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Arbitrarily primed-PCR based diversity assessment reflects hierarchical groupings of Indian tetraploid wheat genotypes

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Abstract Genetic diversity analysis using PCR with arbitrary decamer primers (RAPD — random amplified polymorphic DNA) was carried out in a set of 63 tetraploid wheat genotypes which comprised 24 durum landraces, 18 durum cultivars, nine dicoccum cultivars, ten less commonly cultivated species and two wild tetraploid species. The durum and dicoccum wheat genotypes are a part of the germplasm used in Indian tetraploid wheat breeding programs. A total of 206 amplification products were obtained with 21 informative primers, of which 162 were polymorphic. The highest degree of polymorphism was seen in the wild and less commonly cultivated species (68.9%). Durum released cultivars showed greater polymorphism (50.6%) than landraces (44.8%), while dicoccum cultivars showed a considerably low level of polymorphism (23.6%). Cluster analysis led to the separation of wild and cultivated genotypes, and among cultivated emmer wheat distinct groups were formed by the durum cultivars, durum landraces and dicoccum cultivars. The subgroupings of landraces had no relation to their geographical distribution. The durum cultivars formed subgroups based on common parentage in their pedigree. Among species, wild timopheevi wheat (*T. araraticum*) and its cultivated form (*T. timopheevi*) formed a distinct group distant from all other genotypes. The present study is a first attempt at determining the genetic variation in Indian tetraploid wheats at the molecular level.

Key words RAPD · Tetraploid wheat · Genetic diversity

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Introduction

The tetraploid group of species in genus *Triticum* constitutes an important genetic resource for wheat improvement. Although a number of botanical species have been described in this group by earlier workers, in more recent classifications only two broad species groups, *T. turgidum* (AABB) and *T. timopheevi* (A^tA^tGG), are recognized, with all other botanical species included as cultivated subspecies of these two species (Kimber and Feldman 1987). The most commonly cultivated tetraploid wheat, durum wheat (*T. durum*), valued for pasta products, accounts for about 10% of the world's wheat production and is under cultivation in many parts of the world. In India, prior to 1960, several local genotypes were selected from varietal mixtures which were cultivated in the deep, black soils of central and peninsular India under arid conditions with residual soil moisture. Breeding work using exotic germplasm resulted in the release of many improved varieties for dryland areas as well as for irrigated land. India also has the distinction of being one of the few countries in world where another tetraploid wheat, dicoccum wheat (*T. dicoccum*, locally known as Khapli wheat) is under cultivation in some parts; this wheat is preferred for specific food products.

The study of genetic diversity is important in a breeding program for the selection of suitably diverse parents to obtain heterotic hybrids as well as for the conservation and characterization of germplasm. Genetic diversity in wheat has been studied using markers such as isozymes (Asins 1983; Asins and Carbonell 1986, 1989) and seed storage proteins (Branlard et al. 1989; Metakovsky et al. 1989; Liu and Shepherd 1996) and DNA-based markers such as sequence tagged site (STS) markers (Chen et al. 1994), ribosomal DNA probes (May and Appels 1987), restriction fragment length polymorphisms (RFLPs, Autrique et al. 1996; Mori et al. 1995,1997) and repeat sequences (Harcourt and Gale 1991). However, both isozymes and RFLPs reveal low levels of polymorphism in wheat owing to its high proportion of repetitive DNA (Ranjekar et al. 1976; Flavell and Smith 1976) and contin-

uous inbreeding due to the mode of self-pollination. The RFLP technique is also time- and labor-intensive and often involves use of radioisotopes. Random amplified polymorphic DNA (RAPD, Williams et al. 1990; Welsh and McClelland 1990), on the other hand, has been shown to be an effective method for detecting polymorphism in wheat (Vierling and Nguyen 1992; Joshi and Nguyen 1993a, b; Sun et al. 1998) and various other crops (see Virk et al. 1995; Penner 1996 for review). Factors such as speed, efficiency and amenability to automation make RAPD the most suitable method for effective germplasm management with respect to estimating diversity, monitoring genetic erosion and removing duplicates from germplasm collections (Virk et al. 1995). Since breeding programs mainly depend on developing hybrids through conventional hybridization, a knowledge of genetic variation in the germplasm from which the parents are chosen for crossing is crucial. To date, no information is available on variation in Indian tetraploid wheat at the molecular level. The study presented here, therefore, was undertaken with the objective of evaluating genetic diversity in a representative set of germplasm from the Indian tetraploid wheat breeding program.

