

Acta Horticulturae  
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**Proceedings of the  
International ISHS-ProMusa Symposium  
on  
Global Perspectives on Asian Challenges**

**Editors  
I. Van den Bergh  
M. Smith  
R. Swennen  
C. Hermanto**



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**PROCEEDINGS OF THE  
INTERNATIONAL ISHS-PROMUSA SYMPOSIUM  
ON  
GLOBAL PERSPECTIVES ON ASIAN  
CHALLENGES**

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**Guangzhou, China**

**September 14-18, 2009**

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1. Banana fields on the island of Hainan. Photograph by courtesy of Nicolas Roux.
2. Visit to the Maosheng orchard, Shenwan Town, Zhongshan City. Photograph by courtesy of Inge Van den Bergh.
3. Banana at the South China Botanical Garden. Photograph by courtesy of Inge Van den Bergh.
4. *Musella lasiocarpa* at the South China Botanical Garden. Photograph by courtesy of Inge Van den Bergh.
5. *Musella lasiocarpa* at the South China Botanical Garden. Photograph by courtesy of Inge Van den Bergh.
6. Banana at the South China Botanical Garden. Photograph by courtesy of Inge Van den Bergh.

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## PREFACE

The papers contained in this volume of *Acta Horticulturae* report the peer reviewed Proceedings of the International ISHS-ProMusa Symposium on Global Perspectives on Asian Challenges. Keynote speakers and authors of selected contributed oral and poster presentations were given the opportunity to submit a manuscript for publication.

The manuscripts were reviewed by the Editors and members of the Editorial Board. Only those papers judged suitable for publication following the authors' consideration of reviewer suggestions appear in this volume of *Acta Horticulturae*.

The ISHS acknowledges and appreciates the contribution of all editors and reviewers. They have made a significant contribution to improving the quality of this publication.

*The ISHS Board of Directors*

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# Microsatellite Markers for Classifying and Analysing Genetic Relationship between Banana Cultivars in Indonesia

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**Keywords:** *Musa acuminata*, *Musa balbisiana*, SSR

## Abstract

In this study, microsatellite markers were used to identify *Musa* genomic groups and to investigate genetic relationships among banana accessions from various areas in Indonesia. One hundred sixteen banana accessions were analysed using MaCIR108 and Ma-3-90 primer pairs for identifying genomic groups. Six additional SSR primer pairs were used for genetic relationship analysis. The results showed that 73 accessions should be classified in the AA/AAA and AAA genomic groups, two accessions in the BB genomic group, 21 accessions in the AAB genomic group and 20 accessions in the ABB genomic group. Ninety-nine out of the 116 accessions were unique genotypes while the rest were synonyms. The dendrogram generated by UPGMA analysis separated the 116 banana accessions into two main clusters with a similarity of 0.13. All banana accessions belonging to the BB, ABB and AAB genomic groups clustered in the first main cluster, together with the majority of the accessions containing the pure A genome. The second main cluster was formed of 11 accessions of AA/AAA and AAA genome. Within the first main cluster, the accessions containing the B genome were clustered according to their genomic group, except four AAB accessions clustering with accessions containing the A genome alone. The ABB genomic group appeared closer to the BB than to the AAB genomic group. The AA and AAA banana accessions could not be significantly distinguished, although the majority of accessions tended to be clustered according to their ploidy level.

## INTRODUCTION

Most edible bananas originated from two wild species, *Musa acuminata* Colla (2n) and *Musa balbisiana* Colla (2n). Scientifically, banana cultivars are often referred to by their genomic groups, which are categorised on the basis of their ploidy levels and the genomes they contain. Ploidy in bananas includes diploids, triploids and rare tetraploids, resulting in genomic groups: AA, BB, AAA, AAB, ABB, AAAB ... with the letters A and B representing the contribution of *M. acuminata* and *M. balbisiana*, respectively. The hybrid bananas that evolved from the two natural species mostly formed triploid cultivars (Heslop-Harison and Schwarzacher, 2007).

