Evaluation of Genetic Diversity in Indian Durum Wheat with RAPD Markers

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Genetic variability among 26 released varieties/local cultivars of *Triticum durum* was estimated by RAPD marker analysis and the results discussed in view of their centre of breeding and the pedigree. A total of 4 series of primers were used to screen the polymorphic primers and the profiles generated by 15 of such types were used for analysis. The primers OPA3 and OPP6 were the most polymorphic primers and can be used in conjugation with the morphological markers for the identification of the varieties. The varieties developed by the selection among the local race Bansi were grouped together. The highest degree of polymorphism was between the local landrace Malvaraj with Motia and Jay. The clustering on the basis of RAPD markers in the present study was clearly on the pattern of their parentage. The varieties developed through the selection in the CIMMYT material were widely dispersed and therefore can be used along with the local landraces in the breeding programme to diversify the genetic base. The grouping of the germplasm particularly the landraces by RAPD markers can be used in developing the Indian durum wheat cultivars with wider genetic base.

Key words: Diversity, Genetic Similarity, RAPD Markers, *Triticum turgidum* (durum)

Durum wheat, [*Triticum turgidum* var. *durum* Dest.] is a tetraploid species with extremely hard grains and generally used for macaroni, spaghetti and other pasta products. Knowledge about the extent of genetic variation in cultivated durum and its comparison with that of landraces is important for conservation of genetic resources and breeding. Durum wheat has longer evolutionary history than *T. aestivum* and also have been exploited to lesser extent and therefore, likely to possess sufficient polymorphism. The germplasm collection have been selected and crossed in search of new gene or gene combinations and in the process yield increase have been accompanied by increase in total biomass production, harvest index and changes in yield components such as number of spikes/unit area and spike fertility (CIMMYT, 1988). In India, durum breeding did not receive as much emphasis as aestivum wheat as it is less widely grown because of its non-suitability for *chapati* and bread making. Breeders have largely used the landraces either directly for selection or for improving the adaptability of the high yielding exotic lines. Therefore, the extent of genetic diversity present in the Indian released varieties have always been a debatable issue. The morphological characters have been used both for plant variety identification and for the diversity analysis (Yang *et al.*, 1991). Greater variation was found among the characters but not among the countries by Porceddu (1976). The use of morphological descriptors have been criticised because of environmental and epistatic interactions and the unknown genetic control of the traits (Smith and Smith, 1989). The use of biochemical markers involving seed storage protein and isozymes have been questioned due to poor genomic sampling. The pedigree analysis of the varieties released have been used by many workers to account for genetic variation and in many cases have found the narrow genetic base (CIMMYT, 1987, Martin *et al.*, 1991). Random amplified polymorphic DNA (RAPD) is one of the several multiple arbitrary amplicon profiling technique used to characterize plant varieties at the molecular level. It uses a single primer of arbitrary nucleotide sequence to generate relatively complex DNA profile (William *et al.*, 1990, Welsh and McClelland,1990). The degree of polymorphism generated by PCR based techniqu es ensures the higher level of differentiation of plant varieties in comparison to morphological and biochemical markers. The germplasm used in durum breeding in India largely comprises landraces, released Indian cultivars and improved material received from CIMMYT and ICARDA. The breeders, generally, involve these germplasm in their breeding on the performance *per se* or the pedigree which many times does not lead to highly segregating population of desired types. The characterization and grouping of the germplasm in the distinct clusters is very important for improving the breeding efficiency. The objective of the present research was to study the level of diversity present in local and released cultivars of Indian durum wheat and the results are then discussed in view of the pedigree record of the variety.

Material and Methods

Plant Material

A total of 26 local and released cultivars of *T. turgidum* var. *durum* were used for the present study. Seeds were obtained from the collections maintained at Directorate of Wheat Research Karnal, Haryana. The plant material include the varieties bred by different centres of durum breeding and the local cultivars. A complete list of the varieties along with their origin and status is presented in Table 1.

