Genome Classification of Banana Cultivars Found in Manipur, India using IRAP and RAPD

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ABSTRACT
The genome groups of 34 wild and cultivated banana samples collected from 9 districts of Manipur, India were analyzed using IRAP and RAPD. IRAP-PCR analysis of the banana genome revealed that 32 out of 34 banana samples have shown presence of multiple polymorphic bands in amplified products. There were 5 genome specific bands (2 for B genome and 3 for A genome) in all the samples analyzed by using IRAP marker. Analysis of A and B genome among plant samples using RAPD-PCR generated a total of 1425 polymorphic bands by six primers (OPF-6, OPX-6, OPK-12, RAPD-6, RAPD-14 and RAPD-17). The highest number of bands was generated by OPK12 (318) followed by RAPD6 (308 bands), OPX6 (263 bands), RAPD 17 (215 bands), RAPD 14 (165 bands) and OPF6 (156 bands) respectively. Among these primers, RAPD 14 displayed specific bands per sample thereby showing higher delineating power than other primers.

Key words: IRAP, Musa acuminata, Musa balbisiana, RAPD.

INTRODUCTION
North East India is rich in genetic diversity of banana germplasm. Bidyut et al. (2012) reported that maximum genetic variability of Musa acuminata and M. balbisiana occurs in NE India and Musa flaviflora is localized to Manipur and Meghalaya only. Among NE India, Assam is the highest producer of banana. The region in north-eastern India (Assam) lies at a point where Musa balbisiana, from the Indian subcontinent, meets Musa acuminata from South East Asia (INIBAP, 2000). These two species and other wild relatives have mingled naturally to form a distinctive level of genetic diversity. The hilly terrain was clothed by different types of forest; most of the wild banana germplasm occurs in semi-evergreen and subtropical forest (INIBAP, 2000). A total of 28 accessions of wild species and cultivated varieties of banana from the north eastern states of India were reported for the first time by a group of scientists from National Research Centre for Bananas (NRCB) which comprised of M. acuminata and its clones and M. balbisiana and its clones along with 5 members of ornamental species (Uma et al. 2001). In Manipur, Tamenglong District has produced highest amount other than the remaining Districts (Annonymous, 2010). Banana is also one of the important food crop plant in Manipur and it is used in different ways. Banana plant (Pseudostem) and male bud is used as vegetable food for making curry and leaves are used as plate as well as container for holistic items. In Manipur, most of banana varieties are of wild type and seeded in nature. Assam in north eastern region (NER) ranked ninth with 857000 tonnes among top 10 banana producers of the country while Manipur produced only 90450 tonnes was listed at the bottom along with other NER states. One of the primary reasons for lower production capacity of banana in the region could be small area of plantation and dependence of local communities on local varieties of banana with lower yields. Therefore, the present study was undertaken to explore and analyze wild and cultivated bananas of Manipur. This can help to identify and suggest best edible cultivars and potential banana groups suitable for commercial production for local consumption and export prospects in the near future.

MATERIALS AND METHODS
An extensive field survey and sample collections were conducted to study phenotypic and genotypic characterization of wild and cultivated banana cultivars covering all the 9 districts of Manipur. Field data of key characters useful in identification of Musa species were collected using the standard format of descriptor for banana. Young cigar leaves were collected, cut into small size and immediately stored in the CTAB storage buffer.

DNA of the samples was extracted using CTAB method. The genome types of cultivated and wild bananas were investigated using the molecular markers such as IRAP-PCR (Nair et al. 2005) and RAPD-PCR (Devi et al. 2009) on the isolated genomic DNA as well as PCR-amplified DNA samples.
After gel electrophoresis the DNA samples were amplified by PCR using IRAP primer pair 5’-AGGGTTCGAAGTATAG GTTCCG-3’ and 5’-AATGTTTAAGTAGGGCAAGGAGG-3’ obtained from SIGMA-ALDRICH CHEMICALS Pvt. Ltd. 50 µl of reaction mixture contain DNA (100 ng/µl), 5.0 µl of 10 x Taq buffer, 1.0 µl of 10 mM dNTPs, 0.5µl of 100 pmol/µl single primer, 0.5 µl of 5 U/µl Taq DNA polymerase and added ddH2O to bring volume upto 50µl. Amplification was performed using Eppendorf Mastercyler (Eppendorf, Germany). Initial denaturation was done at 94º C for 2 minute, Denaturation 94ºC 30 seconds, Primer annealing 51ºC 30 seconds, Ramp 0.5ºC per second to 72ºC, Primer extensions 72ºC 2 minutes, Cycling repeat steps 2-5 for 29 cycles, final extension 72ºC 8 minutes. 3 µl of amplified product added 5 x loading buffer containing dye loaded into on 2% agarose gel. Stained gel with ethidium bromide, visualized and photographed the bands under UV light.

