Journal of Plant Biology, March 2005, 48(1) : 96-105

Genome Constitution and Classification Using Retrotransposon-Based Markers in the Orphan Crop Banana

Chee How Teo[1,5], Siang Hee Tan[2]*, Chai Ling Ho[1], Qamaruz Zaman Faridah[3], Yasmin Rofina Othman[4], John Seymour Heslop-Harrison[5], Ruslan Kalendar[6], and Alan Howard Schulman[6,7]

[1]Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Molecular Biology Department, Sime Darby Technology Centre Sdn Bhd, 2, Jalan Tandang 46050 Petaling Jaya, Selangor, Malaysia
[3]Department of Biology, Faculty of Science and Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Institute of Biological Sciences, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[5]Department of Biology, University of Leicester, Leicester LE1 7RH, United Kingdom
[6]MTT/BI Plant Genomics Laboratory, Institute of Biotechnology, University of Helsinki, P. O. Box 56, Viikinkaari 9, FIN-00014 Helsinki, Finland
[7]Plant Breeding Biotechnology, MTT Agrifood Research Finland, Myllytie 10, FIN-00810 Jokioinen, Finland

We have exploited the repetitive and dispersed nature of many long terminal repeat (LTR)-retrotransposon families for characterizing genome constitutions and classifying cultivars of the genus *Musa*. Insertional polymorphisms of the elements were studied using seven published and two newly designed primers facing outwards from the LTRs and reverse transcriptase (RT) domain of the retrotransposon. The primers generated specific amplification patterns showing the universal applicability of this marker type. The Inter-Retrotransposon Amplified Polymorphism (IRAP) markers distinguished the A and B genomes of the banana species (*Musa acuminata* Colla and *Musa balbisiana* Colla) and between banana cultivars. The IRAP markers enabled phylogenetic analysis of 16 Malaysian banana cultivars and determination of the genome constitution of hybrid banana (AAB, ABB, AABB, and AAAB), and gave information about ancestral genotypes of the hybrids. In addition, the IRAP detected new retrotransposon insertions into the genome of tissue culture regenerants. This PCR-based IRAP assay is amenable to large-scale throughput demands in screening breeding populations and is applicable for any crop.

Keywords: genome constitution, Inter-Retrotransposon Amplified Polymorphism, long terminal repeat, *Musa*, phylogenetic analysis, retrotransposon

Retrotransposons are highly abundant and dispersed components of most plant genomes (Flavell et al., 1992; Voytas et al., 1992; Kumar and Bennetzen, 1999). Ubiquitous distribution, high copy number and widespread chromosomal dispersion of these mobile elements provide excellent potential for developing DNA-based marker systems.

Retrotransposons flanked by long-terminal repeats (LTR-retrotransposons) are abundant in plants (Pearce et al., 1996) and propagate within the genome via RNA intermediates in the cycle of transcription, reverse transcription, and integration (Kumar and Bennetzen, 1999). Integration of new copies typically produces a 5-10 kb genomic insertion in which the LTRs, conserved within a retrotransposon family, lie next to anonymous host sequences. These new copies are inserted but, unlike DNA transposons, not transpositionally removed, which facilitates phylogenetic analyses (Shimamura et al., 1997). Over time, accumulation, fixation and incomplete excision of retrotransposon insertions causes genomic diversification.

Retrotransposon insertional polymorphisms can be detected by a variety of PCR-based techniques in which outward-facing primers are designed for conserved domains such as LTRs within the elements (Waugh et al., 1997; Flavell et al., 1998; Kalendar et al., 1999). The polymorphisms may then be used to model the temporal sequence of insertion events in a lineage and to establish phylogenies (Kalendar et al., 1999). Retrotransposons of differing activity profiles

*Corresponding author; fax +603-7781-0230
e-mail sianqtsh@simedarby.com

have varying degrees of phylogenetic resolution, allowing choice of the marker system to match the task.

Of the various techniques available, IRAP and REMAP (Kalendar et al., 1999) detect high levels of polymorphism without the need of DNA digestion, ligations or probe hybridization to generate marker data, thus increasing the reliability and robustness of the assay. The LTRs of retrotransposons contain sequences that are essential for expression (promoter and processing signals) and integration. From the conserved LTR regions, primers for PCR amplification are designed to amplify the DNA fragment between the closely dispersed members of a retrotransposon family. The use of RT primers matching highly conserved domains (Flavell et al., 1992; Voytas et al., 1992; Teo et al., 2002) allows the detection of insertions in virtually any *copia*-like retrotransposon.

In principle, retrotransposons may integrate in either orientation (sense or antisense) into the genome, and hence any two members of a retrotransposon family may be found head-to-tail, head-to-head, or tail-to-tail. Thus, intervening genomic DNA can be amplified from the elements sufficiently close to one another using either 5′ or 3′ LTR primers. A single primer is sufficient to generate PCR products from elements close to one another in either the head-to-head or tail-to-tail orientation. A combination of LTR and RT primers can be used to track nested insertion events: whether a retrotransposon is integrated into the LTR or internal region of another retrotransposon. Such nested patterns of insertions are common at least in barley and maize (SanMiguel et al., 1996; Shirasu et al., 2000). Methods have been developed for rapidly isolating native retrotransposons based on conserved domains within retrotransposons (Pearce et al., 1999). Primers derived from retrotransposons have recently been exploited to study biodiversity and phylogeny in the genus *Brassica* (Tatout et al., 1999), *Hordeum* (Waugh et al., 1997; Kalendar et al., 1999), *Oryza* (Iwamoto et al., 1999), *Spartina* (Baumel et al., 2002), *Pisum* (Pearce et al., 2000) and *Musa* (Teo et al., 2002), and to construct recombinational maps in *Hordeum* (Manninen et al., 2000) and *Triticum* (Boyko et al., 2002).