Genomic constitution plays an important role in the classification of bananas (Pillay et al., 2004). Cultivar identification based on morphological characters is not always easy due to the influence of environmental factors. Molecular approaches have been recognised to be more effective than morphological techniques to distinguish banana cultivars and classify them in genomic groups (Ford-Lloyd et al., 1997).

Microsatellites are one of the most informative molecular markers to reveal genetic diversity of banana cultivars (Creste et al., 2003, 2004). Such markers have been used for genotype identification of many plant species (Perera et al., 2001; Chakravarthi and Naravaneni, 2006). Microsatellites are DNA regions that consist of short repeat units flanked by conserved sequences that are unique to specific loci in the genome (Semagn et al., 2006). In previous studies in banana, several microsatellite primers were shown to be useful in producing diagnostic characters for different genomic groups (Kaemmer et al., 1997; Creste et al., 2005), with a determination key based on application of these markers

constructed (Retnoningsih et al., 2010a).

The objectives of this study were to classify banana accessions from various regions in Indonesia using a microsatellite determination key, and to investigate the genetic relationships among these accessions based on microsatellite markers.

## **MATERIALS AND METHODS**

### **Plant Materials and Primers**

A total of 116 banana accessions were collected from various locations in Indonesia. The genetic relationships among banana germplasm was investigated using eight microsatellite primer pairs. The accessions were classified into their genomic groups using a molecular key developed by Retnoningsih et al. (2010a), presented in Figure 1.

### **DNA Extraction, Microsatellite Region Amplification and Silver Staining**

Total DNA was extracted from fresh young leaves using a modified SDS procedure (Dixit, 1998). Amplification of microsatellite regions was conducted following Kaemmer et al. (1997), with modifications, using a Perkin Elmer 2400 thermocycler (Applied Biosystems, Foster City, CA, USA®). The PCR products were separated on 6% denaturing polyacrylamide gels containing 7 M urea (Sigma-Aldrich Chemicals Germany®) at 45-60 W for 2 to 3 h. Alleles of each microsatellite were visualised by silver staining according to the modified procedure of Creste et al. (2001). The size of each allele was estimated using a 100 bp DNA ladder (Invitrogen®).

### **Data Analysis**

Each variant fragment of microsatellite was considered an allele. Genetic relationship analysis was based on data generated with the eight microsatellite markers, with each allele scored as present (1) or absent (0). Jaccard's coefficient was used to calculate similarity values between accessions using the Similarity of Qualitative Data (SYMQUAL) procedure. This matrix was then analysed via cluster analysis to visualise genetic relationships among the accessions. Cluster analysis was performed using Sequential, Agglomerative, Hierarchical and Nested (SAHN) clustering of the Unweighted Pair-grouping with Arithmetic Average (UPGMA) using Numerical Taxonomy and Multivariate Analysis System (NTSYS) software, version 2.02 for PC (Rohlf, 1998).

## **RESULTS AND DISCUSSION**

All microsatellite markers generated clearly distinguishable alleles and polymorphic patterns. A total of 93 alleles were detected with the eight markers. The number of alleles per locus varied from 5 to 18, with an average of 11.6 alleles per locus. The size of the alleles ranged from approximately 110 to 436 bp with the polymorphism degree per primer ranging from 5 to 18 alleles. The highest degree of polymorphism was observed with MaCIR108 and the lowest was detected with MaCIR327b (Table 1).

### **Classification of Banana Accessions Using a Microsatellite Determination Key**

The molecular determination key used was able to classify the 116 banana accessions effectively into their genomic groups. The key is based on four diagnostic alleles of locus MaCIR108 and one allele of locus Ma-3-90 with a size of 152 bp. A MaCIR108 allele with a size longer than 270 bp was considered a specific allele for the B genome, while an allele equal to or less than 270 bp was considered specific for the A genome (Retnoningsih et al., 2010b).

