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RESEARCH ARTICLE

Efficacy of RAPD, ISSR and DAMD markers in assessment of genetic variability and population structure of wild *Musa acuminata* colla

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Abstract North east India is considered as one of the major biodiversity hotspots worldwide and centre of origin of several plant species including *Musa*. *Musa acuminata* Colla is known to be one of the wild progenitors of cultivated bananas and plantains. Three single primer based DNA marker techniques viz., random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and directed amplification of minisatellites DNA (DAMD) were used for diversity diagnostics among 25 genotypes of wild *M. acuminata* collected from Meghalaya province of north east India. A total of 58 primers (26-RAPD, 21-ISSR, and11-DAMD) yielded 451 DNA fragments, of which 395 (87.58 %) were found to be polymorphic in nature. The polymorphic information content (PIC) values were almost identical for each marker system. The resolving power of the marker system was found to be highest in RAPD (3.96) whereas ISSR resolved highest marker index (16.39) in the study. Selected amplicon data obtained through single primer amplification reactions were utilized for determination of diversity within and among the populations of *M. acuminata*. Nei's genetic differentiation (Gst) value (0.451) indicated higher proportion of the genetic variation within the populations which is supported by the AMOVA analysis (88 %). The study provides insight into the efficacy of RAPD, ISSR and DAMD to analyse the genetic variation existing in the wild *Musa* germplasm, which can further be exploited for quality trait improvement and domestication of such important horticultural crops. The genetic diversity based population structure may shed light on the genetic basis of speciation and evolution of various species within the genus *Musa*.

Keywords *Musa acuminata* · SPAR · Genetic variability · Population structure

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Introduction

Bananas and plantains belong to the genus *Musa* L. and family Musaceae Juss., which are herbaceous, sterile and parthenocarpic. Banana is the fourth most important crop in the developing world; after rice, wheat and maize (Frison et al. 2004), with its production at a staggering 102 million tons annually. Its year round availability, affordability, varietal range, taste, nutritive and medicinal value makes it the favourite fruit amongst all classes of people. Banana is a rich source of carbohydrates and vitamins, particularly vitamins B; it is also a good source of sodium, potassium, calcium and magnesium (Dickinson 2000). The fruit is easy to digest, free from fat and cholesterol. The vast majority of the cultivated bananas are diploids ($2n=2\times=22$), triploids ($2n=3\times=33$) or tetraploids ($2n=2\times=44$) which have derived from inter- and intra-specific crosses involving two diploid ($2n=22$) wild species viz. *Musa acuminata* Colla and *M. balbisiana* Colla (Simmonds and Shepherd 1955).

Southeast Asia is the centre of bananas' origin (Simmonds 1962) but the cultivation has spread throughout the tropics and sub-tropics of the world. *M. acuminata* was introduced into the Indian sub-continent through human movement due to its

multifaceted importance. It also hybridized with the local *M. balbisiana*, a seedy and wild species endemic to north eastern India (Uma et al. 2006). Though north east India is considered to be a mega-biodiversity hotspot centre (Myers et al. 2000) and having rich resources of banana diversity, only few studies have been conducted on the wild *M. acuminata* of the region. The evaluation and conservation of genetic diversity for the progenitors of cultivated plants is imperative to guarantee sustainable development. Wild *M. acuminata* provide important genetic resources for banana breeding because it has numerous agriculturally advantageous characters, such as disease- resistance (Javed et al. 2004). *M. acuminata* has also contributed significantly to many breeding programmes across the globe for developing varieties resistant to sigatoka leaf spot disease (Uma et al. 2006).

The assessment of the genetic variability in wild plant species has pivotal role in the conservation of genetic resources, and molecular markers offer us to demonstrate the genetic diversity. In comparison to traditional methods, DNA fingerprinting revealed plant genetic variability at genetic levels with consistent data and authenticity, a prerequisite for the estimation of genetic diversity. With the advances in plant molecular biology, a number of molecular marker types such as amplified fragment length polymorphism (AFLP; Vos et al. 1995), random amplified polymorphic DNA (RAPD; Williams et al. 1990), inter-simple sequence repeats (ISSR; Meyer et al. 1993; Zietkiewicz et al. 1994) and directed amplification of minisatellite DNA regions (DAMD; Heath et al. 1993) have been developed and effectively utilized in genetic diversity analysis. Different DNA markers were used to study the genetic diversity of *Musa* including isozyme polymorphisms (Bhat et al. 1992). The commonly used polymerase chain reaction (PCR)-based DNA marker for *Musa* variability studies are restriction fragment length polymorphism (RFLP; Jarret et al. 1992), RAPD (Jarret et al. 1993; Onguso et al. 2004), microsatellites (Lagoda et al. 1998; Creste et al. 2003, 2004), and AFLP (Wong et al. 2002). Lately, the single primer amplification reaction (SPAR) method i.e., the combination of RAPD, ISSR and DAMD markers is becoming an important tool for genetic diversity analysis in plants and they collectively provide a comprehensive description of the nature and the extent of diversity, its genetic relationship and germplasm management (Bhattacharya et al. 2005; Kumar et al. 2011, 2013; Ranade et al. 2009; Sharma et al. 2010, 2011). This could be of the fact that RAPD amplicons are generated from amplifications representing widely distributed regions of the genome, whereas ISSR and DAMD amplicons are generated from the microsatellite-rich portions of the genome (Kumar et al. 2014). Thus, the present study aims to compare the efficacies of these three SPAR methods and determine the natural genetic variation in some populations of *M. acuminata* from Meghalaya province of north east India.

