
GENETIC DIVERSITY AMONG MAIZE VARIETIES REVEALED BY PHENOTYPIC DESCRIPTORS AND RAPD PROFILES

Suresh Handi, Sasidharan N., Sudeshna Chakraborty, Sneha Macwana, Ruchi Trivedi, Bhupendra Singh Punwar and Ashish G. Vala

ABSTRACT

Fifty-six genotypes of maize were analyzed for their phenotypic and genotypic differences at Anand Agricultural University, Gujarat, India. The genetic dissimilarities calculated from average taxonomic distance (E_{ij}) matrix among the 56 maize genotypes ranged from 0.59 between the pairs GWC 0310 and GWC 0606 and upto 2.90 between pairs GWC 0400 and Amber. The genotypic study with 40 RAPD primers, 11 gave polymorphic result with 4129 scorable bands and 151 loci, among which 147 loci were found polymorphic, with an average of 97.03 percent polymorphism. The study revealed that morphological characters combined with molecular markers were useful in diversity analysis studies.

Key words: *Genotypic, phenotypic, taxonomic distance, RAPD, polymorphism.*

INTRODUCTION

Maize (*Zea mays* L.) belonging to family Poaceae is a globally important crop and preferred staple food for more than 1 billion people in sub Saharan Africa and Latin America (Gupta *et al.*, 2009). It holds a unique position in world agriculture as a food, feed and industrial crop. It is expected that the demand for maize production in developing countries is destined to surpass the demand for both rice and wheat by the year 2020 (Prasanna and Hoisington, 2003). It is used in the human diet in both fresh and processed forms. The value-added concept has been an economic driver in the specialty corn markets (Aslam *et al.*, 2009). It is also an important cereal in Asia, but more than half of the produce is used for livestock feed primarily due to strong economic growth and rapid urbanization experienced by the subcontinent, including India.

Maize is mainly a cross pollinated crop showing high phenotypic variability among

the species. In any crop improvement programme, existence of variability and selection of genotypes with due selection pressure on yield component characters is of prime importance to generate productive recombinants. However, to date the information on its genetic diversity and phylogenetic relationships has been sparse. In the recent years, although new molecular markers such as sequence related amplified polymorphism (SRAP), simple sequence repeat (SSR) etc. were invented, RAPD technique which was developed by Williams *et al.*, (1990) has been widely applied in either identification of cultivars, analysis of seed purity or estimating genetic relationships and diversity among crop germplasms owing to its easiness, cheapness and quickness in comparison with other molecular markers (Fu *et al.*, 2006). The assessment of genetic variability in the base population and Marker Assisted Selection (MAS) for advancing generations will help

in population improvement. The diversity analysis, at the molecular level is much more effective than at the phenotypic level, since morphological characteristics are often influenced by the environment and therefore, do not always express genetic relationships. In order to gauge the genetic diversity present in 56 maize germplasm lines, they were first assessed phenotypically through morphological characters and then attempts were made to validate the same the RAPD profile.

MATERIALS AND METHODS

Plant materials

Fifty six genotypes of maize supplied from Main Maize Research Station, Anand Agricultural University, Godhra (Panchmahal) were selfed and genotypes developed were utilized for raising the experimental material (Table 01)

Table 01: List of genotypes used in the study

S. no.	Genotype	S. no.	Genotype	S. no.	Genotype
1	GWC-9101	21	GWC-0208	41	GWC-0605
2	GWC-9103	22	GWC-0301	42	GWC-0606
3	GWC-9401	23	GWC-0302	43	GWC-0608
4	GWC-9412	24	GWC-0310	44	GWC-0609
5	GWC-9413	25	GWC-0311	45	GWC-0702
6	GWC-9601	26	GWC-0316	46	GWC-0704
7	GWC-9602	27	GWC-0320	47	Farmsameri
8	GWC-9603	28	GWC-0323	48	GM-2
9	GWC-9604	29	GWC-0325	49	GM-6
10	GWC-9610	30	GWC-0400	50	Narmada Moti
11	GWC-9611	31	GWC-0401	51	African tall
12	GWC-9612	32	GWC-0402	52	Sweta
13	GWC-9623	33	GWC-0501	53	Manokarma
14	GWC-9631	34	GWC-0502	54	Chhindwada
15	GWC-9634	35	GWC-0503	55	D-822
16	GWC-9701	36	GWC-0505	56	Amber (Popcorn)
17	GWC-9802	37	GWC-0506		
18	GWC-0203	38	GWC-0507		
19	GWC-0204	39	GWC-0703		
20	GWC-0207	40	GWC-0510		