DNA Isolation

DNA was isolated from 7-day-old seedlings germinated on towel paper incubated in a seed germinator at 20°C. Plant tissues (5-10 g) were ground in liquid nitrogen and mixed immediately with 20 ml of CTAB extraction buffer 2X(1.4M NaCl, 100 mM Tris, 20 mM EDTA, 2% mercaptoethanol, 1.5% CTAB; pH 8.0). The samples were incubated at 60°C for 1 h. Chloroform: isoamyl alcohol (24:1) was then added and mixed gently by inverting the tubes for 5 min. The aqueous phase was recovered after centrifugation at 17,000 rpm for 10 min. This was followed by DNA precipitation in 2/3rd volume of isopropanol. The precipitated DNA was then spooled out and stored in 70% alocohol at 4°C. DNA was washed twice in 70% alcohol and dried under vacuum. The DNA pellet was dissolved in 10:1 TE (10 mM Tris, 1mM EDTA; pH 8.0) solution. This solution then was treated with RNAase, which was followed by two extractions. DNA was reprecipitated by adding 1/10 volume of 3M NaOAc and 2.5 volume of distilled ethanol. DNA was collected and washed, followed by drying under vacuum and dissolving in TE. The samples were then stored at -20^0 C.

PCR Reaction

For amplification of random genomic sequences in reproducible way, the reaction condition was rigorously optimized (10 mM tris -Cl pH8.3, 500 mM KCl, 200 µmols each of d NTPs, 3.25 mM $MgCl_2$, 0.5 µM primer, 0.75 U Taq DNA polymerase and 20 ng template DNA; total volume 25 µl). The PCR reaction were carried out in Perkin Elmer thermal cycler programmed for initial denaturation at 92°C for 3 min, followed by 40 cycles of 1min at 94°C, 1 min at 35°C and 1 min at 72°C.

Table 1. Durum wheat varieties/lines used in the study with their pedigree and origin

S.No.	Name	Pedigree	Origin and remarks
1	PBW 34	AASIB/FGOSIB	PAU, Ludhiana, released variety selected from the CIMMYT material
2	Jairaj	YAGULATE(Pol)/4/PI/3/ZENATI/BTL//WLS	Jabalpur (MP), released variety selected from the CIMMYT material
3	Motia	Selection from local Bansi	Niphad (Maharastra), released cultivar
4	PDW 215	Raj911//AASIB/D#2E/3/DWL5002	PAU, Ludhiana, released variety
5	Vizapur 8	Local strain	Vizapur, local strain
6	Bijaga Yellow	Bijaga Red Sib	Bijapur (Gujarat), released variety
7	A-9-30-1	A 206/ GAZA	Arnej (Gujarat), released variety
8	A-624	Selection from local	Arnej (Gujarat), released cultivar
9	HI 8381	JO 69 SIB/AASIB//FGOSIB	IARI, Indore, released variety through selection in the CIMMYT material
10	HD 4502	PISIB/2* BY//TC60/3/Zena/TI/BTL//WLS	IARI, released variety through selection in the CIMMYT material
11	Amrut	ANLC/GAZA	Annigeri (Karnataka), released variety
12	N-59	-	Niphad (Maharastra), released variety
13	Malvaraj	Selection from local	Indore (MP), local improved
14	MACS 1967	GULAB/5/ BYE*2/TCTE//4*TC60/3/BYE *2/TC60/STW63/4?AASIB/CITSIB	Pune (Maharastra), released variety
15	Vizapur 10	Vizapur (Gujarat)	Vizapur (Gujarat), local
16	GW2	GS SIB//A206/NP200	Junagarh (Gujarat), released variety
17	Raj 1555	CIT/RAJ911	RAU, Durgapura (Raj), released variety
18	NP 404	GAZA/EKD6	IARI, Indore, released variety
19	MPO 215	Narmada 215	Powerkhera (MP), released variety
20	Gulab	Selection in local Bansi	Niphad (Maharastra)
21	HD4530	TPT/MOGHK/4/PI/TML//2 *TC60/3/3ZENTI/BTL//WLS	IARI, Delhi, released variety through selection in the CIMMYT material
22	NW 51	Unclassified germplasm	Not known
23	PDW 233	-	PAU, Ludhiana, released variety
24	DWL 5023	CR/LDS//PLC/Gaza	PAU, Ludhiana released variety through selection in the CIMMYT material
25	Jay	Motia/KPH	Niphad (Maharastra), released variety
26	GW 1	A206/VSM//A206	Junagarh (Gujarat), released variety