Materials and methods

Study site and sample collection

Several exploration trips were under taken throughout the state of Meghalaya. Collectively, 25 individual samples from seven populations inhabiting different districts of Meghalaya viz. East Khasi Hills, West Khasi Hills, Jaintia Hills, Ri Bhoi, East Garo Hills, West Garo Hills and South Garo Hills district (Table 1; Supplementary Fig. S1 included) were collected. Identification of the wild *M. acuminata* specimens was done following the descriptor based on the morphological characters provided by International Network for the Improvement of Banana and Plantain (INIBAP 1996) and *Musa*logue-INIBAP (Daniells et al. 2001). Specimen vouchers were deposited with the herbarium curator in the Department of Botany, North Eastern Hill University, Shillong (India) and voucher numbers were obtained.

DNA isolation

Total genomic DNA was isolated from young cigar leaves of all the collected samples according to the method described by Gawel and Jarret (1991) with few modifications. The purified total DNA was quantified by gel electrophoresis and verified by UV-spectrophotometry.

PCR optimization and primer selection

Three RAPD kits (OPC, OPH and OPX) comprising of 20 decamer random primers per kit were procured form Operon Technologies, Alameda, CA, USA. A total of 40 ISSR primers and 20 DAMD primers synthesized from Metabion Inc. Ltd., Germany were used. Initially, optimum PCR conditions for RAPD, ISSR and DAMD were standardized with different quantities of template DNA (20, 30, 40, 50 and 60 ng), dNTPs (0.1, 0.2 and 0.3 mM) and $MgCl_2$ (0–5 mM) to assay for their ability to amplify. Amplification with each arbitrary primer was repeated three times and those primers that produced reproducible and consistent bands were only selected for data generation.

RAPD, ISSR and DAMD amplification reactions

Following the results of initial primer screening, 26 RAPD decamer primers, 21 ISSR and 11 DAMD primers were selected for final amplification (Table 2). After final standardization, RAPD amplifications were performed routinely using PCR mixture (25 μl) which contained 50 ng of genomic DNA as template, 1 X PCR buffer, 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$, 1 unit (U) of *Taq* DNA polymerase (Merck) and 30 μM of each primer. PCR was performed using a thermal cycler (Esco, USA) at initial denaturation of 94 °C for 5 min

Table 1 Place of collections of *M. acuminata* from the seven districts of Meghalaya

Pop. ID.	District of collection	Collection ID	Latitude	Longitude	Altitude (feet)
Pop1	East Khasi Hills (EKH)	MA1	N25°31'54.5"	E91°49'11.6"	5821
		MA10	N25°15'29.7"	E91°31'47.9"	3601
		MA11	N25°15'29.3"	E91°31'55.5"	3579
		MA12	N25°14'24.2"	E91°26'08.2"	1784
Pop2	West Khasi Hills (WKH)	MA13	N25°13'25.5"	E91°14'23.2"	236
		MA14	N25°12'55.4"	E91°15'24.7"	633
		MA15	N25°14'00.2"	E91°13'41.2"	453
		MA22	N25°40'49.3"	E91°38'05.2"	4318
Pop3	Jaintia Hills (JH)	MA6	N25°20'49.8"	E92°22'50.5"	3669
		MA8	N25°14'43.2"	E91°58'26.2"	1662
		MA9	N25°13'28.0"	E91°58'57.8"	2037
		MA19	N25°30'01.9"	E92°23'39.5"	4306
		MA20	N25°31'44.3"	E92°32'34.6"	3045
		MA21	N25°32'11.6"	E92°37'09.9"	2512
Pop4	Ri Bhoi (RB)	MA2	N25°41'47.7"	E91°54'05.7"	3017
		MA3	N25°42'41.7"	E91°59'49.4"	2914
		MA4	N25°42'40.3"	E92° 01'37.0"	2888
		MA5	N25°42'37.1"	E92° 01'21.4"	2909
		MA7	N25°54'30.0"	E91°52'29.4"	1912
Pop5	East Garo Hills (EGH)	MA23	N25°53'43.0"	E90°36'10.6"	374
		MA24	N25°54'30.4"	E90°36'26.0"	214
Pop6	West Garo Hills (WGH)	MA17	N25°30'55.8"	E90°12'21.3"	973
		MA18	N25°31'09.3"	E90°12'04.9"	802
Pop7	South Garo Hills (SGH)	MA16	N25°11'35.9"	E90°38'07.1"	330
		MA25	N25°11'42.3"	E90°38'04.2"	287

followed by 35 cycles at 94 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min with a final extension at 72 °C for 7 min.