Seventy-three banana accessions were classified in the AAA or AA/AAA genomic groups, because they possessed only alleles of MaCIR108 with size equal to or shorter than 270 bp. The size of the MaCIR108 allele observed in these accessions ranged from approximately 210 to 268 bp. A-genome accessions with only one or two alleles were grouped in the AA/AAA genomic group whereas the accessions having three alleles were



classified into AAA genomic group. Diploid accessions could not be easily distinguished from triploid accessions, because there are no specific alleles to differentiate the AA from the AAA genomic group, as also reported by Creste et al. (2004). Furthermore, the dosage effects of the microsatellite allele (simplex, duplex, triplex, etc.) cannot be differentiated (Zhang et al., 2000) because most cultivated bananas are naturally polyploid (Stover and Simmonds, 1987). The triploid accessions possessing two alleles in a certain locus could not be differentiated from the diploid also possessing the same allele numbers. Two accessions were classified in the BB genomic group, because they possessed a MaCIR108 allele with a size of 295 bp without any allele size shorter than 270 bp.

Twenty accessions were classified in the ABB genomic group and 21 accessions in the AAB genomic group. A combination of the diagnostic character for the B genome (MaCIR108 allele size of 295 bp alone, or in combination with allele size of 289 or 287 bp) and the diagnostic character for the A genome (one allele size shorter than 270 bp) was found in some accessions of the ABB group. Other accessions with alleles with diagnostic characters for the A and B genomes were also identified as the ABB genomic group due to the presence of a Ma-3-90 allele with a size of 152 bp. In the 21 accessions classified as AAB, some contained a MaCIR108 allele with a size of 287 bp and shorter than 270 bp, but they did not have a Ma-3-90 allele with a size of 152 bp. Other AAB group members possessed a MaCIR108 allele with a size of 275 bp and shorter than 270 bp. In the 73 accessions of pure A genome, the Ma-3-90 allele with a size of 152 bp was not observed. The study also detected a Ma-3-139 allele with a size of 132 bp found in all banana accessions containing the B genome, while none was observed in banana accessions containing the A genome alone. This suggested that the locus Ma-3-139 could be considered as a diagnostic character for distinguishing banana accessions with the B genome from those with the A genome alone.

The number of pure A-genome accessions was greater than the number of B-genome accessions and their hybrids. These results also supported the hypothesis that Indonesia is one of the main centres of diversity and the centre of origin of *M. acuminata* (Daniells et al., 2001).

#### **Analysis of Genetic Relationships among Banana Germplasm in Indonesia**

The dendrogram generated using UPGMA analysis separated the 116 banana accessions into two main clusters with a similarity of 0.13 (Fig. 2). All banana accessions belonging to the BB, AAB and ABB genomic group clustered in the first main cluster together with most banana accessions containing the A genome alone. The second main cluster was formed from 11 banana accessions with the A genome alone.

The polymorphism of microsatellite loci analysed in this study was consistent with a previous study by Creste et al. (2004), but was greater than that observed by Creste et al. (2003). Using nine primers, Creste et al. (2004) observed an average of 12.8 alleles, ranging from 10 to 15 alleles in a group of 58 *Musa* genotypes, including 49 AA and 9 AAB cultivars. However, Creste et al. (2003) detected an average of 6.1 alleles at each of 11 microsatellite loci studied in a group of 35 banana genotypes of various genomic compositions and ploidy levels. One possible reason for this difference is that all accessions used in this study originated from natural cultivars, with a relatively wide genetic base.

Genetic relationship analysis among banana accessions of various genomic groups demonstrated that the accessions containing the B genome were clearly distinct from each other (Fig. 2). The BB genomic group appears closer to the ABB genomic group. All banana accessions containing the B genome were clustered according to their genomic group, except four AAB accessions, namely 'Kepok Amerika', 'Raja Sereh', 'Embok' and 'Susu', clustering with accessions containing the A genome alone.

