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Full Length Research Paper

Comparative results of RAPD and ISSR markers for genetic diversity assessment in *Melocanna baccifera* Roxb. growing in Mizoram State of India

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The genetic similarity among 12 accessions of *Melocanna baccifera* from Mizoram in India was tested using RAPD and ISSR markers. RAPD and ISSR DNA profile shows high polymorphism of DNA fragments. Genetic similarity among accessions was calculated according to Dice similarity coefficient; the mean level of genetic similarity with populations of *M. baccifera* by using RAPD and ISSR markers were 0.600 and 0.650, respectively. The similarity coefficients were then used to construct a dendrogram using the UPGMA cluster analysis. Cluster analysis base on Dice coefficient shows two major groups. Mantel test revealed the different distribution pattern of the polymorphism between RAPD and ISSR markers and the correlation coefficient (r) was found as r = 0.1902.

Key words: ***Melocanna baccifera***, ISSR, RAPD, genetic similarity.

INTRODUCTION

The economy of India and so also of many Asian countries depends on bamboos and their uses are not only in domestic items but also in rural housing and raw materials to several industries. In the state of Mizoram (India), bamboo forest covers 12,54,400 ha, out of the total area of 21,090 sq.km. These bamboo forest areas of Mizoram constitute 14% of the total India's bamboo area, that is, 8.96 million ha. Non-clump forming bamboo ***Melocana baccifera*** is the most abundant and most economical bamboo in Mizoram, contributing 95% of the growing stock of bamboo. It belongs to members of subfamily Bambusoideae of family Poaceae and flowering is at a regular interval of 48 years, the culm die after flowering. Extensive field survey was carried out during the flowering of this bamboo. It was found that, there was a difference in the seeds colour of ***M. baccifera*** in some accessions (shown in Figure 1). In order to analyze variation in their gene pool, some accessions were collected. The places of collection were shown in the map of Figure 2. Traditionally, morphological characters like growth habit, leaf type and floral morphology have been used to define taxa (Nayak et al., 2003) this is still poor taxonomic description. To date no molecular analysis has been performed to test genetic relationship of ***M. baccifera*** period. The random amplified polymorphic DNA (RAPD) marker technique is quick, easy and required less time. This detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Williams et al., 1990), inter-simple sequence repeat (ISSR) permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats. RAPD and ISSR markers have been extensively used for DNA finger-printing (Moreno et al., 1998), genetic diversity studies (S'anchez de la Hoz et al., 1996; Esselman et al., 1999), population genetic studies (Wolfe et al., 1998; Nebauer et al., 1999) and phylogenetic studies (Hess et al., 2000)

The objectives of the present research are to study the genetic similarity within ***M. baccifera,*** to compare RAPD and ISSR markers for the molecular characterization of ***M. baccifera*** and to study phylogenetic relationships in ***M. baccifera***.

MATERIALS AND METHODS

Collection

An extensive field survey was carried out, that is, from March-

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Figure 1. Accessions of ***Melocanna baccifera*** showing different accessions characters; (a) non-flowering accession (green patch) surrounded by brownish-white colour flowering culms, (b) green colour fruit, (c) red colour fruit and (d) blackish brown colour fruit.

August 2007 throughout Mizoram (India) during the flowering of ***M. baccifera***. Twelve accessions of ***M. baccifera*** were collected from the study area. One accession was collected in the form of leaf and the remaining 11 accessions were collected in the form of seeds. All the seeds collected were sown in the experimental field in Hyderabad University campus. The accession names, form of collection and morphological characteristics are given in Table 1.

DNA extraction

Genomic DNA of every sample was isolated from the frozen leaf according to the CTAB protocol of Doyle and Doyle (1990) with minor modification.

RAPD analysis

Forty arbitrary decamer primer sets of Operon Kits A and C (India) were tested on the genomic DNA of two accessions and nine primers showed reproducible amplified products were chosen for this investigation. Primers used are given in the Table 2. Amplification was performed in a 15 µl reaction volume, containing 50 µg template DNA, 1x PCR buffer, 0.3 mM each dNTP, 2 mM of $MgCl_2$, 1.5 µM of each primer and l unit of Taq DNA Polymerase (Sigma Aldrich). Amplification was performed in My-gene^{TM} series peltier thermal cycler (Longgene Scientific Instruments Co., Ltd. Hang zhou). Initial denaturation was for 4 min at 94^{o}C, followed by 45 cycles of 50 s at 94^{o}C, 1 min at 37^{o}C, 2 min at 72^{o}C and 10 min at 72^{o}C for final extension step. The amplified products were