Materials and methods

Plant material

A total of 63 tetraploid wheat genotypes (Table 1) were used in this analysis. These represented: group 1, wild emmer wheat (*T. dicoccoides*), nine less commonly, cultivated species and, wild timopheevi wheat (*T. araraticum*) and its cultivated form (*T. timopheevi*); group 2, 18 released Indian durum cultivars; group 3, 24 local durum varieties (landraces) of India; and group 4, nine dicoccum wheat cultivars, seven of which were Indian and two exotic. In addition, the hexaploid wheat *T. aestivum* var 'Chinese Spring' was used as a standard control variety. Table 1 lists all the genotypes analyzed in this study.

DNA extraction

DNA was isolated from young leaves following the CTAB method described by Rogers and Bendich (1985). All DNAs were diluted to a concentration of 10 ng/µl for use in the polymerase chain reaction (PCR).

PCR analysis

Eighty decamer random primers of series A, F, J and V from Operon Technologies (Alameda, Calif.) were used in PCR analysis.

Table 1: Tetraploid wheat genotypes included in the study

Sr. no.	Genotype	Accession no.	Sr. no.	Genotype	Accession no.
Wild and less commonly cultivated species			Durum landraces (*T. durum*)		
1.	*T. aethiopicum*	176	28.	A-1	674
2.	*T. araraticum*	3496	29.	A-1–8-1	984
3.	*T. carthlicum*	558	30.	A-28	854
4.	*T. dicoccoides*	3515	31.	A-206	42 h
5.	*T. militinae*	2622	33.	Bijapur-370	334
6.	*T. palaeocolchicum*	2623	34.	Dharwad red	1996
7.	*T. persicum*	198	35.	Dharwad yel.	96
8.	*T. polonicum*	205	36.	Navalgund red	2612
9.	*T. pyramidale*	199	37.	Navalgund yel.	2611
10.	*T. timopheevi*	1393	38.	Ekdania-69	1635
11.	*T. turanicum*	2001	39.	Kathia-21	630
12.	*T. turgidum*	223	40.	Malvi local	1634
			41.	Narsimgarh 111	1633
Durum released cultivars (*T. durum*)			42.	Ujjain prog.-9	1632
13.	A-9–30–1	894	43.	Bansi-202	–
14.	GW-2	3493	44.	Baxi 288–18	1816
15.	Bijaga yellow	1433	45.	Bhalegaon-3	483
16.	HD-4530	1114	46.	Chandur biswa-7	487
17.	Meghdoot	1166	47.	Dasarkhed-1	481
18.	Raj-1555	3486	48.	Datala-5	485
19.	MACS-9	694	49.	Gulab	527
20.	MACS-1967	3485	51.	Motia	526
21.	Malavika	857	52.	Vidarbha local	523
22.	N-59	41	54.	Haura	776
23.	N-5749	895			
24.	NI-146	429	Dicoccum varieties (*T. dicoccum*)		
25.	PBW-34	3487	55.	Arbhavi local	1597
26.	PDW-215	3488	56.	Ex-7	33a
27.	PDW-233	3489	57.	Ex-33	33b
32.	Vishram	2012	58.	Felted khapli	33c
50.	Jay	42 k	59.	Yellow khapli	37
53.	Vijay	421	60.	KDH	36
			61.	Farrum	572
			62.	Rufrum	397
			63.	NP-200	1096
			64.	*T. aestivum* var Chinese spring (CS)	

A standard 25-µl reaction contained 20 ng template DNA, 0.5 units of *Taq* DNA polymerase (Bangalore Genei, India), 1 x PCR reaction buffer containing 1.5 mM $MgCl_2$, 5 picomoles primer and 100 µmoles of each dNTP. DNA amplifications were performed in a Perkin Elmer DNA thermal cycler 480. The thermal cycling protocol of Eastwood et al. (1994) with a few modifications as described in Naik et al. (1998) was used. Amplified products were separated on 2% agarose gels and visualized by ethidium bromide staining.

Reproducibility of RAPD data

Amplifications were repeated at least three times to ensure reproducibility. RAPD bands were considered reproducible only when they were observed in three separate amplifications using different DNA isolations. Bands seen against a heavy background smear were not scored. Each set of amplifications was accompanied by a control reaction which contained all of the reaction components except template DNA. In rare cases, bands were also obtained in the control reaction. However, these bands did not match with those in the experimental reactions.