In this study, we aimed to test the transferability of retrotransposon markers from one crop to another, and in particular, barley-derived sequences for identification and characterization of banana cultivars and classification of *Musa* genome constitutions. Banana is the fourth most important staple crop in developing countries (FAO, 2003) and a foundation of subsistence agriculture in many of the poorest. The genus *Musa* can be divided into four sections: *Callimusa*, *Australimusa*, *Eumusa* and *Rhodochlamys* (Horry et al., 1997). The *Eumusa* section is the largest in the genus and the most geographically widespread with species being found throughout India, South East Asia and the Pacific Islands (Horry et al., 1997). The most important cultivated bananas arose from this section. Bananas consist of two groups of plants; the cultivars which are clones maintained exclusively through vegetative propagation and the wild plants. The wild plants are seeded diploid and are classified into two main species (*M. acuminata* Colla and *M. balbisiana* Colla).

Systematic scoring of characters diagnostic of the two parental species together with chromosome counting suffices to diagnose the main cultivated groups (Stover and Simmonds, 1987). They are designated by genome constitution: AA, AAA, AB, AAB, and ABBB. The other groups (ABB, AAAB and AABB) have yet been fully classified but should present no great difficulty. Here, we aimed to apply IRAP methods to generate molecular markers which characterise the genome constitution and diversity of species in the banana genus (*Musa*) and banana cultivars. We also evaluated the method for analysing the relationships of Malaysian banana cultivars.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Sixteen banana cultivars were used (Table 1). Twelve cultivars were from the germplasm collection of Dr. Siti Hawa Jamaluddin from Malaysia Agricultural Research and Development Institute (MARDI), Malaysia. Four cultivars (Berangan, Abu Baru, Gold Finger, and Mas), propagated by tissue culture, were kindly provided by Tropbio Research Sdn. Bhd., Technology Park Malaysia, Serdang, Malaysia. Genomic DNA of the banana cultivars was extracted from young leaves as previously described in Teo et al. (2002).

Inter-Retrotransposon Amplified Polymorphism (IRAP)

The IRAP method was carried out as previously described, using LTR primers derived from barley (*Hordeum vulgare*) (Kalendar et al., 1999; Kalendar et al., 2000; Manninen et al., 2000; Vicient et al., 2001; Boyko et al., 2002). The primer sequences, retrotrans-

Table 1. The accession, ploidy level and genome constitution of 16 cultivars of banana.

Accession	Ploidy	Genome Constitution
Musa ornata Roxburgh	2*x*	Ornamental
Musa acuminata Colla subsp. *malaccensis*	2*x*	AA (Seeded)
Pisang Kra (7732)	2*x*	AA (Seeded)
Pisang Bulin	2*x*	AA (Edible)
Pisang Lemak Manis Terengganu	2*x*	AA (Edible)
Pisang Mas tissue culture	2*x*	AA (Edible)
Musa balbisiana Colla subsp. Gala	2*x*	BB
Pisang Montel	3*x*	AAA
Pisang Berangan	3*x*	AAA
Pisang Berangan tissue culture	3*x*	AAA
Pisang Nangka Pulau Tioman	3*x*	AAB
Pisang Nipah	3*x*	ABB
Pisang Abu Siam Selangor (7765)	4*x*	ABBB
Pisang Abu Baru	4*x*	AABB
Pisang Abu Baru tissue culture	4*x*	AABB
Gold Finger tissue culture	4*x*	AAAB

Table 2. Primers for IRAP.

Name	T_m	Retrotransposon source, orientation	Sequence	Accession, position
LTR 6149	61	*BARE*-1 →	CTCGCTCGCCCACTACATCAACCGCGTTTATT	Z17327 1993-2012
LTR 6150	62	*BARE*-1 ←	CTGGTTCGGCCCATGTCTATGTATCCACACATGTA	Z17327 418-439
5′ LTR1	63	*BARE*-1 ←	TTGCCTCTAGGGCATATTTCCAACA	Z17327 1-26
5′ LTR2	58	*BARE*-1 ←	ATCATTGCCTCTAGGGCATAATTC	Z17327 314-338 7417-7441
3′ LTR	69	*BARE*-1 →	TGTTTCCCATGCGACGTTCCCCAACA	Z17327 2112-2138
Sukkula	66	*Sukkula* →	GATAGGGTCGCATCTTGGGCGTGAC	AY054376 4301-4326
Nikita	58	***Nikita*** →	CGCATTTGTTCAAGCCTAAACC	AY078073 AY078074 AY078075 1-22
Reverse TY1	42	W1, W3, W7, W8 ←	CCYTGNAYYAANGCNGT	AF416815 AF416816 AF416817 AF416818 1-17
Reverse TY2	36	W1, W3, W7, W8 →	TRGTARAGRAGNTGRAT	AF416815 AF416816 AF416817 AF416818 252-269

poson source, and orientation are shown in Table 2. Two additional IRAP primers (Reverse TY1 and Reverse TY2) were designed facing outward from the highly conserved reverse transcriptase (RT) priming sites of the banana Ty1-*copia*-like retrotransposons (Teo et al., 2002) and corresponded to the TAFLHG and YVDDML conserved regions of RT priming sites, respectively.

