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Phylogenetic Relationship to Study the Ploidy Status and Resistance to Karnal Bunt in Indian Wheat Cultivars Using RAPD Technique

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Abstract: Tetraploid and hexaploid Indian wheat accessions were analyzed for genetic polymorphism using RAPD-PCR in order to study the gene rearrangements during polyploidization. Ten RAPD primers were employed to establish the evolutionary relationship amongst 10 tetraploid and 17 hexaploid wheat accessions. Tetraploid accession showed 75.7% polymorphism whereas hexaploid accessions showed 65.3% polymorphism. The Genetic Distance (GD) value of the tetraploid accessions ranged 0.400 to 0.966 was significantly higher than the GD values of the hexaploid accessions (0.630 to 0.952). RAPD primers clearly categorized tetraploid to hexaploid accessions in different groups according to similarity coefficient. Nearly all tetraploid accessions were grouped together. The same set of primers was also able enough to establish polymorphism amongst 20 Karnal bunt susceptible and resistant tetra and hexaploid wheat varieties. Like tetraploid accessions, susceptible varieties showed low genetic relationship. Some of the primers gave a distinct RAPD pattern for the discrimination between resistant and susceptible varieties but none of the primer was able to discriminate the resistant, susceptible and moderately susceptible wheat varieties. The phylogenetic polymorphism according to ploidy level and resistant status to KB is interpreted as the result of alteration in gene loci during polyploidization, genetic drift during inter and intra specific breeding and/or selection pressure for improved fertility.

Key words: Genetic evolution, karnal bunt resistance, polyploidy, RAPD, wheat

INTRODUCTION

Wheat (*Triticum* spp.) is the world's leading cereal grain and the most important food crop. Its diverse uses, nutritive content and storage qualities have made wheat as a staple food for more than one third of the world population. It has been cultivated in southwestern Asia, its geographic centre of origin, for more than 10,000 years. Related wild species still grow in Lebanon, Syria, Northern Israel, Iraq and Eastern Turkey. Man began breeding wheat in the early 1800s. Since then, there have been improvements in yield and grain quality, modifications in the plant architecture and increased resistance to drought, lodging, insect pests and pathogens (Poehlman and Sleper, 1995). Polyploidization has played a major role in higher plant evolution. Over the last century, important information has been generated on many aspects of population biology, speciation and polyploid genetics (Hancock, 2005). Plant evolutionary theory has been greatly enriched by studies on crop species and a majority of angiosperms (70-80%) (Masterson, 1994) including some of the most important crops (wheat, maize, potato, cotton, sugarcane) are polyploid. Polyploidization allows novel genetic interactions and its role in plant genome evolution is highly relevant (Wendel, 2000). The genetic origin of wheat is a classic example of how closely related species combine in nature to form a polyploid series.

The species of *Triticum* are grouped into three ploidy classes; diploid (2n = 14), tetraploid (2n = 28) and hexaploid (2n = 42). Currently, 11 diploid, 12 tetraploid and 6 hexaploid species of *Triticum* are recognized. Only two species of *Triticum* are commercially important, the tetraploid *T. turgidum* and the hexaploid *T. aestivum*. *T. turgidum* evolved as an alloploid combing genomes from the diploid species, *T. uratu thum ex gandil* (AA) and an unknown species (BB) related to *Aegilops speltoides* (SS). Bread wheat (*T. aestivum*) is hexaploid (2n = 42) with three (A, B and D) sub genome each containing seven pair of homologous chromosomes. Hexaploid wheat, which arose approximately 10,000 years ago (Feldman *et al*., 1995) is a classical example of allopolyploidization. It originated from the hybridization

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of tetraploid wheat *T. turgidum* (AABB) and *Aegilops tauschii* (DD) (Kihara, 1944; McFadden and Sears, 1946; Poehlman and Sleper, 1995; Friebe and Gill, 1996). Recent studies have shown that allopolyploidization triggers rapid genome changes (revolutionary changes) through the instantaneous generation of a variety of cardinal genetic and epigenetic alterations and the allopolyploid condition also facilitates sporadic genomic changes during the life of the species (evolutionary changes) that are not attainable at the diploid level. These phenomena, emphasizing the plasticity of the genome with regards to both structure and function, might improve the adaptability of the newly formed allopolyploids and facilitate their rapid and successful establishment in nature (Feldman and Levy, 2005).

