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Genome constitution for *Musa beccarii* (Musaceae) varieties

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Abstract *Musa beccarii* N.W.Simmonds var. *beccarii* and *Musa beccarii* N.W.Simmonds var. *hottana* Häkkinen were described earlier in Acta Phytotaxonomica et Geobotanica based on their morphological characteristics. In order to distinguish the genomes between the two *M. beccarii* varieties and *M. coccinea* Andrews, we have now studied them with the Inter-Retrotransposon Amplified Polymorphism (IRAP) marker analyses. The high levels of IRAP polymorphism detected in this study showed that *M. beccarii* var. *beccarii* and *M. beccarii* var. *hottana* are two distinct varieties. Additional IRAP bands found in *M. beccarii* var. *beccarii* indicated that *M. beccarii* var. *hottana* might be evolutionarily more ancient than *M. beccarii* var. *beccarii* because LTR retrotransposons are not transpositionally removed.

Key words genome constitution, inter-retrotransposon amplified polymorphism, *Musa beccarii* var. *beccarii*, *Musa beccarii* var. *hottana*, *Musa coccinea*, phylogenetic analysis.

Borneo is a large island in Southeast Asia. It is divided politically into three parts: the kingdom of Brunei on the north central coast; the Malaysian states of Sarawak and Sabah to the west and east; with Kalimantan of Indonesia making up the larger part to the south. Located on the equator, it has a rainy humid equatorial climate. Borneo, being part of the primary banana diversity center, has a large number of wild endemic banana species. As banana plants prefer an open exposure, their growth is usually confined to rather small, isolated populations. They consequently manifest much genetic variation. The number of wild species of *Musa* L. (Linnaeus, 1753) in Borneo may now total 20, though only 17 species have been previously described (Häkkinen, 2004, 2006). *Musa beccarii* N.W.Simmonds is one of the wild species native to Borneo (Häkkinen et al., 2005). Wild *Musa* species are generally grouped into four sections: *Australimusa* Cheesman 2*n*=20, *Callimusa* Cheesman 2*n*=20 including *Musa beccarii* 2*n*=18, *Eumusa* (Baker) Cheesman 2*n*=22 and *Rhodochlamy*s (Baker) Cheesman 2*n*=22 (Baker, 1893; Cheesman, 1947; Simmonds & Weatherup, 1990; Häkkinen, 2004).

Wild populations of *Musa beccarii* var. *beccarii* have been reduced enormously due to massive land clearing for oil palm plantations in the eastern part of Sabah. Only a few *M. beccarii* populations could be found during the field study. *M. beccarii* var. *hottana* Häkkinen is an extremely rare new variety and was only found in one location at the lower Kinanbatangan River, Sabah.

These two varieties evolved in different directions, as adaptation to their growth conditions. *M. beccarii* var. *beccarii* can only grow in open exposure. Under the canopy it very soon shrivels and dies. *M. beccarii* var. *hottana* with contrary characteristics can only grow under the canopy.

A well-known banana researcher Prof. N.W.Simmonds, who never visited Borneo,

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originally described *M. beccarii* from a cultivated plant, which he grew in Trinidad from seeds imported from Sabah. He quoted that "This interesting little plant recalls *Musa coccinea* Andrews in general appearance but is quite distinct from it in the deciduous basal bracts, the large green fruits, and the long-lived male bud and, above all, in the seeds which are not of the barrel- or top-shaped type characteristic of section *Callimusa*. The chromosome number, 2*n*=18, is new to the genus *Musa*. In the herbarium the plant looks like section *Rhodochlamys* and I took it to be allied to *M. laterita* (2*n*=22, authors' note) E. E. Cheesman when I first saw specimens in the Singapore collections." Simmonds (1956) also added "The species grows well in Trinidad (which is rather unusual for bananas from Borneo) but sets fruit only sporadically. The description below is based on living plants grown in Trinidad, except for fruit characters, which are described from the Singapore specimens. So far as other characters can be determined in the herbarium, the Trinidad and Singapore plants agree well and there is no reasonable doubt that all the specimens cited represent but one species." (Simmonds, 1956) [(*M. coccinea*, Andrews, 1797; sect. *Callimusa*, Cheesman, 1947, Häkkinen, 2004; and *M. laterita*, Cheesman, 1949)].

