GENETIC DIVERSITY OF SOME BANANAS IN THAILAND USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM

Varinthorn Yeerong[1], Supachai Vuttipongchaikij[1,2], Somsak Apisitwanich[1,2], Saowanee Suputtitada[1,2]*

[1]Department of Genetics Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[2]Center for Advanced Studies in tropical Natural Resource, National Research University, KasetsartUniversity, Bangkok 10900, Thailand
*e-mail: fscisns@ku.ac.th

Abstract

Bananas are important crop in tropical region, especially Africa and Asia. Twenty-four countries from Africa, Asia, Australia and Europe are involved in the Musa Genomic Consortium (AGC). They try to reveal all banana genomes. Our study, classification and genetic relationships of some Thai bananas using 2 pairs of restriction enzymes, *Eco*RI/*Msp*I and *Eco*RI/*Hpa*II were introduced. Twenty primer pairs with 3 selective bases of each AFLP set were applied to amplify on 17 bananas. Genetic similarlity among samples ranged from 0.64-1.00 in *Eco*RI/*Msp*I and 0.60-1.00 in *Eco*RI/*Hpa*II. Bananas were separated into 2 main groups. The first group were bananas having *A* genomes, as *AA, AAA* and *AAB*. The second group were bananas having *B* genomes, as *BB, ABB* and *ABBB* etc. Moreover, 2 phylogenetic trees showed 8 banana accessions in the same subgroups. These results were confirmed by each other. AFLP technique is fruitful for genetic classification and revealing genetic relationship among banana cultivars with accurate and repeatable results. The PIC score of our research ranged from 0.12-0.30 and its average was 0.23. Six primer pairs provided higher value than the average were selected. These primers would be useful for further study on specific primer to indicate genome or species identification.

Keywords: AFLP, genetic diversity, banana, Musa

Introduction

Bananas and plantains are ones of the most important crops in tropical and subtropical areas. They are perennial herbs and belong to the Musa genera. The Musa genus is divided into five sections: Rhodochamy, Australimusa, Callimusa, Ingentimusa and Eumusa. Most cultivated bananas and widely distributied bananas are in Eumusa section. Banana cultivars are expected to be originated from two wild diploid species, *M. balbisiana* Colla (*B* genome) and *M. accuminata* Colla (*A* genome). Bananas are divided into two groups, dessert sweet banana group, having *A* genome and cooking banana group having *B* genome. The species type of *A* genome is *M. accuminata*, and *M. balbisiana*is the species type for *B* genome (Valmayor et al. 2000)**.**

Recently, molecular markers have been used to identify and study genetic diversity in many plants. Genetic diversity in banana genomes has been revealed by different types of molecular markers, including restriction fragment length polymorphism (RFLP), random

amplified polymorphic DNA (RAPD), suppression subtractive hybridization (SSH), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP). Among molecular markers, the AFLP is widely used for analyzing genetic diversity. Many scientists used the AFLP technique to study and classify samples in the *Musa* genus and *M. accuminata*subspecies (Wong et al. 2001; Ude et al. 2002) and *M. balbisiana* (Wang et al. 2007). Moreover, this technique provides genetic diversity and relationships among banana cultivars (Ude etal. 2002; Phothipanet al. 2006). The objective of this study was to verify the genetic relationship of some bananas in Thailand by comparing two sets of AFLP markers.

Methodology

Plant materials
Seventeen accessions of bananas containing two samples of *M. accuminata*, two samples of *M. balbisiana* and 13 samples of banana cultivars (Table 1) were collected from Pakchong research station, Kasetsart University, NakornRachasima. Genomic DNA of all samples were extracted from cigar leaves by modified CTAB method.

AFLP and the genetic relationships
DNA samples were digested and two pairs of adapters *Eco*RI/*Msp*I and *Eco*RI/*Hpa*II were ligated into both ends of DNA fragments. Each 20 primer pairs were used for selective amplification. PCR products were separated by polyacrylamide electrophoresis and stained with silver nitrate. The AFLP bands werescored as "1" and "0" for presence and absence of band. All binumeric data were analyzed with the software NTSYS-pc 2.10. Similarity coefficients were calculated using simple matching. Clustering was grouped by the unweighted pair group method with arithmetic average (UPGMA) and constructed phylogenetic tree using SAHN. The polymorphic information content (PIC) of each marker was analyzed by the software PowermarkerV3 (Botstein et al.1980).

