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Short Communication

Quantifying diversity in wheat using D^2 technique and molecular profiling

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A group of 48 wheat varieties have been used in the present study to assess the morphological and molecular diversity. These wheat genotypes belonging to *durum* and *aestivum* species were grown in RBD with two replications in three rows of 2m length with spacing of 23 cm between rows. Observations on five randomly selected plants were recorded in each entry for quantitative traits *viz*., days to 50 per cent flowering, days to maturity, plant height, peduncle length, flag leaf length, flag leaf width, number of productive tillers per meter, spike length, awn length, number of spikelets per spike, number of grains per spike, 1000-grain weight and grain yield per plot. Mahalanobis D^2 statistic was used for assessing the genetic divergence between genotypes [1]. For assessing the molecular diversity, the DNA was extracted from 10-12 days old wheat seedlings following CTAB extraction method [2]. Thirty random decamer primers *viz*., OPA 01 to 12, OPP 01 to 10, OPK 03 and 07 and OPF 01, 02, 03, 05, 10, 15 were used to screen the wheat genotypes. As many as 11 primers exhibited polymorphism and 2 primers showed monomorphism, while the remaining primers did not produce detectable bands. RAPD profiles were visually scored for the presence or absence of bands across the lanes. The 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.

Among the quantitative characters studied, number of grains per spike (23.58%), awn length (22.16%), flag leaf length (18.53%) and plant height (15.34%) contributed considerably towards diversity (Table 1). Similarly, previous workers [3] also reported considerable contribution of characters such as number of grains per spike, 1000 grain weight and number of productive tillers towards diversity. Based on D^2 analysis, 48 genotypes were grouped into 12 clusters (Table 2). Five of them (VIII, IX, X, XI, XII) were solitary. The formation of solitary clusters may be due to total isolation preventing the gene flow or intensive natural/artificial selection for diverse adaptive niches. The genotypes belonging to different species (*T. aestivum* and *T. durum*) and different geographical conditions grouped together in the same cluster. Similar results were reported earlier on non-parallelism between geographic and genetic diversity [4].

Table 1. Per cent contribution of different traits towards total diversity

Character	Times ranked first	Per cent contribution
Days to 50% flowering	2	0.18
Days to maturity	0	0.00
Plant height (cm)	173	15.34
Peduncle length (cm)	1	0.09
Flag leaf length (cm)	209	18.53
Flag leaf width (cm)	0	0.00
Number of productive tillers/meter	105	9.31
Spike length (cm)	1	0.09
Awn length (m)	250	22.16
Number of spike lets per spike	1	0.09
Number of grains per spike	266	23.58
1000 grain weight (g)	16	1.42
Grain yield per plot (g)	104	9.22

Eleven random decamer primers produced high degree of polymorphism with an average of 99.04 per cent (Table 3). All the primers except OPA-03 gave the highest polymorphism (100%). On an average 9.45 bands per primer were amplified. The primer OPP-05 demonstrated nine distinct polymorphic bands (Fig. 1). The diversity ranged from 13 to 57.50 per cent indicating

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diverse nature of the genotypes used. Maximum diversity was observed between HY-278 and Lok-1 (57.50). High level of polymorphism based on RAPD has been reported among the genotypes of wheat [5]. The clustering pattern did not follow any definite character base, geographic area of adoption or ploidy level. The set of primers used were not able to group the genotypes into phenotypically intended categories.

The grouping of genotypes based on morphological diversity i.e. D^2 analysis and DNA fingerprinting is not concurrent. The genotypes, which exhibited low diversity at phenotypic level, exhibited higher diversity at molecular level. For instance, the genotypes Amrut, DWR-16, GW-18, HUW-510 and LOK-1 grouped together in cluster-I, indicating morphological similarity among themselves whereas, same genotypes were grouped in different clusters at molecular level. The genotypes HUW-510 and LOK-1 exhibited higher genetic diversity at molecular level. Similar results were observed for all the clusters formed by D^2 analysis. Even though the genotypes HD 2285, LOK-1 and LOK-45 grouped in different clusters at phenotypic level, they grouped together and formed a separate cluster at molecular level, indicating high degree of genetic similarity at molecular level. The difference between morphological and molecular diversity may be due to the screening or use of limited number of RAPD markers. The grouping of genotypes or

