BIOLOGIA PLANTARUM 53 (4): 711-714, 2009

BRIEF COMMUNICATION

Analysis of genetic variability in two diploid *Musa* cultivars using RAPD

P. PADMESH[1]*, J.V. REJI[2], C. BENADICT PAUL[3], S. MUKUNTHAKUMAR[1], G. PRAVEEN[1] and S. SEENI[2]

Biotechnology and Bioinformatics Division, Tropical Botanic Garden and Research Institute, Palode-695562, Thiruvananthapuram, Kerala, India[1]
Department of Biotechnology, Sathyabama University, Chennai-600119, India[2]
Department of Biotechnology, College of Biomedical Science, Technology and Research, Sri Ramachandra University, Chennai-600116, India[3]

Abstract

Twenty accessions of the sparsely cultivated diploid *Musa* cultivars Matti (AA) and Rasakadali (AB) were subjected to random amplified polymorphic DNA (RAPD) assay. A total of 14 random primers were used for the estimation of inter- and intracultivar variations. Out of 86 bands generated, 64 were polymorphic (74.4 % polymorphism). The cluster analysis grouped the cultivars into two major clusters: cluster I with 10 accessions of Matti and 2 of Rasakadali and cluster II comprising the remaining 8 accessions of Rasakadali. The coefficient of genetic similarity (GS) was from 0.73 to 0.99, suggesting low level of intercultivar variation. The accessions of Rasakadali with mean GS of 0.89 were genetically more diverse than those of Matti (GS = 0.93).

Additional key words: banana, genetic similarity coefficient, UPGMA phenogram.

Diversity within the cultivars of banana, which consist mainly of diploids, is complex with combinations of different degrees of expression of the parental species. Among the various marker systems used to identify cultivars, the random amplified polymorphic DNA (RAPD) is capable of characterizing the cultivars, varieties and intergeneric genome analysis (Padmesh *et al.* 2006, Zhang and Zhou 2009). Biochemical markers like isozymes and molecular markers like RFLP are also reported to offer quick, easy and reliable system for classification and identification of cultivars (Bhat *et al*. 1992). RAPD markers are detected by the use of short oligonucleotides of arbitrary sequence as primers for the amplification of segments of the target genome. Generally, 10-mer primers with 50 - 80 % G+C content are preferred. However, complex banding patterns were also generated with primers as short as 5 bases (Caetano-Anolles *et al*. 1991). There are few reports on the use of long primers (over 12 bases) (Rafalski *et al*. 1991). Amplified fragments within the 0.5 - 5.0 kb range are separated by gel electrophoresis and polymorphisms are detected as the presence or absence of bands of particular size. These polymorphisms are considered to be primarily due to variation in the primer annealing sites (Bhat and Jarret 1995). The RAPD technique has been widely used in bananas for the construction of genetic maps of the species (Faure *et al*. 1993) for genotype identification and taxonomic studies (Castiglione *et al*. 1993) in distinguishing diverse *Musa* germplasm (Kaemmer *et al*. 1992, Howell *et al*. 1994, Bhat and Jarret 1995). The present study was carried out to find possible genotypic differences within selected banana cultivars if any, as they exhibit considerable phenotypic changes.

Accessions of two elite South Indian diploid *Musa* cultivars Matti and Rasakadali used in the present investigation were collected from plantations of Kanyakumari district of Tamil Nadu and Thiruvanathapuram district of Kerala. Total DNA was isolated from fresh leaves of 22 accessions (11 from each cultivar) following modified Murray and Thompson method (1980) using cetyltrimethylammonium bromide (CTAB). After ethanol precipitation DNA was resuspended in

Received 3 April 2007, *accepted* 8 June 2008.
Abbreviations: RAPD - random amplified polymorphic DNA; UPGMA - unwighed pair-group method analysis.
Acknowledgements: The authors are highly indebted to Kerala State Council for Science Technology and Environment (KSCSTE) for their financial assistance and to Mr.Gireeshkumar and Mr. K.Gopakumar for their technical assistance.
* Corresponding author; fax: (+91) 472 2869646 , e-mail: padmeshpad@rediffmail.com

0.1 cm^3 of 1× TE buffer (pH 8.0). The DNA was quantified spectrophotometrically by taking the absorbance at 260 nm. RAPD assay was carried out in 0.025 cm^3 reaction mixture containing 0.2 mM dNTP's, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 % Triton X-100, 1.0 U Taq DNA polymerase (*Finnzymes*, Helsinki, Finland), 15 pmol primers from kit '*C*' (*IDT*, Coraville, USA) and 50 ng of genomic DNA. PCR amplification was performed in a thermal cycler (*MJ Research PTC-100*, Watertown, USA). After the initial cycle of 2 min at 94 °C, 2 min at 36 °C and 2 min at 72 °C, 38 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C were performed. The last cycle was followed by 7 min extension at 72 °C. Reaction mixture wherein template DNA replaced by distilled water was used as negative control. Amplified products were resolved in 1.4 % agarose gel (1× TBE) followed by EtBr staining.