Scoring and data analysis

For each genotype, the presence of a band (1) or its absence (0) was entered into the RAPDISTANCE computer program (Armstrong et al. 1994). Pairwise similarity coefficients (s) were calculated using the coefficient of Jaccard (1908) and subsequently converted to distance measures (*d*) using a formula *d* = 1-s of this program. Cluster analysis was performed from the distance matrix using the UPGMA (Unweighted pair group method with arithmatic averages, Sneath and Sokal 1973) and Neighbor Joining method to obtain separate dendrograms. To observe interrelationships of the wild and less commonly cultivated species, we excerpted band data of these genotypes from the original datafile and processed these in the PHYLIP program version 3.2c (Felsentein 1993) to obtain a cladogram. Nucleotide diversity value (π), which is an average genetic distance (*d*) for all of the genotype pairs in a group of interest, was calculated for each group of genotypes studied.

Results

Level of polymorphism in Indian tetraploid wheat

Of the 80 primers used for the initial screening for polymorphism using tetraploid species, 13 gave no amplification at all while 21 primers amplified polymorphic products. These 21 primers were then used for RAPD analysis of local and cultivated durum wheats and dicoccum wheats. Amplification of 64 genotypes with these primers yielded a total of 206 scorable bands, of which 162 were polymorphic (Table 2). An average of 10 bands were obtained per primer, and the amplification products ranged in size from 450 bp to 4 kb. The highest number of bands (15) was obtained with primers OPA-17 and OPV-14 while the lowest number, 5, was obtained with primer OPJ-13 (Table 2).

Figure 1 shows a representative amplification pattern obtained using primer OPV-15. This primer amplified 7 scorable bands, of which 6 were polymorphic. As in the case of most of the primers, wild and less commonly cultivated species (Fig. 1A) show more bands than the other groups. The former group reveals a total of 7 amplification products, while durum cultivars (Fig. 1B) and durum landraces (Fig. 1C) amplified 6 bands each and dicoccum wheats (Fig. 1D) amplifed 5 bands. The band $OPV15_{870}$ is monomorphic across all the genotypes.

Table 2: Percent polymorphism revealed by random primers

Sr. no.	Primer	Total number of bands	Polymorphic bands	Percentage polymorphism
1	OPA-17	15	10	66.6
2	OPF-04	14	12	85.7
3	OPF-06	07	06	85.7
4	OPF-07	12	10	83.3
5	OPF-09	08	07	87.5
6	OPF-12	08	07	87.5
7	OPF-14	11	10	90.9
8	OPJ-01	08	05	62.5
9	OPJ-04	13	10	76.9
10	OPJ-06	13	11	84.6
11	OPJ-09	07	06	85.7
12	OPJ-13	05	03	60.0
13	OPJ-14	07	07	100.0
14	OPJ-15	09	07	77.7
15	OPJ-18	10	09	90.0
16	OPV-01	08	06	75.0
17	OPV-06	11	06	54.5
18	OPV-07	06	04	66.6
19	OPV-14	15	13	86.6
20	OPV-15	07	06	85.7
21	OPV-18	12	07	58.3

The polymorphism obtained in the 63 tetraploid wheat genotypes with the 21 informative primers showed a distinct variation. In general, the first group containing wild and less commonly cultivated species showed the highest degree of polymorphism (68.9%), while durum released cultivars and local durum wheats had lower levels of polymorphism (50.6% and 44.8%, respectively). A remarkably low level of polymorphism (23.60%) was observed in dicoccum wheats. Different primers varied in their ability to detect polymorphism. For example, primer OPJ-14 revealed the highest level of polymorphism (100%) with all its amplification products being polymorphic, while primer OPV-06 revealed the lowest polymorphism (54.5%). Three primers (OPJ-01, OPJ-14 and OPV-01) did not detect any polymorphism in dicoccum wheats.

Group and genotype-specific markers

A close examination of the RAPD gel patterns revealed a number of amplification products that could distinguish the four groups of wheat genotypes. Among them, amplification product $OPJ\text{-}18_{2000}$ was specific to group 3 (landraces). A few other products were present only in two of the four groups. Band $OPA\text{-}17_{2300}$ was present only in groups 3 and 4, while 3 other products, $OPJ\text{-}04_{1850}$, $OPV\text{-}07_{1000}$ and $OPV\text{-}07_{1175}$, were present only in groups 2 and 3. Similarly, $OPF\text{-}14_{1000}$ was present in groups 1 and 3, while bands $OPF\text{-}14_{1650}$ and