Amplification of genomic DNA using RAPD - PCR

The PCR amplification of genomic DNA by RAPD primer was done in Eppendorf Mastercyler (Eppendorf, Germany). The RAPD primers OPX-6 (5’ACGCCAGAGG 3’), OPF-6 (5’GGGAATTCGG 3’), OPK-12 (5’TGGCCCTCAC 3’), RAPD-6 (5’GAACAGCGG 3’), RAPD-14 (5’GCCGTCTACG 3’) and RAPD-17 (5’GGCATCGGCC 3’)obtained from SIGMA-ALDRICH CHEMICALS Pvt. Ltd. were used for the genomic DNA amplification. Each 25µl reaction volume contain about 50ng of template DNA, 2.5µl of 10X PCR buffer (15mM MgCl2, 0.5M KCl, 100mM Tris-Cl), 1µl of 10mM dNTP Mix, 1µl of 0.6µM single primers,1µl of 1U/µl taq DNA polymerase. The thermocycler was programme for an initial denaturation step of 4 min at 94ºC, followed by 35 cycles of 1 min denaturation at 94ºC, 50 sec annealing at 36ºC, 1 min and 30 sec extension at 72ºC, final extension of 5 min at 72ºC and a hold temperature of 4ºC at the end for RAPD primers OPX, OPF, RAPD-6, 14 and 17. 10ul PCR products mixed with 3ul dye were electrophoresed on 1% agarose gel in 100 ml 0.5X TBE buffer stained with 5 µl ethidium bromide at 70V for 2 hours. Gel with amplification fragment were visualized and photographed under UV light.

RESULTS AND DISCUSSION

Documentation of cultivated and wild bananas in Manipur

A total of 34 banana (Musa) samples were collected from 9 districts of Manipur. Twenty six (26) samples were recorded as cultivated varieties while 8 samples including a member

| Accession No. | Site         | Vernacular/Local Name | Cultivar  | Total of 34 |
|---------------|--------------|------------------------|-----------|-------------|
| MN002         | Bishnupur    | ChangbiLangou          | Wild      |             |
| MN010         | Bishnupur    | Heiren                 | Cultivated|             |
| MN021         | Bishnupur    | ChangbiLarong          | Cultivated| 5           |
| MN030         | Bishnupur    | Laphulamang            | Wild      |             |
| MN022         | Bishnupur    | Laphulempra            | Wild      |             |
| MN013         | Chandel      | Mapalheijao            | Cultivated|             |
| MN025         | Chandel      | ChangbiLukhoibi        | Wild      |             |
| MN029         | Chandel      | Chandel-2              | Wild      |             |
| MN038         | Chandel      | Chandel-1              | Wild      |             |
| MN031         | Churchandpur | Khuga Dam laphoi       | Wild      |             |
| MN032         | Churchandpur | Lachang pong           | Cultivated|             |
| MN033         | Churchandpur | Lachang Nat            | Cultivated| 3           |
| MN006         | Imphal East  | Hei Kola               | Cultivated|             |
| MN009         | Imphal East  | Champa cola            | Cultivated|             |
| MN005         | Imphal East  | Hangou                 | Cultivated| 5           |
| MN014         | Imphal East  | Maringheii             | Cultivated|             |
| MN020         | Imphal East  | Ompopot                | Cultivated|             |
| MN001         | Imphal West  | BamdiaHeijao           | Cultivated|             |
| MN007         | Imphal West  | Tamhei (Heijao-2)      | Cultivated|             |
| MN012         | Imphal West  | LaphuThambal           | Wild      |             |
| MN016         | Imphal West  | Heijao-1               | Cultivated|             |
| MN023         | Imphal West  | ChangbiLammu           | Cultivated| 8           |
| MN024         | Imphal West  | ChangbiLakai           | Cultivated|             |
| MN027         | Imphal west  | Kamongheijao           | Cultivated|             |
| MN028         | Imphal west  | Tera laphoi            | Cultivated|             |
| MN026         | Senapati     | Jahajimacha            | Cultivated| 1           |
| MN016         | Tamenglong   | Mayangheii             | Cultivated| 1           |
| MN004         | Thoubal      | Gera hei               | Cultivated|             |
| MN008         | Thoubal      | Meitei Hei             | Cultivated| 4           |
| MN009         | Thoubal      | Hei-Laax               | Cultivated|             |
| MN011         | Thoubal      | Korb                  | Cultivated|             |
| MN017         | Ukhrul       | Ukhrul he-1            | Cultivated|             |
| MN018         | Ukhrul       | Ukhrul he-2            | Cultivated| 3           |
| MN019         | Ukhrul       | Ukhrulheijao           | Cultivated|             |