Genomic DNA samples were diluted with sterile and deionized H_2O to 50 ng/µL. The IRAP PCR was performed in a 20 µL reaction mixture containing 50 ng DNA, 1X PCR buffer (Promega, USA), 2 mM $MgCl_2$, 5 pmol of each primer, 200 µM dNTP mix, 1 U *Taq* polymerase (Promega, USA). Amplification was performed using PTC-225 DNA Engine Tetrad (MJ Research, USA). The PCR reaction parameters consisted of: 95°C, 2 min; 30 cycles of 95°C, 60 s, annealing at the T_a specified in Table 3 for 60 s, ramp +0.5°C s^{-1} to 72°C, and 72°C for 2 min+3 s per cycle; a final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis on 2% (w/v) agarose gel and detected by ethidium bromide staining. The IRAP banding pattern was scored using Quantity One version 4 for Windows (Bio-Rad, USA).

Phylogenetic Analyses

Phylogenetic trees were constructed by a distance-based method. Evolutionary distances were calculated, using RAPDistance Package Version 1.04, from tables consisting of scores for presence or absence of bands of particular mobility in accessions. Only clear and distinguishable bands were scored. A value of '1' indicates the presence of a band of a particular mobility compared to a DNA ladder, and '0' indicates the absence of that particular band. Phylogeny was analysed using neighbour-joining method (Saitou and Nei, 1987) implemented in a DOS-based program, NTSYS-pc, version 1.80 (Exeter Software, Australia).

RESULTS

Amplification of Genomic DNA with IRAP Primers

All 16 PCR universal primer combinations (Table 4) generated multiple fragments of defined sizes from the genomic DNA of all banana cultivars (Fig. 1, Table 4). On average, single LTR primer yielded 12 bands, two LTR primers from the same retrotransposon fam-

Table 3. IRAP primer combinations and the respective annealing temperatures. ⃠ indicates unsuccessful primer combinations for amplification screening of designated varieties.

	Reverse TY1	Reverse TY2	LTR 6149	LTR 6150	5′ LTR1	5′ LTR2	3′ LTR	Sukkula LTR	Nikita LTR
Reverse TY1	⃠	⃠	⃠	⃠	45.5	⃠	47.0	45.5	45.5
Reverse TY2		⃠	⃠	⃠	45.5	45.5	47.0	48.4	45.5
LTR 6149			40.5	40.5	41.5	45.5	45.5	45.5	45.5
LTR 6150				40.5	45.5	40.0	45.5	48.4	45.5
5′ LTR1					41.5	43.2	⃠	43.2	40.5
5′ LTR2						45.5	48.4	43.2	45.5
3′ LTR							48.4	47.0	47.0
Sukkula LTR								45.5	45.5
Nikita LTR									48.4