Compared with other allopolyploids, wheat is considered to be a young polyploid. The identity, organization and the evolution of different genomes constituting wheat have been intensively studied in last decades (Flavell *et al*., 1987; Kimber and Sears, 1987; Feldman *et al*., 1995; Feldman and Levy, 2005). These studies were performed using a number of techniques such as cytogenetics, protein and isozyme electrophoresis, comparative mapping and molecular markers or DNA sequence comparisons. In addition, several tools that allow quick and efficient chromosomal localization in hexaploid wheat were developed, including a series of aneuploid lines (deletion, addition or substitution lines) of the variety Chinese spring (Sears, 1966; Endo and Gill, 1996). These features, combined with the possibility of producing synthetic polyploids (Feldman *et al*., 1997), make wheat a model plant to study the mechanism of evolution in polyploid species.

Polyploidization events can have many consequences on genome evolution, particularly on gene expression and gene organization (Wendel, 2000). In wheat, studies with synthetic polyploids have indicated that genome reorganization probably occurs rapidly after the polyploidization event and the coding and non-coding regions might be differentially affected (Liu *et al*., 1998a, b). So far, few studies have been performed to follow the rate and type of changes of individual loci after polyploid formation. The timing and rate of genomic variation induced by allopolyploidization in the intergeneric wheat-rye (*Triticum* spp.- *Secale cereale* L.) hybrid triticale (x Triticosecale Wittmack) was studied using Amplified Fragment Length Polymorphism (AFLP) analyses (Ma and Gustafson, 2006). Their result showed that allopolyploidization induced genome sequence variation in triticale and a great degree of the genome variation occurred immediately following wide hybridization. The data suggested that the cytoplasm and the degree of relationship between parental genomes were key factors in determining the direction, amount, timing and rate of genomic sequence variation occurring during intergeneric allopolyploidization. Seventy-two Xinjiang Triticum and *Triticum polonicum* accessions were subjected to AFLP analyses to discuss the origin of *Triticum petropavlovskyi* by Akond *et al*. (2007) and findings of their study reduced the probability of an independent allopolyploidization event in the origin of *T. petropavlovskyi* and indicated a greater degree of gene flow between *T. aestivum* and *T. polonicum* leading to *T. petropavlovskyi*.

A key question in studying gene evolution arises whether the genes have evolved independently or there was a concerted evolution (Doyle and Gaut, 2000). The identification of resistant genes in several wheat cultivars, having different ploidy levels, will provide an insight to understand the impact of polyploidization in altering these resistant loci. Demeke *et al*. (1996) have characterized the Bt-10 gene in the resistant European wheat lines. The STS markers J13, Gb and J09 were used for screening wheat accessions for leaf rust resistance genes (Tyryshkin *et al*., 2006) and a leaf rust resistance gene Lr19 on the chromosome 7DL of wheat was tagged with random amplified polymorphic DNA (RAPD) (Gupta *et al*., 2006). RAPD based gene loci allow us to analyze the evolutionary relationship between wheat genotypes of different ploidy and identify resistance of genotypes against Karnal Bunt (KB).

Knowledge of genetic diversity and relationship among a set of germplasm and the potential merits of genetic diversity would be beneficial to all the phases of crop improvement. Assessment of genetic diversity of the elite germplasm have been sought and used by plant breeding for numerous reasons e.g., genetic relationships, parent selection, germplasm management and protection among others (Lee, 1995). To date, no information is available on variation in Indian tetraploid and hexaploid wheat genotypes at the molecular level. There is an urgent need that the germplasm should be well maintained and efforts should be made to organize research programs on germplasm characterization, utilization and enhancement including molecular characterization. The investigation presented here, therefore, was undertaken with the objective to evaluate and compare genetic diversity between and within tetraploid and hexaploid wheat genotypes.