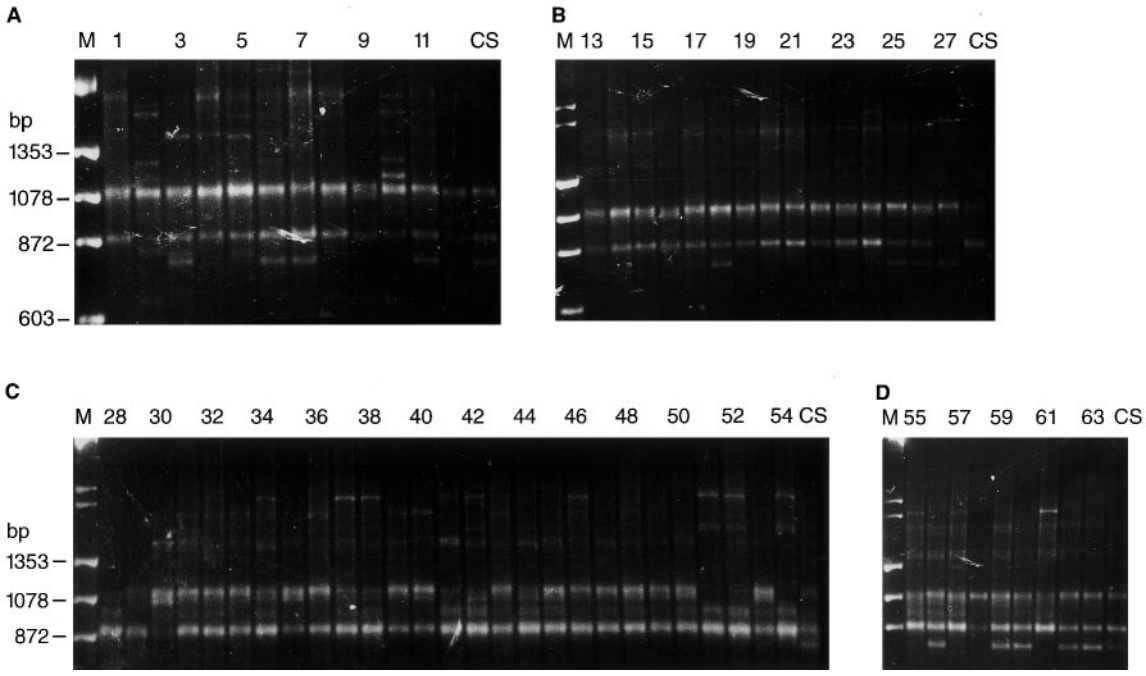

Fig. 1 A–D. RAPD profile of tetraploid wheat genotypes using the primer **OPV-15**. Wild and less commonly cultivated species (**A**), durum cultivars (**B**), durum landraces (**C**, except 32, 50 and 53 which are durum cultivars) and dicoccum cultivars (**D**). *Lane numbers* correspond to the serial numbers in Table 1. *M* molecular weight markers λ phage DNA *Hind*III digest and ϕX174 phage DNA *Hae*III digest, *CS Triticum aestivum* var 'Chinese spring'

OPJ-18_{2700} were present only in groups 1 and 2. On the other hand, the absence of bands in certain groups was also noted, for example, 2 bands, OPF-12_{550} and OPF-06_{620}, were absent in group 1 while 2 other bands, OPF-04_{870} and OPJ-15_{2000}, were absent in durum cultivars (group 2). Dicoccum wheats (group 4) in particular showed the absence of a maximum number of products, i.e 11 products (OPF-12_{775}, OPF-12_{700}, OPF-14_{1700}, OPJ-06_{1250}, OPJ-15_{2500}, OPV-01_{950}, OPV-01_{900}, OPV-14_{2000}, OPV-14_{1370}, OPV-15_{1000} and OPV-18_{700}), while they were present in the other groups.

A number of genotype-specific products could also be identified. Of these, 14 amplification products were specific to genotypes belonging to wild and less commonly cultivated species. Among them, many were specific to the two timopheevi wheats. Work is in progress to characterize these bands.

Genetic relationships of different groups based on cluster analysis

Two dendrograms showing genetic relationships among the 63 tetraploid and one hexaploid genotype were obtained using Jaccard's similarity measure and based on the UPGMA and Neighbor Joining methods, respectively. Our objective was to observe changes, if any, in the clusters of the dendrogram upon employing two different clustering algorithms. The dendrogram based on the UPGMA method and using Jaccard's similarity measure (Fig. 2) showed three important features: first, the wild and less commonly cultivated emmer wheat genotypes stood out from the cultivated ones; secondly, the two timopheevi wheats appeared separately, distant from other genotypes (d=0.123), all of which are turgidum wheats; the third important feature was that the three groups of cultivated emmers, i.e dicoccum wheats, durum landraces and durum cultivars, each formed separate clusters in the dendrogram. The wild emmer wheat *T. dicoccoides* formed a distinct cluster along with *T. palaeocolchicum* at a distance of 0.132. The only hexaploid wheat in the set, *T. aestivum* var 'Chinese spring' separated out from the tetraploid wheats at a genetic distance of 0.159.