Morphological study

In the present investigation 29 different phenotypic traits were studied for 56 genotypes

of Maize according to the guidelines for the conduct of DUS test (distinctiveness, uniformity, stability) (Table 02).

Table 02: Frequency distribution of genotypes belonging to different phenotypic classes

S. No.	Characteristics	State of expression	Number of genotypes belonging to each class
1.	First leaf: Shape of tip	pointed	0
		pointed to round	24
		round	29
		round to speculate	3
		speculate	0
2.	Leaf: attitude of blade (on leaf just above upper ear)	straight	15
		recurved	38
		strongly recurved	4
3.	Stem: Anthocyanin coloration of brace roots	absent or very weak	19
		medium	36
		very strong	1
4.	Tassel: Anthocyanin coloration at base of glume (in the middle third of main axis)	absent or weak	32
		medium	13
		strong	11
5.	Tassel: Density of spikelets (in middle third of main axis)	lax	32
		medium	20
		dense	4
6.	Tassel: Attitude of lateral branches (in lower third of tassel)	straight	10
		slightly recurved	27
		recurved	15
		strong recurved	4
		very strong recurved	0
7.	Ear: Anthocyanin coloration of silk (on day of emergence)	absent	40
		present	16
8.	Ear: Shape	conical	6
		conical-cylindrical	37
		cylindrical	13
9.	Ear: Type of grain (in middle third of ear)	flint	25
		semi - flint	27
		dent	4

10.	Ear: Colour of top of grain	pure white	8
		yellowish	40
		yellow	8
11.	Ear: Colour of dorsal side of grain	pure white	1
		yellowish	54
		yellow	1
12.	Ear: Anthocyanin coloration of glumes of cob	white	55
		white purple	1
		dark purple	0
13.	Kernel row arrangement (middle of ear)	straight	54
		spiral	0
		irregular	2
14.	Leaf: angle between blade and stem (on leaf just above upper ear)	very small (< 30 ^o)	0
		small (30 - 45 ^o)	28
		medium (45 - 60 ^o)	26
		large (61-75 ^o)	2
		very large (75- 90 ^o)	0
15.	Tassel: Time of anthesis (on middle third of main axis)	very early (< 40 days)	0
		early (40 - 45 days)	8
		medium (45 - 55 days)	42
		late (55 - 65 days)	6
		very late (>65 days)	0
16	Tassel: Angle between main axis and lateral branches (lower third of tassel)	very small (<15 ^o)	0
		small (15-30 ^o)	15
		medium (31-60 ^o)	32
		large (61-90 ^o)	7
		very large (>90 ^o)	2
17.	Tassel: Number of primary lateral branches	absent	0
		few (1-5 branches)	1
		medium (6-10 branches)	8
		many (11 -15 branches)	45
		very many(>15branches)	2
18.	Tassel: Length of main axis above lowest side branch	very short (<15 cm)	0
		short (15-20 cm)	25
		medium (21-30 cm)	29
		long (31-35 cm)	2
		very long (>35 cm)	0