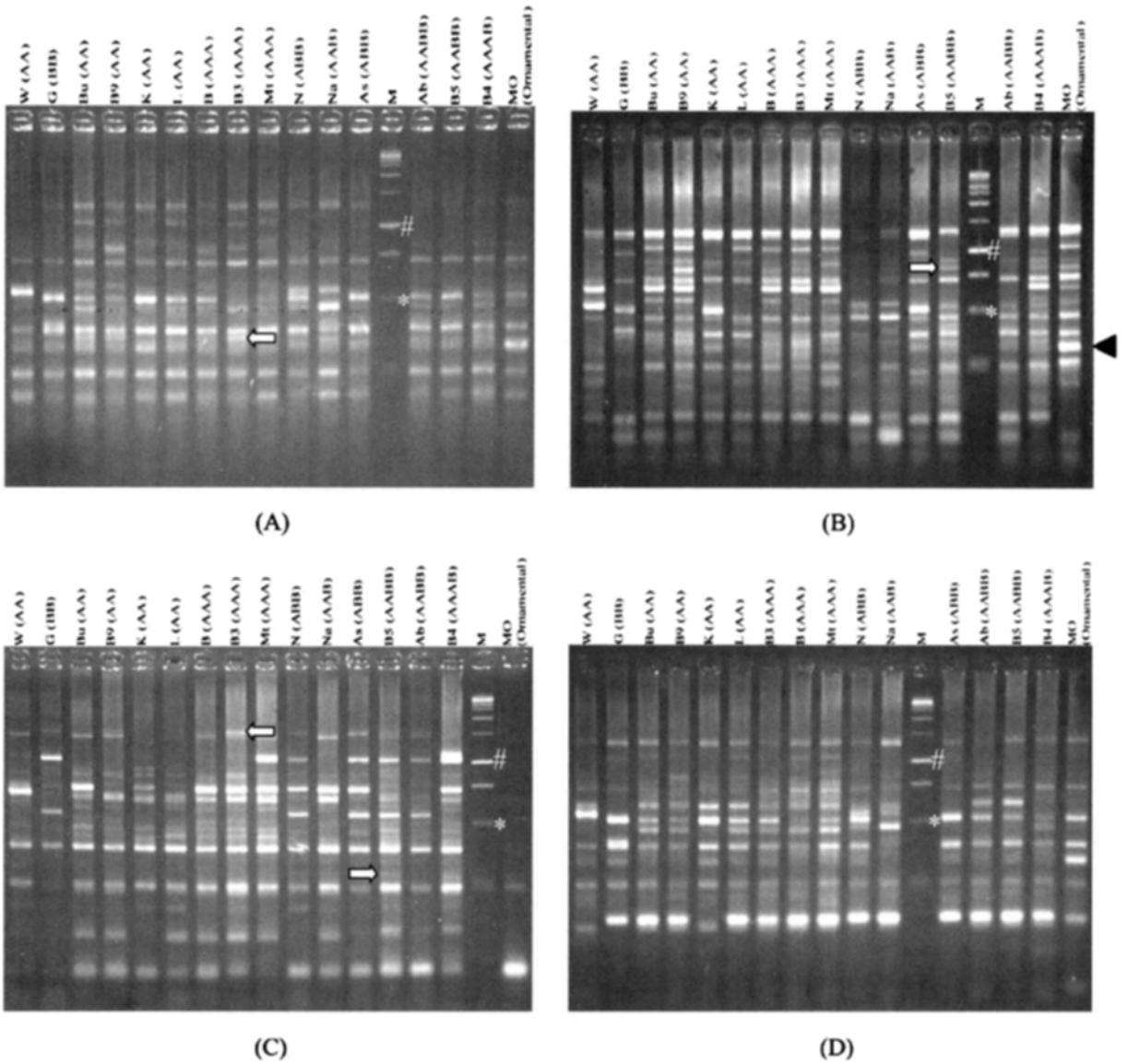

Figure 1. Polymorphism patterns from sixteen banana varieties generated by IRAP. **(A)** IRAP with a single 3′ LTR primer. The arrows represent new bands only found in tissue-cultured material. **(B)** IRAP with 6150 LTR primer and 3′ LTR primer. The arrowhead represents the unique band only found in *M. ornata* Roxburgh. The arrows represent new bands only found in tissue-cultured material. **(C)** IRAP with 6150 LTR primer and *Nikita* LTR primer. The arrows represent new bands only found in tissue-cultured material. **(D)** IRAP with 3′ LTR primer and LTR primer 5′ LTR2.

ily yielded 13 bands, a combination of LTR primers from different families yielded 15 bands, and an LTR primer in combination with a degenerate RT primeryielded 10 bands. There were 118 polymorphic bands among the cultivars; with shorter fragments amplified from most or all cultivars, suggesting conservation of the internal organization of parts of retroelements. These results show the transferable nature of the retrotransposon-based marker system.

Analysis of the cultivars showed groupings in accordance with known relationships. *M. ornata* Roxburgh, taxonomically distant to the *M. acuminata* Colla and *M. balbisiana* Colla species in section *Eumusa*, showed smaller number of fragments of different sizes from the A and B genomes (MO track in Fig. 1). Five unique bands were identified (arrowheads in Fig. 1). There was a high level of polymorphism between the A and B genome accessions. The IRAP analysis of all the hybrid-

