Available Online at ESci Journals
Journal of Plant Breeding and Genetics
ISSN: 2305-297X (Online), 2308-121X (Print)
http://www.escijournals.net/JPBG

EVALUATION OF GENETIC DIVERSITY AMONG EDIBLE BANANA VARIETIES FOUND IN MIZORAM, INDIA USING RANDOMLY AMPLIFIED POLYMORPHIC DNA

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A B S T R A C T

Genetic variations in the edible non-seeded banana cultivars found in Mizoram, India was studied using RAPD markers. A total of seven edible cultivars belonging to different genome groups were analyzed using six standardized UBC-primers. Out of six primers used, two primers namely UBC 416 and 450 generates 100% polymorphism. The average polymorphism per primer was 92.7%. The polymorphism information content (PIC) of the primers in used ranged from 0.58 to 0.89. The resolving power of primer, UBC-418 was the highest (9.4) while that of primer UBC-419 was the lowest (3.7). The dendrogram analysis revealed the grouping of the cultivars into two major groups. The first group was constituted by all the six hybrid cultivars of A and B genomes while the second group was represented by the lone triploid AAA genome sample. The result of the principal co-ordinate analysis also supported the dendrogram grouping of the samples studied. Thus, the use of RAPD technique for the study of genetic variations in the banana cultivars was demonstrated. It was also shown that significant amount of genetic diversity existed in the samples studied, thereby providing clear genetic information for future breeding and conservation strategies.

Keywords: Edible banana, Mizoram, RAPD, genetic variation.

INTRODUCTION

Banana is regarded as one of the favorite fruit crops of the world and is widely distributed throughout the tropics in more than 120 countries with a total production of approximately 106 million tonnes per year (Molina and Kudagamage, 2002). It is the fourth most important crop after rice, wheat, and maize (Uma and Sathiamoorthy, 2002). Banana originated from southeast Asia where they occur from India to Polynesia (Simmonds, 1962; Sathiamoorthy *et al*., 2001; Daniells et al., 2001). Nearly half of the world banana production is cultivated in Asia, while almost 75% of the world's plantains are cultivated in Africa. Edible banana along with ornamental ones and plantains belong to the family of *Musaceae*, which consists of two genera: *Musa* and *Ensete*. The taxonomy of the approximately 50 species within the genus Musa remains poorly resolved, not least because of the widespread vegetative reproduction and natural occurrence of many hybrids (Heslop and Schwarzacher, 2007). Most frequently, the genus is divided into four (sometimes five) sections, Eumusa and Rhodochlamys with a basic chromosome number of x = 11, Australimusa (x = 10), and Callimusa (x = 10 or x = 9) (after Cheesman, 1947; Simmonds and Weatherup, 1990; Dolezel and Bartos, 2005). Various minor and major regroupings have been suggested (Wong et al., 2002). At the species level, the number of species and the status of subspecies have been debated (Taxonomic Advisory Group for Musa, 2007). Eumusa constitutes the sources of edible banana chiefly belonging to *M. acuminata* Colla and *M. balbisiana* Colla originating from the inter- and intra-specific hybridization (Ashalatha *et al*., 2005). Depending on the contribution of *M. acuminata* (AA) and *M. balbisiana* (BB), the cultivars have been classified into genomic groups (AA, AAA, AAB, ABB, BB, AB, BBB, AAAA and ABBB). The cultivated edible bananas and plantains differ from their wild relatives by being seedless and parthenocarpic. The genetic basis of the mutation (or mutations) in the A genome that gives rise to parthenocarpy has not been characterized, and no parthenocarpy has been identified

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in B genome diploids, although hybrids of A and B show the character (Heslop and Schwarzacher, 2007). Most of

the cultivars are wild collections made by farmers of spontaneously occurring mutants with parthenocarpic fruit production, which were brought into cultivation and then multiplied and distributed by vegetative propagation. India is the leading country in global banana production with an annual production of 13.5 mt from an area of 4.0 lakh ha and is further focusing to achieve 25 mt by the end of 2020 in production level (Sathiamoorthy *et al*., 2001). India has a rich genetic diversity of banana with more than 90 distinct clones. The northeastern region of India have been considered as the richest sources of natural banana diversity where *M. balbisiana* from Indian subcontinent meet *M. acuminata* from South East Asia (Molina and Kudagamage, 2002). However, the vast genetic resource of wild and cultivated banana in the region remains unexplored and untapped for scientific studies. Mizoram is located in the northeastern corner of India, enjoying the tropical, sub-tropical and temperate region in the state. Wild and edible banana varieties are abundantly distributed throughout Mizoram. Therefore, the understanding of the level of genetic diversity of edible banana in the state will provide useful information for the status and breeding purposes.