The 24 Indian durum landraces obtained from five states of the country formed 2 major subgroups in the dendrogram, one containing the majority (18) of the landraces while the other had 4 genotypes (Fig. 2). Two landraces, namely A-1 and Malvi local, formed a third subgroup.

The 18 Indian durum cultivars formed 2 main subgroups in the dendrogram (Fig. 2). The first subgroup had 11 cultivars, while the second subgroup had 4 cultivars. The remaining 3 cultivars, namely 'Vishram', 'Jay' and 'Vijay', appeared along with the landraces, the first being in the subgroup containing the majority of the landraces and the other 2 in the second subgroup of landraces.

The cluster in the dendrogram comprising nine dicoccum cultivars showed 1 cultivar, 'Felted Khapli', at a distance of 0.058, standing out from the rest of the cultivars. The other cultivars formed 2 subgroups: the first containing 'Arbhavi local' and 'Ex-7', and the second containing the remaining 6 cultivars.

The nucleotide diversity values for wild and less commonly cultivated species, durum landraces, durum culti-

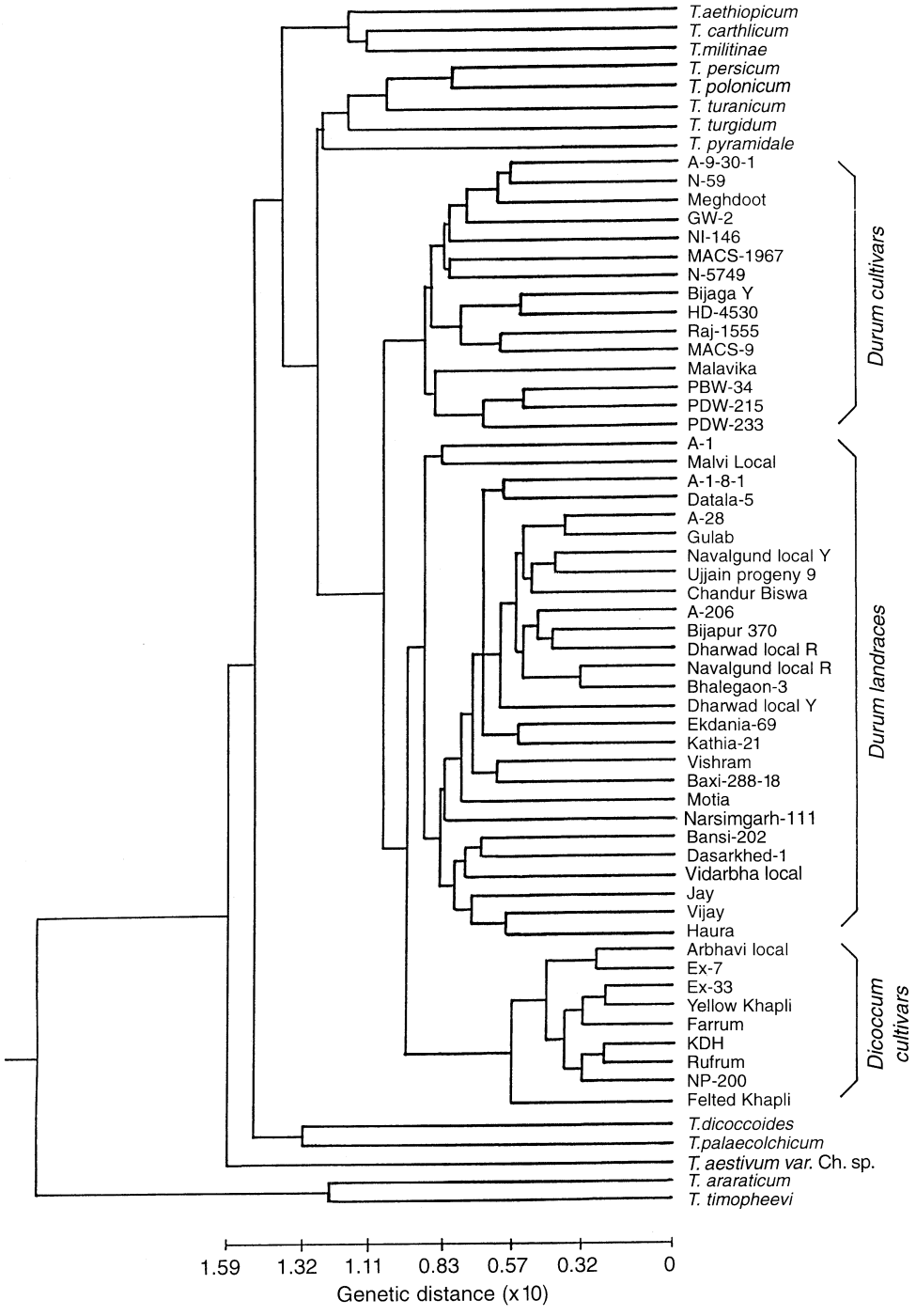

Fig. 2 Dendrogram showing interrelationships of 63 tetraploid wheat genotypes. Names of wild and less commonly cultivated species are indicated in *italics*.

vars and dicoccum cultivars were 0.314, 0.151, 0.181 and 0.086, respectively.

The band data of 12 wild and less commonly cultivated species was also processed separately to obtain a cladogram (Fig. 3). In this phylogenetic tree, timopheevi wheats separated out in the first bifurcation. *T. dicoccoides*, the progenitor of present-day cultivated tetraploid wheats and *T. palaeocolchicum* separated out in the next branching. The subsequent bifurcation showed the formation of two distinct groups, one comprising *T. persicum, T. polonicum, T. pyramidale, T. turanicum* and *T. turgidum* and the other group containing *T. aethiopicum, T. carthlicum* and *T. militinae*.

Discussion

RAPD as an efficient tool for variability analyses

Several doubts have been raised regarding the suitability of RAPD for diversity studies, the most important one being that comigrating bands may not be allelic or com-

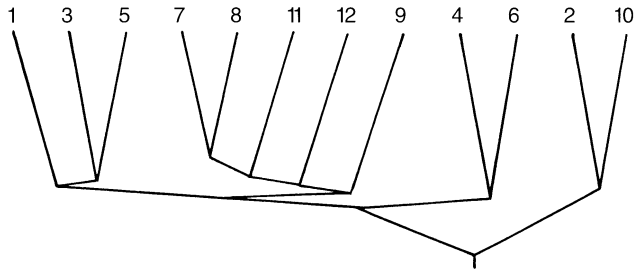