In case of ISSR and DAMD primers, PCR mixture (25 µl) contained 50 ng of template DNA, 1 X PCR buffer, 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Merck), 30 and 15 µM of each primer for ISSR and DAMD, respectively. Reactions were set for PCR cycle at 94 °C for 5 min followed by 35 cycles at 94 °C for 0.40 min, 1 min of specific annealing temperature, and 72 °C for 1 min and 30 s, with a final extension at 72 °C for 7 min in a thermal cycler (Esco, USA).

The PCR products obtained were separated on 2 % agarose gel, stained with ethidium bromide and visualized using a gel documentation system BIO-RAD (Biorad, USA). The size of the amplification products was estimated from 100 bp DNA ladder (NEB).

Data analysis

Reproducible, well resolved and non-ambiguous amplicons were scored manually as presence (1) and absence (0) across all samples and a binary data matrix was constructed which is used for all analysis. Faint bands that could not be scored unambiguously were not considered for analysis. Molecular sizes of the amplicons were estimated by using 100 bp DNA ladder (NEB). Discriminatory power of each of the three markers (RAPD, ISSR and DAMD) was evaluated by means of three parameters, viz. Polymorphic information content (PIC), the probability in detecting polymorphism by a primer or primer combination between two randomly drawn genotypes was calculated using the formula $PIC=1-\Sigma pi^2$, where *pi* is the frequency of the *i*th allele (Sehgal et al. 2009); Resolving power (Rp) which is the ability of each primer to detect level of variation between individuals was calculated according to Prevost and Wilkinson (1999): $Rp=\Sigma Ib$ where Ib (band informativeness) takes the values of: $1–[2|0.5–p|]$, where *p* is the proportion of individuals containing the band, and marker index (MI) for each primer was also calculated as a product of two functions - the polymorphic information content and effective multiplex ratio (EMR) (Varshney et al. 2007) i.e., $MI=PIC\times EMR$.

To estimate population genetic structure, genetic diversity parameters including the percentage of polymorphic loci (Lynch and Milligan 1994), observed number of alleles, effective number of alleles (Kimura and Crow 1964), Nei's gene diversity (Nei 1973) and Shannon index (Lewontin 1972)

Table 2 RAPD, ISSR, and DAMD primers used for amplification. TB, total band; PB, polymorphic band; MB, monomorphic band; PPB, percentage polymorphic band; PIC, polymorphic information content; RP, resolving power; MI, marker index. In case of ISSR primers: R = A/G; Y = C/T

Sl. No.	Primer	Primer sequences (5'-3')	TB	PB	MB	PPB	PIC	Rp	MI
1	OPC-1	TTC GAG CCA G	7	7	0	100.00	0.41	4.96	14.00
2	OPC-2	GTG AGG CGT C	12	12	0	100.00	0.42	8.88	
3	OPC-4	CCG CAT CTA C	8	8	0	100.00	0.45	3.28	
4	OPC-7	GTC CCG ACG A	7	6	1	85.71	0.39	3.92	
5	OPH-1	GGT CGG AGA A	12	10	2	83.33	0.40	6.00	
6	OPH-3	AGA CGT CCA C	11	10	1	90.90	0.39	5.52	
7	OPH-4	GGA AGT CGC C	6	6	0	100.00	0.43	3.60	
8	OPH-5	AGT CGT CCC C	11	10	1	90.90	0.37	5.04	
9	OPH-6	ACG CAT CGC A	9	4	5	44.44	0.19	2.40	
10	OPH-8	GAA ACA CCC C	8	8	0	100.00	0.43	4.64	
11	OPH-12	ACG CGC ATG T	9	7	2	77.77	0.34	5.28	
12	OPH-13	GAC GCC ACA C	8	7	1	87.50	0.36	4.32	
13	OPH-17	CAC TCT CCT C	6	4	2	66.66	0.31	3.04	
14	OPH-18	GAA TCG GCC A	8	5	3	62.50	0.26	2.80	
15	OPH-19	CTG ACC AGC C	6	4	2	66.66	0.29	1.68	
16	OPH-20	GGG AGA CAT C	9	6	3	66.66	0.28	2.64	
17	OPX-3	TGG CGC AGT G	9	7	2	77.77	0.32	5.20	
18	OPX-4	CCG CTA CCG A	6	5	1	83.33	0.38	2.88	
19	OPX-5	CCT TTC CCT C	7	7	0	100.00	0.43	2.32	
20	OPX-6	ACG CCA GAG G	6	6	0	100.00	0.40	1.92	
21	OPX-7	GAG CGA GGC T	7	6	1	85.71	0.34	3.04	
22	OPX-8	CAG GGG TGG A	5	4	1	80.00	0.21	2.40	
23	OPX-11	GGA GCC TCA G	10	9	1	90.00	0.38	4.88	
24	OPX-15	CAG ACA AGC C	11	8	3	72.72	0.31	4.48	
25	OPX-19	TGG CAA GGC A	5	5	0	100.00	0.43	2.80	
26	OPX-20	CCC AGC TAG A	9	9	0	100.00	0.46	4.96	
Total			212	180	32				
Average/primer			8.15	6.92	1.23	85.09	0.36	3.96	
ISSR									
27	I-807	AGA GAG AGA GAG AGA GT	7	6	1	85.71	0.30	3.52	16.39
28	I-810	GAG AGA GAG AGA GAG AT	6	5	1	83.33	0.25	2.00	
29	I-813	CTC TCT CTC TCT CTC TT	6	4	2	66.66	0.25	2.16	
30	I-814	CTC TCT CTC TCT CTC TA	5	4	1	80.00	0.37	1.52	
31	I-815	CTC TCT CTC TCT CTC TG	8	6	2	75.00	0.34	3.04	
32	I-818	CAC ACA CAC ACA CAC AG	8	7	1	87.50	0.44	4.96	
33	I-825	ACA CAC ACA CAC ACA CT	6	4	2	66.66	0.22	1.76	
34	I-826	ACA CAC ACA CAC ACA CC	5	5	0	100.00	0.45	2.16	
35	I-827	ACA CAC ACA CAC ACA CG	8	6	2	75.00	0.34	2.40	
36	I-834	AGA GAG AGA GAG AGA GYT	7	7	0	100.00	0.33	2.00	
37	I-835	AGA GAG AGA GAG AGA GYC	9	9	0	100.00	0.41	4.24	
38	I-840	GAG AGA GAG AGA GAG AYT	6	6	0	100.00	0.39	3.52	
39	I-843	CTC TCT CTC TCT CTC TRA	8	8	0	100.00	0.35	5.68	
40	I-844	CTC TCT CTC TCT CTC TRC	7	6	1	85.71	0.32	3.68	
41	I-847	CAC ACA CAC ACA CAC ARC	7	6	1	85.71	0.32	2.32	
42	I-848	CAC ACA CAC ACA CAC ARG	7	7	0	100.00	0.34	4.00	
43	I-854	TCT CTC TCT CTC TCT CRG	5	5	0	100.00	0.36	3.52	
44	I-855	ACA CAC ACA CAC ACA CYT	6	6	0	100.00	0.38	4.00	
45	I-857	ACA CAC ACA CAC ACA CYG	6	6	0	100.00	0.40	2.56	