19.	Tassel: Length of main axis above upper side branch	very short (<15 cm)	0
		short (15-20 cm)	4
		medium (21-30 cm)	45
		long (31-35 cm)	6
		very long (>35 cm)	1
20.	Tassel: Length of side branches	very short (<15 cm)	0
		short (15-20 cm)	7
		medium (21-30 cm)	42
		long (31-35 cm)	7
		very long (>35 cm)	0
21.	Plant: Length	< 50 cm	0
		50-75 cm	0
		75-100 cm	0
		100-125cm	0
		125-150 cm	1
		150-175 cm	8
		175-200 cm	25
		200-225 cm	18
		>225 cm	4
22.	Plant: Ratio height of insertion of upper ear to plant length (ear placement)	short (<40 %)	39
		medium (40-50 %)	17
		medium long (51-60%)	0
		long (61-70%)	0
		very long (>70%)	0
23.	Leaf: Width of blade	very small (<5 cm)	0
		small (5-7 cm)	18
		medium (7.1-9 cm)	38
		large (9.1-11 cm)	0
		very large (>11 cm)	0
24.	Ear: Time of silk emergence	very early (< 52 days)	5
		early (52 -57 days)	18
		medium (58-62 days)	23
		late (63-67 days)	10
		very late (>67 days)	0
		short (5-10 cm)	30
		medium (10.1-15 cm)	25
		long (15.1-20 cm)	1
		very long (>20.1 cm)	0

25.	Ear: Length of peduncle	very short (<5 cm)	0
		short (5-10 cm)	30
		medium (10.1-15 cm)	25
		long (15.1-20 cm)	1
		very long (>20.1 cm)	0
26.	Ear: Length	<5 cm	0
		5-7.5 cm	0
		7.6-10 cm	0
		10.1-12.5 cm	0
		12.6-15 cm	28
		15.1-17.5 cm	23
		17.6-20 cm	5
		20.1-22.5 cm	0
		>22.5 cm	0
27.	Ear: Diameter without husk	very small (<2.5 cm)	0
		small (2.6-3.5 cm)	56
		medium (3.6 - 5.0 cm)	0
		large (5.1 - 5.5 cm)	0
		very large (>5.5 cm)	0
28.	Ear: Number of rows of grain	very few (8 rows)	0
		few (8.1-10 rows)	0
		medium (10.1-14 rows)	34
		many (14.1-16 rows)	22
		very many (>16 rows)	0
29.	Kernel: 100 kernel weight	<10 g	0
		10-15 g	0
		15.1-20 g	0
		20.1-25 g	20
		25.1-30 g	30
		30.1-35 g	6
		35.1-40 g	0
		40.1-45 g	0
		>45 g	0

RAPD analysis

Total genomic DNA extracted from fifteen day old seedlings grown in field was used for DNA isolation (following CTAB method, George and Regalado, 2004). The Taq DNA polymerase from Fermentas was used for

carrying out the amplification by PCR. A total of 59 decamer primers (MWG, India) were screened for RAPD analysis. Thereafter, 11 primers producing clear, distinct and consistent amplification products were utilized for

further DNA amplification of each genotype. PCR reaction was performed using 30 µl reaction mixture containing 3.0 µl 10X PCR buffer, dNTP 0.5µl, Taq DNA polymerase 0.3 µl, primer 1.5 µl and template DNA 5.0 µl and nuclease free water 19.7 µl. The total reaction volume for DNA amplification was 30 µl. The DNA amplification was performed in a thermal cycler Eppendorf and the reaction program was set as follows: the first step, 1 cycle of 5 min at 94°C for initial strand separation; the second step, 40 cycles of 45 second at 93 °C for denaturation, 1 min at 36 °C for annealing and 7min at 72 °C for final extension. After DNA amplification, the PCR products were analyzed by electrophoresis in 2% agarose gels.

Cluster analysis

In order to identify the amplification products, the profiles obtained were analyzed with an image analyzer G-box from Syngene (v.7.07.01) and each amplification product was regarded as a characteristic unit and all of the accessions were scored for the absence (0) or presence (1) of a specific band and per cent polymorphism was calculated using the following formula.

$$\text{Per cent polymorphism (\%)} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

The polymorphic information content (PIC) value for each locus of RAPD provides an estimate of the discriminating power of a locus by taking into account not only the number of alleles that are expressed but also their relative frequencies. Calculations were made using the following formula (Anderson *et al.*, 1993):

$$\text{PIC}_i = 1 - \sum P_{ij}^2$$

Where P_{ij} is the frequency of j^{th} allele for marker i , and summation extends over n alleles.