Fig. 3 Cladogram showing genetic relationships of tetraploid wheat species. *1 T. aethiopicum* (AABB), *2 T. araraticum* (A^tA^tGG), *3 T. carthlicum* (AABB), *4 T. dicoccoides* (AABB), *5 T. militinae* (AABB), *6 T. palaeocolchicum* (AABB), *7 T. persicum* (AABB), *8 T. polonicum* (AABB), *9 T. pyramidale* (AABB), *10 T. timopheevi* (A^tA^tGG), *11 T. turanicum* (AABB), *12 T. turgidum* (AABB)

posed of similar sequences. However, the homology of comigrating RAPD bands has been demonstrated in some species of *Glycine* and *Allium* (Williams et al. 1993; Wilkie et al. 1993). In addition, conformity of phylogenetic groupings based on RAPD data to those based on conventional approaches like morphology, cytology and isozyme analysis is in itself indirect, but significant evidence in support of the allelism of comigrating RAPD bands (Virk et al. 1995). Some such specific examples include studies in genome relationships of *Musa* and *Brassica* (Howell et al. 1994; Demeke et al. 1992) and between species of *Stylosanthes* (Kazan et al. 1993). Secondly, the use of a large number of polymorphic markers would minimize the skewing of results due to non-allelism. Another problem often encountered in RAPD analysis is that of reproducibility of band patterns between different PCR reactions. This aspect can be overcome by using a thoroughly optimized PCR protocol and by scoring only reproducible bands. The RAPD method has been employed in the past successfully for genetic diversity analysis in wheat (Joshi and Nguyen 1993a,b; Vierling and Nguyen 1992; Castagna et al. 1997; Sun et al. 1998). Our study on Indian tetraploid wheat genotypes has also shown that RAPD is a robust and reliable method to detect genetic diversity and study the genetic relationships of tetraploid wheat. Recently it has been reported that the data on genetic relationships among wheat genotypes obtained on the basis of RAPD markers are very similar to data obtained using other markers. Castagna et al. (1997) analyzed 49 accessions of diploid wheat and found genetic similarity values for intraspecific comparisons to be very similar using RFLP and RAPD markers. Nagaoka and Ogihara (1997), in their study using six wheat accessions, found that genetic relationships estimated by ISSR (inter-simple sequence repeat) markers were identical with those inferred by RFLP and RAPD markers.