Table 1: The details of collected banana samples.
Genome Classification of Banana Cultivars Found in Manipur, India using IRAP and RAPD

Fig 1(a-c): IRAP amplified PCR products of 34 banana samples. (a) Lane Nos. 02 to 28 represents Sample nos. MN02 to MN28; M1=100 bp DNA ladder; M2=1 kb DNA ladder. (b). Lane Nos. 01 to 38 represents Sample nos. MN01 to MN38; M1=100 bp DNA ladder; M2=1 kb DNA ladder. (c) Lane Nos. 09 to 32 represents Sample nos. MN09 to MN32; M1=100 bp DNA ladder; M2=1 kb DNA ladder. (Lanes X1 to X6 are banana samples which are not included in the analysis).

Fig 2: RAPD14-PCR amplified polymorphic bands of genomic DNA of 34 banana samples. (Lanes 1 to 34 represents Sample Acc. Nos MN01 to MN38; M=100 bp DNA ladder).

of Ensete were collected from the wild habitats. The details of 26 banana cultivars based on accession numbers, vernacular name in Manipuri (M), place and date of collection are shown in the Table 1. Majority of the cultivated samples were collected from the backyards and home gardens. Seven (7) of the cultivars were collected from Imphal West district followed by 5 from Imphal East, 4 from Thoubal, 3 from Ukhrul, 2 from Bishnupur, 2 from Churchandpur and 1 each from Chandel, Tamenlong and Senapati districts respectively.

Eight banana samples including a member of the genus Ensete were collected from 4 districts of Manipur. Three (3) samples each were collected from Bishnupur and Chandel districts while one each samples were collected from Imphal West and Churachandpur districts. Ensete glaucum (Laphu lempra, MN022 was the only member of the genus Ensete collected from Leimaram Hill range of Bishnupur district.
Differentiation of A and B genome of cultivated and wild bananas (Musa spp) using molecular markers (IRAP and RAPD):

Analysis of A and B genome among cultivated and wild bananas using IRAP-PCR

The amplified IRAP-PCR products of the 34 banana samples have shown presence of multiple polymorphic bands (Fig 1a-c). All samples except sample MN10 and MN27 have shown amplification of IRAP bands. A specific clear band of ~350 bp which is characteristic of B genome was observed in 11 samples (MN01,02,12,14,15,20,23,24,25,29 and 30). There was another B genome specific band (~250 bp) in 24 banana samples (MN 01, 02,04,05,07,08,09,11,13,15,16,18,19,21,23,24,25,28,29,30,31,32,33,38). Two IRAP bands at 900bp and 100bp were found to be specific in bananas of AAA group, particularly Jahaji (Granile, MN26). These bands were also found in 12 other samples (MN03,05,06,07,08,11,13,16,17,18 and 28). Another A genome specific band was also observed at ~1400 bp in 11 of the banana samples (MNO1,04,14,19,21,24,25,26,28,29 and 38).