Table 2 (continued)

Sl. No.	Primer	Primer sequences (5'-3')	TB	PB	MB	PPB	PIC	Rp	MI
46	I-868	GAA GAA GAA GAA GAA GAA	6	6	0	100.00	0.38	3.12	
47	I-873	GAC AGA CAG ACA GAC A	6	6	0	100.00	0.40	3.92	
Total			139	125	14				
Average/Primer			6.62	5.95	0.66	90.06	0.35	3.15	
DAMD									
48	D-6	ATG TGT GCG ATC AGT TGC TG	11	11	0	100.00	0.42	4.48	12.08
49	D-7	TAC ATC GCA AGT GAC ACA GG	8	8	0	100.00	0.35	5.28	
50	D-8	AAT GTG GGC AAG CTG GTG GT	10	9	1	90.00	0.32	6.00	
51	D-10	GGA CAA GAA GAG GAT GTG GA	6	4	2	66.66	0.31	2.24	
52	D-12	AAG AGG CAT TCT ACC ACC AC	10	9	1	90.00	0.41	4.64	
53	D-13	GGT GTA GAG AGG GGT	12	11	1	91.66	0.37	5.36	
54	D-14	CCT CCT CCC TCC T	6	5	1	83.33	0.33	2.88	
55	D-16	GAG GGT GGC GGT TCT	7	5	2	71.43	0.29	3.84	
56	D-17	CTC TGG GTG TCG TGC	9	9	0	100.00	0.39	4.24	
57	D-18	ACA GGG GTG TGG GG	11	11	0	100.00	0.40	5.52	
58	D-19	CCC GTG GGG CCG CCG	10	8	2	80.00	0.30	2.32	
Total			100	90	10				
Average/Primer			9.09	8.18	0.91	88.46	0.35	4.25	
RAPD, ISSR, DAMD (SPAR) Total			451	395	56	87.58	0.35	3.79	14.16

were calculated using the software PopGene version 1.31 (Yeh et al. 1999). Estimate of gene flow (Nm) was calculated as $Nm=0.5\times(1-Gst)/Gst$ (McDermott and McDonald 1993), where Gst is the gene differentiation. Analysis of molecular variance (AMOVA) input files were created using AMOVA-PREP version 1.01 (Miller 1998) and the analyses were made using WinAMOVA version 1.5 (Excoffier et al. 1992). A dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) with the sequential, agglomerative, hierarchical, and nested (SAHN) module for analysis of genetic relationships for all the collections (Sneath and Sokal 1973).