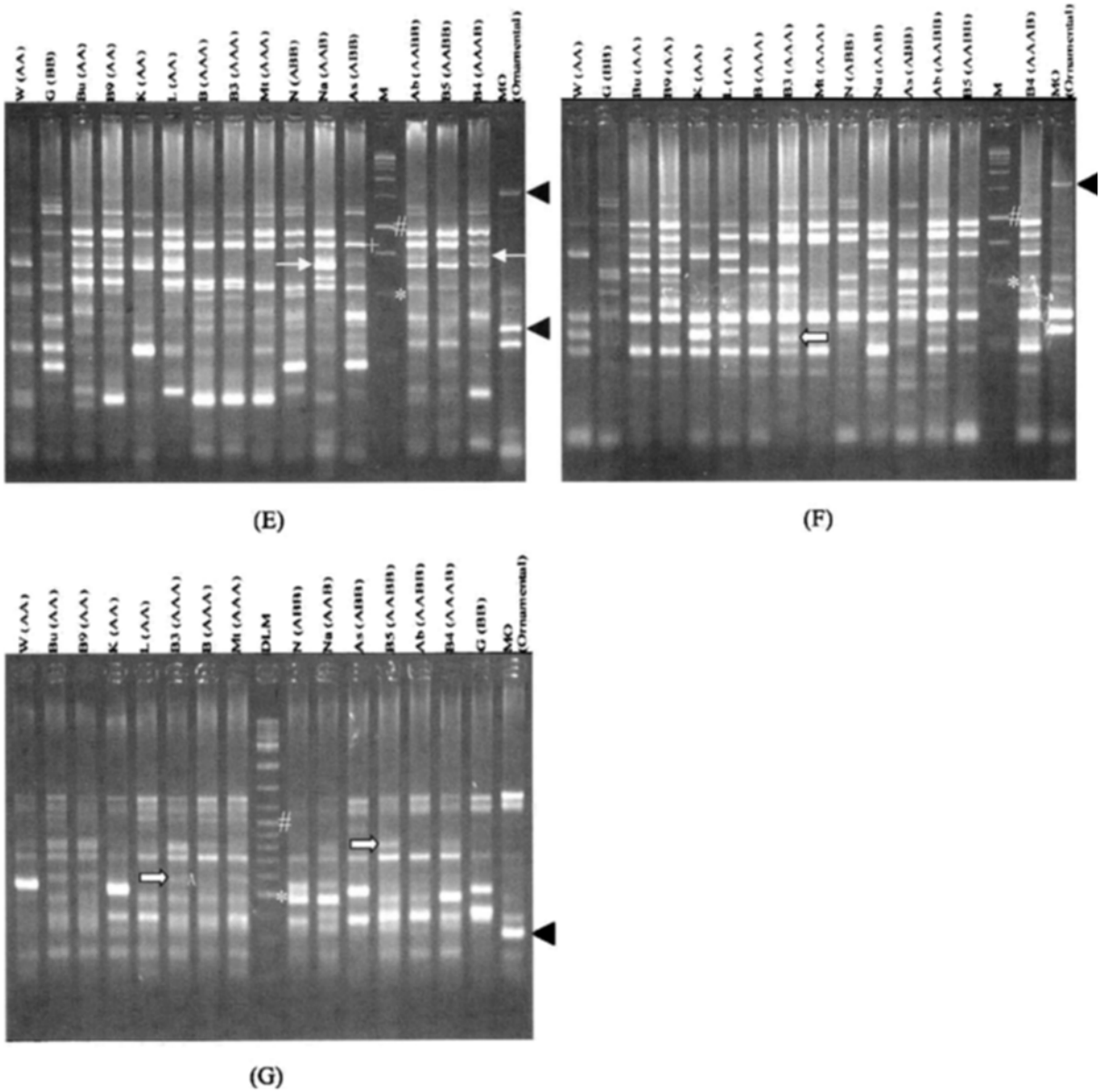

Figure 1. continued

(E) IRAP with 6149 LTR primer and *Sukkula* LTR primer. The arrowheads represent the unique bands only found in *M. ornata* Roxburgh. The arrows represent two bands that were absent in all AAA varieties but present in Gold Finger (AAAB). + indicates the ~833 bp bands. **(F)** IRAP with 6150 LTR primer and *Sukkula* LTR primer. The arrowhead represents the unique band only found in *M. ornata* Roxburgh. The arrows represent new bands only found in tissue-cultured material. **(G)** IRAP with 3′ LTR primer and reverse transcriptase primer RTY1. The arrowhead represents the unique band only found in *M. ornata* Roxburgh. The arrows represent new bands only found in tissue-cultured material. The sixteen banana varieties are: W=*Musa acuminata* Colla subsp. *malaccensis*, Bu=Bulin, B9=Mas tissue culture, K=Kra 7732, L=Lemak Manis Terengganu, B3=Berangan tissue culture, B=Berangan, Mt=Montel, N=Nipah, Na=Nangka Pulau Tioman, As=Abu Siam Selangor 7765, B5=Abu Baru Tissue Culture, Ab=Abu Baru, G=*Musa balbisiana* Colla subsp. Gala and MO=*Musa ornata* Roxburgh. Commercial standards used are: M, 1 kb DNA ladder from Promega; DLM, GeneRuler™ DNA ladder mix from MBI Fermentas, USA. Both * and # indicate the 500 bp and 1000 bp bands of the commercial standard.

origin accessions included products from both the A and B genomes, allowing them to be correctly classified. The relative proportion of A and B genomes seemed to affect the banding pattern, perhaps due to template competition during PCR or to differences or heterozygosity in the retrotransposons in ancestral

Table 4. The degree of polymorphism of the IRAP products within the banana accessions. *indicates primer combinations that used in Fig. 1.

Primer combination	Total bands	Polymorphic bands	Degree of polymorphism (%)
LTR 6149 + LTR 6149	16	15	93.75
*3′ LTR + LTR 6150	24	23	95.83
3′ LTR + Nikita LTR	21	20	95.24
5′ LTR2 + LTR 6150	17	17	100.0
*LTR 6149 + Sukkula LTR	24	23	95.83
LTR 6149 + Nikita LTR	21	21	100.0
*LTR 6150 + Nikita LTR	16	16	100.0
*LTR 6150 + Sukkula LTR	19	18	94.74
5′ LTR2 + Sukkula LTR	17	17	100.0
3′ LTR + LTR 6149	18	17	94.44
5′LTR1 + 5′ LTR2	20	20	100.0
LTR 6150 + LTR 6150	24	23	95.83
*3′ LTR + Reverse TY1	17	15	88.24
3′ LTR + Reverse TY2	16	15	93.75
*3′ LTR + 5′ LTR2	17	14	82.35
*3′LTR + 3′ LTR	14	9	64.29
TOTAL	301	283	94.02