Genetic diversity in Indian tetraploid wheat cultivars

We analyzed genetic diversity in a set of 63 tetraploid wheat genotypes consisting of wild tetraploid wheat species, less commonly cultivated species, durum landraces, durum cultivars and dicoccum cultivars. Although investigations on diversity analysis of tetraploid wheats have been reported (Asins 1983; Asins and Carbonell 1989; Autrique et al. 1996; Joshi and Nguyen 1993a; Mori et al. 1995, 1997), most did not analyze all of the above-mentioned subgroups of tetraploid wheats together. Therefore, to our knowledge, this is the first report, in which all of these subgroups have been analyzed together at the same time.

Of the total amplification products scored in the RAPD analysis, 78.6% were polymorphic and detected varied levels of polymorphism in the different categories of tetraploid wheats included in the study. We found the level of polymorphism in wild and less commonly cultivated emmer species to be much greater than that in cultivated durum wheats, which is consistent with the findings of Joshi and Nguyen (1993a). Lower levels of polymorphism in cultivars could be attributed to a narrow genetic base and the frequent inbreeding involved in breeding programs. However, the percentage of polymorphic fragments obtained in cultivated durum wheats in our study was lower than that reported by Joshi and Nguyen (1993a). In another work on genetic diversity analysis in durum wheat using RFLP markers, morphophysiological traits and coefficient of parentage, lower genetic variation was found in improved durum cultivars than in durum landraces (Autrique et al. 1996). Genetic variation in landraces could be attributed to the considerable amount of natural outbreeding that occurs in these genotypes (Tsegaye 1996). In our study, however, the durum cultivars showed a higher degree of diversity than the landraces, though one would expect the reverse, as landraces are mere selections from local germplasm and not products of repeated inbreeding like the released cultivars. The higher diversity in the cultivars may be a consequence of the fact that exotic germplasm (mostly from the CIMMYT-International Maize and Wheat Improvement Center, Mexico) was involved in the pedigree of almost all the cultivars released for cultivation in India.

The dicoccum cultivars, on the other hand, appeared to be a rather homogeneous group. The very low level of genetic diversity among dicoccum cultivars could be due to limited selection pressure. These wheats are under cultivation in only a few parts of the world. In addition to India, the dicoccum wheats are still grown on a limited scale in Ethiopia, Iran, Transcaucasia, eastern Turkey and the Balkans (Feldman, 1976). The only odd cultivar, ‘Felted khapli’ (d=0.058), which stood out from the rest, was also the only cultivar in this group with felted glumes.

No significant correlation could be drawn between the clusters in the dendrogram and the geographical distribution of durum landraces, cultivars and dicoccum cultivars. However, in the case of durum cultivars, all the genotypes from Punjab (PBW-34, PDW-215 and PDW-233) appeared in one cluster.

Table 3 Pedigrees of durum cultivars included in the study

Name	Pedigree	State
A-9-30-1	A206/Gaza	Gujarat
GW-2	GS“S”//A206/NP200(DM)	Gujarat
Bijaga yellow	Mysore local/Gaza	Karnataka
HD-4530	TPT/MOGHK//4/PI/TML//2*TC60/3/ Zenati/BTL//WLS	Madhya Pradesh
Meghdoot	Gaza/GD11//A098/Gaza/3/NP404	Madhya Pradesh
Raj-1555	CIT/Raj-911	Rajasthan
MACS-9	N59/F185(*T. polonicum*)	Maharashtra
MACS-1967	Gulab//5/BYE*2/TCTE//4*TC60/3/BYE*2/ TC60/ STW63/4/AA “S”/CIT “S”	Maharashtra
Malavika	PI “S”/2*BY//TC60/3/Zenati/BTL//WLS	Maharashtra
N-59	Gaza/Motia	Maharashtra
N-5749	G-4-48/N-59	Maharashtra
NI-146	Gaza/BAX 23	Maharashtra
PBW-34	AA “S”/FGO “S”	Punjab
PDW-215	Raj-911//AA “S”/D#2 E/3/DWL5002	Punjab
PDW-233	YAV “S”/TEN “S”	Punjab
Vishram	Jay/ *T. polonicum*	Gujarat
Jay	Motia/KPH(DM)	Maharashtra
Vijay	Natural cross of Motia/KPH(DM)	Maharashtra

Several group- and genotype-specific amplification products were observed in this study. Twenty-three amplification products could differentiate the four groups of genotypes. Some products were specific to the timopheevi wheats (A^tA^tGG). In an earlier study on diploid perennial species in Triticeae representing eight different genomes, Wei and Wang (1995) reported 29 genome-specific and 11 species-specific RAPD markers.