Amplification with each random primer (Table 1) was repeated 3 times and those primers that produced reproducible and consistent bands were selected for data generation. Reproducible RAPD products were scored against the presence (+) or absence (–) of a fragment. Bands of equal molecular mass and mobility generated by the same primer were considered as of identical locus. The distance matrix was formulated by Nei's genetic distance analysis method (1972) using the *Popgene* software version *3.5* and the phenogram constructed using UPGMA with the software *Popgene* version *1.32*. The normalized statistic methods of Nei were taken for determining the level of association between the matrices.

Table 1. List of primers and its sequence used for RAPD analysis.

Primer	Primer sequence 5'→3'	Number of bands	Number of polymorphic bands
C65	GAT GAC CGC C	13	9
C66	GAA CGG ACT C	4	3
C67	GTC CCG ACG A	4	3
C68	TGG ACC GGT G	11	9
C69	CTC ACC GTC C	4	4
C70	TGT CTG GGT G	7	7
C71	AAA GCT GCG G	7	6
C74	TGC GTG CTT G	4	2
C76	CAC ACT CCA G	8	7
C77	TTC CCC CCA G	6	5
C78	TGA GTG GGT G	3	0
C79	GTT GCC AGC C	4	3
C80	ACT TCG CCA C	8	4
C61	TTC GAG CCA G	3	2

Out of 86 bands generated, 64 were polymorphic (74.4 % polymorphism). On an average, the primers generated 6.1 products and 4.6 polymorphisms per primer. The number of bands generated by these arbitrary 10-mer primers was 3 - 13 with primer C65 giving the maximum while primers C61 and C78 giving the minimum number of amplicons. The number of amplicons so generated can be arbitrarily grouped under 3-band classes: 3 - 4, 6 - 7 and 8 - 13. The primers C61, 78, 74, 69, 67 and C66 produced lower numbers of bands while a higher number of products were produced by primers C65, 68, 70, 71, 76 and 80. Interestingly, primers C69 and C70 produced 100 % polymorphism and primer C78 produced all monomorphic bands across the accessions. A representative RAPD profile of the cultivars obtained with primer C65 is shown in Fig. 1. The moderate levels of polymorphism detected in the cultivars may be attributed to the vegetative propagation. Although higher levels of polymorphic bands are useful for easy and rapid resolution of intervarietal variations and development of cultivar specific markers, lower to moderate levels of polymorphism contribute to accurate and reliable estimation of genetic variability, thereby helping in defining the nature of existing gene pools of the cultivars (Iqbal *et al*. 1997). It has also been reported that banana genomes are abundantly rich in dinucleate repeat motifs like (CA)n and (AC)n when compared with other plant genomes and hence a few tri- and tetranucleotide repeat anchored primers will produce reproducible bands (Ray *et al*. 2006). Although 22 accessions were subjected to RAPD analysis (11 from each cultivar), samples 3 and 13 were consistent in giving no amplicons with any of the primers

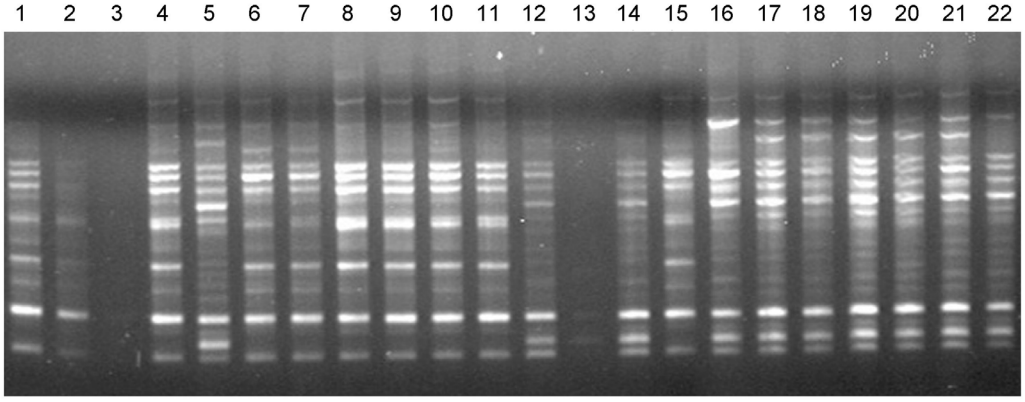

Fig. 1. RAPD profile of cultivars of *Musa*. *Lanes* 1 - 11: cv. Matti, 12 - 22 cv. Rasakadali.