MATERIALS AND METHODS

Collection of wheat lines: Seventeen accessions of bread wheat (*Triticum aestivum*) and ten of durum wheat (*T. durum*) (Table 1), used to study genetic diversity, were

Table 1: The place of collection of the accessions used to study of genetic divergence during polyploidization

Accession No.	Village	State	Class
IC-78754	Jhunjhunu	Rajasthan	*T. aestivum*
IC-78868	Pali	Rajasthan	*T. aestivum*
IC-78944	Jhunjhunu	Rajasthan	*T. aestivum*
IC-79037	Hamirpur	Himachal Pradesh	*T. aestivum*
IC-79105	Hamirpur	Himachal Pradesh	*T. aestivum*
IC-82198	Shimla	Himachal Pradesh	*T. aestivum*
IC-82199	Shimla	Himachal Pradesh	*T. aestivum*
IC-82202	Shimla	Himachal Pradesh	*T. aestivum*
IC-82233	Shimla	Himachal Pradesh	*T. aestivum*
IC-82234	Kinnaur	Himachal Pradesh	*T. aestivum*
IC-82272	Kinnaur	Himachal Pradesh	*T. aestivum*
IC-82280	Kullu	Himachal Pradesh	*T. aestivum*
IC-82249	Chittorgarh	Rajasthan	*T. aestivum*
IC-82256	Ahmedabad	Gujarat	*T. aestivum*
IC-99785	Chamoli	Uttaranchal	*T. aestivum*
IC-104522	-	-	*T. aestivum*
IC-104637	Alwar	Rajasthan	*T. aestivum*
IC-35102-D	Bijapur	Karnataka	*T. durum*
IC-35107-D	Bijapur	Karnataka	*T. durum*
IC-35144-D	Bellary	Karnataka	*T. durum*
IC-35148-D	Dharwar	Karnataka	*T. durum*
IC-35149-D	Dharwar	Karnataka	*T. durum*
IC-35140-D	Raichur	Karnataka	*T. durum*
IC-35141-D	Raichur	Karnataka	*T. durum*
IC-35161-D	Bijapur	Karnataka	*T. durum*
IC-35177-D	Bijapur	Karnataka	*T. durum*
IC-35720-D	Palampur	Himachal Pradesh	*T. durum*

collected from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Eighteen varieties of bread wheat (*T. aestivum*) and two varieties of durum wheat (*T. durum*) were collected from CRC, Pantnagar and Department of Plant Breeding, Punjab Agricultural University, Ludhiana, India for the study of Karnal bunt resistance RAPD loci. Out of these 20 genotypes of hexaploid and tetraploid wheat, 6 were highly susceptible to KB, 10 were moderately susceptible and 4 were resistant to KB (Table 2).

Genomic DNA isolation: Genomic DNA of all 47 wheat samples (27 accessions and 20 varieties of wheat) was isolated using SDS method (Dellaporta *et al*., 1983). Isolated genomic DNA was purified (Sambrook *et al*., 1989) and quantified by both UV spectrophotometer (DU640B spectrophotometer, Beckman) and DyNA Quant 2000 flourimeter (Hoefer).

Polymerase chain reaction (RAPD): Ten decamer primers (Table 3) were selected (Teshale *et al*., 2003) for the PCR (RAPD) which was carried out in 25 μL of a reaction mixture containing 20 ng of genomic DNA, 200 μM each dNTPs, 1.25 U *Taq* DNA polymerase, 10 mM Tris-Cl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin and 0.2 μM random decamer primer. The PCR amplification was conducted in Biometra thermal cycler programmed as initial denaturation at 94°C for 5 min; remaining 45 cycles with 94°C denaturation for 1 min., 37°C annealing for 2 min and 72°C extension for 2 min. Final extension was given at 72°C for 5 min. PCR amplified products were analyzed on 1.5% agarose gel in TAE (pH 8.0) buffer (Sambrook *et al*., 1989).