The first author has also studied the Singapore herbarium collection and agrees with Simmonds' observations. The first author has also written articles on the section *Rhodochlamys* (Häkkinen & Sharrock, 2002) and on *M. laterita* (Häkkinen, 2001). *M. beccarii* was treated as *incertae sedis* until Simmonds and Weatherup's numerical taxonomic analysis of wild bananas placed it in section *Callimusa* (Simmonds & Weatherup, 1990).

The aim of this study is to prove the distinction between *M. coccinea*, originating in Guangxi, China and *M. beccarii* varieties, which originate in Northern Borneo.

LTR-retrotransposons are abundant in plants (Pearce et al., 1996) and propagate within the genome via RNA intermediates by a cycle of transcription, reverse transcription, and integration (Kumar & Bennetzen, 1999). Integration of new copies typically produces a 5–12 kb genomic insertion. These new copies are inserted and not transpositionally removed, which facilitates phylogenetic analyses (Shimamura et al., 1997). Accumulation, fixation and incomplete excision of retrotransposon insertions cause genomic diversification. The ubiquitous distribution, high copy number and widespread chromosomal dispersion of retrotransposon families provide excellent potential for developing DNA-based marker systems (Teo et al., 2005; Ashalatha et al., 2005).

In this study, we have explored the repetitive, dispersed nature of many long terminal repeat (LTR) retrotransposon families for characterizing genome constitutions and classifying varieties of *M. beccarii* (Häkkinen et al., 2005). The retroelements' insertional polymorphisms were studied using banana Ty3-*gypsy*-like LTR (Ashalatha et al., 2005) and barley Ty1-*copia*-like LTR sequences (Kalendar et al., 1999; Teo et al., 2005) as outward primers to amplify the sequences between two LTR retrotransposons. The primers generated specific fingerprinting patterns, which distinguish the *M. coccinea, M. beccarii* var. *beccarii*, and *M. beccarii* var. *hottana* genomes of each species/variety and between species/varieties.

1 Material and methods

1.1 Plant materials

The first author collected fresh leaf samples of *M. beccarii* var. *beccarii* and *M. beccarii* var. *hottana* during an expedition to Sabah in 2004. The sample of *M. coccinea* was from the collection of the University of Helsinki.

Musa species and varieties studied:

Musa coccinea Andrews, accession 2001-0387 (H, Herbarium of University of Helsinki, Finland).

Musa beccarii N.W.Simmonds var. *beccarii*, accession: 2004-05-18, *M. Häkkinen* & *J. Gisil 11* (BORH, Herbarium of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah).

Musa beccarii N.W.Simmonds var. *hottana*, accession: 2004-05-20, *M. Häkkinen* & *J. Gisil 12* (BORH).

Leaf tissue was used for IRAP methods to generate molecular markers, which characterize the genome constitution and diversity of species and varieties.

1.2 DNA extraction

Plant DNA was extracted using a modified CTAB method (Teo et al., 2002, 2005; Ashalatha et al., 2005). The percentage of polyvinylpyrrolidone with molecular weight 40,000 (PVP-40, SIGMA) was increased to 6% due to the high concentration of phenolic compounds in banana leaves.

1.3 Polymerase chain reaction

IRAP amplifications were carried out according to parameters described in Teo et al. (2005) and Ashalatha et al. (2005). Total genomic DNA samples were diluted with sterile H_2O to 25 ng/μL. IRAP was performed in a 25 μL reaction mixture containing 50 ng DNA, 1×Promega PCR buffer, 1.5 mmol/L $MgCl_2$, 5 pmol of each primer, 200 μmol/L dNTP mix, 1 U *Taq* polymerase (Promega, USA). Amplification was performed using a Tgradient thermocycler (Whatman Biometra, Germany). The PCR reaction parameters consisted of: 95 °C, 5 min; 30 cycles of 95 °C, 30 s, annealing at the Ta specified in Table 1 for 30 s, ramp +0.5 °C s^{-1} to 72 °C, and 72 °C for 2 min + 3 s extension per cycle; a final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis on 1.5% (w/v) agarose gel and detected by ethidium bromide staining.