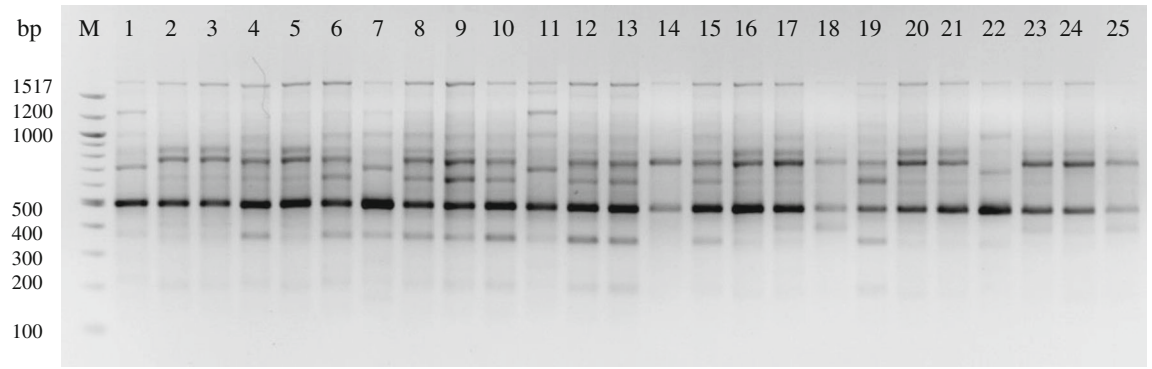
Results

RAPD analysis

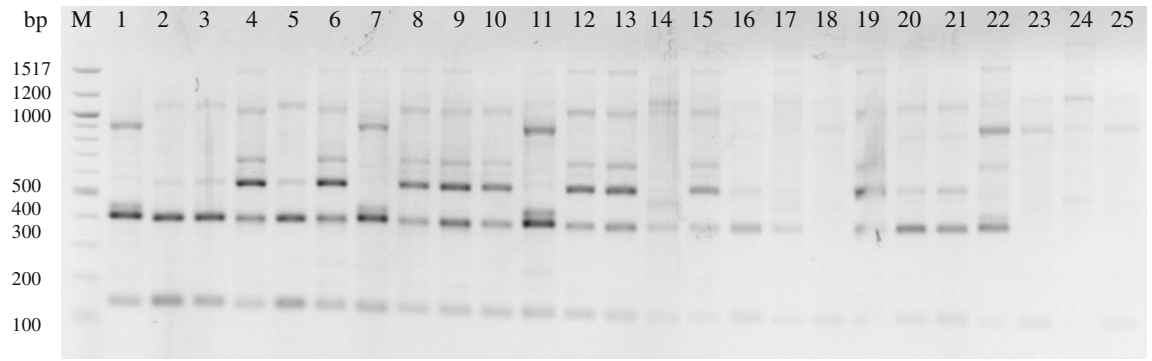
A total 60 RAPD primers were screened of which 26 primers showed reliable banding patterns with high reproducibility and clear band resolution. These 26 primers produced a total of 212 distinct amplification products ranging in size from 0.2 to 1.5 kb (Fig. 1; Supplementary Fig. S2 included). The number of products generated per primer by these RAPD primers was found to range from 5 to 12 amplicons with primer OPC-2 being the highest at 12 and that of OPX-8 and OPX-19 at a minimum of 5 amplicon. Out of the 212 products produced, 180 (84.91 %) were found to be polymorphic and only 32 (15.09 %) were monomorphic in nature (Table 2). The percentage of polymorphism ranged from 44.44 % for OPH-6 to 100 % for OPC-1, OPC-2, OPC-4, OPH-4, OPH-8, OPX-5, OPX-6, OPX-19 and OPX-20 with an average of 85.09 % polymorphism per primer. The PIC values ranged from a high of 0.46 (OPX-20) to a low of 0.19 (OPH-6) with an average of 0.36, indicating hypervariability among the individuals studied. The estimates of Rp were found to be highest for primer OPC-2 (8.88) and the lowest with the primer OPH-19 (1.68) with an average Rp of 3.96 per primer. To determine the overall usefulness of a given marker system, the marker index (MI) was calculated for RAPD and a value of 14.00 was determined.

ISSR analysis

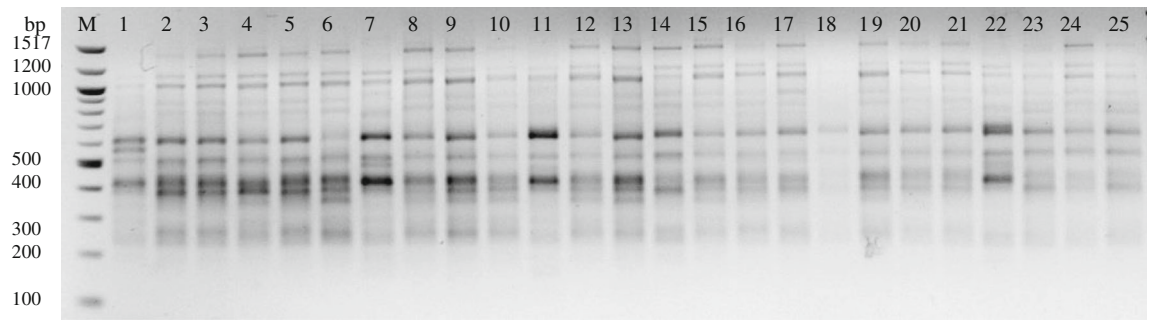
The 25 samples of *M. acuminata* representing seven populations were amplified with the 21 selected primers (Table 2). The number of products generated per primer was found to range from 5 to 9 of different sizes in the range of 0.2 to 1.5 kb (Fig. 1; Supplementary Fig. S2 included). The primer UBC-835 exhibited the maximum (9) product whereas primers UBC-814, UBC-826 and UBC-854 gave the least (5) number of products. A total of 139 amplified products were produced with an average of 6.62 products per primer, of which 125 (89.93 %) were polymorphic in nature and 14 (10.07 %) products were monomorphic (Table 2). The percentage of polymorphic bands ranged from 66.66 % for primers UBC-813



