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Genetic Relationship among Bananas in AA, AAB and BB Groups Using Random Amplified Polymorphic DNA (RAPD) and Sequence Related Amplified Polymorphism (SRAP) Techniques

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ABSTRACT

RAPD and SRAP techniques were introduced to analyse the genetic relationship among 29 accessions of banana in AA, AAB and BB groups at Department of Genetics and Department of Horticulture, Kasetsart University, Thailand. The genetic similarity and UPGMA were determined. The results showed that the SRAP technique was very similar to the RAPD technique for detecting genetic polymorphism and genetic relationship. The phylogenetic tree of RAPD and SRAP data showed two main clusters, AA-AAB and BB banana genome accessions. The BB group consisted of two subgroups with 8 cultivars. Within this group, Kluai Pa from Nakhon Si Thammarat and Kluai Pa from Na Khom, Kluai Tani Eisan and Kluai Tani Dam showed very close relationships. In the AA-AAB, it consisted of 21 cultivars. It was divided into three subgroups. First subgroup consisted of 6 from 10 AAB bananas. These cultivars were placed close to Kluai Pa Phrae. The second subgroup consisted of 4 AAB bananas, Kluai Klai, Kluai Nga Chang, Kluai Khom and Kluai Nom Sao, and all AA bananas except only 'Kluai Pa Abisiania' which was in the forth subgroup. The cultivar Kluai Klai and Kluai Nga Chang (AAB) were more closely related to Kluai Flava and Kluai Pa Pli Som while Kluai Khom and Kluai Nom Sao were closer to acuminata cultivars than the others.

Keywords: bananas, AA group, AAB group, BB group, RAPD technique, SRAP technique

INTRODUCTION

Bananas and Plantains (*Musa* spp.) are one of the most important crops in Thailand. They belong to Musaceae family consisting of three genera, *Musa*, *Ensete* and *Musella*. The genus *Musa* is divided into five sections according to the basic chromosome number and morphological characters. They are *Australimusa, Callimusa, Eumusa, Rhodochlamys* and *Ingentimusa* (Silayoi, 2002). The section *Eumusa* is the largest and most diversified. It includes the diploid wild ancestors of modern bananas, *M. acuminata* (AA) and *M. balbisiana* (BB) Colla, which contribute the A and B genomes, respectively, to the cultivated bananas.

Five subspecies of *Musa acuminata* are s*iameae, burmanica, microcarpa, malaccensis* and *banksii*. There is no record of any subspecies in *Musa balbisiana.* (Silayoi, 2002)

The classification was done by using Simmond and Shepherd's scoring method together with the chromosome number. They were grouped

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into *AA, AAA, AB, AAB, ABB, ABBB, BB* and *BBB* genomic constitutions. AAB group is divided into two subgroups, edible or sweet banana and plantain or cooking banana.

Recently, DNA markers that are more abundant than morphological characters and free from environmental influence have been used in genetic diversity studies of plant including *Musa*. Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) or sequence-related amplified polymorphism (SRAP) have been particularly useful in *Musa* diversity studies. But these techniques need technically skillful persons.

The objectives of this study were to use RAPD and SRAP techniques to analyse the genetic relationship among bananas in AA, AAB and BB groups.

MATERIALS AND METHODS

Plant materials

Twenty nine accessions (Table 1) of 11 *M. acuminata* (*AA* genome), 8 *M. balbisiana* (*BB* genome) and 10 triploid hybrids AAB group were collected from Pak Chong Research Station, Nakhon Ratchasima and Department of Horticulture, Kasetsart University, Bangkok, Thailand.

DNA extraction

Genomic DNAs were extracted from the banana's leaves of all accessions according to Agrawal *et al*. (1992) and quantified. DNA concentrations were measured according to the absorbance at λ 260 nm by a UV spectrophotometer.