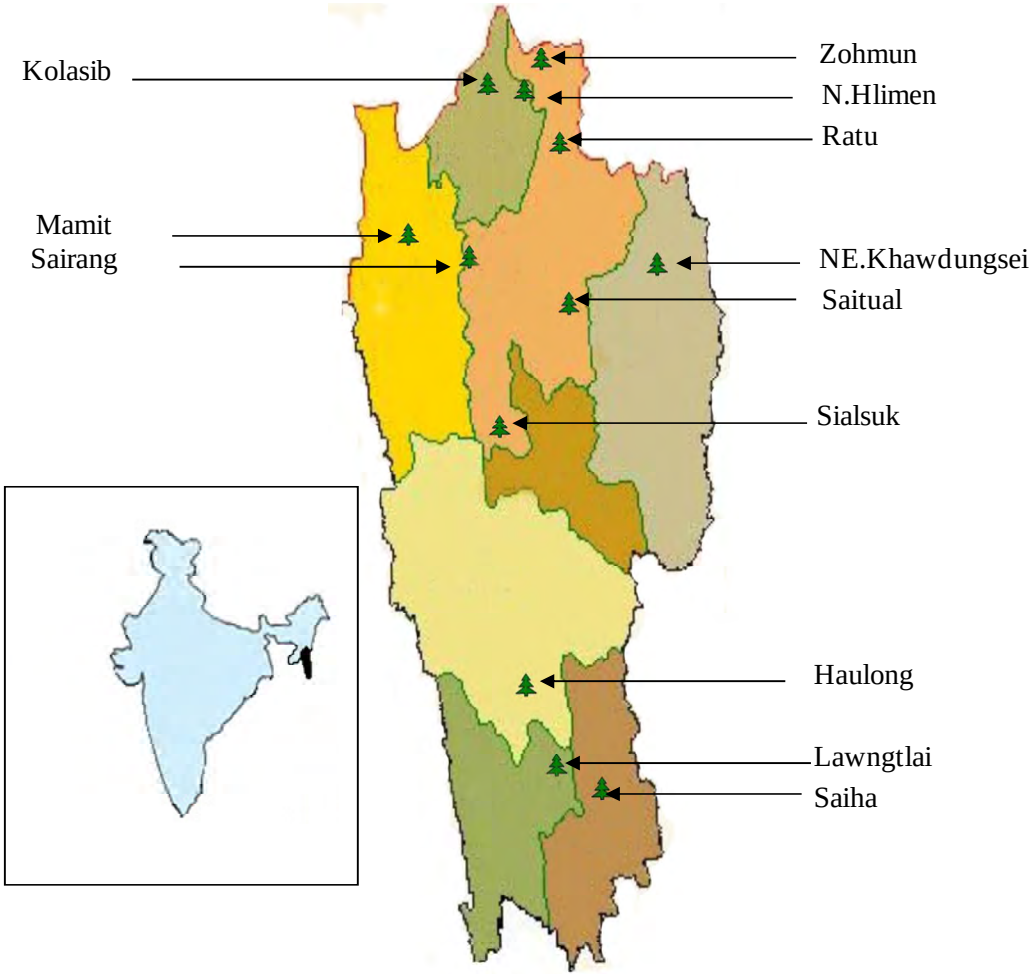

Figure 2. Hydrographic map of the Mizoram, showing the collection sites of ***Melocanna baccifera***.

Table 1. Bamboo (***Melocanna baccifera*** Roxb.) accessions collected in different regions of Mizoram.

S/N	Accessions name	Sample	Accessions character
1.	N.Hlimen	Seeds	Culm bears red colour fruit
2.	Haulong	Seeds	Culm bears green colour fruit
3.	Kolasib	Seeds	Culm bears green colour fruit
4.	Lawngtlai	Seeds	Culm bears green colour fruit
5.	Mamit	Seeds	Culm bears green colour fruit
6.	NE. Khawdungsei	Seeds	Culm bears green colour fruit
7.	Ratu	Seeds	Culm bears green colour fruit
8.	Saiha	Seeds	Culm bears green colour fruit
9.	Sairang	Seeds	Culm bears green colour fruit
10.	Sialsuk	Seeds	Culm bears blackish-brown colour fruit
11.	Saitual	Seeds	Culm bears green colour fruit
12.	Zohmun	Leaves	Non-flowering culms

separated on 1.2% agarose gels and stained with ethidium bromide. Images were captured using Uvidoc (Uvitech, UK) gel documentation system.

ISSR analysis

Forty five ISSR primers sets from University of British Columbia

Table 2. The statistical data for 9 RAPD and 17 ISSR primers used for analyzing 12 accessions of ***M. baccifera***.