Pair wise genetic similarities (S_{ij}) between

genotypes were estimated by Jaccard's similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and clusters obtained on Unweighted Pair Group Arithmetic Mean (UPGMA) using SAHN (Sequential, Agglomerative, Heirarchical Nested clustering method) module of NTSYS-pc version 2.02i (Rohlf, 1998).

RESULTS AND DISCUSSION

During the present work, 29 phenotypic traits of different genotypes were studied and characterized (Table 02). The genetic dissimilarities calculated from average taxonomic distance (E_{ij}) matrix among the 56 maize genotypes ranged from 0.59 between the pairs GWC 0310 and GWC 0606 and upto 2.90 between pairs GWC 0400 and Amber. All the genotypes showed diversity among themselves indicating number of phenotypic descriptors were able to discriminate between them. However, as reported by Kumar *et al.*, (2003) and Rana *et al.*, (2005) in cotton and Kwon *et al.*, (2005) in pepper, the genetic diversity estimates were found to be of low magnitude for maize. The maize genotypes were clustered mainly into four groups such as A, B, C and D (Table 03 and Figure 01) (Sneath and Sokal, 1973). Similar results were reported by Giancola *et al.*, (2002) in soybean, which clustered 100 soybean varieties into two clusters. Kwon *et al.*, (2005) reported three major clusters obtained from the analysis of phenotypic characters in pepper.

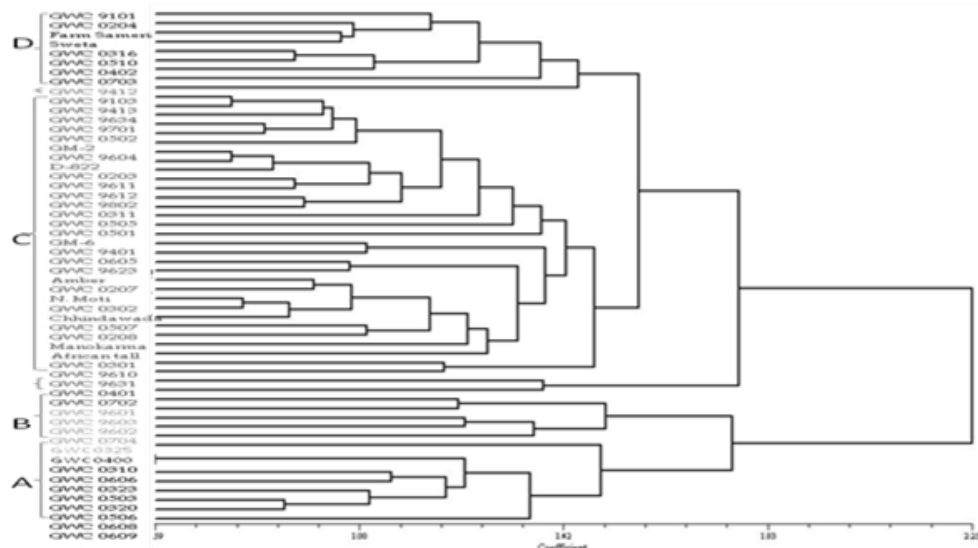


Figure 01. Dendrogram of genetic relationships among 56 varieties of maize based phenotypic traits

During the present investigation 40 random primers (Operon Technologies, USA) of OPA and OPK series were screened sequentially for RAPD, out of which 11 primers giving reproducible results were used for screening 56 genotypes (Table 04, Table 05 and Figure 02). The dendrogram generated from RAPD fingerprints were analyzed using NTSYS-pc software. The pooled RAPD analysis of all the 56 maize genotypes using 11 arbitrary oligonucleotide primers of two primer series viz. OPA and OPK, generated a total of 4129 scorable bands with 151 loci, among which, 147 loci were found polymorphic, showing average 97.03 percent polymorphism. The

PIC value was 0.898. In the present study 100 percent polymorphism was recorded by primers OPA 3, OPA 10, OPA 11, OPA 12, OPA 18, OPK 14 and OPK 19 while the lowest polymorphism (88.89 percent) was exhibited by primer OPA 15. The PIC value ranged from 0.853 (OPA 15) to 0.938 (OPA 18) with an average of 0.898. Dendrogram based on symmetric matrix of Jaccard's similarity coefficient (Table 06) by UPGMA method formed three major clusters designated as A, B and C (Figure 03). The two main clusters A and B were separated at 0.44 Jaccard's similarity coefficient, whereas Cluster B and C were separated at 0.45 coefficient.