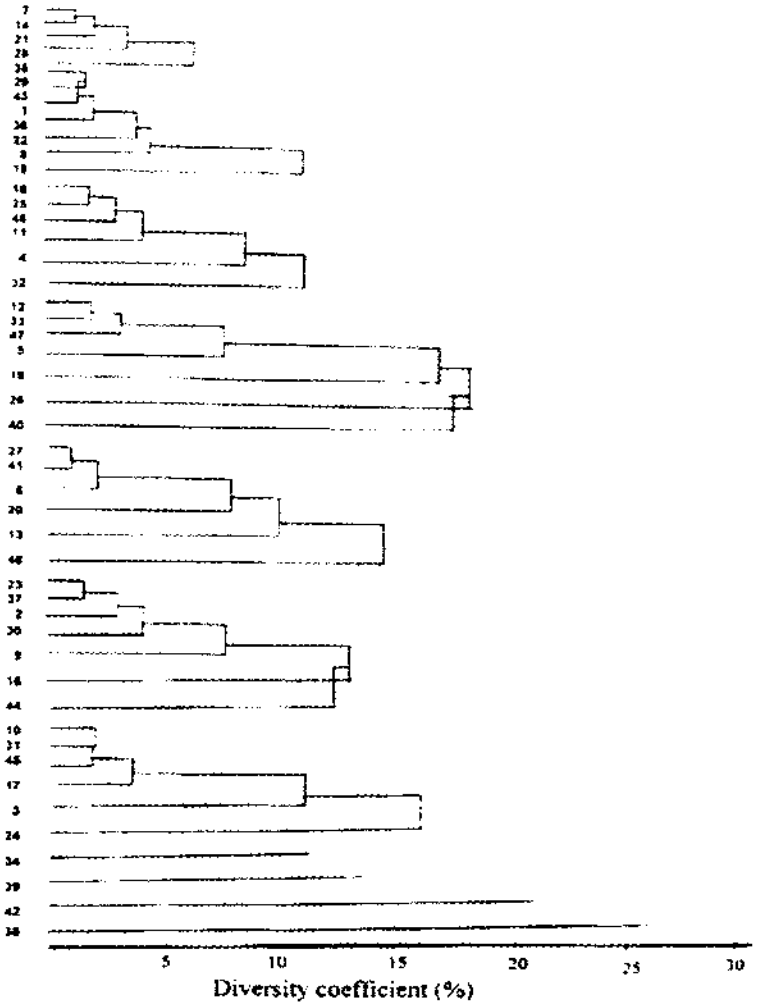

Fig. 1. Dendrogram obtained from D^2 analysis of wheat genotypes

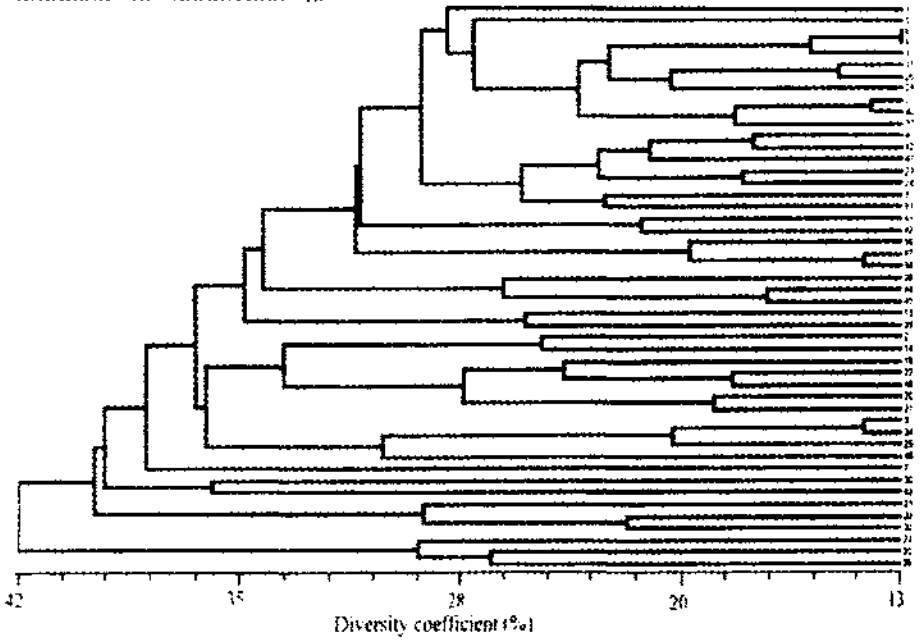

Fig. 2. Dendrogram obtained from pooled data of RAPD profiles of wheat genotypes

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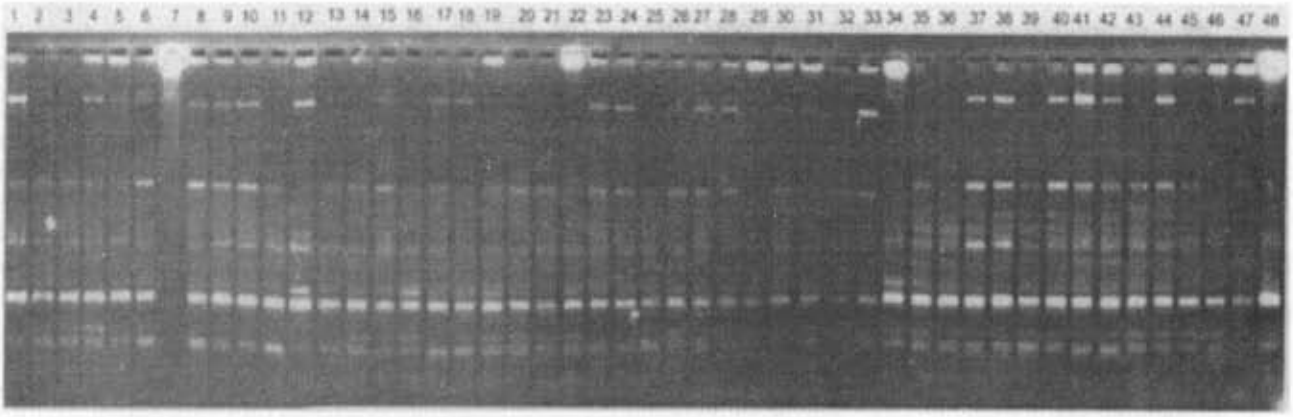

Fig. 3. RAPD profiles of 48 wheat genotypes

Table 2. Clustering pattern of 48 wheat genotypes

Cluster No.	Genotypes
I	Amrut, DWR-16, GW 18, HUW 510, LOK 1
II	HY 5, NP 52, 8A, LOK 45, GW 190, BA × 1288-18, DWR-39
III	DWR-185, HD 2501, NP 770, C 285, Ajanta, Kalyanasona
IV	C 306, Kharchia 65, NP 799, AKW-381, Gulab, HD 4502, N 8223
V	HPW 155, Narbada 4, AKW-1071, GW 10, DL 788-2, NP 836
VI	HD 2285, MACS 9, A-9-30-1, HY 278, Bijaga yellow, DWR 137, NP 404
VII	C 281, Jay, NP 715, DWR-162, A-206
VIII	HD 2329
IX	Lal Bahadur
X	MACS 2496
XI	NP 4
XII	MACS 1967

Table 3. Analysis of RAPD patterns generated using 11 random primers for wheat genotypes

Primer	Total number of bands	Polymorphic bands	Per cent polymorphism (%)
OPK-03	10.00	10.00	100.00
OPP-10	11.00	11.00	100.00
OPA-10	08.00	08.00	100.00
OPA-11	17.00	17.00	100.00
OPP-05	09.00	09.00	100.00
OPP-06	06.00	06.00	100.00
OPA-02	05.00	05.00	100.00
OPA-03	07.00	06.00	85.70
OPP-09	08.00	08.00	100.00