Primer	Sequence	Annealing temperature	TNB	NPB	PB(%)
RAPD					
OPA-05	5′-AGGGTCTTG-3′	37^{0}C	5	4	80%
OPA-09	5′-GGGTAAGGCC-3′	37^{0}C	8	7	87.5%
OPA-10	5′-GTGATCGCAG-3′	37^{0}C	20	19	95%
OPA-17	5′-GACCGCTTGT-3′	37^{0}C	20	19	95%
OPC-02	5′-GTGAGGCGTC-3′	37^{0}C	7	7	100%
OPC-04	5′-CCGCATCTAC-3′	37^{0}C	5	5	100%
OPC-07	5′-GTCCCGACGA-3′	37^{0}C	9	8	88.8%
OPC-11	5′-AAAGCTGCGG-3′	37^{0}C	11	10	90.9%
OPC-15	5′-GACGGATCAG-3′	37^{0}C	9	9	100%
Total			94	88	
Average			10.4	9.7	98.02%
ISSR					
UBC-810	5′-GAGAGAGAGAGAGAGAT-3′	52^{0}C	10	9	90%
UBC-813	5′-CTCTCTCTCTCTCTCTT-3′	55^{0}C	11	10	90.9%
UBC-814	5′-CTCTCTCTCTCTCTCTA-3′	52^{0}C	5	4	80%
UBC-815	5′-CTCTCTCTCTCTCTCTG-3′	52^{0}C	4	2	50%
UBC-820	5′-GTGTGTGTGTGTGTGTC-3′	57^{0}C	5	5	100%
UBC-821	5′-GTGTGTGTGTGTGTGTT-3′	55^{0}C	7	5	71.4%
UBC-822	5′-TCTCTCTCTCTCTCTCA-3′	55^{0}C	8	8	100%
UBC-823	5′-TCTCTCTCTCTCTCTCC-3′	55^{0}C	8	7	87.5%
UBC-824	5′-TCTCTCTCTCTCTCTCG-3′	55^{0}C	4	4	100%
UBC-827	5′-ACACACACACACACACG-3′	57^{0}C	8	7	87.5%
UBC-828	5′-TGTGTGTGTGTGTGTGA-3′	57^{0}C	5	4	80%
UBC-830	5′-TGTGTGTGTGTGTGTGG-3′	57^{0}C	5	4	80%
UBC-846	5′-CACACACACACACACART-3′	55^{0}C	8	8	100%
UBC-847	5′-CACACACACACACACARC-3′	56^{0}C	9	7	77.7%
UBC-856	5′-ACACACACACACACACYA-3′	55^{0}C	7	6	85.7%
UBC-857	5′-ACACACACACACACACYG-3′	56^{0}C	12	12	100%
UBC-859	5′-TGTGTGTGTGTGTGTGRC-3′	56^{0}C	4	2	50%
Total			120	104	
Average			7.05	6.1	84.1%

R = A/G; Y = C/T; TNB = total number of bands; NPB = number of polymorphic bands; PB (%) = percentage of polymorphic band.

(Vancouver, Canada) were used to amplify the genomic DNA of two accessions and 17 primers were selected (Table 2). Amplification was preformed in a 15 µl reaction volume, containing 50 ng template DNA. 1X PCR buffer, 0.4 mM each of dNTP, 2 mM of $MgCl_2$, 1.0 µM each of primer and 1 unit of Taq. DNA polymerase (Sigma Aldrich). Amplification was performed in My-Gene^{TM} series peltier thermal cycle (Longgene Scientific Instruments Co., Ltd. Hangzhou). Initial denaturation was for 7 min at 94^{0}C, followed by 45 cycles of 1 min at 94^{0}C, 1 min at 54^{0}C, 2 min at 72^{0}C and a 10 min final extension step at 72^{0}C. The amplified products were separated on 1.2% agarose gels and stained with ethidium bromide. Images were captured using Uvidoc (Uvitech, UK) gel documentation system.

Data analysis

The amplified DNA fragments for each accession were scored as present (1) or absent (0). Data generated by nine RAPD and 17 ISSR primers were used to compile a binary matrix for cluster analysis. Genetics similarity among accessions was calculated according to Dice similarity coefficient (Dice, 1945). The similarity co-efficients were then used to construct a dendrogram using the UPGMA (Unweighted Pair-Group Method with Arithmetical average) cluster analysis (Sneath and Sokal, 1973). Similarity matrices based on RAPD and ISSR markers were compared using the mantel matrix-correspondence test (Mantel, 1967). All procedures were computed with the computer package NTSYS-pc

(Numerical Taxonomy and Multivariate Analysis System, Biostatistics, New York, USA, software version 2.02j package) (Rohlf, 1993).

