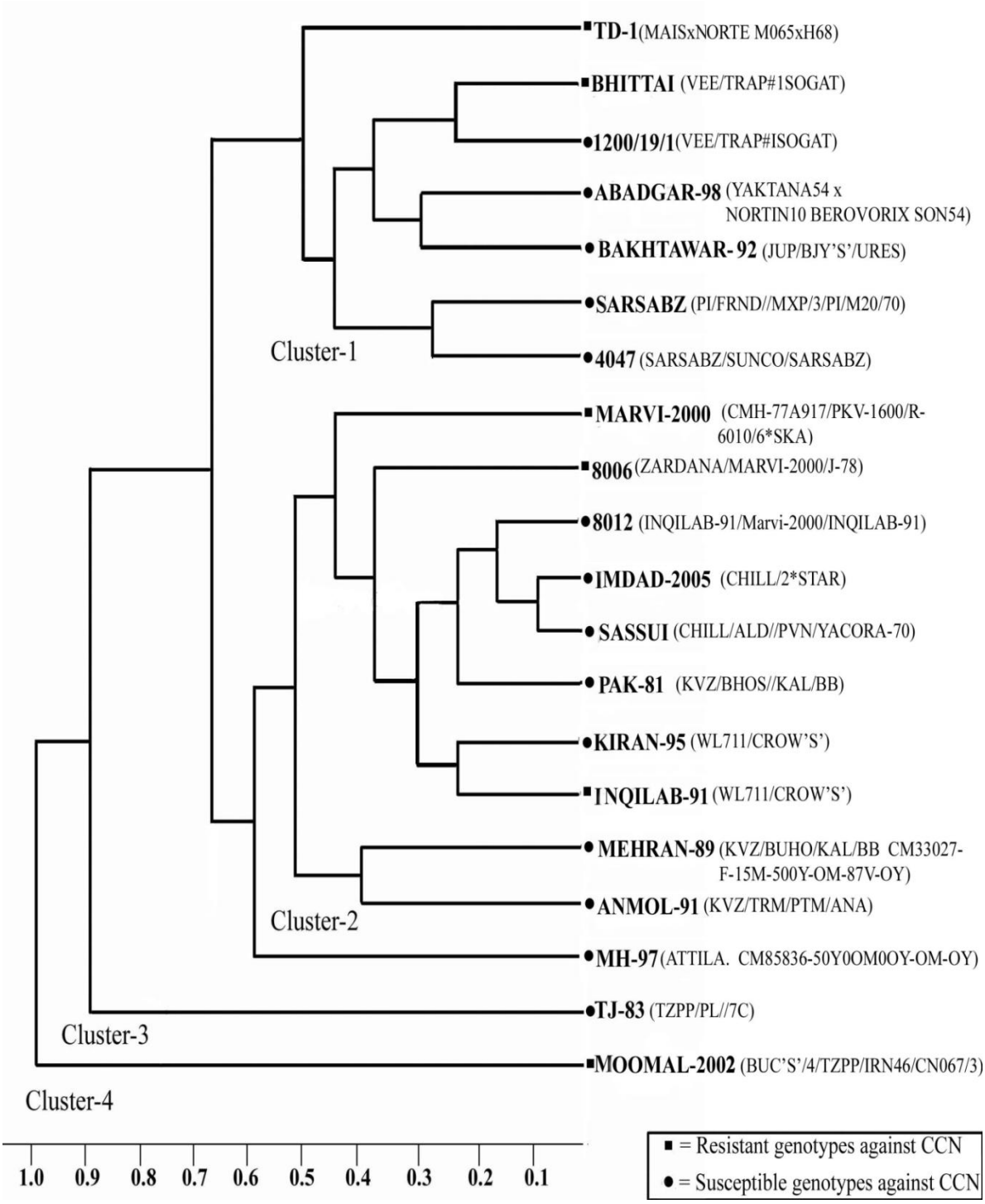

Fig. 3. Dendrogram based Nei's (1972) genetic distance constructed using UPGMA method.

Table 4. Summary of genic variation statistics for all loci and gene flow in subdivided population.