Table 2: Wheat varieties used for the study of RAPD loci of the KB resistance gene

Name of variety	Status of resistance
C 306	Highly susceptible
HD 2329	Highly susceptible
WH 452	Highly susceptible
HD 2687	Highly susceptible
UP 2003	Highly susceptible
PDW 377	Highly susceptible
UP 2425	Moderately susceptible
UP 2378	Moderately susceptible
UP 2382	Moderately susceptible
PBW 343	Moderately susceptible
Raj 3077	Moderately susceptible
Raj 3765	Moderately susceptible
Sonalika	Moderately susceptible
UP 2338	Moderately susceptible
HD 2285	Moderately susceptible
PBW 455	Moderately susceptible
Lok 1	Resistance
HD 29	Resistance
HD 30	Resistance
PBW 215	Resistance

Table 3: Arbitrary decamers primary used for divergence study

Operon code	Sequence 5'-3'	G-C-content (%)
UBC 18	GGGCCGTTTA	60
UBC 337	TCCCGAACGG	70
UBC 350	TGACGCGCGCTC	70
UBC 386	TGACGCGCGCTC	50
UBC 532	TTGAGACAGG	50
UBC 534	CACCCCCTGC	80
UBC 535	CCACCAACAG	60
UBC 552	CTAAATGGCG	50
UBC 572	TTCGACCATC	50
UBC600	GAAGACCGC	60

Data analysis: All gels were scored twice, independently and manually. Presence of bands has been indicated by 1 and absence by 0. All monomorphic bands were also scored and included in the analysis. Presence or absence of unique and shared polymorphic as well as monomorphic products was used to generate a similarity coefficient. These data matrices were submitted to NTSYS-PC (Numerical Taxonomy and Multivariate system programme) (Rohlf, 1992) and were analyzed using SIMUQAL program to generate Jaccard's similarity coefficients (Sokal and Sneath, 1963). These similarity coefficients were used to construct dendrogram using the unweighted pair-group method with arithmetic average (UPGMA) using NTSYS programme.

RESULTS

The genomic DNA band of all the accessions and wheat varieties were intact and showed good quality that was preferable for RAPD analysis as the $OD_{260/280}$ ratio of

purified genomic DNA was approximately 1.8 for each sample and the concentration ranged between 880 to 1585 ng μL^{-1}. The template DNA concentration for the optimum amplification was found to be 20 ng per 25 μL reaction for all the primers. The optimized amplification protocol for all 10 primers was the same.

RAPD based study of genetic divergence during polyploidization: The numbers of RAPD loci generated by ten primers with 27 wheat accessions (genotypes) were 103 (Table 4). Out of 103 loci, 82 (79.6%) were polymorphic for one or more genotypes and remaining 21 (20.4%) were monomorphic for all the genotypes. The size of amplified products ranged between 0.3 to 3.0 kb. All the ten primers used, were informative and gave polymorphic bands for one or more genotypes. The results gave an average of 8.2 polymorphic and 2.1 monomorphic bands per primer. Primer UBC 552 gave highest 14 RAPD loci, of which 11 were polymorphic and rest were monomorphic. Primer UBC 535 and UBC 534, both gave 13 RAPD loci, out of which 12 and 10 were polymorphic, respectively. UBC 535 was able to distinguish accession IC-99785, IC-35161-D, IC-35177-D and IC-35720-D by giving unique bands, while UBC 534 gave unique bands with accession IC-35161-D and IC-35177-D only. 12 RAPD loci were observed with primer UBC 600, out of which 10 were polymorphic and a unique band of size 1.5 kb was obtained with accession IC-35616-D only.

Primer UBC 572 and UBC 386, both gave 11 RAPD loci in which only 4 polymorphic bands were obtained with UBC 572 while second primer gave 10 polymorphic bands but a unique band of size 0.5 kb was observed in primer UBC 572 only with accession IC-35720-D. 10 RAPD loci with 9 polymorphic bands were obtained with primer UBC 18 beside this a unique band of size 0.7 kb was also observed in accession IC-35177-D. Primer UBC 337, UBC 532 and UBC 350 gave 8, 6 and 5 RAPD loci, respectively. Primer UBC 532 gave 50% (i.e., 3) polymorphic bands while 100% polymorphic bands were observed with the rest two primers despite of, a unique band of size 0.85 kb was also observed in accession IC-82233 with primer UBC 337.

Genetic variation: Data of RAPD markers scanned from 27 genotypes of wheat with ten RAPD (decamer) primers was used to generate similarity coefficients. The similarity coefficient among hexaploid and tetraploid wheat ranged from 0.361 to 0.828. The results of pair-wise combinations indicated that two different accessions of tetraploid wheat (IC-35107-D and IC-35144-D) were highly related with highest value of similarity coefficient (0.966), followed by two different accessions of hexaploid wheat (IC-82280 and IC-82526) with 0.952 similarity coefficient. None of the two accessions had identical patterns. Accession IC-35177-D (tetraploid) and IC-78868 (hexaploid) were highly unrelated, showing the lowest similarity coefficient value of 0.361.