(a) RAPD profile with primer OPH-18 of the 25 individuals of wild *M. acuminata*.



(b) ISSR profile with primer I-815 of the 25 individuals of wild *M. acuminata.*



(c) DAMD profile with primer D-19 of the 25 individuals of wild *M. acuminata.*

Fig. 1 Banding profiles obtained with **a** RAPD primer **b** ISSR primer and **c** DAMD primer with all 25 individuals of *M. acuminata*. Lane M – 100 bp marker (NEB). Lanes 1–25 are different individuals of wild *M. acuminata* in terms of collection ID given in Table 1

and UBC-825 to 100 % for primers UBC-834, UBC-835, UBC-840, UBC-843, UBC-848, UBC-854, UBC-855, UBC-857, UBC-868 and UBC-873 with an average polymorphism of 90.06 % per primer. These ISSR primers gave a high PIC value of 0.44 for primer UBC-818 and low PIC value of 0.22 for primer UBC-825, with an average PIC value of 0.35 per primer. An average Rp of 3.15 per primer was obtained with the highest Rp value of 5.68 being that of primer UBC-843 and the lowest value of 1.52 for primer UBC-814 (Table 2). The average of the products of PIC and EMR gave a value of 16.39 as the marker index.

DAMD analysis

Out of 19 DAMD primers tested, 11 primers of 12–20 bp produced reproducible, well resolved and non-ambiguous amplicons (Table 2). The number of amplified products generated by these DAMD primers is in the range of 6–12 bands with primer D-13 being the highest (12) and primer D-10 and D-14 giving the minimum (6) number of amplicons. The total number of loci amplified was 100 in different sizes ranging from 0.2–1.5 kb (Fig. 1; Supplementary Fig. S2 included). Out of these 100 amplicons, 90 (90 %) products are polymorphic and

10 (10 %) products are monomorphic. The percentage of polymorphic ranged from 66.66 % for primer D-10 to 100 % for that of primer D-6, D-7, D-17 and D-18 with an average of 88.46 % polymorphism per primer. The PIC values ranged from 0.29 to 0.42 with the highest being for primer D-6 and the lowest for primer D-16 at an average PIC value of 0.35 were obtained (Table 2). While the highest Rp value was recorded at 6.00 (D-8), the lowest was at 2.24 (D-10) with an average of 4.25 per primer. Marker index (MI), the overall usefulness of a given marker system was calculated and a value of 12.08 was determined.

SPAR analysis

The molecular markers used in the present study have revealed polymorphism independent of each other at various levels. A combined analysis was also performed in order to judge the efficacy of the best marker, either individually or in combination, for diversity studies in *M. acuminata*. In this present study, a total of 58 primers were used and this yielded 451 fragments, of which 395 (87.58 %) were polymorphic in nature. The mean values of PIC, Rp and MI revealed from the combination of these three markers were 0.35, 3.79 and 14.16, respectively (Table 2).

The percentage of polymorphic loci when pooled together from among the individuals of all the seven districts varied in the range of 18.18 % (EGH) to 59.87 % (WKH) at the population level, while at the species level it exhibited a average value of 89.36 %. The samples of WKH region have the highest (1.598±0.490) observed number of alleles (Na) and that of EGH (1.181±0.386) having the lowest, at an average of 1.893±0.308 (Table 3). The effective number of alleles (Ne) was invariably less than Na values for each population showing a variation in the range of 1.128 (EGH) to 1.463 (WKH) with an average of 1.589±0.331. The Nei's gene diversity (H) and Shannon index (I) among all the populations were highest in populations collected from WKH (H=0.252± 0.220; I=0.364±0.310) and the lowest was recorded for EGH (Table 3). Therefore, among all these seven populations used in the study, the collections from WKH exhibited the greatest level of variability. Mean coefficient of gene differentiation (Gst) value 0.451 indicated a high level of population differentiation while estimate of gene flow (Nm) in the population was found as 0.607 (Table 3). AMOVA analysis of pairwise distances indicated high variation (88.12 %) within populations and 11.88 % variance occurred among populations (Table 4).