RESULTS

Identification of genetic relationships in ***M. baccifera*** is very difficult because of the lack of morphological differences. The traditional methods of identifying DNA profiling are largely used for identification because of several limitations of morphological data. In recent years, RAPD and ISSR technology is a rapid and sensitive technique which can be used to estimate relationship between closely, and more distantly accessions.

Polymorphisms detected by RAPD and ISSR markers

The polymorphism of the ***M. baccifera*** germplasm was high. A total of 40 arbitrary RAPD primers were used for screening, out of these 9 primers were selected out for twelve accessions of ***M. baccifera*** DNA amplification, which produced clear bands for further analysis. The nine arbitrary primers used for RAPD analysis detected a total of 94 fragments, with an average of 10.4 fragments per primers and 98.02% fragments were polymorphic. 45 arbitrary ISSR primers were used for screening, out of these 17 primers were selected. A Total of 120 fragments were detected, with an average of 7.05 fragments per primer and 84.1% fragments were polymorphic. The DNA fragments produced by RAPD and ISSR markers were shown in Figure 3.

Genetics similarities base on RAPD and ISSR markers

The genetic similarities values based on RAPD and ISSR markers were different. The maximum genetic similarity by RAPD was 0.872 between Saiha and Sairang, while the lowest genetic similarity of 0.430 was between Mamit and N. Hlimen (Table 3). The maximum genetic similarity observed by ISSR was 0.769 between Kolasib and Sialsuk, while the lowest genetic similarity of 0.534 was between Saitual and N. Hlimen.

Phylogenetic analysis based on RAPD and ISSR

The phylogenetic relationships among 12 accessions of ***M. baccifera*** were analyzed by an UPGMA method (Figure 4). The cluster result indicated that all the 12 accessions could be distinguished by RAPD and ISSR markers, respectively. But the relationship among the accessions revealed by RAPD analysis were somewhat different from that revealed by the analysis of ISSR data. The cluster analysis of RAPD marker showed variation of a geographic distribution as all the accessions could be separated into two major groups. Majority of accessions, that is, 11 out of 12 accessions formed one cohesive cluster and the one N. Hlimen was resolved in another cluster. The bigger group was again sub-grouped in two smaller clusters containing Haulong, Saiha and Sairang in cluster I and Kolasib, Lawngtlai, Mamit, NE. Khawdungsei, Saitual, Sialsuk, Ratu and Zohmun in cluster II. It can be observed from the dendrogram that the variation between accessions Saiha and Sairang was the least in cluster I with similarity coefficient of 0.87. It can be further noted that N. Hlimen was the most diverse accession within the cluster.

The dendrogram obtained from UPGMA cluster analysis of genetic distance based on 17 ISSR markers is shown in Figure1b; the cluster analysis resolved 12 accessions into two major groups. Only Saiha accession form group I and the group II contained other eleven accessions. Group II could be further divided into 3 clusters. Cluster I consist of Saitual, NE. Khawdungsei and Ratu accessions. Cluster II consists of Sairang, Mamit, Zohmun, Sialsuk and Kolasib accessions and cluster III consists of Haulong, Lawngtlai, N. Hlimen. It can be observed from the dendrogram, the variation between Kolasib and Sialsuk accessions was the least with similarity coefficient of 0.77. Saiha was the most diverse accession.

Comparison of RAPD and ISSR markers

It is important to understand that different markers have different properties and will reflect different aspects of genetic diversity (Karp et al., 1995). RAPD (William et al., 1990) and SSR (Zietkiewicz et al., 1994) markers have advantages and disadvantages for assessing genetic diversity. In this work, the RAPD and ISSR surveys among 12 accessions of ***M. baccifera*** revealed 98.02 and 84.1% of polymorphic bands, respectively (nearly same). Mantel test revealed the different distribution patterns of the polymorphism between RAPD and ISSR markers and the correlation coefficient(r) was found as $r = 0.1902$.

DISCUSSION

RAPD and ISSR markers are proved to be efficient and inexpensive way to provide molecular data. They have been used successfully in determining genetic relationship and used for DNA fingerprinting (Moreno et al., 1998; Blair et al., 1999; Divaret et al., 1999; Gilbert et al., 1999). Researchers who have compared RAPD and ISSR methods have found that ISSR markers exhibit higher level of polymorphism or reproductivity compared