As the similarity matrix showed, the tetraploid genotypes had more wider genetic distance value (0.4 to 0.966) than hexaploid genotypes which showed a relatively narrow genetic distance value, 0.630 to 0.952. An association among the 27 wheat accessions (genotypes) revealed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis (Fig. 1). The dendrogram puts these 27 genotypes into two clusters (Cluster I and II).

Cluster I comprised of 2 sub-clusters, of which sub-cluster I consisted of 6 hexaploid accessions with similarity percentage of 75. In this sub-cluster accession IC-82199 and IC-82202 showed higher genetic similarity followed by accessions IC-78754 and IC-78868. Sub-cluster II could be classified into 2 groups. Group 1 is further divided into two sub-groups of hexaploid wheat accessions having 3 and 8 accessions per sub-group, respectively. Group 2 included 7 tetraploid wheat accessions and in turn it could be divided into two

Table 4: RAPD based study of genetic divergence of different wheat accessions (genotypes)

	Total RAPD	Polymorphic loci		Monomorphic loci		Unique bands		Mol. wt. of bands (bp)		
Primer	loci	No.	(%)	No.	(%)	No.	Size	Lowest	Highest	Genotypes distinguished
UBC-18	10	9	90.0	1	10.0	1	0.7 kb	300	2100	IC-35177-D
UBC-337	8	8	100.0	0	0.0	1	0.85 kb	300	2800	C-82233
UBC-350	5	5	100.0	-	-	-	-	500	3000	
UBC-386	11	10	91.0	1	9.0	-	-	500	2000	
UBC-532	6	3	50.0	3	50.0	-	-	300	2500	
UBC-534	13	10	77.0	3	23.0	2	0.8 kb	400	2500	IC-35161-D, IC-35177-D
UBC-535	13	12	92.3	1	7.7	4	< 0.9 kb	300	2000	IC-99785, IC-35161-D, IC-35177-D, IC-35720-D
UBC-552	14	11	78.6	3	21.4	-	-	400	3000	
UBC-572	11	4	36.4	7	63.6	1	0.5 kb	400	1800	IC-35720-D
UBC-600	12	10	83.3	2	16.7	1	1.5 kb	300	2500	IC-35161-D
Total/Average	103	82	79.6	21	20.4	10	-	-	-	5 out of 27

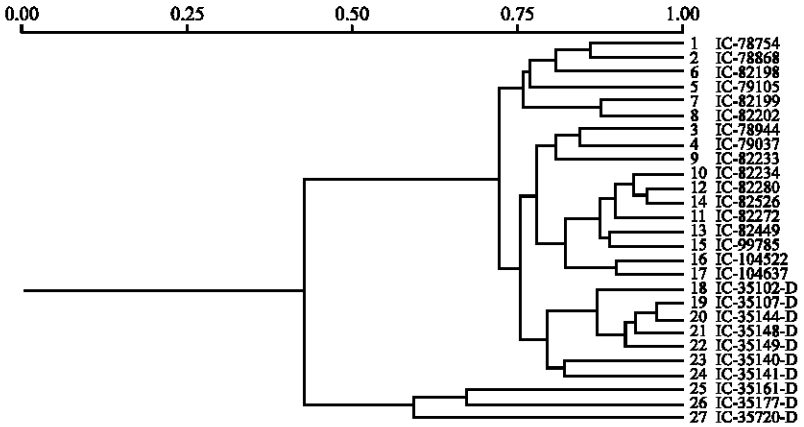

Fig. 1: Dendrogram of wheat genotypes constructed using UPGMA based on Jaccard's similarity coefficients. (Scale on top is Jaccard's coefficient of Similarity; 1 to 17 are hexaploids and 18-27 are tetraploids)