Table 04: List of RAPD primers and their sequence

Sl. No.	Name of Primer	Oligo-nucleotide primer sequence
1	OPA – 02	5'- TGC-CGA-GCT-G – 3'
2	OPA – 03	5'- AGT-CAG-CCA-C – 3'
3	OPA – 04	5'- AGT-CAG-CCA-C -3'
4	OPA – 10	5'- GTG-ATC-GCA-G -3'
5	OPA – 11	5'- CAA-TCG-CCG-T -3'
6	OPA – 12	5' – TCG-GCG-ATA-G - 3'
7	OPA - 15	5' – TTC-CGA-ACC-C – 3'
8	OPA – 18	5'- AGG-TGA-CCG-T -3'
9	OPK – 14	5'- CCC-GCT-ACA-C – 3'
10	OPK – 16	5'- GAG-CGT-CGA-A – 3'
11	OPK – 19	5'- CAC-AGG-CGG-A - 3'

Table 05: Analysis of RAPD patterns generated using 11 arbitrary primers for maize genotypes.

Sr. No.	Name of Primer	Maximum Scorable Band	Polymorphic loci (P)	Total loci (T)	Percentage Polymorphism (P/T)X 100	PIC Value
OPA series						
1.	OPA - 2	391	10	11	90.9	0.890
2.	OPA - 3	314	11	11	100.0	0.870
3.	OPA - 4	451	15	16	93.75	0.913
4.	OPA - 10	284	14	14	100.0	0.898
5.	OPA - 11	362	14	14	100.0	0.906
6.	OPA - 12	431	15	15	100.0	0.911
7.	OPA - 15	279	8	9	88.89	0.853
8.	OPA - 18	500	21	21	100.0	0.938
OPK series						
9.	OPK - 14	262	11	11	100.0	0.856
10.	OPK - 16	494	15	16	93.75	0.937
11.	OPK - 19	380	13	13	100.0	0.905
	Range	262-500	8-21	9-21	88.89-100	0.853-0.938
	Average	375.36	13.36	13.73	97.03	0.898
	Pooled	4129	147	151	--	--

