Research Paper

ADVANCE RESEARCH JOURNAL OF
C R P
IMPROVEMENT
Volume 3 | Issue 2 | December, 2012 | 135-138

Quantifying diversity through morphological and molecular markers in wheat genotypes

■ SHASHIKALA S. KOLAKAR, R.R. HANCHINAL[1] AND SADASHIV NADUKERI[2]

AUTHORS' INFO

Associated Co-author :
[1]University of Agriculutral Sciences, Krishi Nagar, DHARWAD (KARNATAKA) INDIA

[2]College of Horticulture, U.H.S.(B) MUDIGERE (KARNATAKA) INDIA

Author for correspondence :
SHASHIKALA S. KOLAKAR
Department of Crop Improvement and Biotechnology, College of Horticulture, U.H.S. (B), MUDIGERE (KARNATAKA) INDIA
Email : shashikala_kolakar@yahoo.co.in

ABSTRACT : Genetic diversity among 37 wheat genotypes belonging to *Triticum aestivum*, durum and dicoccum from DWR Karnal was studied using Mahalanobis D^2 analysis and random amplified polymorphic (RAPD) analysis. A total of 10 clusters were formed from morphological studies, with the major contribution from the characters like days to 50 per cent flowering and spike length. RAPD analysis with 10 primers, generated 56 amplification products of which 54 amplicons were polymorphic, however, the extent of polymorphism varied with each primer. The values of diversity coefficient ranged from 4.00 to 51. Cluster analysis showed considerable amount of diversity in the material used. The clustering pattern revealed that nine clusters formed from RAPD analysis showed that the genotypes which exhibited low diversity at phenotypic level, exhibited higher diversity at molecular level, *i.e.* D^2 analysis and DNA finger printing was not fully concurrent. The difference between morphological and molecular diversity showed that grouping of genotypes or diversity is independent of geographical location and ploidy level or even phenotypic markers.

Key Words : RAPD, Diversity, DNA finger printing

How to cite this paper : Kolakar, Shashikala S., Hanchinal, R.R. and Nadukeri, Sadashiv (2012). Quantifying diversity through morphological and molecular markers in wheat genotypes, *Adv. Res. J. Crop Improv.,* **3** (2) : 135-138.

Paper History : Received : 01.09.2012; **Revised :** 01.10.2012; **Accepted :** 15.11.2012

Wheat is the world's second most important cereal crop. It is aptly described as the 'king of cereals' because of the acreage it occupies, high productivity and the prominent position it holds in the international food grain trade. Genetic diversity plays an important role in plant breeding either to exploit heterosis or to generate productive recombinants. The choice of parents is of paramount importance in breeding programme. Hence, the knowledge of genetic diversity and relatedness in the germplasm is a pre-requisite for crop improvement programmes.

Assessment of genetic diversity at the molecular level is more meaningful than at phenotypic level as the later involves data on morphological traits which are environmental dependent. Though, they significantly contribute towards phenotypic variation but cannot be accurately phenotyped. Hence, the study of polymorphism is best done at DNA level, the primary source of all biological information. At this level, even seemingly identical accessions could display enormous differences, if appropriate DNA profiling techniques are employed. Randomly amplified polymorphic DNA (RAPD) is one such method (Welsh and McClelland, 1990; Williams *et al.*, 1990) of identifying polymorphism that can be used to elicit information on genetic differences among individuals of a population, between lines or germplasm accessions or any breeding material. In the present study, 37 released and unique varieties were used for assessing the diversity at morphological and molecular level.

RESEARCH PROCEDURE

Morphological diversity:

The experiment was laid out in a Simple lattice design with two replications. Each genotype was grown in a plot of three meter rows of two meter length each with a spacing of 23 cm between rows at wheat Improvement Project, Main Agricultural Research station, Dharwad.The recommended package of practices was followed. The data on morphological characters were recorded on a five competitive plants in each plot.