Table 5: RAPD pattern in the context of Karnal Bunt

	Resistant varieties					Moderately susceptible varieties					Highly susceptible varieties				
	Total RAPD	Polymorphic loci		Monomorphic loci		Total RAPD	Polymorphic loci		Monomorphic loci		Total RAPD	Polymorphic loci		Monomorphic loci	
Primer	loci	No.	(%)	No.	(%)	loci	No.	(%)	No.	(%)	loci	No.	(%)	No.	(%)
UBC 337	9	6	66.6	3	33.3	11	10	90.9	1	9.1	8	5	62.5	3	37.8
UBC 350	5	3	60.0	2	40.0	5	3	60.0	2	40.0	7	5	71.4	2	28.5
UBC 386	9	4	44.4	5	55.5	11	7	63.6	4	36.3	11	7	63.0	4	47.0
UBC 535	4	1	25.0	3	75.0	5	2	40.0	3	60.0	7	5	71.4	2	28.5
UBC 552	8	4	50.0	4	50.0	5	3	60.0	40	40.0	7	5	71.4	2	28.5
UBC 572	6	4	66.6	2	33.3	11	8	72.7	3	27.3	5	3	40.0	3	60.0
UBC 600	4	2	50.0	2	50.0	4	2	50.0	2	50.0	4	2	50.0	2	50.0
Total	45.0	24.0	53.3	21.0	46.7	52.0	35.0	67.3	17.0	32.7	48.0	30.0	62.5	18.0	37.5
Average	6.4	3.4		3.0		7.4	3.5		2.4		6.8	4.2		2.58	

sub-groups having 2 and 5 accessions per sub-group, respectively. Cluster II comprised of 3 unique tetraploid wheat accessions, which could be divided into 2 sub-clusters. Accession IC-35720-D is one sub-cluster and the rest two accessions (IC-35177-D and IC-35720) were in other cluster.

Genetics relationship with KB resistance: The RAPD pattern with seven primers (3 primers not gave informative RAPD pattern), among resistant, moderately susceptible and highly susceptible genotypes, showed high polymorphism (Table 5). Total 45 RAPD loci were observed with KB resistant varieties, out of which, 24 (53.3%) were polymorphic and 21 (46.7%) were monomorphic. Nine RAPD loci with 6 (66.6%) polymorphic bands were obtained with primer UBC 337. 9 RAPD loci but with 4 (44.4%) polymorphic bands were also observed with UBC 386 primer. Eight RAPD loci with 50% polymorphism, 6 RAPD loci with 66.6% polymorphism and 5 RAPD loci with 60% polymorphism were recorded with primer UBC 552, UBC 572 and UBC 350, respectively. Primer UBC 535 and UBC 600, both gave 4 RAPD loci but with 25 and 50% polymorphic bands, respectively. In terms of average, 6.4 RAPD loci with 3.4 polymorphic and 3.0 monomorphic loci were generated for each primer.

The RAPD amplification for 10 moderately susceptible wheat varieties for KB gave a total of 52 RAPD loci (Table 5). Out of which, 35 (67.3%) were polymorphic and 17 loci (32%) were monomorphic. The highest number of RAPD loci, 11 was generated from the primer UBC 386, UBC 572 and UBC 337, while the highest number of the polymorphic loci, 10 was generated from the primer UBC 386 again. Lowest number of RAPD loci, 4 (with 50% polymorphic bands) were generated from the primer UBC 600, while 5 RAPD loci were obtained by primer UBC 350, UBC 535 and UBC 552 with 60, 40 and 60% polymorphic bands. Thus, an average of 7.4 RAPD loci was generated for each primer. In terms of average, 3.5 loci were polymorphic and 2.4 loci were monomorphic for each primer. Wheat varieties from different genetic lineage were included in the moderately susceptible group, therefore they display greater polymorphism.

The RAPD amplification with 6 highly susceptible wheat varieties for KB with all 7 primers gave 48 RAPD loci, in which 30 loci (62.5%) were polymorphic and 18 loci (37.5%) were monomorphic, giving an average of