After the last cycle, the sample were kept at 72°C for additional 3 min and then cooled to 4°C. The amplified products with the loading buffer were electophoresed in 1.8% agarose gel at a constant voltage. The gel was stained with ethidium bromide and photographed. Four series of oligonucleotide primers of operon were screened and the primers giving the good polymorphic and reproducible banding pattern were selected and these are presented in Table 2.

RAPD profiles were visually scored for the presence or absence of bands across the lanes. The combined data from all the 15 primers were used to calculate the similarity coefficient for all possible pair-wise combinations. These similarity coefficients were used for cluster analysis to depict the relationship among the genotypes using unweighed pair group method for arithmetic average (UPGMA). All the computational and statistical analyses were performed using NTSYS programme.

Results and Discussion

Four series of the Operon primers, namely, OPA, OPP, OPD and OPM were screened. Most of the primers were found to give average or poor resolution of durum wheat cultivars. Limited polymorphism in wheat in general have also been reported by Devos and Gale, (1996). Moreover, in the present study, the material chosen was from India only and, thus, has a narrow genetic base. Natural selection causes RAPD to have ecogeographical (Fahima *et al.*,1999) adaptation and therefore similar kind of environment in the central and South-West India from where most of landraces originated resulting in poor polymorphism and differentiation in the present study. None of the primers alone or in combination were able to identify all of the varieties.

Table 2. List of arbitrary primers used and the resolution achieved in durum wheat

Primer	Resolution
OPA2	Average
OPA3	Good
OPA10	Average
OPA11	Average
OPA12	Poor
OPP3	Average
OPP6	Good
OPP7	Poor
OPP9	Poor
OPP15	Poor
OPD5	Poor
OPD12	Average
OPD13	Average
OPD15	Average

However, some of the primers like OPA3 and OPP6 gave good resolution and, therefore, could be used in conjugation with the morphological markers for identification of the variety. The phenogram resulting from cluster analysis using UPGMA is shown in Fig.1. The largest divergence was observed between the local race Malvaraj (No.13) selected from the Malva tract of central India with the two other local cultivars of India i.e, Motia (No.3) and Jay (No.25). Except the two entries i.e., PBW34 (No.1) and Jayraj (No.2), the released varieties developed through selection in the CIMMYT advance material were genetically diverse and spread under different nodes and sub-nodes. International organizations like CIMMYT have global collection of germplasm, and therefore, generally the material generated by them are more likely to have the wide genetic base in comparison to the national programme. Three varieties, namely, Motia (No. 3), Gulab (No. 20) and Jay (No. 25) appeared under one node. These varieties have markedly low polymorphism among themselves. This might be due to their common origin and common ancestry as all of these were developed through selection from Local Bansi accession. Similarly, two other varieties like A-9-3-1 and GW1 showed very high similarity. It might also be due to their common ancestry as both of them involve A 206 in their pedigree. However, the close proximity of PDW 215(No. 4) and B.Yellow (No. 6) was quite surprising as both of these neither have the ancestry or their centre of origin common. The limited number of primers used in the study may be one of the reasons and therefore, needs to be confirmed through further screening with large number of primers. The two local cultivars from Vizapur also appear close. Others like Amrut (No. 11) and N 59 (No.12) formed a separate subgroup and both of these cultivars were having the strain GAZA in their pedigree. The clustering on the basis of RAPD markers was clearly on the basis of parentage of the entries involved in the study. Pujar *et al.* (1999) in their study on Indian tetraploid wheat found the highest divergence in wild and less commonly cultivated species along with the sub grouping of Indian released durum cultivars on the basis of their common parentage. The unclassified accessions could not be assigned to any of the groups and therefore the entry is either exotic or some other centre of durum breeding in India with entirely different parentage. The genetic base of improved cultivars of durum wheat in India is largely represented by the local cultivars and landraces and is very narrow, therefore, more vulnerable to biotic and abiotic stresses. Widening of genetic base of any