Morphological characters have been used for classifying genome constitution and ploidy levels in banana (Pollefeys et al., 2004). However, phenotyping is difficult because of the size of the plants, and long life cycle and thus molecular marker methods are widely used for germplasm characterization. RAPD, AFLP and microsatellites (Onguso *et al*., 2004; Wong *et al*., 2002) have been used for the characterization of banana. Uma *et al*., (2005) have also reported the evidence of genetic variation successfully in Indian wild *Musa balbisiana* (BB) population by using RAPD markers. The use of RAPDs in molecular biology has the advantage that the material is processed by an efficient and inexpensive technique without requiring prior knowledge of the genome (Bhat *et al*., 1995). RAPD assay has the advantage of being easy to use, requiring very small amount of genomic DNA without the need for blotting and radioactive detection and are moderately reproducible (Cipriani *et al*., 1996). Thus, the present study was undertaken to analyze the genetic variation of edible cultivated banana varieties maintained in the field gene bank of the Department of Biotechnology, Mizoram University using RAPD markers.

MATERIALS AND METHODS

Plant Material: For the present study, seven cultivated non-seeded edible banana cultivars maintained in the field gene bank of the Department of Biotechnology, Mizoram University, Aizawl were selected (Table 1).

Table 1 List of edible non-seeded banana cultivars of Mizoram chosen for the study.

Accession (Local name)	Code	Species	Genome group
Vaibalhla	VB	*Musa acuminata*	AAA
Banria	BR	*Musa sp.*	ABB
Lawngbalhla	LB	*Musa sp.*	AAB
Kawlbalhla	KB	*Musa sp.*	ABBB
Banthur	BT	*Musa sp.*	AAB
Banpawl	BP	*Musa sp.*	ABB
Balhlasen	BS	*Musa sp.*	AAB

DNA extraction and PCR amplification: The young cigar leaves were collected from the banana plants and used for DNA extraction. Leaves weighing approximately about 200 mg were used in the DNA extraction using the modified CTAB method protocols of Thangjam *et al.* (2003).

The quantity of the extracted DNA was estimated using Biophotometer plus (Eppendorf, Germany) by taking their absorbance at 260 nm and 280 nm. For each sample, a 100 ng/µl stock was prepared from the original mother stocks for further analytical procedures. The PCR reaction mixture was assembled in a 25 µl volume containing 50 ng of template DNA, 1X Taq buffer, 0.5 µM dNTPs, 2 µM $MgCl_2$, 1U Taq polymerase (Bangalore Genei Pvt. Ltd., India) and 50 pmol UBC RAPD primer set # 5 (University of British Columbia, Canada). The thermocycler (Applied Biosystems, USA) was programmed at 1 cycle of 94 ^{0}C for 1 min and then 35 cycles of 94 ^{0}C for 1min 36 ^{0}C for 50 sec and 72 ^{0}C for 2 min. The last extension cycle was programmed at 72 ^{0}C for 4 min. The amplified products were electrophoresed on a 1.8% ethidium bromide stained agarose gel in TBE buffer and then photographed using a gel documentation system (UVP Ltd., UK).

Analysis of the RAPD profiles: The amplified DNA fragment generated was treated as a separate character and scored as a discrete variable, using 1 to indicate presence, and 0 for absence of DNA fragments. Accordingly rectangular binary data matrix was generated from the RAPD profiles.