Results

AFLP analysis revealed 20 primer pairs that could generate polymorphic bands. Twenty primer pairs provided 347 bands and the average was of 18 bands per primer pair. Bands ranging from 75 to 530 bp were scored. Genetic similarity among samples ranged from 0.64-1.00 in AFLP-*Eco*RI/*Msp*I and 0.60-1.00 in AFLP-*Eco*RI/*Hpa*II. Two phylogenetic trees were constructed and showed the similarity among banana accessions, according to bands of AFLP (Table 1). The first phylogenetic tree (Figure 1A) was constructed from genetic relationship metrics of AFLP-*Eco*RI/*Msp*I and the second phylogenetic tree (Figure 1B), was constructed from genetic relationship metrics of AFLP-*Eco*RI/*Hpa*II. Both AFLPs gave the same result of two main groups. The first main group consisted of bananas which were *AA*, *AAA* and *AAB*, the other main group consisted of bananas which were *BB*, *ABB*, *BBB* and *ABBB*. Both sets of AFLP could classify bananas in 3 same subgroups. They were *AA* and *AAA* subgroup, *AAB* subgroupand *ABB* and *ABBB* subgroup.The first subgroup consisted of three bananas (10, 13 and 15),The second group consisted of 2 bananas (16 and 17) and the last group consisted of 3 bananas (4, 6 and 8). There were 9 left bananas which were in different subgroups of both phylogenetic trees.

PIC values of two primer sets in this study ranged from 0.12-0.30, and the mean PIC was 0.23 and the only 6 primer pairs were higher than the mean. The minimum PIC value was

from the E-ACC+Msp-GAA primer pair and the maximum PIC value was fromE-ACG+Msp-GGA primer pair. (Table 2)

Discussion

Genetic relationship of some Thai bananas has been assessed by AFLP technique using two pairs of restriction enzymes. Similarity indices and phylogenetic trees showed two main groups of, *AA, AAA, AAB* and *BB, BBB, ABB, ABBB* bananas. This result conforms to the previous reports of Ude et al. (2002) and Phothipan (2006). Two accessions of *M. balbisiana* (*BB* genome) were separated into two subgroups, as reported by Arjcharoen (2010) and they were proposed to be B_N andB_E.genomes. This research, the *Msp*I and *Hpa*II were used. They were recognized the same recognition site (5'-CCGG-3') but were differently sensitive to methylation. The *Hpa*II is sensitive to double strand C-methylated DNA but *Msp*I is sensitive to the external single and double strand C-methylated DNAs.This epigenetic phenomena could be fruitful for complex genome or closed related genome analysis (Cervera et al. 2000; Peraza-Echeverria et al. 2001). For PIC value of primer study, 6 primer pairs provided higher value than the average. These primers would be used for further study on specific primer for indicated genome or species identification.