Table 3. Jaccard's Similarity Coefficients among 26 wheat cultivars

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
01	1.00																									
02	0.83	1.00																								
03	0.60	0.57	1.00																							
04	0.70	0.71	0.54	1.00																						
05	0.53	0.52	0.60	0.54	1.00																					
06	0.66	0.67	0.59	0.64	0.60	1.00																				
07	0.64	0.72	0.65	0.63	0.54	0.76	1.00																			
08	0.61	0.70	0.61	0.64	0.58	0.57	0.71	1.00																		
09	0.63	0.63	0.62	0.64	0.58	0.58	0.58	0.73	1.00																	
10	0.53	0.57	0.64	0.60	0.59	0.60	0.65	0.57	0.63	1.00																
11	0.59	0.68	0.61	0.63	0.57	0.59	0.68	0.64	0.58	0.68	1.00															
12	0.57	0.62	0.61	0.61	0.62	0.57	0.62	0.63	0.59	0.62	0.77	1.00														
13	0.62	0.62	0.43	0.59	0.45	0.50	0.52	0.51	0.52	0.49	0.54	0.56	1.00													
14	0.57	0.61	0.61	0.59	0.62	0.63	0.66	0.63	0.60	0.59	0.62	0.65	0.55	1.00												
15	0.53	0.51	0.59	0.52	0.71	0.48	0.56	0.60	0.59	0.61	0.53	0.65	0.47	0.65	1.00											
16	0.57	0.61	0.62	0.59	0.51	0.64	0.72	0.61	0.56	0.6	0.60	0.52	0.45	0.65	0.51	1.00										
17	0.57	0.57	0.59	0.63	0.51	0.52	0.62	0.62	0.55	0.56	0.59	0.49	0.54	0.59	0.54	0.70	1.00									
18	0.54	0.55	0.63	0.61	0.61	0.57	0.59	0.62	0.68	0.62	0.54	0.58	0.51	0.70	0.66	0.55	0.60	1.00								
19	0.52	0.57	0.63	0.54	0.54	0.47	0.57	0.66	0.63	0.59	0.57	0.55	0.52	0.60	0.55	0.61	0.65	0.62	1.00							
20	0.54	0.53	0.89	0.49	0.60	0.56	0.61	0.57	0.57	0.60	0.56	0.55	0.38	0.54	0.60	0.56	0.54	0.59	0.60	1.00						
21	0.56	0.58	0.59	0.50	0.48	0.46	0.53	0.57	0.53	0.49	0.55	0.53	0.46	0.57	0.57	0.54	0.66	0.56	0.60	0.61	1.00					
22	0.62	0.57	0.50	0.53	0.46	0.67	0.60	0.45	0.46	0.51	0.46	0.54	0.61	0.51	0.49	0.51	0.47	0.49	0.44	0.46	0.52	1.00				
23	0.52	0.61	0.57	0.56	0.44	0.47	0.55	0.57	0.48	0.62	0.75	0.53	0.54	0.61	0.49	0.66	0.73	0.55	0.61	0.55	0.55	0.46	1.00			
24	0.52	0.60	0.55	0.51	0.53	0.47	0.55	0.55	0.51	0.54	0.54	0.56	0.55	0.60	0.57	0.58	0.66	0.57	0.58	0.49	0.55	0.47	0.68	1.00		
25	0.57	0.55	0.86	0.50	0.58	0.54	0.63	0.57	0.57	0.64	0.60	0.59	0.43	0.55	0.61	0.57	0.57	0.60	0.59	0.85	0.56	0.46	0.62	0.58	1.00	
26	0.56	0.64	0.70	0.60	0.51	0.64	0.79	0.62	0.52	0.61	0.64	0.60	0.55	0.62	0.53	0.65	0.64	0.57	0.56	0.62	0.55	0.54	0.64	0.67	0.74	1.00