Primer banding characteristics such as number of scored bands (NSB), number of polymorphic bands (NPB), and percentage of polymorphic bands (PPB) were obtained. Resolving power and marker index for the primers were also calculated. Polymorphism information content (PIC_i) of a band was calculated according to Anderson *et al.* (1993) as follows:

$$PIC_i = 1 - \sum_j f_{ij}^2$$

Where f_{ij} is the frequency of the j^{th} pattern of the i^{th} band (note that dominant markers have two patterns for a band as being present and absent). Then, the PIC of each primer was calculated as:

$$PIC = 1/n - \sum_{i=1}^{n} PIC_i$$

where n is the NPB for that primer. Informativeness of a band (BI_i) was calculated as:

BI_i = 1- (2 × | 0.5 – p |)

where p is the proportion of the seven accessions containing the band. Then, the resolving power (RP) of each primer was calculated as:

$$RP = \sum_{i=1}^{n} IB_i$$

where n is the NPB for that primer (Prevost and Wilkinson, 1999). Further we calculate mean resolving power for each primer as:

MRP = 1/n RP

Following Milbourne et al. (1997), marker index (MI) was calculated as product of PIC and effective multiplex ratio (EMR), which is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci (Seyit *et al.,* 2010).

Statistical analysis: Rectangular binary data matrix of RAPD was used for statistical analyses. Pairwise dissimilarity matrix was generated using Nei's coefficient (Nei, 1978) and principal coordinate analysis (PCoA) of the doubled centre distance matrix was performed. A dendrogram using neighbor joining method (unweighted) was constructed on the basis of the dissimilarity matrix data. All the analysis was conducted using the software NTSYS-pc version 2.20a (Numerical Taxonomy and Multivariate Analysis for Personal Computer, Rohlf, 2000).

RESULTS AND DISCUSSION

The present study was carried out to evaluate the level of genetic variations in the cultivated non-seeded banana cultivars of Mizoram using RAPD markers. The details of the RAPD profile generated in the banana samples studied using 6 RAPD primers were given in Table 2. Only the distinct and reproducible bands were scored for the analysis. A total of 94 fragments were scored out of which 84 were polymorphic (92.5%). This amplification result indicates that the randomly arranged nucleotides sequences in UBC- primers have their complementary nucleotides with the isolated DNA from the samples. The patterns of their dissimilarity in amplifications signify that they are genetically asymmetric. Selective amplification products in PCR condition by using random primers established that the tested DNA samples were diversified from one another. Different samples produce different numbers of bands with a particular primer. Two primers UBC-416 and -450 generated 100% polymorphism. RAPD profile generated by the UBC-416 was represented in Fig. 1.

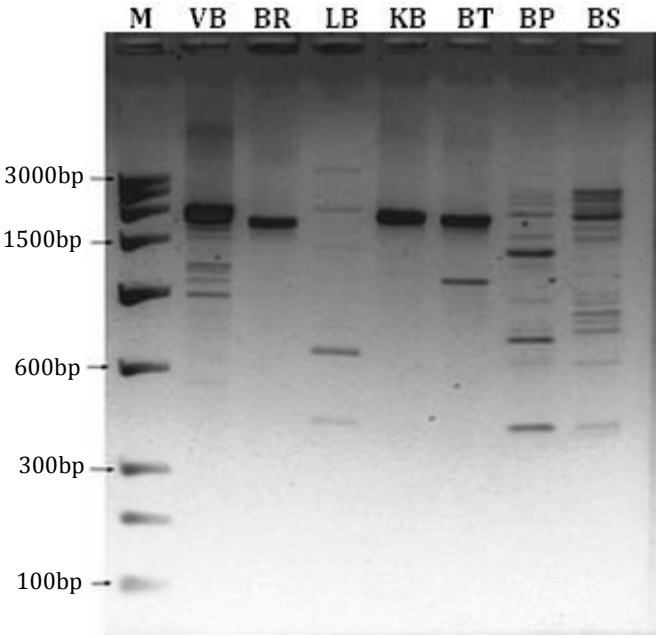

Fig. 1 RAPD profile generated by the primer UBC-416 (5′-GTGTTTCCGC-3′) in the banana samples studied. M- Low range DNA Ladder; VB- Vaibalha; BR- Banria; LB- Lawngbalha; KB- Kawlbalha; BT- Banthur; BP- Banpawl; BS- Balhlasen.