RAPD technique

PCR amplifications were performed using eleven of 10-mer oligonucleotide primers (Operon Technologies Inc., Alameda, USA). The PCR was carried out with the initial cycle at 94°C for 3 min then 40 cycles of 94°C for 1 min, 35°C or 45°C for 1 min, 72°C for 2 min and final extension at 72°C for 5 min. The PCR products were separated by eletrophoresis on 1.5% agarose gel, stained with ethidium bromide, detected under UV transilluminator and photographed.

SRAP technique

Forty-one primer pairs were used and amplified by SRAP-PCR. The PCR was carried out with the initial cycle at 94°C for 3 min, 5 cycles of 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, another 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min and the final extension at 72°C for 5 min. The PCR products were separated by polyacrylamide gel electrophoresis

Genetic relationship Analysis

Selected bands from DNA fingerprint of RAPD and SRAP. Techniques were changed to binary data (0 and 1) and were analyzed with the software NTSYS-pc 2.01. Similar coefficients were calculated using simple matching. Clustering was grouped using unweighted pair group method with arithmetic average (UPGMA).

RESULTS

RAPD analysis

RAPD could generate 76 polymorphic bands among the 29 accessions of bananas. Four out of eleven primers, OPA-03, OPA-04, OPA-10 and OPA-13, showed highly polymorphic bands. Primer OPA-04 revealed that K. Pa Abisiania could be distinguished from the other cultivars by the absence of a 450 bp band (Figure 1). Amplification with primer OPC-15 showed that cultivar K. Leb Mu Nang, K. Nam Nom, K. Sa and K. Hom Champa had a clear band at 1,000 bp (Figure 1).

The Phylogenetic tree from RAPD (Figure 2) showed two main clusters, AA-AAB and BB banana genome accessions. The BB group

consisted of two subgroups with 8 cultivars. Within this group, some cultivar were closely related such as K. Tani Nua and K. Tani Dam, K. Tani from Ubon Ratchathani and K. Tani Eisan, K. Na Khom and K. Tani from Phrae. The AA-AAB cluster consisted of 21 cultivars. It was divided into three subgroups. The first subgroup consisted of 6 from 10 AAB bananas. The second subgroup consisted of 4 AAB bananas, K. Klai, K. Nga Chang, K. Khom and K. Nom Sao, and all AA bananas except only K. Pa Abisiania which was in the forth subgroup. The cultivar K. Klai and K. Nga Chang (AAB) were closer to K. Flava and K. Pa Pli Som than to the others.

Table 1 List of *Musa* accessions used in this study.

No.	Genome	Scientific name
A. Wild balbisiana		
1.	*BB*	*Musa balbisiana* (Kluai Pa) from Nakhon Si Thammarat
2.	*BB*	*Musa balbisiana* (Kluai Pa) from Na Khom, Lampang
3.	*BB*	*Musa balbisiana* (Kluai Tani) from Phrae
4.	*BB*	*Musa balbisiana* (Kluai Tani Nua) from Phrae
5.	*BB*	*Musa balbisiana* (Kluai Tani) from Ubonratchathani
6.	*BB*	*Musa balbisiana* (Kluai Tani Eisan) from Buri Ram
7.	*BB*	*Musa balbisiana* (Kluai Tani Tai) from Nakhon Si Thammarat
8.	*BB*	*Musa balbisiana* (Kluai Tani Dam) from the Philippines
B. Hybrids		
9.	*AAB*	*Musa x paradisiaca* 'Kluai Namphad'
10.	*AAB*	*Musa x paradisiaca* 'Kluai Chin'
11.	*AAB*	*Musa x paradisiaca* 'Kluai Rowi'
12.	*AAB*	*Musa x paradisiaca* 'Kluai Nue Mu Nang'
13.	*AAB*	*Musa x paradisiaca* 'Kluai Khai Boran'
14.	*AAB*	*Musa x paradisiaca* 'Kluai Nam'
15.	*AAB*	*Musa x paradisiaca* 'Kluai Klai' (Plantain subgroup)
16.	*AAB*	*Musa x paradisiaca* 'Kluai Nga Chang' (Plantain subgroup)
17.	*AAB*	*Musa x paradisiaca* 'Kluai Khom'
18.	*AAB*	*Musa x paradisiaca* 'Kluai Nom Sao'
C. Acuminata cultivars		
19.	*AA*	*Musa acuminata* 'Kluai Khai'
20.	*AA*	*Musa acuminata* 'Kluai Leb Mu Nang'
21.	*AA*	*Musa acuminata* 'Kluai Homchan'
22.	*AA*	*Musa acuminata* 'Kluai Nam Nom'
23.	*AA*	*Musa acuminata* 'Kluai Sa'
24.	*AA*	*Musa acuminata* 'Kluai Hom Champa' or 'Kluai Pa Phatthalung'
D. Wild acuminata		
25.	*AA*	*Musa acuminata* (Kluai Pa Phrae or Kluai Pa Rongkwang Phrae)
26.	*AA*	*Musa acuminata* (Kluai Pa Abisiania)
27.	*AA*	*Musa acuminata* (Kluai Flava)
28.	*AA*	*Musa acuminata* (Kluai Pa Pli Som)
29.	*AA*	*Musa acuminata* (Kluai Pa Pli Lueang)