Table 2. Similarity matrix of *Musa* cultivars analysed using Nei's original measures of genetic identity (M - Matti, R - Rasakadali).

	M1	M2	M3	M	M5	M6	M7	M8	M9	M10	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
M1	1.00	0.98	0.94	0.95	0.93	0.95	0.98	0.96	0.99	0.93	0.96	0.89	0.98	0.91	0.92	0.90	0.88	0.87	0.86	0.78
M2		1.00	0.90	0.94	0.94	0.93	0.96	0.92	0.96	0.90	0.96	0.86	0.96	0.88	0.87	0.85	0.83	0.83	0.84	0.80
M3			1.00	0.89	0.90	0.94	0.92	0.94	0.92	0.96	0.89	0.84	0.93	0.87	0.88	0.91	0.83	0.82	0.89	0.77
M4				1.00	0.95	0.91	0.97	0.89	0.97	0.89	0.96	0.88	0.94	0.89	0.95	0.93	0.90	0.90	0.86	0.85
M5					1.00	0.94	0.95	0.86	0.95	0.95	0.94	0.83	0.92	0.86	0.88	0.91	0.83	0.83	0.87	0.86
M6						1.00	0.95	0.95	0.95	0.97	0.95	0.87	0.93	0.89	0.89	0.93	0.85	0.85	0.90	0.82
M7							1.00	0.95	0.99	0.94	0.96	0.88	0.95	0.90	0.93	0.90	0.88	0.89	0.87	0.79
M8								1.00	0.95	0.91	0.93	0.86	0.95	0.90	0.88	0.86	0.84	0.85	0.87	0.73
M9									1.00	0.93	0.96	0.88	0.96	0.89	0.92	0.90	0.88	0.89	0.87	0.78
M10										1.00	0.89	0.82	0.91	0.84	0.86	0.92	0.82	0.83	0.91	0.79
R1											1.00	0.88	0.96	0.92	0.92	0.89	0.87	0.87	0.88	0.84
R2												1.00	0.86	0.94	0.95	0.90	0.98	0.97	0.88	0.76
R3													1.00	0.91	0.89	0.87	0.85	0.83	0.86	0.78
R4														1.00	0.90	0.84	0.93	0.92	0.95	0.80
R5															1.00	0.96	0.97	0.96	0.85	0.78
R6																1.00	0.91	0.89	0.84	0.80
R7																	1.00	0.97	0.88	0.76
R8																		1.00	0.90	0.75
R9																			1.00	0.82
R10																				1.00

and therefore they were eliminated in the data scoring and subsequent analysis step. However, the reproducibility of RAPD profiles for the rest of the samples as observed in the present study indicates genetic stability of the cultivars. The coefficient of similarity value in terms of genetic relatedness obtained from RAPD data analysis was found to range from 0.73 to 0.99 with mean value of GS = 0.91, suggesting low levels of intercultivar variation. The cultivars of Rasakadali with mean GS of 0.89 is genetically more diverse than those of Matti with mean value of GS = 0.93 (Table 2). It is expected as Rasakadali with its characteristic AB genome is considered to be more diverse than the AA genome present in Matti. It is reported that BB genome harbors many of the agronomically important traits like tolerance to biotic stress, resistance to pathogens, *etc*. (Morgante *et al*. 2002) and therefore Rasakadali with AB heterozygous genome type must have inherited some of the useful and diverse traits of ancestral BB type and hence genetically more diverse than homozygous AA genome type.

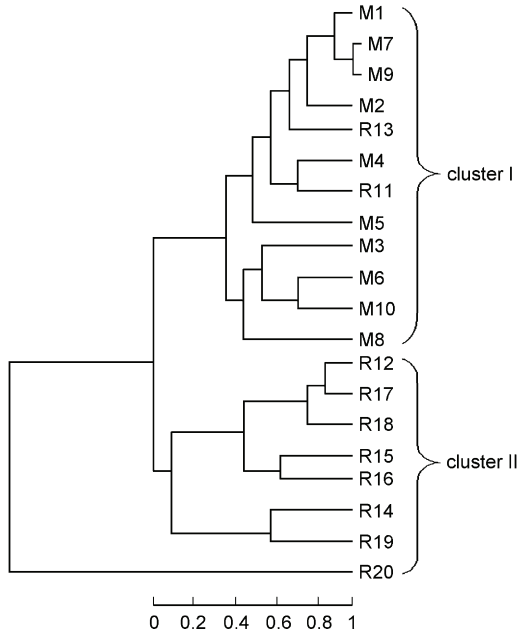

Fig. 2. UPGMA phenogram showing grouping between the two diploid *Musa* cultivars.