genomes leading to the A/B varieties. Lower ramping rate of annealing temperature and longer annealing time during the PCR probably reduced the possibility of template competition during amplification. The differences or heterozygosity in the retrotransposons in ancestral genomes provided advantages to retrotransposon-based markers for distinguishing the product of cross between different ancestral genomes. An example of such differences can be traced for a particular IRAP band: a fragment (~ 833 bp, Fig. 1E) was present in all the pure A (AA and AAA) and A/B (AAB, AABB, ABBB and AAAB) cultivars tested except for *M. acuminata* Colla subsp. *malaccensis* (AA) and Kra 7732 (AA), suggesting an integrational event after the divergence of Kra 7732 and *M. acuminata* Colla subsp. *malaccensis* (see also Fig. 2).

Phylogenetic Analyses of Genome Diversity

Phylogenetic analyses of IRAP polymorphisms separated *M. balbisiana* Colla subsp. Gala and *M. ornata* Roxburgh from *M. acuminata* Colla subsp. *malaccensis* and provided a fairly good discrimination between species with dissimilarity values (RAPDistance Package) ranging from 0.446 to 0.792 (Fig. 2).

All A/B cultivars used in this study were not included in the phylogenetic tree due to the fact that the tree construction only assumes dichotomous branching but no hybrids. The identification of ancestral banana genotypes was carried out by comparing the banding pattern of the IRAP fragments and reconfirmed with the description of Stover and Simmonds (1987). Further development of analytical methods is required to show positions of interspecies hybrids and to quantify their ancestral relationships in phylogenies.

Different subgroups of bananas are clustered based on the genotypes and ploidy level. When the IRAP fragments were analysed from the accessions known to include both the A and B genomes, inferences on their likely ancestral genotypes can be obtained. Abu Baru (AABB) and Abu Baru (AABB, tissue culture) and Gold Finger (AAAB), which are new interspecies hybrids produced by the Honduran Foundation for Agricultural Research (FHIA), formed a sister group to subgroup III (Fig. 2). The MB1 interspecies hybrid Gold Finger (AAAB, FHIA-01), produced in the Honduras and introduced in 1977, originated from multiple crosses between the female Brazilian apple-flavored "Prata Ana" (AAB) clone with a wild Southeast Asian banana (AA, SH-3142) as the recurrent parent. The absence of two bands (~688 bp and ~ 750 bp, Fig. 1E) in all the AAA genome cultivars and the presence of these two bands in the Gold Finger (AAAB) is consistent with Gold Finger (AAAB, FHIA-01) being the product of multiple crosses between a

M. acuminata Colla (AA, SH-3142) derivative and AAB-bearing plant. This furthermore suggests that Abu Baru (AABB, FHIA-03) may have a similar origin. Nangka Pulau Tioman (AAB) was a sister group in subgroup II suggesting that Nangka Pulau Tioman (AAB) was derived from diverse edible *M. acuminata* Colla cultivars and *M. balbisiana* Colla (Fig. 2). This was supported by the close relationships found in this study between the different *M. acuminata* Colla cultivars (Bulin, Mas, and Lemak Manis Terengganu). Nipah (ABB) and Abu Siam Selangor 7765 (ABBB) formed a sister group to group B (Fig. 2). Nipah and Abu Siam Selangor 7765 had similar band patterns to *M. balbisiana* Colla subsp. Gala (BB) on the one side and to Kra 7732 (AA) on the other, suggesting possible ancestral genotypes for these triploid and tetraploid varieties. Using pedigree analysis of the IRAP band patterns, it appears that Abu Siam Selangor 7765 (ABBB), which may be the product of Kra 7732 (AA) and *M. balbisiana* Colla subsp. Gala (BB), may have preceded Nipah (ABB). Kra 7732 itself might be derived from *M. acuminata* Colla subsp. *malaccensis* under human selection for parthenocarpy and seed-sterility.