The average polymorphism was found to be 92.7%. The polymorphism information content (PIC) for the RAPD primer, UBC-416 was the highest (0.894) while that of primer UBC-421 was the lowest (0.582). The mean resolving power (MRP) of the primers ranges from 0.46 (UBC-419) to 0.63 (UBC-418). Resolving Power (Rp) provided a modest indication of the ability of these primers to distinguish between cultivars. Marker index (MI) which is the discriminating power for a marker was also calculated for each primer and found to be highest in the primer UBC-416 (16.1). Rp of a primer has been found to correlate strongly with its ability to distinguish between cultivars. The function is well suited for comparing primers, primer-enzyme combinations or probe-enzyme combinations generated by RAPD, ISSR-PCR, AFLP or RFLP analyses. Crucially, Rp provides quantitative data allowing comparisons between primers (or probe-enzymes etc.), including those that are able to distinguish all genotypes examined in a study. It can also be used to predict the performance of groups of primers.

Table 2 Details of amplification bands and their associated polymorphic fragments obtained with different UBC decamer oligonucleotides utilized as random primers in the banana samples. NSB- Number of scored band; NPB- Number of polymorphic band; PPB-Percentage of polymorphic band; PIC- Polymorphism information contents; RP- Resolving power; MRP- Mean resolving power; MI- Marker index.

UBC Primer (set # 5)	Sequence (5´ - 3´)	Fragment size range (bp)	NSB	NPB	PPB	PIC	RP	MRP	MI
UBC-416	TGTTTCCGC	2000-350	18	18	100	0.894	8.5	0.47	16.1
UBC-418	AGGAAGCTA	2000-300	16	15	93.7	0.758	9.4	0.63	10.6
UBC-419	ACGTGCCCG	2700-440	9	8	88.9	0.775	3.7	0.46	5.51
UBC-420	CAGGGTTCT	2400-270	15	14	93.4	0.821	8.3	0.59	10.7
UBC-421	CGGCCCACC	3000-400	20	16	80	0.582	8.8	0.55	7.45
UBC-450	GGAGAGCCA	2000-450	16	16	100	0.665	8.0	0.50	10.6

The Nei's (1978) dissimilarity matrix between the samples was given in Table 3. The highest similarity was obtained between the two cultivars (BR and BP) belonging to the ABB genome with a dissimilarity index of 0.156 and the lowest similarity was found between the AAA cultivar (VB) and ABB cultivar (BP) with a score of 0.754.

Table 3 Dissimilarity matrix based on Nei's coefficient (1978) obtained from RAPD profile in the banana samples studied. VB- Vaibalha; BR- Banria; LB- Lawngbalha; KB- Kawlbalha; BT- Banthur; BP- Banpawl; BS- Balhlasen.

Sample	VB	BR	LB	KB	BT	BP	BS
VB	0.00						
BR	0.739	0.000					
LB	0.724	0.455	0.000				
KB	0.647	0.468	0.693	0.000			
BT	0.668	0.346	0.559	0.531	0.000		
BP	0.754	0.156	0.449	0.618	0.324	0.000	
BS	0.611	0.467	0.734	0.583	0.474	0.376	0.000

The scores obtained from the RAPD profiles were used to construct a dendrogram using unweighted method of the Neighbor Joining cluster analysis. The resulted dendrogram revealed the clustering of the seven samples into two distinct groups (Fig. 2). The first group was sub-divided into 2 clusters, with 3 cultivars namely – KB (ABBB genome), BS (AAB genome) and BT (AAB genome) in the first cluster and another 3 cultivars namely – LB (AAB genome), BP (ABB genome) and BR (ABB genome) in the second cluster. The lone AAA genome sample (VB) was place in the second group.

The analysis of the principal co-ordinate analysis was represented by the 3-D projection diagram (Fig. 3). The extracted co-ordinates was also in accordance with the dendrogram, projecting VB alone in one different dimension while BR, BP and LB were clustered together and likewise BT, BS and KB also form one cluster.