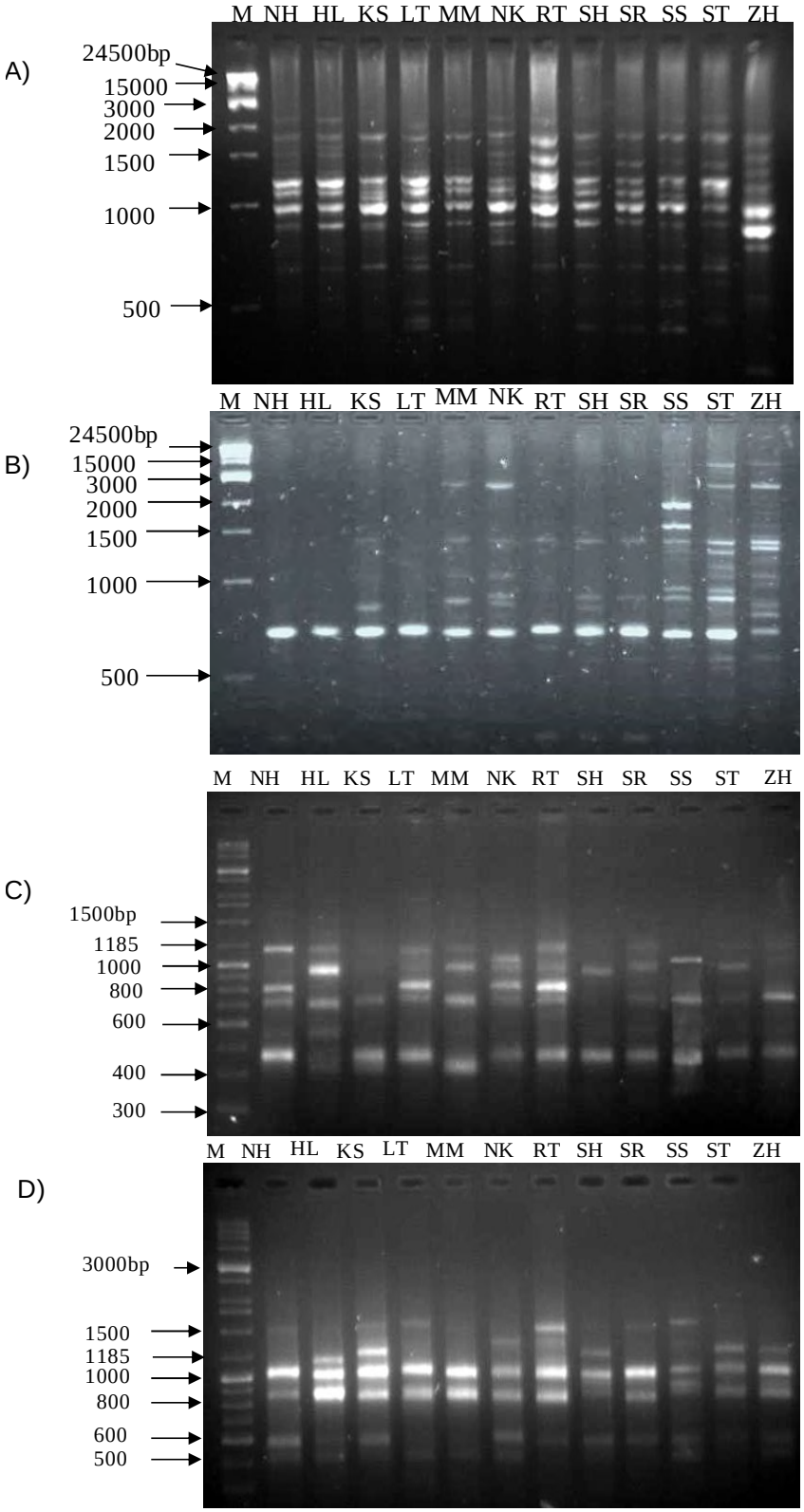

Figure 3. (A) RAPD bands amplified by the primer OPA-10. (B) RAPD bands amplified by the primer OPA-17. (C) ISSR bands amplified by the primer UBC-821. (D) ISSR bands amplified by the primer UBC-823. M = marker, NH = N.Hlimen, HL = Haulong, KS = Kolasib, LT = Lawngtlai, MM = Mamit, NK = NE. Khawdungsei, RT = Ratu, SH = Saiha, SR = Sairang, SS = Sialsuk, ST = Saitual, ZH = Zohmun.

Table 3. (A) Similarity matrix of ***M. baccifera*** accessions based on RAPD marker, and (B) similarity matrix of M. baccifera based on ISSR marker generated from Dice estimation of similarity based on the number of shared fragments.