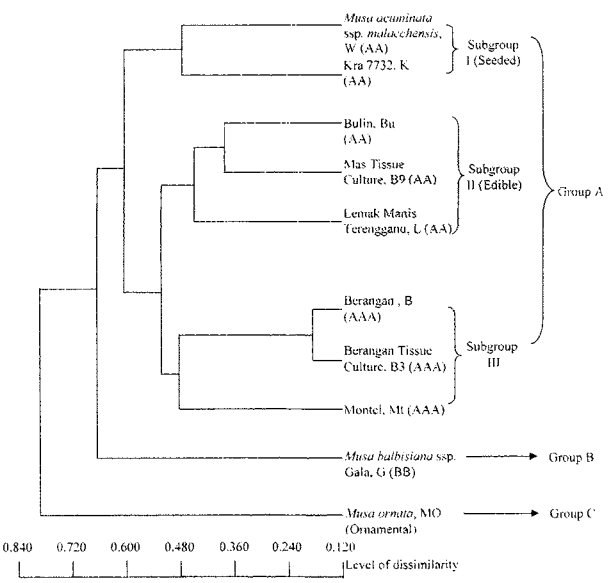

Figure 2. Phylogenetic model of the IRAP data based on sixteen primer combinations for 16 Malaysian banana cultivars. This phylogenetic model suggests that *M. ornata* Roxburgh is the taxon most distantly related to *M. acuminata* Colla subsp. *malaccensis* (AA) followed by *M. balbisiana* Colla subsp. Gala (BB). Group A comprises the largest set of cultivars including seeded bananas Kra 7732 (AA) and *M. acuminata* Colla subsp. *malaccensis* in Subgroup I. Subgroup II contains all the edible bananas (AA) and Nangka Pulau Tioman (AAA). The triploid A-genome cultivars Montel, Nangka Pulau Tioman and Berangan cluster into Subgroup III. Group B comprises the diploid B banana *M. balbisiana* Colla subsp. Gala. Group C comprises *M. ornata* Roxburgh from *Rhodochlamys* section of *Musa* that used as outgroup taxon in this study. Consistent with their origins, the tissue culture derivatives showed high bootstrap or low level of dissimilarity values around the nodes separating them from their parental stocks.

The association of bands with particular genome types can be explained as the result of the integration of new retrotransposon copies after divergence of the ancestral genomes. The power of IRAP to identify the genome type was confirmed by comparing our results with genome classifications of local bananas based on morphological techniques (Stover and Simmonds, 1987). While the conventional phylogenetic analysis assuming divergence from a common ancestor cannot correctly identify the relationships of individual genomes in hybrids, individual bands from retrotransposons analysed here are clearly of value in characterizing the genomes present (Fig. 1), and close analysis of larger numbers of genome-specific bands may enable the relationships of the individual genomes to be ascertained. This will help breeders to clearly identify and screen the genotypes with better production value and manage the genotype resources of bananas that sometime made difficult by synonym names.

Comparison between Tissue Culture and Field (*ex Situ*) Materials

The two accessions of Berangan from different sources had dissimilarity value of 0.180 showing their close relationship but divergence during culture. Tissue culture of plants is an important means to propagate genetically identical individuals asexually and to produce transgenic plants. However, tissue culture-induced mutations have been reported in many plant species and seem to be ubiquitous (Hirochika et al., 1996). The first active retrotransposon to be identified in plants, *Tnt*-1 of tobacco, was isolated by virtue of its mutagenicity in tissue culture (Grandbastien et al., 1989), and this property of retrotransposons was also reported in rice (Hirochika et al., 1996). Here, we have used IRAP with *BARE*-1 and RT primers to track the integration of new retrotransposons into the genome of bananas during tissue culture. Total genomic DNA was isolated from tissue-cultured banana plantlets of Berangan and Abu Baru, and new bands were observed compared to the source material (arrows in Fig. 1A, B, C, F and G). This was similar to the results reported by Nakayashiki and coworkers (2001) in *Magnaporthe grisea* that showed new bands

appeared during the tissue culture process. Not only did the IRAP marker system distinguish *Musa* cultivars, it also detected new insertions in lines regenerated from tissue culture. Retrotransposons are generally thought to be activated by tissue culture. Among the *Tos10*, *Tos17* and *Tos19* elements of rice (Hirochika et al., 1996), *Tos17* is in fact only active in tissue culture and its copy number increased with prolonged culture. The copy number of tobacco retrotransposon *Tto1*, normally an inactive element, can increase 10-fold during tissue culture (Hirochika, 1993).

DISCUSSION

The degree of polymorphism of the IRAP products using barley primers in *Musa* was high (~94.02%, Table 4), similar to that observed in sixteen species of *Hordeum* (Kalendar et al., 1999). Table 3 shows that all the primer combinations gave high degree of polymorphism, except for the 3'LTR-3'LTR combination. This suggests that the integration level of *copia*-retrotransposons in tail-to-tail orientation in *Musa* is lower than the other two orientations (head-to-tail and head-to-head). Although retrotransposon markers have previously been applied in the study of cereals (Kalendar et al., 1999; Kalendar et al., 2000; Manninen et al., 2000; Boyko et al., 2002) and Poaceae (Baumel et al., 2002), the work reported here is the first application for banana or non-grass monocots.

The utility and polymorphism of barley-based primers within the *Musa* genome are perhaps surprising. One might expect that retrotransposons sufficiently ancient to be conserved between such divergent genera would not be recently active and therefore not display insertional polymorphism within non-native genome such as in *Musa*. However, a recent survey of transcribed retrotransposons represented within the expressed sequence tag (EST) databases (Vicient and Schulman, 2002) indicates that grasses, and therefore monocots more generally, may share active and conserved families of retrotransposons. The insertion of new *BARE*-1 elements in the Berangan and Abu Baru regenerants demonstrates that a retroelement family is active in *Musa* as well, consistent with its widespread translation in the grasses (Vicient et al., 2001), and provides a means of isolating newly inserted active members for further analysis. The practical implication is that retrotransposon-based primers may be applied directly across divergent genera in orphan crops.

Received August 25, 2004; accepted January 5, 2005.