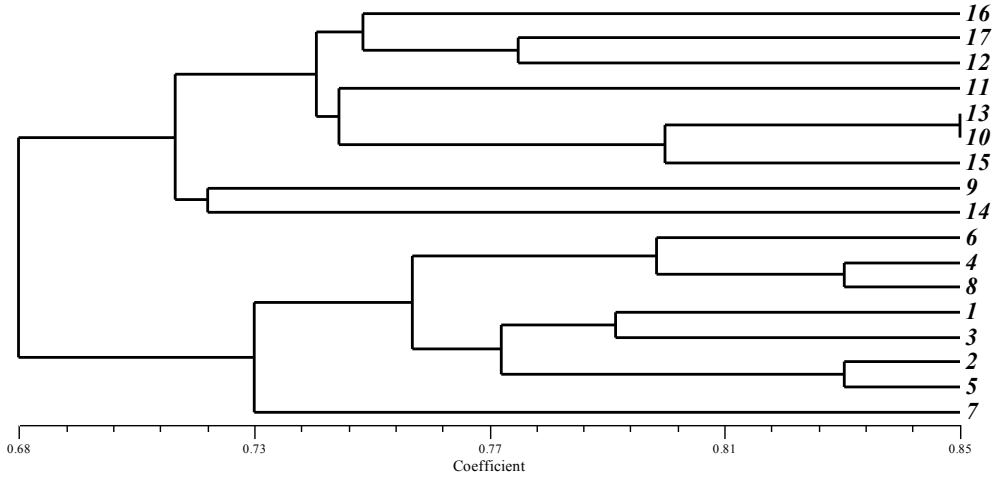
Conclusion

The genetic diversity of 17 banana accessions using 2 sets of AFLP, genetic similarities generated 2 phylogenetic trees with the same main groups. This showed the efficacy of AFLP. PIC analysis was introduced to select 6 primer pairs that were higher than the average for further study on other cultivated bananas in Thailand.

Table 1 Banana accessions used in this study and their genomes

No	Scientific name	Genomes
1	*Musa balbisiana* (KluaiTaniEisan)	*BB*
2	*Musa balbisiana* (KluaiTaniNuea)	*BB*
3	*Musa x paradisiaca* 'KluaiNamwaNaun'	*ABB*
4	*Musa x paradisiaca* 'KluaiNamwaKhom'	*ABB*
5	*Musa x paradisiaca* 'KluaiHakmuk Thong'	*ABB*
6	*Musa x paradisiaca* 'KluaiHakmukKhiao'	*ABB*
7	*Musa balbisiana* (KluaiLeb Chang Kud)	*BBB*
8	*Musa x paradisiaca* 'KluaiThep Pharos'	*ABBB*
9	*Musa accuminata* (Kluai Pa Pare)	*AA*
10	*Musa accuminata* 'KluaiHomJumpa'	*AA*
11	*Musa accuminata* (Kluai Pa Presom)	*AA*
12	*Musa accuminata* 'KluaiKhai'	*AA*
13	*Musa accuminata* (KluaiHom Thong)	*AAA*
14	*Musa accuminata* (KluaiHomKhiaoKhom)	*AAA*
15	*Musa accuminata* (KluaiNak)	*AAA*
16	*Musa x paradisiaca* 'KluaiRoiwee'	*AAB*
17	*Musa x paradisiaca* 'KluaiNamfad'	*AAB*

A.



B.

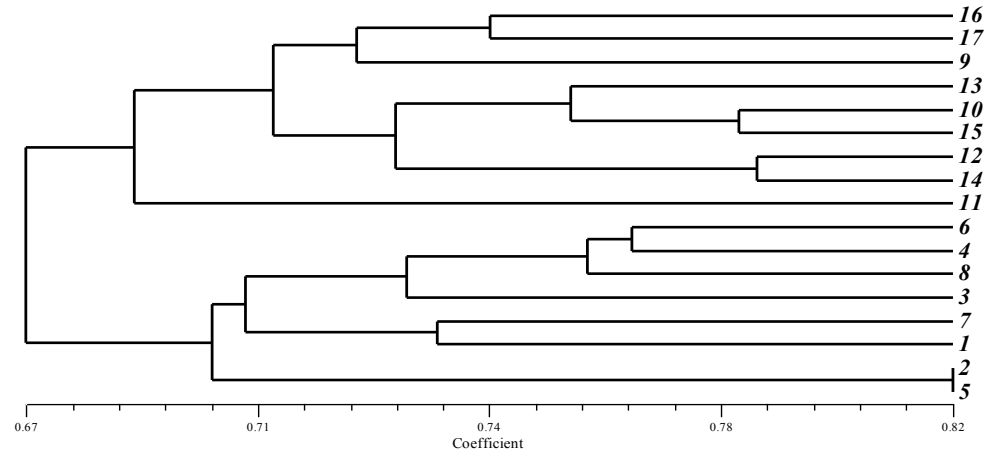


Figure1. Phylogenetic tree of 17 banana accessions from 2 genetic similarity-data sets of A. AFLP-*Eco*RI/*Msp*I and B. *Eco*RI/*Hap*II. Numbers 1-17 refer to the *Musa* accessions in Table 1.