Locus	Sequence	Ne*	h*	I*	Nm*
OPA-01	CAGGCCCTTC	1.3066	0.2347	0.3972	18.463
OPA-02	TGCCCAGCTG	1.0210	0.0206	0.0579	6.7320
OPA-04	AATCGGGCTG	1.2113	0.1744	0.3174	11.107
OPA-07	GAAACGGGTC	1.3034	0.2328	0.3948	9.4590
OPA-08	GTGACGTAGG	1.2838	0.2210	0.3797	26.390
OPA-09	GGGTAACGCC	1.4399	0.3055	0.4836	82.121
OPA-10	GTGATCGCAG	1.1806	0.1529	0.2871	3.3623
OPA-11	CAATCGCCCG	1.1142	0.1025	0.2106	4.2345
OPA-12	TCGGCGATAG	1.2910	0.2254	0.3854	25.578
OPA-14	TCTGTGCTGG	1.2300	0.1870	0.3347	15.518
OPB-03	CATCCCCCTG	1.3931	0.2822	0.4558	11.155
OPB-06	TGCTCTGCCC	1.3331	0.2499	0.4163	9.4403
OPD-04	TCTGGTGAGG	1.2513	0.2008	0.3532	5.3039
OPD-11	AGCGCCATTG	1.2994	0.2304	0.3918	18.413
Mean		1.2613	0.2014	0.3475	11.094
St. Dev		0.1076	0.0730	0.1080	

Sample size=200; Observed number of alleles=2.0000;
* ne = Effective number of alleles;* h = Nei's (1973) gene diversity;* I = Shannon's Information index * Nm = estimate of gene flow in subdivided populations (The number of polymorphic loci is: 14; the percentage of polymorphic loci is: 100.00).

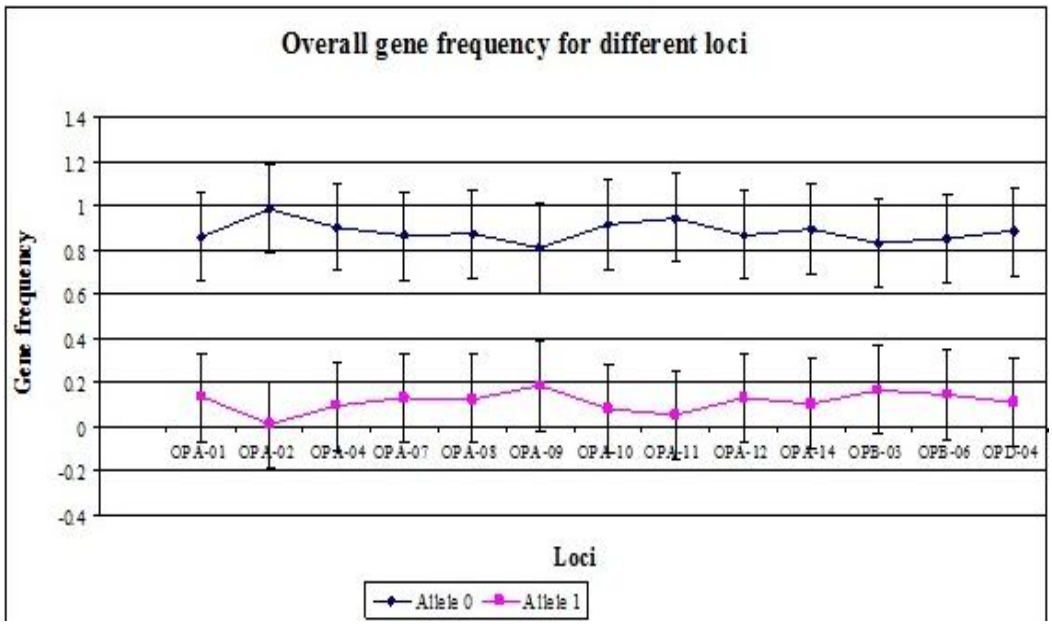

Fig. 4. Gene frequency of different loci for heterozygous individuals.

Table 5. Population structure estimations using Chi-square and likelihood ratio using Hardy-Weinberg equilibrium at each locus.