Thus, the RAPD analysis of 63 emmer wheat genotypes and subsequent cluster analysis of RAPD data led to two notable observations in our study: (1) the wild and cultivated species separated out clearly in the dendrogram and (2) among the cultivated species, durum landraces, durum cultivars and dicoccum cultivars formed distinct clusters. The main clusters remained largely similar in the dendrogram obtained using UPGMA method (Fig. 2) and Neighbor-Joining method (data not shown). Only subgroups of durum landraces and wild and less commonly cultivated species showed some differences in the two dendrograms.

Correlation of cluster analysis with pedigrees of durum cultivars

An examination of the pedigrees of the 18 durum cultivars (Table 3) used in this study revealed considerable correlation between the groups formed by the cultivars in the dendrogram and their parentage. Four of the five varieties which grouped together, namely ‘A-9–30–1’, ‘N-59’, ‘Meghdoot’ and ‘NI-146’ had a Palestinian variety, ‘Gaza’, in their pedigree. The fifth cultivar in this subgroup, ‘GW-2’, did not have ‘Gaza’ in its pedigree, however it shared a common parent, ‘A-206’, with ‘A-9–30–1’. Another subgroup consisted of 4 cultivars, namely ‘Malavika’, ‘PBW-34’, ‘PDW-215’ and ‘PDW-233’, all of which were derived from CIMMYT germplasm. The 3 durum cultivars,’Vishram’, ‘Jay’ and ‘Vijay’, which clustered along with the landraces, all had a pedigree which involved a single cross, and one of the parents in the cross was an Indian durum landrace. However, there were a few exceptions to the above correlation between grouping and common parentage. The cultivar, ‘Bijaga Yellow’, which was the result of a cross between an Indian landrace and ‘Gaza’, was placed in a different cluster. Similarly, ‘MACS-9’ and ‘Vishram’ share tetraploid species *T. polonicum* as a common parent, but they were placed in different subgroups.

Phylogenetic relationships of wild and less commonly cultivated species and cultivated emmer wheats

Cluster analysis of the RAPD data led to the clear distinction of the wild and less commonly cultivated species from the cultivated wheats. A similar observation by Mori et al. (1997) led them to speculate that this could be due to the limited number of domestication events during the evolution of cultivated emmers. The same logic could be extended to explain the formation of distinct groups by the durum and dicoccum wheats. However, as pointed out by Bekele (1985), genetic changes in cultivated plants during domestication coincide with genetic changes in their progenitors. Due to this complex genetic buildup, it is difficult to interpret the differences obtained in this study between wild species and their cultivated forms.

Among the wild and less commonly cultivated tetraploid species, the groupings were consistent with the currently understood path of their evolution. A clear separation of timopheevi wheats from the emmer wheats reiterates the different evolutionary history of these two groups. The evidence to date, based on cytological and molecular work, indicates that the two wheats have evolved from different hybridization events (Jiang and Gill 1994; Tsunewaki 1995; Brown-Guedira et al. 1996; Ogihara and Tsunewaki 1988). In the emmer group, *T.*

dicoccoides and *T. palaeocolchicum*, both non-free threshing wheats, were separated from all of the other free-threshing subspecies (see Fig. 3). The free threshing types in turn formed 2 groups, one containing *T. persicum*, *T. polonicum*, *T. pyramidale*, *T. turanicum* and *T. turgidum* and the other group containing *T. aethiopicum*, *T. carthlicum* and *T. militinae*. The former group had *T. persicum* as the odd member as it is considered to be a synonym for *T. carthlicum*. However, a study of chromosome pairing of the above two species by Deodikar et al. (1979) indicated that the two are not genetically identical.

In summary, our assessment of genetic diversity in Indian tetraploid wheats revealed a greater diversity in durum landraces than in durum cultivars and considerably less diversity in dicoccum cultivars. The 3 groups formed separate clusters in the dendrogram. A good correlation between the groupings of durum cultivars and their pedigrees based on our RAPD data indicates that RAPD diversity data can be used in selecting diverse parents in breeding improved cultivars and in maintaining genetic variation in the germplasm. Evaluation of genetic variation among the germplasm, particularly of wild species and landraces, is crucial in harnessing the genetic potential of these genotypes for improvement of traits needed for adaptation to various stress conditions. A previous knowledge of genetic variability of a crop is thus useful in order to limit the number of potential parents in the early stages of a breeding program. To our knowledge, this study is the first attempt in using molecular markers for assessing genetic diversity in Indian tetraploid wheat germplasm, and the outcome of this work could be useful for future breeding programs involving this germplasm.

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