Table 2 The polymorphic information content (PIC) of selected primer pairs

primer pairs	PIC		primer pairs	PIC	
	*Msp*I	*Hpa*II		*Msp*I	*Hpa*II
E-AGC+Msp-GTT	0.18	0.23	E-ACA+Msp-GCT	0.19	0.22
E-ACC+Msp-GGT	0.19	0.21	E-ACC+Msp-GCT	0.29	0.28
E-ACT+Msp-GGT	0.24	0.26	E-ACT+Msp-GCT	0.26	0.28
E-ACG+Msp-GGA	0.3	0.29	E-AAG+Msp-GCA	0.13	0.12
E-AAC+Msp-GGA	0.26	0.26	E-AAG+Msp-GAA	0.17	0.23
E-AGC+Msp-GGA	0.22	0.21	E-AGG+Msp-GAA	0.17	0.13
E-AGG+Msp-GAT	0.29	0.27	E-ACA+Msp-GAA	0.13	0.12
E-ACG+Msp-GAT	0.2	0.23	E-AGC+Msp-GAA	0.22	0.22
E-AAC+Msp-GAT	0.22	0.22	E-ACC+Msp-GAA	0.23	0.25
E-ACT+Msp-GAT	0.23	0.26	E-ACT+Msp-GAA	0.21	0.23

Acknowledgments

This research is partially supported by Kasetsart University Research and Development Institute.

References

1. Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage mapping man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314-331
2. Arjcharoen A, Silayoi B, Wanichkal K, Apisitwanich S (2010) Variation of *B* Genome in Musa Accessions and Their New Identifications. Kasetsart J.(Nat. Sci.) 44:392–400
3. Cervera MT, Remington D, Frigerio JM, Storme V, Ivens B, Boerjan W, Plomion C. (2000) Improved AFLP analysis of tree species. Can. J. For. Res 30:1608-1616
4. Peraza-Echeverria S, Herrera-Valencia VA, Kay AJ (2001) Detection of DNA methylation changes in micropropagated banana plants using methylation-sensitive amplification polymorphism (MSAP). Plant Sci 161: 3359-367.
5. Simmonds NW, K Shepherd (1955) The taxonomy and origin of the cultivated bananas.J. Linn. Soc. (Bot.) 55:302-312.
6. Phothipan S (2006) Ascertaining Genetic Lineage of Banana Varieties in *AA, AAB* and *BB* group using Specific-PCR, RAPD, SRAP and AFLP technique. M.S.Thesis, Kasetsart University, Bangkok.
7. Ude G, Pillay M, Nwakanma D, Tenkouano A (2002) Genetic diversity in *Musa acuminata*Colla and *Musa balbisiana*Colla and some of their natural hybrids using AFLP markers. Theor. Appl. Genet 104:1246-1252.
8. Ude G, Pillay M, Ogundiwin E, Tenkouano A (2003) Genetic diversity in an African plantain core collection using AFLP and RAPD markers. Theor. Appl.Genet 107: 248-255
9. Valmayor RV, Jamaluddin SH, Silayoi B, Kusumo S, Danh LD, Pascua OC, Espino RRC (2000) Banana Cultivar Names and Synonyms in Southeast Asia. INIBAP Asia and Pacific Office, Los Banos, Laguna,Philippines. 24 pp.
10. Wang XL, Chiang TY, Roux N, Hao G, Ge XJ (2007) Genetic diversity of wild banana (*Musa Balbisiana*Colla) in China as revealed by AFLP markers.Genet. Resour. Crop Evol 54:1125-1132
11. Wong C, Kiew R, Loh JP, Gan LH, Set O, Lee SK, Ohn S, Lum S, Gan YY (2001) Genetic diversity of the wild banana *Musa acuminata*Colla in Malaysia as evidenced by AFLP. Ann. Bot. (Lond) 88:1017-1025