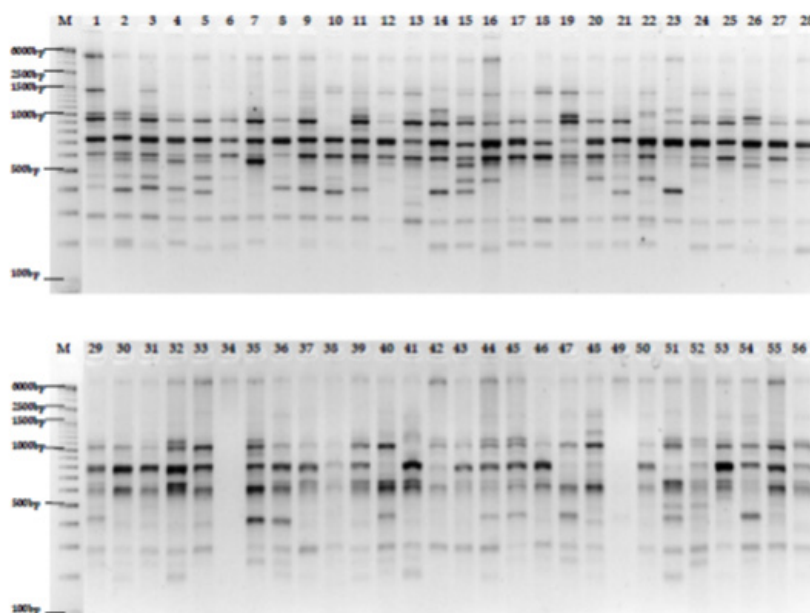


Figure 02. RAPD profile of maize genotypes generated by primer OPA-12

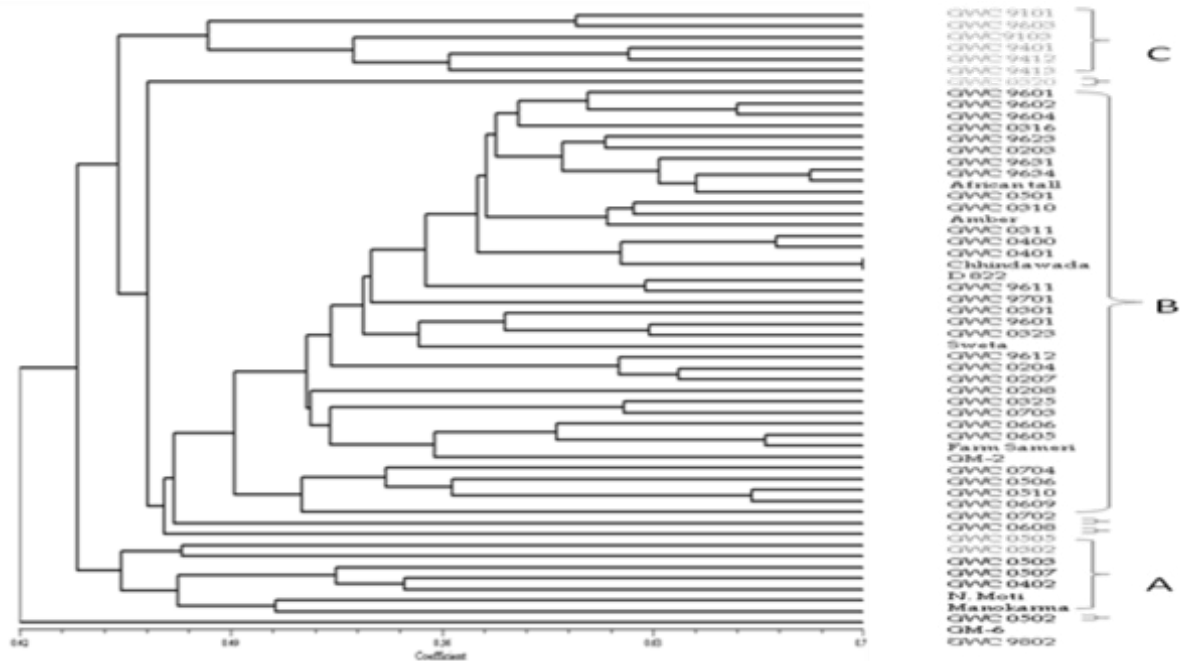


Figure 03. Dendrogram of genetic relationships among 56 varieties of maize based on RAPD analysis

These results showed the ability of RAPD to discriminate among genotypes and suggests their application for cultivar identification. The present investigation was in accordance with the study of Bauer *et al.*, (2005), who clustered early maturing hybrids of maize into three distinct sub clusters on the basis of their genetic similarity which showed good separation of hybrids. Asif *et al.*, (2006) concluded that RAPD is a powerful tool for purity detection of seed lots of six hybrid corn samples. Carvalho *et al.*, (2004) constructed dendrogram based on genetic similarity using the UPGMA method which grouped the 81 maize accessions into two clusters which were correlated according to kernel colours. Moeller and Schaal (1999) analysed 16 accessions of native american maize using RAPDs for 11 primers which grouped the accessions into four groups based on cluster analysis and revealed that RAPDs are successful in confirming hypothesized relationships and in identifying misclassified specimens.

It was observed that the genetic diversity in maize may be related to genetic distances

obtained from morphological characters and RAPD analysis. The diversity revealed at morphological and molecular level can be used for selecting parents for attempting better hybrid combinations. The information generated from such studies has considerable value for cataloging the genetic diversity in the breeding material, without the limitation of environmental factors associated with the conventional breeding programs. Although the phenotypic markers employed in the current study could not fully unravel the diversity present in the maize population, if more number of morphological markers are utilized and also if the genes responsible for that particular character expression can be tagged with the help of molecular markers, genetic diversity analysis with phenotypic markers can be made more informative and meaningful. Use of morphological marker methods in variety identification are however questionable because morphological traits are strongly affected by environmental conditions. In addition, this approach is relatively inefficient because of the time and cost involved.