With a similarity of 0.18, the dendrogram showed that the first main cluster was divided into two subclusters. The first subcluster contained diploid and triploid accessions containing the A genome alone (AA/AAA and AAA) and four AAB accessions. Cluster analysis based on 93 alleles of microsatellites could not significantly distinguish diploid

from triploid banana accessions of the A genome alone due to the dosage effects of microsatellite alleles. However, the majority of accessions, especially AAA accessions, tended to be clustered according to their ploidy levels. Similar results were reported by Creste et al. (2004) and in a previous study by Retnoningsih et al. (2010b) investigating genetic relationships among A-genome cultivars. In addition to the dosage effect, characteristics of the AA and AAA accessions were not clearly distinct, possibly due to both groups originating from the same parents.

Four banana accessions with an AAB genome were separated from the second cluster which contained all banana accessions with the B genome. This is because these accessions had one specific allele of the MaCIR108 microsatellite with a size of 275 bp. According to the microsatellite marker determination key, this allele was not detected in the other genomic groups. They were closely related to banana accessions containing the A genome alone. Seventeen AAB accessions had a MaCIR108 allele with a size of 287 bp that was also observed in several accessions of ABB. Thus, this AAB genomic group was closely related to ABB.

Cluster analysis showed that microsatellite markers could discriminate each accession, except accessions which were known to be phenotypically similar. Ninety-nine unique genotypes were identified from the analysis of 116 accessions. In identical accessions, some had the same local name, whereas other accessions were synonymous.

In conclusion, 116 banana accessions were successfully classified into their genomic groups using a molecular key based on two SSR primer pairs. The key is only applicable to accessions that contain A and B genomes. For other cultivated bananas of S and T genomes, identification using molecular markers could likely be performed if primers were available. Cluster analysis based on 93 microsatellite alleles could not significantly distinguish diploid from triploid banana accessions. Incorporation of several *M. acuminata* subspecies in the analysis could likely reveal further genetic relationship with the cultivars.

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## Tables

Table 1. Alleles size, number of alleles and observed heterozygosity value produced from analysis of 116 banana accessions using eight microsatellite markers.

Primer	Allele size range (bp)	Number of allele	Observed heterozygosity
MaCIR327b	388-436	5	0.45
Ma-1-132	330-378	9	0.51
MaCIR332a	260-296	11	0.85
MaCIR108	220-295	18	0.84
Ma-3-139	132-177	17	0.83
Ma-3-90	132-172	14	0.86
Ma-1-27	122-142	8	0.57
Ma-1-17	110-154	11	0.91
Total		93	
Mean		11.6	0.73



**Figures**

1a.	MaCIR108 alleles size equal to or shorter than 270 bp .....	2
2a.	The number of alleles, one to two .....	AA/AAA
2b.	The number of alleles, three .....	AAA
1b.	MaCIR108 alleles size longer than 270 bp .....	3
3a.	Allele size 295 bp only .....	<b>BB</b>
3b.	Alleles combination: 295 bp; or 295 bp and 289 bp; or 295 bp and 287 bp; with one allele size equal to or shorter than 270 bp .....	<b>ABB</b>
3c.	Alleles combination of 287 bp with alleles size equal to or shorter than 270 bp .....	4
4a.	Alleles combination of 287 bp with two alleles size equal to or shorter than 270 bp .....	<b>AAB</b>
4b.	Alleles combination of 287 bp with one allele size equal to or shorter than 270 bp .....	5
5a.	The Ma-3-90 alleles of 152 bp, present .....	<b>ABB</b>
5b.	The Ma-3-90 alleles of 152 bp, absent .....	<b>AAB</b>
3d.	Alleles combination of 275 bp with alleles size equal to or shorter than 270 bp .....	<b>AAB</b>

Fig. 1. The molecular determination key of banana genomic groups (Retnoningsih et al., 2010a).



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