Table 1 IRAP primers

Figure number	Primer combination	T_a (°C)
1A	GyLTRev + GyLTRev	62.0
1B	Sukkula LTR + Sukkula LTR	45.5
1C	LTR 6150 + Nikita LTR	45.5
1D	LTR 6149 + Sukkula LTR	45.5

2 Results

Inter-retrotransposon amplification polymorphism (IRAP) has been extensively used in different plant species to study the genome diversity (Kalendar et al., 1999; Baumel et al., 2002; Teo et al., 2005; Ashalatha et al., 2005).

Musa coccinea (2*n*=20), which is in the same section as both *M. beccarii* varieties, was used as a reference species for *Musa* sectional comparison. *M. laterita* (2*n*=22), which is in a different section (sect. *Rhodochlamys*), is not suitable for this type of comparison. Bartoš et al. (2005) showed that *M. laterita* formed a different subgroup, from *M. beccarii*, with species from sect. *Eumusa* and sect. *Rhodochlamys* in a phylogenetic analysis based on the genome size, number of chromosomes, and number of 45S rDNA loci.

In this study, we expand the uses of banana Ty3-*gypsy*-like LTR (Ashalatha et al., 2005) and barley Ty1-*copia*-like LTR sequences (Kalendar et al., 1999; Teo et al., 2005) on the section *Callimusa* to distinguish the genome of *M. beccarii* var. *beccarii* and *M. beccarii* var. *hottana*. The IRAP polymorphic patterns generated from Ty3-*gypsy*-like and Ty1-*copia*-like LTRs showed clearly distinct differences between var. *beccarii* and var. *hottana*. Four LTR primer combinations (Table 1) generated multiple fragments of defined sizes from total

genomic DNA of var. *beccarii* and var. *hottana* and *M. coccinea* (Fig. 1: A–D). Species-specific IRAP bands were observed, which enable the clear distinction of var. *beccarii* and var. *hottana* from *M. coccinea* using molecular markers (arrowheads, Fig. 1: A–D). Unique bands were observed in var. *hottana*, which allow var. *hottana* to be distinguished from var. *beccarii* (arrows, Fig. 1: A–D).