CONCLUSIONS

Moreover, morphological criteria alone are not able to detect differences between some varieties that are phenotypically similar but have different agronomical behaviour since some of the traits will be encoded by more than one gene (Giancola *et al.*, 2002 and Kwon *et al.*, 2005). Morphological characters

combined with molecular markers were useful in diversity analysis studies. Hence, developing passport data for maize cultivars using phenotype markers in conjunction with molecular data will be a proper approach for genotyping maize genotypes.

REFERENCES

- Anderson JA, Churchill JA, Autrique JE, Tanksley SD and Vosman B. (1993). Optimizing parental selection for genetic linkage maps. *Genome* 36:181-186.
- Asif M, Rahman MU and Zafar Y. (2006). Genotyping analysis of six maize hybrids using DNA fingerprinting technology. *Pakistan Journal of Botany* 38(5):1425-1430.
- Aslam M, Awan HS, Khan IA and Khan AI. (2009). Estimation of genetic distance between 10 maize accessions with varying response to different levels of soil moisture. *Genetics and Molecular Research* 8(4):1459-1465.
- Bauer I, Drinic SM, Filipovic M and Konstantinov K. (2005). Genetic characterization of early maturing maize hybrids (*Zea mays* L.) obtained by protein and RAPD markers. *Genetika* 37 (3): 235-243.
- Carvalho VP, Ruas CF, Ferreira JM, Moreira MP and Ruas M. (2004). Genetic diversity among maize (*Zea mays* L.) landraces assessed by RAPD markers. *Genetics and Molecular Biology* 27(2): 228-236.
- Fu J, Zhang MF and Qi XH. (2006). Genetic diversity of traditional Chinese mustard crops *Brassica juncea* as revealed by phenotypic differences and RAPD markers. *Genetic Resources and Crop Evolution* 53:1513-1519.
- George MLC and Regalado ES. (2004). Laboratory handbook, Protocols for Maize Genotyping using SSR Markers and Data Analysis, CIMMYT.
- Giancola S, Poltri SM, Lacaze P and Hopp HE. (2002). Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. *Euphytica* 127:95-113.
- Gupta HS, Agrawal PK, Mahajan V, Bisht GS, Kumar A, Verma P, Srivastava A, Saha S, Babu R, Pant MC and Mani VP. (2009). Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular assisted breeding. *Current Science* 96(2): 230-237.
- Kumar P, Singh K, Vikal Y, Randhawa LS and Chahal GS. (2003). Genetic diversity studies of elite cotton germplasm lines using RAPD markers and morphological characteristics. *Indian Journal of Genetics* 63(1): 5-10.

- Kwon YS, Lee JM, Yi GB, Yi SI, Kim KM, Soh EH, Bae KM, Park EK, Song IH and Kim BD. (2005). Use of SSR markers to complement tests of Distinctiveness, Uniformity and Stability (DUS) of Pepper (*Capsicum annuum* L.) varieties. *Molecules and Cells* 19(3): 428-435.
- Moeller DA and Schaal BA. (1999). Genetic relationships among Native American maize accessions of the Great Plains assessed by RAPD. *Theoretical Applied Genetics* 99: 1061–1067.
- Prasanna BM and Hoisington D. (2003). Molecular breeding for maize improvement: An overview. *Indian Journal of Biotechnology* 2: 85-98.
- Rana MK, Singh VP and Bhat KV. (2005). Assessment of genetic diversity in upland cotton (*Gossypium hirsutum* L.) breeding lines by using amplified fragment length polymorphism (AFLP) markers and morphological characteristics. *Genetic Resources and Crop Evolution* 52: 989–997.
- Rohlf FJ. (1998). NTSYS-pc numerical taxonomy and multivariate analysis, ver. 2.02. Applied Biostatistics, New York.
- Sneath PHA and Sokal RR. (1973). Numerical Taxonomy, The principles and practice of numerical classification, Freeman, San Francisco, pp 573.
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* 18: 6531-6535.