(A)	NH	HL	KS	LT	MM	NK	RT	SH	SR	SS	ST	ZH
NH	1											
HL	0.597	1										
KS	0.567	0.696	1									
LT	0.542	0.608	0.724	1								
MM	0.430	0.625	0.656	0.656	1							
NK	0.542	0.695	0.724	0.583	0.656	1						
RT	0.513	0.657	0.657	0.526	0.507	0.578	1					
SH	0.500	0.677	0.711	0.580	0.631	0.677	0.545	1				
SR	0.507	0.741	0.677	0.615	0.700	0.646	0.521	0.872	1			
SS	0.500	0.586	0.693	0.564	0.575	0.666	0.585	0.647	0.647	1		
ST	0.485	0.579	0.550	0.527	0.626	0.722	0.473	0.612	0.646	0.692	1	
ZH	0.512	0.545	0.623	0.550	0.533	0.575	0.476	0.485	0.493	0.534	0.525	1
(B)	**NH**	**HL**	**KS**	**LT**	**MM**	**NK**	**RT**	**SH**	**SR**	**SS**	**ST**	**ZH**
NH	1											
HL	0.660	1										
KS	0.701	0.673	1									
LT	0.683	0.672	0.623	1								
MM	0.626	0.647	0.635	0.654	1							
NK	0.579	0.597	0.606	0.549	0.640	1						
RT	0.656	0.626	0.615	0.666	0.661	0.618	1					
SH	0.538	0.659	0.625	0.585	0.556	0.606	0.560	1				
SR	0.691	0.742	0.707	0.666	0.760	0.630	0.672	0.696	1			
SS	0.696	0.686	0.769	0.691	0.723	0.680	0.643	0.638	0.762	1		
ST	0.534	0.615	0.623	0.604	0.638	0.627	0.596	0.626	0.674	0.681	1	
ZH	0.615	0.691	0.678	0.642	0.690	0.607	0.650	0.646	0.725	0.747	0.650	1

with RAPD markers (Fang and Roose, 1997; Esselman et al., 1999; Parsons et al., 1997; Qian et al., 2001). The result of the present study showed that ISSR markers were not more informative than RAPD markers, however, both of the markers worked effectively. The polymorphism of the ***M. baccifera*** germplasm was high. Based on RAPD and ISSR markers data, the percentage of the polymorphic fragments were 98.02 and 84.1% respectively. The reason why the level of polymorphism in ***M. baccifera*** was high might be that the accessions are located in the different region for rather long time. In the process of the natural selection, the different populations with different genotypes adapted to the different environment were preserved and the accessions of ***M. baccifera*** mainly depend on their underground rhizomes as the propagating organs, since the flowering cycle is 48 - 50 years. Thus the genetic diversity of ***M. baccifera*** was well conserved; this might lead to the abundant genetic diversity of ***M. baccifera*** in this region.

The mean level of genetic similarity with populations of ***M. baccifera*** by using RAPD and ISSR markers are 0.600 and 0.650, respectively. This pattern of genetic diversity may be caused and maintained by effective gene flow within populations and high fecundity. From this, it is evident that the accessions from different geographical locations exhibited a wide range of genetic distance, which did not show any correlation with geographical distances between the collection sites, negating to 'spatial autocorrelation' concept. If the population is more or less continuous, allele frequencies will fluctuate with distance due to genetic drift and selection (Sokal et al., 1989). Wright (1965) predicted that every finite population will experience genetic drift. But that the effects of such drift become more pronounced as population size decreases. Population with continually small effective population size will be especially susceptible to the loss and the re-organization of variation by genetic drift (Barrett and Kohn, 1991; Frankel and Soule, 1981).

Cluster analysis based on Dice coefficient by RAPD markers given in Figure 1a show two major groups. The accessions collected from Zohmun and N.Hlimen were a relative distance of 30 km was found in separate groups. Though most geographically closer localities found in separate groups showed that they are genetically distant.