OPP-07	11.00	11.00	100.00
OPP-08	12.00	12.00	100.00
Pooled	104.00	103.00	99.04
Average	9.45	9.36	

iversity is independent of geographical location and ploidy level or even phenotypic markers like presence or absence of awns. Other reason could be the genotypes selected for fingerprinting were also limited.

References

1. **Mahalanobis P. C.** 1936. On the generalized distance in statistics. Proceedings of National Institute of Science, India, **2**: 49-55.
2. **Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W.** 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of National Academy of Science (USA), **81**: 8014-8018.
3. **Nimbalkar C. A., Navale P. A. and Biradar A. B.** 2002. Generalized D^2 and genetic diversity in wheat. Journal of Maharashtra Agricultural Universities, **27**: 43-45.
4. **Bergale S., Billore M., Holkar A. S., Ruwali K. N., Prasad S. V. S. and Mridula B.** 2001. Genetic variability, diversity and association of quantitative traits with grain yield in bread wheat (*Triticum aestivum* L.). Madras Agricultural Journal, **88**: 457-461.
5. **Mandoulakani B. A., Tabatabaei B. E. S., Bushehri A. A. S., Ghannadha M. R. and Omidi M.** 2003. Assessment of genetic diversity among wheat cultivars by RAPD-PCR. Iranian Journal of Agricultural Sciences, **34**: 447-454.