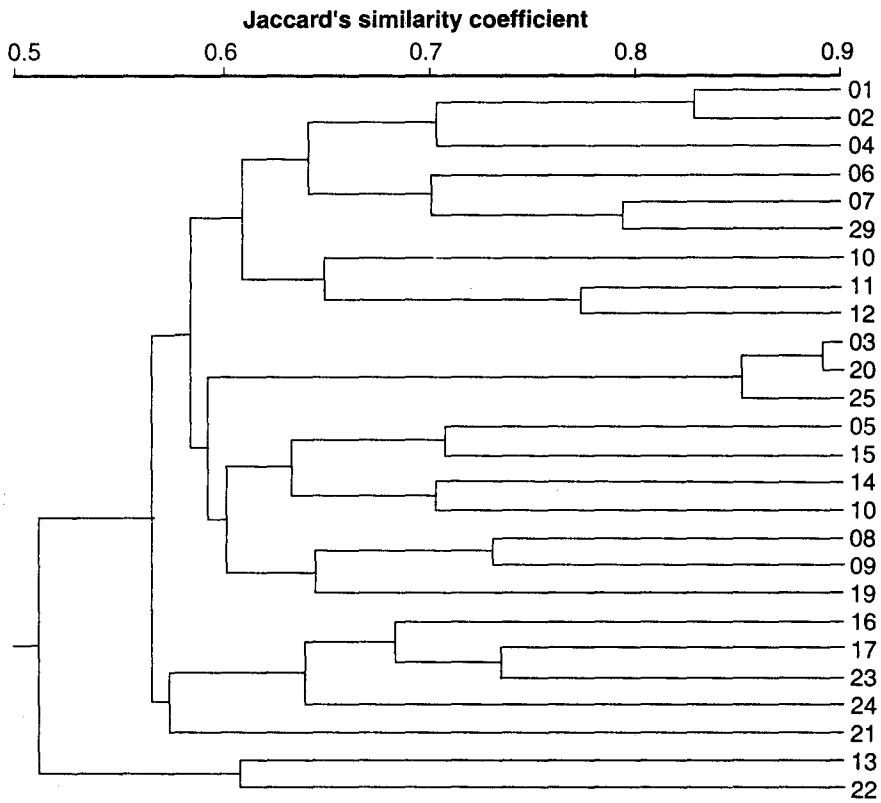

Fig. 1. Jaccard's Similarity Coefficients among 26 wheat cultivars

of the cultivated crop species is very important and the breeding lines/varieties involved in the breeding programme first needs to be characterized for the genetic diversity and due to the narrow range of the descriptors, diversity analysis on the basis of RAPD markers offer a viable alternative. Evaluation of the local cultivars or landraces for morphological and physiological characters is critical to exploit the genetic traits needed for adaptation. Identification of the diverse parents on the basis of agronomic traits, confirming it with the RAPD analysis and using them in crossing programme can maximise the level of variation in the segregating populations. The results confirm the utility of RAPD markers in the estimation of genetic diversity even in the closely related races/varieties. It is clear from the present study that the genetic base of the Indian durum cultivars can be very easily widened by making the crosses between the Indian local landraces and the improved germplasm from CIMMYT.

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