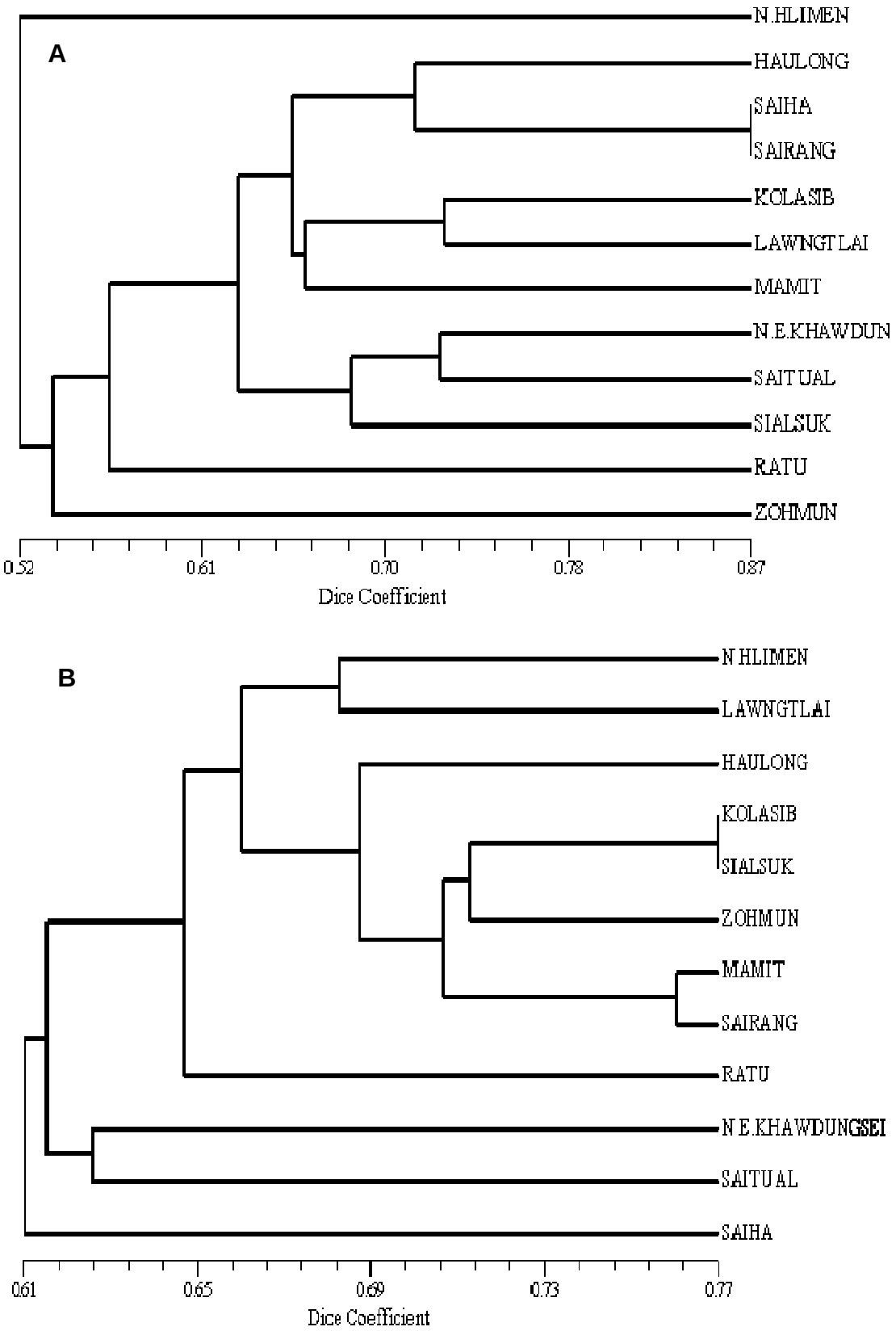

Figure 4. (A) Dendrogram generated using UPGMA method illustrating the genetic diversity relationships among 12 accessions of ***M. baccifera*** by RAPD marker. (B) Dendrogram generated using UPGMA method illustrating the genetic diversity relationships among 12 accessions of ***M. baccifera*** by ISSR marker.

This shows that there is no correlation between genetic make up and geographical distances. The genetic uniformity or non-uniformity is reflected by the genetic distance value as described by Das and Mukherjee, (1997.)

Similarly, from cluster analysis based on Dice coefficient by ISSR markers shown in Figure 4b, two main groups were observed. The accessions collected from Kolasib and Sialsuk showed closest affinities; exhibit a genetic identity of 0.77. Though they are quite distant apart geographically and link with Zohmun, Mamit and Sairang to form a well defined cluster. The possible explanation for the accessions diversity found in ***M. baccifera*** populations is somatic mutation. This bamboo plants can live very long and somatic mutations may occur in their gene pool which may eventually lead to some variations. Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral (Bachmann, 1997; Landergott et al., 2001). Similar opinions have been reported with regard to ISSR markers (Esselman et al., 1999). Some studies have shown that RAPD markers are found throughout the genome and may be associated with functionally important loci (Penner, 1996).

The RAPD and ISSR surveys between 12 accessions of ***M. baccifera*** revealed 98.02 and 84.1% of polymorphic bands, respectively. The results obtained from cluster analysis based on RAPD and ISSR data sets were different. This was also reflected in the correlation coefficient calculated for the elements of RAPD and ISSR similarity matrices by using Mantel test. Although the value of correlation coefficient between RAPD and ISSR markers was significant as $r = 0.1902$, it shows a very poor fit between two markers, according to its interpretation ($0.7 \leq r < 0.8$ poor fit; $r < 0.7$ very poor fit). This inferred that the two sets of markers explore genetic variation differently. It is likely that RAPD and ISSR target different regions of genome which are subjected to different mechanisms generating genetic variation. Genomic regions sampled by the RAPD and ISSR markers maintain a different evolutionary process under selection. In summary RAPD and ISSR markers were a powerful dominant DNA markers, they are effective and promising markers for assessing genetic variation in ***M. baccifera*** species.