A dendrogram was constructed using SAHN clustering through UPGMA from the three marker data (RAPD, ISSR and DAMD). The dendrogram separated the seven populations into two distinct clusters, one with a single population and the other with six populations. Cluster I is a representation of the samples collected from EGH. Cluster II again subclusters into cluster IIa, comprising collections from the hilly terrain of Khasi, Jaintia and Ri Bhoi districts; whereas cluster IIb consists of samples from the somewhat plain or sloppy hills of Garo hills (Fig. 2).

Discussion

There are several powerful DNA-based molecular marker approaches currently available for genetic analysis of plant genomes. The choice of the most appropriate strategy for any specific study is open to debate and it mostly depends on the purpose and nature of genetic structure of the species. Therefore, comparisons are needed, in addition to resources available to the scientist, to decide which approach is most appropriate for the taxon being examined (Scariot et al. 2007). This is the first study to collectively use RAPD, ISSR and DAMD molecular markers both individually and in combination to examine the extent of genetic variability among natural populations of *M. acuminata* from north east India.

A comparison of the level of polymorphism and discriminating efficacy of RAPD, ISSR and DAMD showed that each of the three marker techniques is capable of detecting genetic

Table 3 Genetic variations as revealed through combined RAPD + ISSR + DAMD markers among seven populations of *M. acuminata*. N, sample size; Na, Observed No. of alleles; Ne, Effective No. of alleles; H, Nei's genetic diversity; I, Shannon's information index; n, Number of polymorphic loci; P%, Percentage of polymorphic loci; Gst, Diversity among populations; Nm, Gene flow; SD, Standard deviation

Population	*N*	Na (SD)	Ne (SD)	H (SD)	I (SD)	*n*	P%	Gst)	Nm
Pop1 (EKH)	4	1.545 (±0.498)	1.397 (±0.411)	0.221 (±0.215)	0.322 (±0.306)	246	54.55		
Pop2 (WKH)	4	1.598 (±0.490)	1.463 (±0.429)	0.252 (±0.220)	0.364 (±0.310)	270	59.87		
Pop3 (JH)	6	1.587 (±0.492)	1.434 (±0.421)	0.239 (±0.218)	0.347 (±0.307)	265	58.76		
Pop4 (RB)	5	1.572 (±0.495)	1.395 (±0.407)	0.222 (±0.211)	0.326 (±0.300)	258	57.21		
Pop5 (EGH)	2	1.181 (±0.386)	1.128 (±0.273)	0.075 (±0.159)	0.109 (±0.233)	82	18.18		
Pop6 (WGH)	2	1.317 (±0.465)	1.224 (±0.329)	0.131 (±0.193)	0.191 (±0.281)	143	31.71		
Pop7 (SGH)	2	1.399 (±0.490)	1.282 (±0.346)	0.165 (±0.203)	0.241 (±0.296)	180	39.91		
Total population	25	1.893 (±0.308)	1.589 (±0.331)	0.338 (±0.160)	0.499 (±0.215)	403	89.36	0.451	0.607

Table 4 Analysis of molecular variance (AMOVA) for seven populations of *M. acuminata*

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation
Among populations	6	583.286	8.929	11.88
Within populations	18	1192.633	66.257	88.12

variation in the populations of *M. acuminata*. All the three marker approaches used were found to show high level of polymorphism. It may be observed here that ISSRs have generated highest (90.06 %) number of polymorphic bands per primer, on an average. Comparatively the range of Rp values obtained in all these markers was similar confirming their genotype discriminating power (Prevost and Wilkinson 1999; Smith et al. 1997). However, for a marker to be considered the best for a species, including *M. acuminata*, the concern complete genome should have been probed, with fine focus on repetitive sequences. Therefore, a combination of RAPDs (spanning the entire genome of the DNA) and ISSRs/DAMDs (spanning selected repetitive sequences) can be considered as best markers for more meaningful and reliable analysis of genetic variability.

The population diversity indices like allelic frequency (Na and Ne), gene flow (Nm) and Nei's genetic differentiation (Gst) defines the genome composition of a population. In present analysis, the range of the values for observed (Na) and effective number of alleles (Ne) of pooled data for the three markers (RAPD, ISSR and DAMD) (Table 3) was always lesser than the corresponding values reported in earlier works (Mukunthakumar et al. 2013; Padmesh et al. 2012). This may be attributed to the differences in the marker techniques used in the present study and also due to the inherent genotypic differences. Values of Nei's genetic differentiation among populations (Gst)