Table 6: Analysis of amplicons defining Karnal bunt resistance

Primer	Unique band(s)	Size	Variety	Disease status
UBC 337	1	0.4 kb	UP 1109	Highly susceptible
UBC 350	2	0.7 kb	HD 2687	Highly susceptible
		0.6 kb	Raj 3077	Moderately susceptible
UBC 386	5	0.8 kb	Sonalika	Moderately susceptible
		2.5 kb	Sonalika	Moderately susceptible
		0.7 kb	Sonalika	Moderately susceptible
		0.4 kb	Raj 3765	Moderately susceptible
		1.0	C- 306	Highly susceptible
UBC 535	2	1.2 kb	HD 2687	Highly susceptible
		2.0 kb	UP 2003	Highly susceptible
UBC 552	1	3.5 kb	HD 29	Resistant
UBC 572	2	0.8 kb	UP 2338	Moderately susceptible
		1.0 kb		
UBC 600	2	0.7 kb	HD 2329	Moderately susceptible
		3.5 kb	HD 30	Resistant

6.8 RAPD loci for each primer with 4.2 polymorphic and 2.58 monomorphic loci (Table 5). The highest number of RAPD loci, 11 and polymorphic loci, 7 was generated by UBC 386 while primer UBC 337 gave 8 RAPD loci with 62.5% polymorphism. Three primers, UBC 350, UBC 535 and UBC 552, gave 7 RAPD loci with 71.4% polymorphic bands. Primers UBC 572 and UBC 600 gave 5 and 4 RAPD loci with 40 and 50% polymorphic bands, respectively. Maximum number of monomorphic RAPD loci was present in the resistant group, if compared with susceptible and moderately susceptible varieties.

Although, PCR amplification data showed a greater polymorphism but nearly all major bands were monomorphic. Two unique bands of sizes 0.7 and 0.6 kb were obtained with primer UBC 350 that can distinguish highly susceptible wheat variety HD 2687 and moderately susceptible variety Raj 3077, respectively (Table 6). Primer UBC 337 and UBC 552 gave single unique band of sizes 0.4 kb and 3.5 kb with highly susceptible UP 1109 and resistant HD 29 wheat variety, respectively. Primer UBC 535 differentiated HD 2687 and UP 2003 (both highly susceptible) by giving unique band of sizes 1.2 and 2.0 kb, respectively. Primer UBC 600 gave unique band of sizes 0.7 and 3.5 kb and able to discriminate moderately susceptible HD 2329 and resistant HD 30. Highest 5 number of unique bands were observed with primer UBC 386, out of which 3 bands of sizes 2.5, 0.8 and 0.7 kb were obtained with moderately susceptible variety Sonalika, rest two unique bands of sizes 0.4 and 1.0 kb were observed with moderately susceptible variety Raj 3765 and highly susceptible variety C-306. Primer UBC 572 gave two unique bands of sizes 0.8 and 1.0 kb with moderately susceptible wheat variety UP 2338.

DISCUSSION

In this investigation, the genetic analysis of 17 hexaploid and 10 tetraploid Indian wheat varieties was done. Besides this, RAPD based gene loci were also studied to analyze the evolutionary relationship among 20 wheat varieties having different levels of Karnal bunt resistance, using the same set of primers. Although investigations on diversity analysis of tetraploid and hexaploid wheat have been reported (Joshi and Nguyen, 1993; Sun *et al*., 1998; Pujar *et al*., 1999) to assess the phylogenetic divergence amongst wheat according to ploidy status. However, study of changes in resistance loci during polyploidization is an interesting area to investigate the consequences on genome evolution.

Of the total amplification products scored in the RAPD analysis of this study, 82% were polymorphic and detect various levels of polymorphism between tetraploid and hexaploid Indian wheat accessions. Tetraploid accessions showed 75.7% polymorphism whereas hexaploid accessions showed 65.3% polymorphism with 10 RAPD primers, which is consistent with the findings of Joshi and Nguyen (1993) and Pujar *et al.* (1999). Low levels of polymorphism in genotypes could be attributed to a narrow genetic base and the frequent inbreeding involved in breeding programmes. Lower genetic variation was found in improved durum cultivars than in durum landraces. Genetic variation in landraces could be attributed to the considerable amount of natural outbreeding that occurs in these genotypes. The very low level of genetic diversity among cultivars could be due to limited selection pressure.