Locus	Chi-square	Probability	G-square	Probability
OPA-02	13.82	0.79	8.31	0.98
OPA-04	8.61	0.97	12.03	0.88
OPA-07	10.04	0.95	7.37	0.99
OPA-08	3.71	0.99	3.90	0.99
OPA-09	1.21	1.00	1.19	1.00
OPA-10	25.89	0.13	28.41	0.07
OPA-11	21.12	0.33	22.50	0.25
OPA-12	3.83	0.99	3.85	0.99
OPA-14	6.24	0.99	6.32	0.99
OPB-03	8.57	0.97	8.90	0.97
OPB-06	10.06	0.95	14.07	0.77
OPD-04	17.22	0.57	23.56	0.21
OPD-11	5.28	0.99	6.67	0.99

Df = 19

Discussion

RAPD-PCR revealed diversity of genomic DNA of 20 commercially grown wheat genotypes by producing multiple fragments. Three individual plants of each genotype were tested separately; all have shown similar banding patterns, indicating that the genotypes have high degree of homozygosity. Pedigree analysis, morphological traits or advance technique i.e. molecular markers are different ways to get information about genetic diversity (Pejic *et al*., 1995). RAPD technique is used to detect DNA polymorphism (William *et al*., 1990; Welsh & McClelland, 1990) and works as efficiently as marker assisted selection (MAS) to screen genotypes for desired traits (Prasad *et al*., 2002).

The dendrogram effectively demonstrated the performance of RAPD markers, it finds out the genetically close related genotypes, a huge amount of genetic variation and to trace the different degrees of genetic distances. Genotypes Imdad-2005 and Sassui were distinguished with the least GD, by using 14 primers. As per pedigree information, these two cultivars share the part of same parentage. In cluster-1 Bhittai and SD-1200/19/1 represented sister lines of a single parentage (Vee/ trap # isogat) whereas the genotype Sarsabz was placed at 0.3 GD from SD-4047, which involved in its parentage.

In cluster-2, SD-8012 and SD-8006 had 0.23 distance; both shared Marvi-2000 and Inqilab-91 as their parents, where as Inqilab-91 and Kiran-95 had 0.25 GD and shared same parentage (W1-711/Crow S). Two closely linked genotypes in cluster-2, Imdad-2005 and Sassui belonged to the same group of parentage at GD = 0.15. Further, Mehran-89 and Anmol-91 were at 0.53 GD from the rest of the genotypes of the cluster and have 0.4 distance between them due to the involvement of same parents in previous generation; whereas, MH-97, is the only genotype separated from all others as its parentage not shared with any of the genotype of the same cluster. Despite the differences, this proves that the genotype Pak-81 is much close to Mehran-89, on the basis of the parental information, but the RAPD technique vigorously separated these genotypes on random multiple-locus basis. The third cluster contained TJ-83 while in cluster-4, Moomal-2002 existed which were separated from all the other genotypes studied.

When the genetic diversity between closely related species is to be studied, it is necessary to eliminate or neglect the effect of heterozygosity.

Nei & Li (1979) developed a rigorous mathematical model of genetic divergence. Under these analyes, genotypes Imdad-2005 and Sassui have shown minimum genetic distance led to infer that these varieties were completely homozygous for most of the loci studied as compared to rest of the genotypes. A highest value of heterozygosity was shown by TJ-83 and Moomal-2002 as these varieties have minimum genetic similarities. It was pronounced that the three resistant genotypes TD-1, Marvi-2000 and Moomal-2002 have maximum genetic diversity, among these three genotypes, Moomal-2002 was the most diverse one. Therefore, it is suggested that due to the distinguishing genetic makeup and CCN resistance nature, Moomal-2002 can be recommended as selected genotype against CCN.

In population genetics, it is customary to measure the genic variations of a population in term of heterozygosity or gene diversity from gene differentiation relatives to the total population to relative degree of differentiation with the subpopulation. The variation in gene frequency among sub populations analyzed by the fixation indices or F-statistics, derived formula: $1-F_{IT}= (1-F_{IS}) (1-F_{ST})$ (Wright, 1978). For the parameters, gene frequency, genetic diversity, gene diversity and gene flow values the loci OPA-09 has maximum values indicating that this loci has maximum capacity of crossing-over.

Under Mendalian inheritance system of multiple alleles behaves as follows: The probability that a random individual from the next generation will be (ai/aj), heterozygous is q^2i (i=j) or 2qiqi (i≠j) which is known as Hardy–Weinberg ratio holds against the alternative hypothesis that disturbing forces decrease the number of homozygote. The least likely hood and Chi-square value of loci OPA-09 and OPA-10 PA, respectively both parameters constitute the Hardy Weinberg equilibrium.

It is concluded that the RAPD analysis is a valuable DNA marker system to evaluate genetic diversity. The information generated about genetic relationship among resistant and susceptible genotypes against CCN will be helpful to recommend CCN resistant improved genotype is Moomal-2002. In this way, efficiency and promotion of plant breeding programs can be accelerated by marker assisted system and permit persistent progress in advancement of selected genotypes.

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