The phenogram obtained using cluster analysis (Fig. 2) following the standard statistical procedure of UPGMA grouped the cultivars into two major clusters. Cluster I with a total number of 12 accessions had all the 10 accessions of Matti and 2 of Rasakadali while the remaining 8 accessions of Rasakadali were grouped under cluster II. As the samples were collected from different plantations and the farmers were not able to ascertain the pedigree of plant materials, it is rather difficult to comment on the possible concordance/discordance relationship between the observed genetic relatedness with geographical distribution of the accessions. However, it is likely that grouping in these cultivars to a considerable extent is in accordance with their geographical distribution pattern, as selection of the accessions is not based on any identified agronomical or morphological trait shared by these accessions but on random basis. The similarity matrix identified measures of genetic identity between the cultivars.

The deviant Rasakadali accessions (11 and 13) placed in cluster I along with Matti may be either due to residual heterozygocity in ancestral materials or due to missing data points in the analysis as the number of loci screened

is relatively less. However, as reported by Vidhya *et al*. (2002), comparative genome analysis will be more effective and reliable if more regions of the genome are scanned by way of using more number of primers either in single or in combinations.

Musa with numerous cultivar groups and varieties has wide geographical distribution particularly in the tropics characterized by considerable ecological and morphological variations. The high genetic homogeneity in the *Musa* germplasm may be regarded as an index of 'genetic erosion' to which the crop has been exposed in the recent times due to domestication. Such limited diversity might be detrimental to future breeding programmes. The present study provides valuable information on the nature and pattern of genetic variation existing in two important cultivars of *Musa* at the inter- and intra-cultivar levels.

References

Bhat, K.V., Bhat, S.R., Chandel, K.P.S.: Survey of isozyme polymorphism for clonal identification in *Musa*. I. Esterase, acid phosphatase and catalase - J. hort. Sci. **67**: 501-507, 1992.

Bhat, K.V., Jarret, R.L.: Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. - Genet. Resour. Crop. Evol. **42**: 107-118, 1995.

Caetano-Anolles, G., Bassam, B.J., Gresshoff, P.M.: DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. - Bio/Technology **9**: 553-557, 1991.

Castiglione, S., Wang, G.G., Damiani, C., Bandi, S., Bisoffi, S., Sala, F.: RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. - Theor. appl. Genet. **87**: 54-59, 1993.

Faure, S., Noyer, J.L., Horry, J.P., Bakry, F., Lanaud, C., De Leon, D.G.: A molecular marker-based linkage map of diploid bananas (*Musa acuminata*). - Theor. appl. Genet. **87**: 517-526, 1993.

Howel, E.C., Newbury, H.J., Swennen, R.L., Withers, L.A., Ford-Lloyd, B.V.: The use of RAPD for identifying and classifying *Musa* germplasm. - Genome **37**: 328-332, 1994.

Iqbal, M.J., Aziz, N., Saeed, N.A., Zafar, Y., Malik, K.A.: Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. - Theor. appl. Genet **94**: 139-144, 1997.

Kaemmer, D., Afza, R., Weising, K., Kahl, G., Novak, F.J.: Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* spp.). - Bio/Technology **10**: 1030-1035, 1992.

Morgante, M., Hanafey, M., Powell, W.: Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. - Nat. Genet. **30**: 194-200, 2002.

Murray, M.G., Thompson, W.F.: Rapid isolation of high molecular-weight plant DNA. - Nucl. Acids Res. **8**: 4321-4325, 1980.

Nei, M.: Genetic distance between populations.- Amer.Natur. **106**: 283-292, 1972.

Padmesh, P., Reji, J.V., Jinish Dhar, M., Seeni, S.: Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. - Biol. Plant. **50**: 367-372, 2006.

Rafalski, J.A., Tingey, S.V., William, J.G.K.: RAPD markers-a new technology for genetic mapping and plant breeding. - Agr. Biotech. News Inform. **3**: 645-648, 1991.

Ray, T., Dutta, I., Saha, P., Das, S., Roy, S.C.: Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. - Plant Cell Tissue Organ Cult. **85**: 11-21, 2006.

Vidhya, R., Nair, S.A.: Molecular analysis of somaclonal variation in *Musa accuminata* (AAA) cv. Red. - Phytomorphology **52**: 293-300, 2002.

Zhang, H.-Q., Zghou, Y.-H.: Genetic relationships among *Hystrix patula*, *H. duthiei* and *H. longearistata* according to meitoc studies and genome-specific RAPD assay. - Biol. Plant. **53**: 45-52, 2009.