Significant genetic variation within tetraploid wheat existed as revealed by RAPD analysis although only ten accessions were used in this study. The Genetic Distance (GD) values of tetraploid accessions ranged from 0.400 to 0.966, which was significantly higher than the GD values of hexaploid accessions which ranged from 0.630 to 0.952. The narrowness of genetic basis in the modern improved wheat cultivars is widely accepted and demonstrated by both pedigree (Cox *et al*., 1986) and molecular analysis (Sun *et al*., 1996). The availability of high levels of genetic variation could be useful to diversify the genetic basis of the genotype of interest. The pair-wise comparisons indicated that within hexaploids (accession IC-104522 and IC-82199), within tetraploids (accessions IC-35161-D and IC-35102-D) and between hexaploids and tetraploids, accessions IC-35177-D and IC-78868 showed least similarity. On the other hand, within hexaploids, accession IC-82526 and IC-82280, within tetraploids, accessions IC-

35107-D and IC-35144-D and between hexaploids and tetraploids, both IC-35149-D and IC-104637 and IC-35144-D and IC-I04637 were highly associated, as indicated by the large value of the similarity coefficient.

The six hexaploid accessions grouped together in the dendrogram formed one cluster, while the rest 11 accessions stood out and grouped with the 7 tetraploid accessions. This may probably be because of interspecific hybridization of different tetraploid and hexaploid accessions due to out crossing or because of the majority of the BB genome portion of these 11 hexaploid accessions might largely come from these tetraploids or their ancestors or due to use of small number of RAPD primers for outgrouping of tetraploids and hexaploids. Wheat genome is too large and extremely complex in nature besides having different ploidy levels, so, scanning this large and complex genome and differentiating one genotype from the other is too complex. Even though information on single-nucleotide polymorphisms (SNPs) in hexaploid bread wheat is still scarce. Ravel *et al.* (2006) detected 64 single-base polymorphisms in approximately 21.5 kb (i.e., 1 SNP every 335 bp) using 26 bread wheat line. The level of polymorphism is highly variable among the different genes studied. Fifty percent of the genes studied contained no sequence polymorphism, whereas most SNPs detected were located in only 2 genes and concluded that the genome size of hexaploid wheat and its low level of polymorphism complicate SNP discovery in this species.

In present study 6 highly susceptible wheat varieties from distinct genetic lineages for KB were analyzed. In this case also polymorphism is not particularly high. Lowest number of RAPDs were produced for the susceptible varieties. For the primer UBC 337, UBC 572 and UBC 386 maximum numbers (3, 3 and 4, respectively) of monomorphic RAPD loci were present. Not all major bands were monomorphic. For the primer UBC 572, above trend was contradictory. The RAPD pattern of primer UBC 572 showed a good contrast between RAPD pattern for susceptible and resistant varieties. Several unique bands were present that could identify the several susceptible varieties from the rest of the twenty genotypes. With some primers, a distinctive RAPD pattern for resistant, susceptible and moderately susceptible was obtained. Primer UBC 572 gave a distinct RAPD pattern for discrimination between resistant and susceptible varieties.

Primer UBC 336 also gave distinct RAPD patterns that distinguish between moderately susceptible and susceptible varieties. However, distinction between susceptible and resistant varieties was not observed by this primer. None of the primers, used in the present study, were able to discriminate the resistant, susceptible and moderately susceptible genotypes. Therefore, in present study none of the molecular markers was identified for differentiation between the resistant, moderately susceptible and susceptible varieties. However, RAPD pattern using one primer (UBC 572) was successfully employed for differentiation between the resistant and susceptible varieties. In an earlier study, few molecular markers were developed for the differentiation between the resistant and susceptible verities for common Bunt (Demeke *et al.*, 1996). After screening 672 primers, one primer was detected that gave one band of size 550 bp which was present in all the resistant varieties and the other primer resulted in a band of 1.0 kb which was found in all susceptible varieties and absent in resistant varieties. The results were reproducible and primers gave the same results in other wheat varieties, resistant/susceptible to common bunt. However, screening of a very large number of decamer may be a tedious method. Other methods like AFLP and analysis based on microsatellite etc. can prove as good tools for developing the DNA markers for the resistant and susceptible varieties. Moreover, analysis of the near isogenic lines in the course of the investigation can further develop reproducible DNA markers. However, Poole *et al.* (2007) described the comparison of the Affymatrix GeneChip wheat genome array and analysis of the data generated revealed little concordance and suggested that global comparison is not possible.

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