LITERATURE CITED

Baumel A, Ainouche M, Kalendar R, Schulman AH (2002) Inter-retrotransposon amplified polymorphism (IRAP), and retotransposon-microsatellite amplified polymorphism (REMAP) in populations of the young allopolyploid species *Spartina angelica* Hubbard (Poaceae). Mol Biol Evol 19: 1218-1227

Boyko E, Kalendar R, Korzun V, Gill B, Schulman AH (2002) Combined mapping of *Aegilops tauschii* by retrotransposon, microsatellite, and gene markers. Plant Mol Biol 48: 767-790

Flavell AJ, Dunbar E, Anderson R, Pearce SR, Hartley R, Kumar A (1992) *Ty1-copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucl Acids Res 20: 3639-3644

Flavell AJ, Knox MR, Pearce SR, Ellis THN (1998) Retrotransposon-based insertion polymorphisms (RBIP) for high throughput marker analysis. Plant J 16: 643-650

Grandbastien M-A, Spielmann A, Caboche M (1989) *Tnt1*, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. Nature 337: 376-380

Hirochika H (1993) Activation of tobacco retrotransposons during tissue culture. EMBO J 12: 2521-2528

Hirochika H, Sugimoto K, Otsuki Y, Tsugawa H, Kanda M (1996) Retrotransposons of rice involved in mutations induced by tissue culture. Proc Natl Acad Sci USA 93: 7783-7788

Horry JP, Ortiz R, Arnaud E, Crouch JH, Ferris RSB, Jones DR, Mateo N, Picq C, Vuylsteke (1997) Banana and plantain. *In* D Fuccillo, L Sears, P Stapleton, eds, Biodiversity in Trust. (Conservation and Use of Plant Genetic Resources in CGIAR Centres), Cambridge University, London, pp 67-81

Iwamoto M, Nagashima H, Nagamine T, Higo T, Higo K (1999) *p-SINE1*-like intron of the *CatA* catalase homologs and phylogenetic relationships among AA-genome *Oryza* and related species. Theor Appl Genet 98: 853-861

Kalendar R, Grob T, Regina M, Suoniemi A, Schulman AH (1999) IRAP and REMAP: Two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 98: 704-711

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH (2000) Genome evolution of wild barley (*Hordeum spontaneum*) by *BARE*-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc Natl Acad Sci USA 97: 6603-6607

Kumar A, Bennetzen J (1999) Plant retrotransposons. Ann Rev Genet 33: 479-532

Manninen O, Kalendar R, Robinson J, Schulman AH (2000) Application of *BARE*-1 retrotransposon markers to the mapping of a major resistance gene for net blotch in barley. Mol Gen Genet 264: 325-334

Nakayashiki H, Ikeda K, Hashimota Y, Tosa Y, Mayama S

(2001) Methylation is not the main force repressing the retrotransposon MAGGY in *Magnaporthe grisea*. Nucl Acids Res 29: 1278-1284

Pearce SR, Harrison G, Li D, Heslop-Harrison JS, Kumar A, Flavell AJ (1996) The *Ty1-copia* group of retrotransposons in *Vicia* species: Copy number, sequence heterogeneity and chromosomal localisation. Mol Gen Genet 205: 305-315

Pearce SR, Knox M, Ellis THN, Flavell AJ, Kumar A (2000) Pea Ty1-*copia* group retrotransposons: transpositional activity and use as markers to study genetic diversity in *Pisum*. Mol Gen Genet 263: 898-907

Pearce SR, Stuart-Rogers C, Knox MR, Kumar A, Ellis THN, Flavell AJ (1999) Rapid isolation of plant Ty1-copia group retrotransposon LTR sequences for molecular marker studies. Plant J 19: 711-717

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425

SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL (1996) Nested retrotransposons in the intergenic regions of the maize genome. Science 274: 765-768

Shimamura M, Yasue H, Ohshima K, Abe H, Kato H, Kishiro T, Goto M, Munechika I, Okada N (1997) Molecular evidence from retroposons that whales form a clade within even-toed ungulates. Nature 388: 666-670

Shirasu K, Schulman AH, Lahaye T, Schulze-Lefert P (2000) A contiguous 66 kb barley DNA sequence provides evidence for reversible genome expansion. Genome Res 10: 908-915

Stover P, Simmonds NW (1987) Bananas, Ed 3. Longman, London

Tatout C, Warwick S, Lenoir A, Deragon JM (1999) Sine insertions as clade markers for wild *Crucifer* species. Mol Biol Evol 16: 1614-1621

Teo CH, Tan SH, Othman YR, Schwarzacher T (2002) The cloning of Ty1-copia-like retrotransposons from 10 varieties of banana (*Musa* sp.). J Biochem Mol Biol Biophys 6: 193-201

Vicient CM, Jääskeläinen M, Kalendar R, Schulman AH (2001) Active retrotransposons are a common feature of grass genomes. Plant Physiol 125: 1283-1292

Vicient CM, Schulman AH (2002) *Copia*-like retrotransposons in the rice genome: few and assorted. Genome Lett 1: 35-47

Voytas DF, Cummings MP, Konieczny AK, Ausubel FM, Rodermel SR (1992) *Copia*-like retrotransposons are ubiquitous among plants. Proc Natl Acad Sci USA 89: 7124-7128

Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT, Powell W (1997) Genetic distribution of *BARE*-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). Mol Gen Genet 253: 687-694