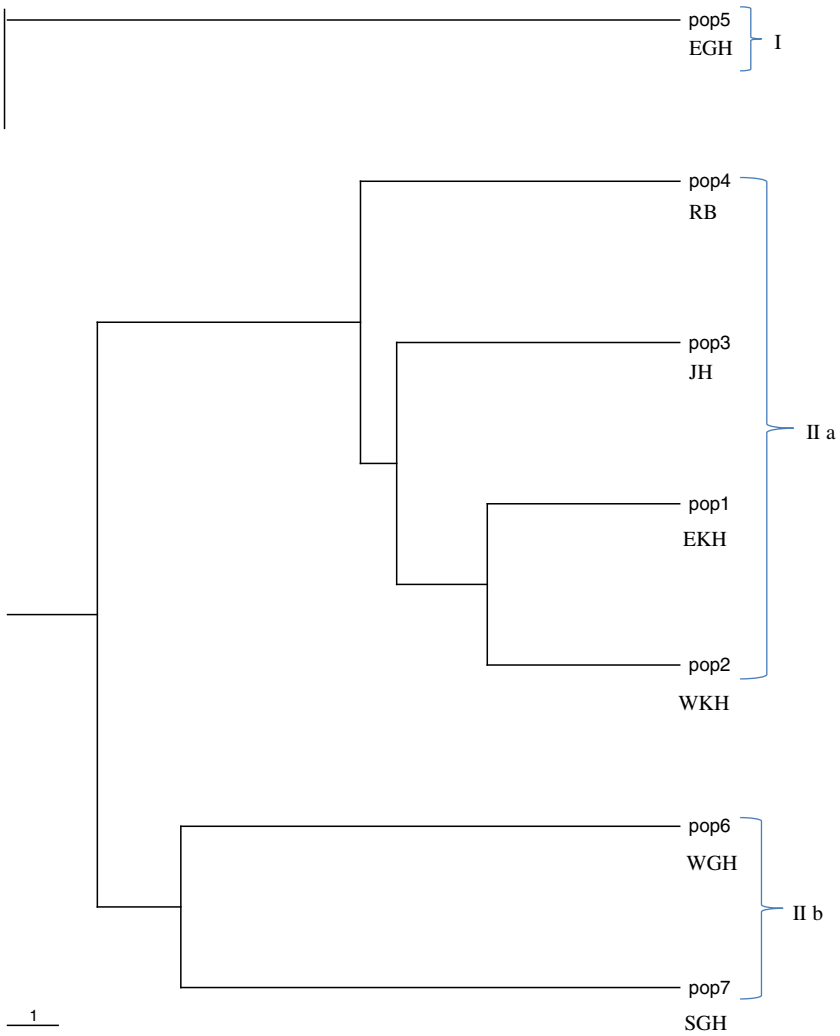


Fig. 2 UPGMA clustering of *M. acuminata* populations from the seven districts

range from zero to one, with high values denoting a large amount of variation among various populations. In the present study, the Gst value has been recorded as 0.451 for all the three markers which indicates that maximum of the variation is within the populations whereas minimum among populations. Such differentiation was lower than those reported for wild *M. balbisiana* using cpDNA PCR-RFLP with Gst value of 0.77 (Ge et al. 2005); but slightly higher than those of *M. acuminata* (Gst=0.39) which used RAPD markers (Mukunthakumar et al. 2013) for wild banana populations from western Ghats of India. This was also supported by the AMOVA analysis which showed that a higher proportion of the genetic variations reside within the populations (88.12 %), while very less degree of genetic variation from differentiation among populations (11.88 %). These corroborate with our present study as most of the out-breeding species usually have higher levels of genetic diversity within populations and low diversity among populations (Hamrick and Godt 1996; Hogbin and Peakall 1999; Zawko et al. 2001). The estimate of gene flow (Nm) has been classified as low (Nm<1), moderate (Nm>1) and extensive (Nm>4) in nature as per the scheme followed by Kumar *et al*. (2014). The value of the estimate of Nm from the pooled data in the present study is 0.607, indicating a low profile. This low level of gene flow (Nm<1) can occur either of habitat fragmentation (natural or human disturbance) which leads to population isolation, and/or lack of suitable animal pollinator for long distance seed/pollen dispersal (because of the terrain characteristic of the region posing as a barrier to normal transfer of pollens). Any or all of these must have delimited the rate of gene flow in this wild *Musa* leading to genetic differentiation among population (Padmesh et al. 2012). Studies also show that genetic variations occurred along elevation gradients because topographical heterogeneity of plants habitat causes substantial changes in the environment (Byars et al. 2009; Kumar et al. 2014; Ohsawa and Ide 2008). It is also reported that at different altitudes strong isolation of populations may occur due to drastic differences in phenology between higher and lower altitudes and mountain barriers which restricts the gene flow between the populations causing complex and varied genetic variations (Arnaud-Haond et al. 2006; Kumar et al. 2014; Liu et al. 2012). This is also supported by the present study where populations of the plain or sloppy belts of Garo hills exhibit low levels of genetic diversity and are distinct from the populations of the hilly terrain of Khasi, Jaintia and Ri Bhoi districts of Meghalaya, where diversity is higher. Such observations are also supported by the clustering pattern of these genotypes as seen in the dendrogram.

Conclusion

This study thus shows the efficacy of RAPD, ISSR and DAMD (SPAR) markers for the analysis of substantial genetic variability and the genetic relationship within *M. acuminata*. The result also signifies that SPAR methods can be used to establish the inter-relationships among different populations, and at times to discriminate even individuals within the populations. Collection and preservation of such genetically diverse germplasm of north eastern India would be of great significance in designing and developing appropriate breeding programmes aim at improvement of the species.

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