Analysis of A and B genome among cultivated and wild bananas using RAPD-PCR:

RAPD analysis of the genomic DNA revealed contrasting results among 6 primers (OPF-6, OPX-6, OPK-12, RAPD-6, RAPD-14 and RAPD-17). A total of 1425 polymorphic bands were generated by the six primers. The highest number of bands was generated by OPK12 (318) followed by RAPD6 (308 bands), OPX6 (263 bands), RAPD 17 (215 bands), RAPD 14 (165 bands) and OPF6 (156 bands) respectively. Among these primers, RAPD 14 displayed specific bands per sample thereby showing higher discriminating power than others. Therefore, detailed analysis of genome types of cultivated and wild bananas in this study is discussed with reference to RAPD 14 only.

The amplified polymorphic band of RAPD 14 produces a total of 165 fragments of genomic DNA for 34 banana samples (Fig 2). The banana sample MN03 (Champa cola) displayed a maximum of 9 bands while lowest number of only one band was observed in MN031 (Wild Khuga). The desert banana with AA/AAA genome (MN11, MN20 and MN26) displayed 4 bands each while MN02 with BB genome produced 8 bands.

Hierarchical analysis of RAPD-14 amplified polymorphism in 34 banana samples:

The hierarchical cluster analysis of the RAPD-14 amplified 165 fragment from 34 banana samples is shown in Fig 3. All the 34 banana samples were classified into two large groups, cluster I comprising of AA and AAA genome groups of 14 banana samples and cluster II comprising of 2 BB genomes. The cluster I was further divided into two clads, Ia comprising of AAA genome groups and Ib comprising of AA groups. On the other hand, the Cluster II was also sub-
divided into two major subgroups, IIA with only one sample of MN012 (*M. laterita*) having AAB genome. The IIb was further divided into clans, IIb (i) with 6 samples of which majority were AB and AAB genomes while the IIb (ii) comprised of 13 samples with BB and ABB genomes respectively.

The molecular technique with RAPD primers has been successfully used to distinguish diverse *Musa* germplasm (Kaemmer *et al.* 1992; Howell *et al.* 1994; Bhat and Jarret, 1995). Random amplified DNA markers were used for determining the genetic diversity of materials from Indian banana germplasms (Jain *et al.* 2007). Venkatachalam *et al.* (2007) reported genome analysis of micropropagated and regenerated plantlets of banana by using RAPD and ISSR markers. Although the reproducibility of RAPDs has been doubted (Collard *et al.* 2005), RAPD markers have been the preferred techniques for analysis of genetic diversity as they require small amounts of DNA, are quick, and are not expensive. The Inter retro transposon amplified polymorphism (IRAP) markers are also very much important and they can identify the presence of B genome in natural hybrid in *Musa* species (Nair *et al.* 2005). IRAP makers can be amplified either by a single primer or a combination of primers based on LTR orientation successfully amplified different retrotransposons dispersed in the *Musa* genome and detected new events of insertions (Muhammad and Othman, 2005). The IRAP primers which was designed based on the LTR sequence of banana Ty3-gypsy-like retroelement (*Musa acuminata* Monkey retrotransposon, AF 143332), has been used only for identifying the B genome in the banana cultivars (Nair *et al.*, 2005). Saraswathi *et al.* (2009) reported that IRAP was more important and robust than RAPD markers to study the intra group diversity within Cavendish clones.

CONCLUSION

In this study, genetic characterization by IRAP-PCR analysis revealed that 32 out of 34 banana samples exhibited presence of multiple polymorphic bands in amplified products. It was found that there were 5 genome specific bands (2 for B genome and 3 for A genome) in all the samples analyzed by using IRAP marker. Molecular analysis using RAPD marker revealed that all 34 Musa species found in Manipur can be classified generally in two major groups *M. acuminata* group (AA/AAA) and *M. balbisiana* (BB/BBB) although there were AB and ABB or AAB were mixed with either of the groups.

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