DNA extraction:

The DNA was extracted from 5g of bulked sample of

leaves from 10-12 days seedlings of each genotype grown in pots. The CTAB-DNA extraction procedure (Sagahai-Maroof *et al*.,1984) was used with some modifications. RNA free samples determined with a Hoefer DNA Flurometer and the DNA samples were diluted in 10:1 TE buffer to a working concentration of approximately 10 ng ml^{-1} and stored at 4^0C until PCR amplification.

DNA amplification and gel electrophoresis:

Protocol PCR was optimized by varying the concentration of template DNA,*Taq DNA polymerase* and magnesium salt, Ten 10-base oligonucleotide primers were selected for final RAPD-PCR amplification. Each reaction mixture (20ml) for PCR amplification consisted of 10X reaction buffer with 15mM, 3mM mgcl2, 3u of Taq DNA polymerase, 2.5mM each of dATP,dTTP,dCTP and dGTP, 15 mM of commercial kit of OPA and OPP primers and approximately 30 ng of genomic DNA template. The standard PCR amplification condition recommended for wheat were followed. The amplification products were electrophoresed on 1.2 per cent agarose gel at 70 volts in 1x TAE buffer. The gel was stained in the presence of ethedium bromide and gels were photographed using gel doc system.

Scoring and data analysis:

For all the genotypes, bands on RAPD gels were scored as present (1) or absent (0). DICE similarity coefficient values were calculated for each pair wise comparison between genotypes and similarity coefficient matrix was constructed. This matrix was subjected to unweighted pair group method for arithmetic average analysis (UPGMA) using SHAN module of NTSYS-pc version 2.0 (Rohlf, 1998), the similarity measurements were converted to genetic distance measurements as (1-SM) X100 (Spooner *et al*., 1996).

RESEARCH ANALYSIS AND REASONING

The results obtained from the present investigation have been duscussed below:

Morphological diversity:

In morphological diversity studies difference was observed in proportion of contribution of each character to total diversity (Table 1). Grain yield per plot contributed highest

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

Sr. No.	Sources	Times ranked first	% contribution
1.	Days to 50 per cent flowering	99	14.86
2.	Days to maturity	28	4.20
3.	Plant height	68	10.21
4.	Peduncle length	71	10.66
5.	Tillers per meter length	28	4.20
6.	Spike length	79	11.86
7.	Spikelets per spike	68	10.21
8.	Grains per spike	45	6.76
9.	1000 grain weight	40	6.01
10.	Protein content	37	5.56
11.	Yield per plot	103	15.47

Table 2 : Analysis of RAPD banding patterns for wheat genotypes

Primers	Total number of bands	Polymorphic bands	Per cent polymorphism (%)
OPA 10	5	5	100
OPA 15	9	9	100
OPP 07	5	3	60
OPP 09	6	6	100
OPP 01	3	3	100
OPP 03	6	6	100
OPA 13	8	8	100
OPA 14	5	5	100
OPA 11	5	5	100
OPA 12	4	4	100
Total	56	54	96
Average	5.6	5.4	-

(15.47%) towards diversity. This was followed by days to 50 per cent flowering (14.86%), spike length (11.86%) and peduncle length (10.66%), plant height and number of spikelet's per spike (10.21%), 1000 grain weight (6.01%), protein content (5.56%), days to maturity and number of tillers per meter length with the value of 4.20 per cent each.

A total of ten clusters (Fig. 1) were formed by grouping of all the 37 genotypes in such a way that genotypes within each cluster had smaller D^2 values than those in other cluster. Cluster pattern revealed that cluster I and III had more number of genotypes where as VII and IX were solitary clusters (Table 3).