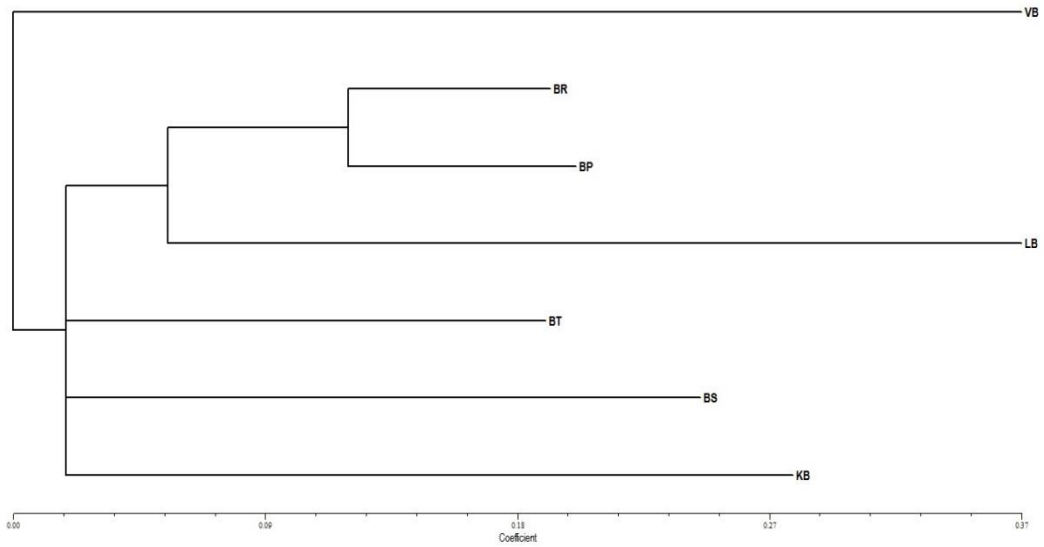

Fig. 2 Dendrogram resulted from the analysis of RAPD data. VB- Vaibalha; BR- Banria; LB- Lawngbalha; KB- Kawlbalha; BT- Banthur; BP- Banpawl; BS- Balhlasen.

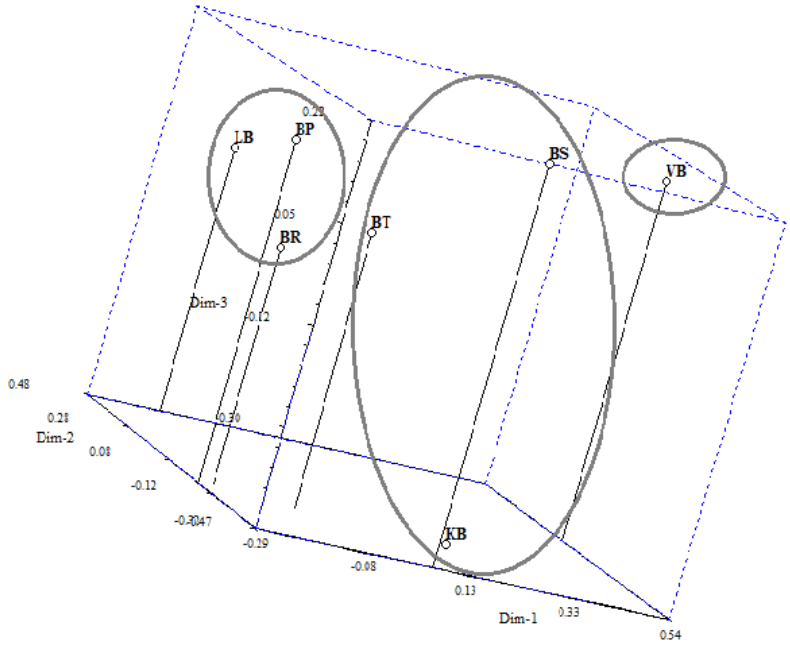

Fig.3 Three-dimensional projection of the RAPD profile generated by the banana samples studied. VB- Vaibalha; BR- Banria; LB- Lawngbalha; KB- Kawlbalha; BT- Banthur; BP- Banpawl; BS- Balhlasen.

The present study was primarily focus on the study of genetic variation among the edible non-seeded banana varieties from locally available banana in Mizoram. Using RAPD technique it was shown that significant amount of genetic diversity existed in the samples studied. Therefore, understanding genetic variation between and within the cultivars is very important for the evaluation of plants with superior traits. The finding derived out of this study will provide useful information for future breeding and conservation strategies of banana genetic resources in the northeast region of India, which is considered as a storehouse of diverse gene-pool.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Department of Biotechnology (DBT), Govt. of India for providing

Bioinformatics Facility (BIF) which was extensively used for the present study and state biotech-hub in Mizoram University.

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