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REFERENCES

Bachmann K (1997). Nuclear DNA markers in plant biosystematic research. Opera. Bot. 132: 137-148.

Barrett SCH, Kohn JD (1991). Genetic and evolutionary consequences of small population size in plants: Implication for conservation. In: Genetics and conservation of rare plants. Oxford University Press, New York, pp. 3-30.

Blair MW, Panaud O, Mccouch SR (1999). Inter- simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (***Oryza sativa*** L.). Theor. Appl. Genet. 98: 780-792.

Das S, Mukherjee KK (1997). Morphological and biochemical investigations on Ipomoea seedlings and their species interrelations. Ann. Bot. 79: 565-571.

Dice LR (1945). Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Divaret I, Margale E, Thomas G (1999). RAPD markers on seed bulks efficiently assess the genetic diversity of a ***Brassica oleraceae*** L. Theor. Appl. Genet. 98: 1029-1035.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Esselman EJ, Jianqiang L, Crawford DL, Windus JL, Wolfe AD (1999). Clonal diversity in the rare ***Calamagrostis porteri*** spp. ***Insperata*** (Poaceae): comperative result for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. Mol. Ecol. 8: 443-451.

Fang DQ, Roose ML (1997). Identification of closely related citrus cultivars with inter-simple repeat markers. Theor. Appl. Genet. 94: 408-417.

Frankel OH, Soule ME (1981). In conservation and evolution. Cambridge University Press, Cambridge.

Gilbert JE, Lewis RV, Wilkinson MJ, Caligari PDS (1999). Developing an appropriate strategy to assess genetic variability in plant germplasm collection. Theor. Appl. Genet. 98: 1125-1131.

Hess J, Kadereit JW, Vargas P (2000). The colonization history of ***Olea europea*** L. in Macaronesia based on internal transcribed apacer 1 (ITS-1) sequence, randomly amplified polymorphic DNAs (RAPD) and inter-simple sequence repeats (ISSR). Mol. Ecol. 9: 857-868.

Landergott U, Holderegger R, Kozlowski G, Schneller JJ (2001). Historical bottlenecks decrease genetic diversity in natural populations of Dryopteris cristata. Heredity, 87: 344-355.

Mantel N (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220.

Moreno S, Martin JP, Ortiz JM (1998). Inter-simple sequence repeat PCR for characterization of closely related grapevine germplasm. Euphytica, 101: 117-125.

Nayak SGR, Rout GR, Das P (2003). Evaluation of the genetic variability in bamboo using RAPD markers. Plant soil Environ. 49: 24-28.

Nebauer SG, Del Castillo Agudo L, Segura J (1999). RAPD variation within and among natural populations of outcrossing willow-leaved foxglove (***Digitalis obscura*** L.). Theor. Appl. Genet. 98: 985-994.

Parsons BJ, Newbury HJ, Jackson MT, Ford-Lloyd BV (1997). Contrasting genetic diversity relationships are revealed in rice (***Oryza sativa*** L.) using different marker types. Mol. Breed. 3: 115-125.

Penner GA (1996). RAPD analysis of plant genome. In: Jauhar PP (Ed.), Methods of genome analysis in plants. CRC Press, Boca Raton, pp. 251-268.

Qian W, Ge S, Hong DY (2001). Genetic variation within and among populations of wild rice ***Oryza granulate*** from China detected by rRAPD and ISSR markers. Theor. Appl. Genet. 102: 440-449.

Rohlf FJ (1993). NYSYS-pc Numerical Taxonomy and Multivariate Analysis System (Version 1.8). Exeter Publishing Ltd., Setauket, New York.

S'anchez de la Hoz MP, Davilla JA, Loarce Y, Ferrer E (1996). Simple sequence repeat primers used in polymerase chain reaction amplification to study genetic diversity in barley. Genome, 39: 112-117.

Sokal RR, Jacues GM, Wooten MC (1989). Spatial autocorrelation analysis of migration and selection. Genetics, 12: 845-855.

Sneath PHA, Sokal RO (1973). Numerical taxonomy. The principles and Practice of Numerical Classification. WH Freeman and Co., San Franciso.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990).

DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid. Res. 18: 6531-6535.
Wolfe AD, Xiang QY, Kephart SR (1998). Assessing hybridization in natural populations of Penstemon (***Svrophulariaceae***) using hypervariable inter-simple sequence repeat (ISSR) bands. Mol. Ecol. 7: 1107-1125.
Wright S (1965). The interpretation of population structure by statistics with special regard to systems of mating. Evolution, 19: 395-420.
Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.