Molecular diversity:

In molecular analysis selection of these 10 primers was done on the basis of their multibanded and easily scorable amplification products. A typical example with primers OPA-13 and OPA-14. Total of 56 amplified products among these 54 were polymorphic and average of 96 per cent polymorphism, with an average of 5.6 bands per primer. The number of bands produced ranged from 3(OPA-01) to 9 (OPA-15) Table2. The diversity coefficient ranged from 4 to 51 and the genotypes Sonalika showed lowest (4.11) diversity coefficient while WH-147 with DDK-1001 and WH-147 with HS-420 showed highest (70.21) diversity coefficient, Similarly, Barcaccia *et al.* (2002) reported the genetic diversity as high as 48 per cent among the local cultivars of Italian emmer wheat. All the genotypes showed diversity among themselves indicating considerable amount of variation in the material used for the study.

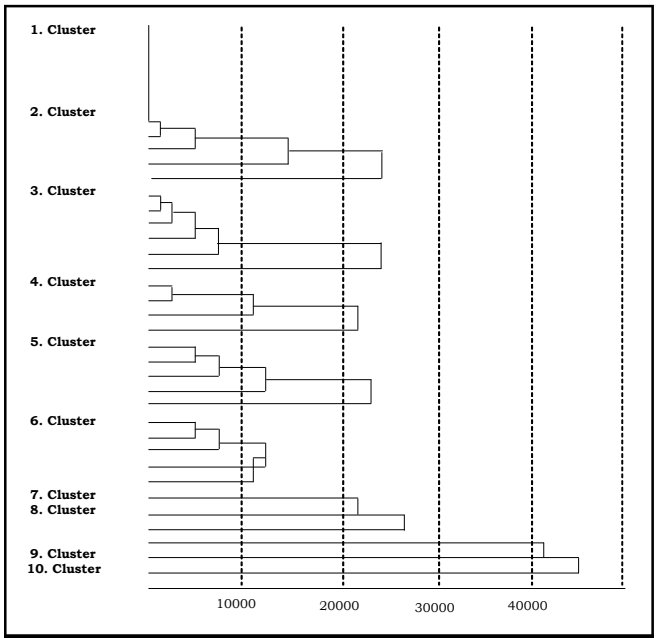

Fig. 1 : Dendrogram obtained from D^2 analysis of wheat genotypes

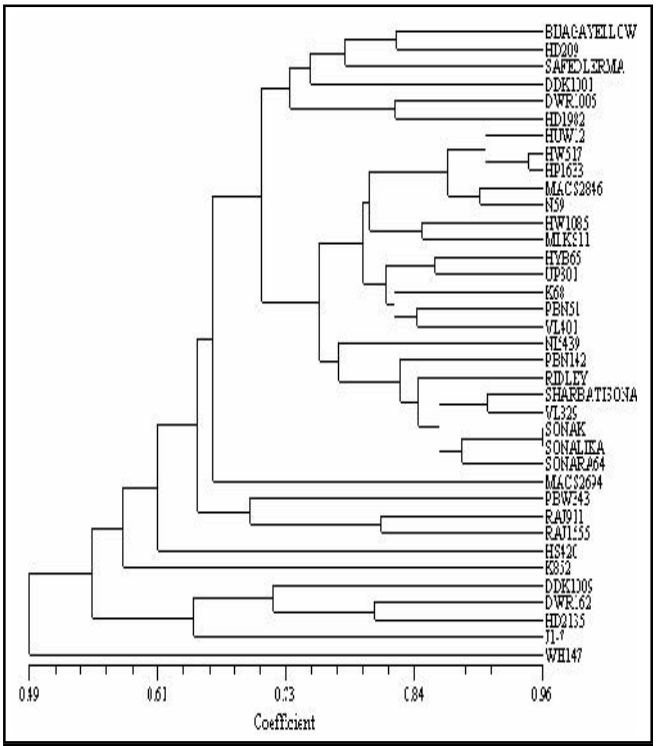

Fig. 2 : Dendrogram obtained from pooled data of RAPD profile of wheat genotypes

Table 3 : Clustering pattern of 37 wheat genotypes

Cluster number	Number of genotypes	Genotypes
I	6	BIJAGA YELLOW, DDK 1001, DDK1009, DWR 162, DWR1006, HD 1982
II	5	MACS 2694, SONAK, PBN 142, WH 147, HW 517
III	6	HW 1085, MACS 2846, PBW 373, RIDLEY, CPAN 1676, SONALIKA
IV	4	HD 2135, RAJ 911, MLKS 11, SONARA 64
V	5	J-1-7, UP 301, RAJ 1555, N59, HP 1633
VI	5	K 68, NI5439, SAFED LERMA, HS 420, VL 401
VII	1	HYB 65
VIII	2	K852, VL829
IX	1	HUW 12
X	2	PBN 51, SHARABATI SONARA