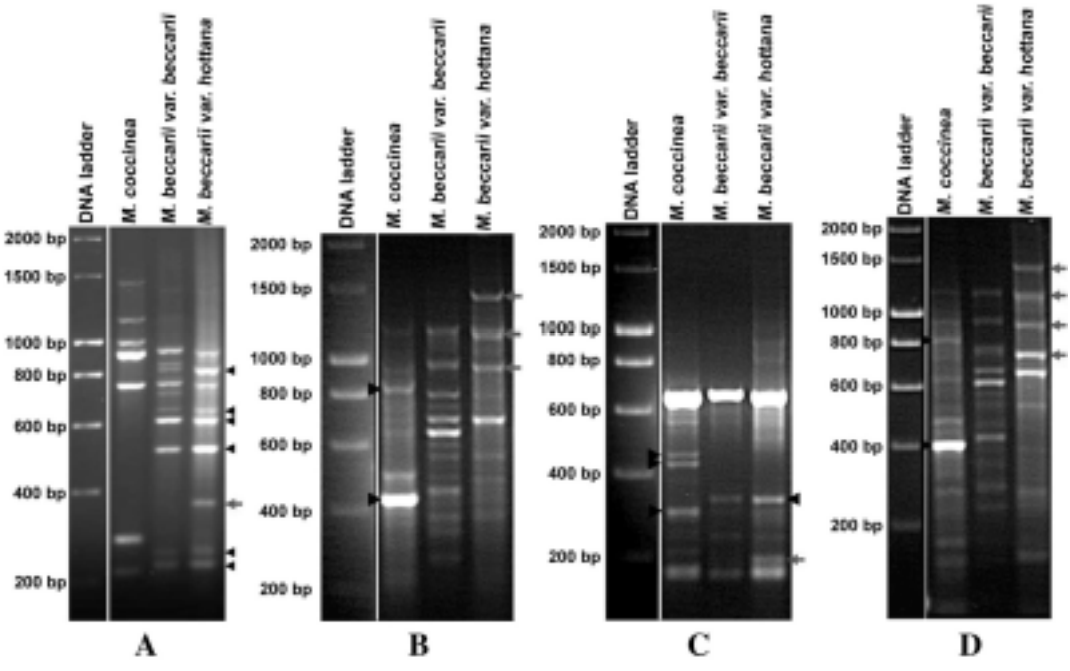

Fig. 1. Polymorphism patterns of *Musa beccarii* var. *beccarii*, *Musa beccarii* var. *hottana*, and *M. coccinea* generated by IRAP. **A,** IRAP with single GyLTRev primer. The arrowheads represent bands that enable the distinction of var. *beccarii* and var. *hottana* from *M. coccinea*. **B,** IRAP with a single Sukkula LTR primer. The arrowheads point to bands that are only found in *M. coccinea*, which distinguish *M. coccinea* from var. *beccarii* and var. *hottana*. **C,** IRAP with LTR6150 and Nikita LTR primer combination. The arrowheads point to bands that enable the distinction of var. *beccarii* and var. *hottana* from *M. coccinea*. **D,** IRAP with LTR6149 and Sukkula LTR primer combination. The arrowheads point to bands that enable the distinction of var. *beccarii* and var. *hottana* from *M. coccinea*. The molecular weight, in base pair (bp), of each DNA ladder is given at the left side of each figure. On the right side of each figure, the arrows point to bands that are only found in var. *hottana*.

3 Discussion

The power of IRAP markers in identifying plant genomes has been demonstrated in different plant species (Kalendar et al., 1999; Baumel et al., 2002; Teo et. al., 2005). Ty1-*copia*-like and Ty3-*gypsy*-like retrotransposons occupy different parts of banana genomes with different copy numbers. In addition, the non-transpositionally removal nature of LTR retrotransposon facilitates the fingerprinting of banana genomes, which allows precise classification of banana genomes. Using a combination of Ty1-*copia*-like and Ty3-*gypsy*-like LTR sequences as primers in this study allows a whole genome study of Musaceae. Balint-Kurti et al. (2000) suggested that Ty3-*gypsy*-like retrotransposons were introduced into *Musa* genus prior to the divergence of *M. acuminata* Colla, *M. balbisiana* Colla and *M. velutina* Wendl. & Drude. They were able to distinguish the A and B genomes using Ty3-*gypsy*-like retrotransposons probe hybridized on *Hin*dIII-digested genomic DNA from eight cultivars of banana with *M. velutina* as the control. Teo et al. (2002) showed that Ty1-*copia*-like

retrotransposons are present with different copy numbers in *M. acuminata*, *M. balbisiana* and *M. ornata* Roxb.

The generally high levels of IRAP polymorphism with all four primer-combinations between *M. beccarii* var. *beccarii* and *M. beccarii* var. *hottana*, together with their distinct morphological characteristics, suggested that they are two distinct varieties (Fig. 1). This is supported by observation of the first author during his extensive field studies in the eastern part of Sabah, Malaysia, where var. *beccarii* can only grow in the open exposure whereas var. *hottana* can only grow under the canopy. These polymorphisms may then be used to model the temporal sequence of insertion events in a lineage and to establish phylogenies (Kalendar et al., 1999).

The additional IRAP bands found in *M. beccarii* var. *beccarii* suggested that *M. beccarii* var. *hottana* might be evolutionarily more ancient than var. *beccarii.* New copies of LTR retrotransposons are not transpositionally removed (Shimamura et. al., 1997) and over time, the accumulation, fixation and incomplete excision of retrotransposon insertions generates more IRAP bands, which signifies genomic diversification. The association of species-specific bands with particular genomes can be explained as the result of integration of new retrotransposon copies after the divergence of ancestral genomes (Teo et al., 2005). The difference in the number of chromosomes between *M. coccinea* (2*n*=20) and *M. beccarii* var. *beccarii* (2*n*=18) might contribute to the presence or absence of species-specific bands in banana genomes.

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