The dendrogram constructed from the pooled data of RAPD revealed nine distinct clusters, in which five were solitary (Fig. 2). Two main clusters were separated at 27 per cent diversity.

It is clear from the D^2 analysis as well as from molecular profiling of genotypes using RAPD markers that, there was sufficient diversity among the genotypes used for the study. But the grouping of genotypes based on morphological diversity *i.e.* D^2 analysis and molecular marker analysis was not fully concurrent. The DNA markers and morphological traits will not necessarily gain closely matching results (Vollmann *et al.*, 2005, Mart2´nez *et al.*, 2005). Semagn (2002) suggested two reasons for low correlation between DNA markers and morphological as well as protein data: (a) DNA markers cover a larger proportion of the genome, including coding and non coding regions, than the morphological markers and (b) DNA markers are less subjected to artificial selection compared with morphological markers. Mart2´nez *et al*. (2005) believed that the correspondence between different methods might be improved by analyzing more morphological characters and DNA markers.

The genotypes that exhibited low diversity at phenotypic level, exhibited higher diversity at molecular level. For instance the genotypes DDK 1001, DDK1009, DWR 162, DWR1006 and HD1982 were grouped together in cluster I, indicating morphological similarity among themselves. Whereas the same genotypes were present in different clusters at molecular level. The 21 genotypes which were distributed in different clusters at morphological level were grouped into the same clusters at molecular level indicating higher 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 diversity is independent of ploidy level or even phenotypic markers.

Accessions with the most distinct DNA profiles are likely to contain the greatest number of novel alleles. The present study indicates that the RAPD markers were suitable to assess genetic diversity. They can be used to identify diverse sources in crop germplasm collections or to select groups of genotypes with desired characters and contrasting phenotypes, if large numbers are employed. Particularly, genetic distance estimates might help in suitable germplasm for introgression programmes.

LITERATURE CITED

Mahalanobis, P.C. (1936). On the generalized distance in statistics. *Proc. National Institute Science, India,* **2**: 49-55.

Mart2´nez, l., Cavagnaro, P. and Masuelli, R. (2005). U.S.A. evaluation of diversity among argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and multivariate analysis system. Exeter software, and aflp markers.*Elect. J. Biotechnol*., **6**:37-45

Rohlf, F.J. (1998). Ntsys-pc numerical taxonomy and multivariate analysis, ver. 2.0. *Applied biostatics,* inc., New York.

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. *Proc. Nat. Academy Sci.* (USA), **81**: 8014-8018.

Semagn, K. (2002). Genetic relationships among, ten endod types as revealed by a combination of morphological, rapd and aflp markers, *Hereditas,***137** : 149-156.

Spooner, D.M., Tivang, J., Nienhis, J., Miller, J.T., Douches, D.S. and Contreas, M.A. (1996). Comparison of four molecular markers in measuring relationships among the wild potato relatives. *Solanum* section *e. tuberosum*. *Theoretical & Appl. Genet.*, **92**: 532-540.

Vollmann J., Grausgruber, H., Stift, G., Dryzhyruk, V. and Lelley, T. (2005). Genetic diversity in camelina germplasm as reavealed by seed quality characteristics and RAPD polymorphism. *Plant Breed.*, **124** : 446-456.

Welsh, J. and Mcclelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, **18**: 7213-7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.I., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphism amplified by arbitary primers are useful as genetic markers. *Nucleic Acids Res.*, **18**: 6531-6535.

****