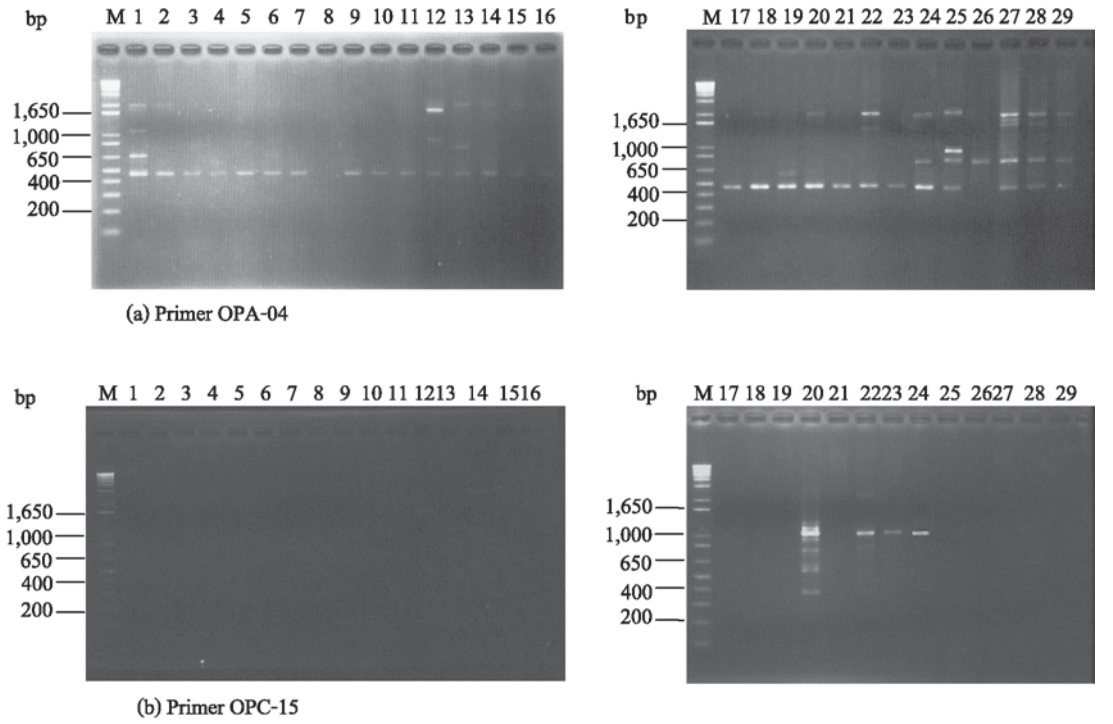

Figure 1 (a) OPA-04 RAPD pattern and (b) OPC-15 RAPD pattern of 29 accessions of banana. Numbers on the top (1-29) refer to the list of *Musa* accessions in Table 1. Lane M is the 1 kb ladder DNA size standard and numbers on the left size are shown in base pairs.

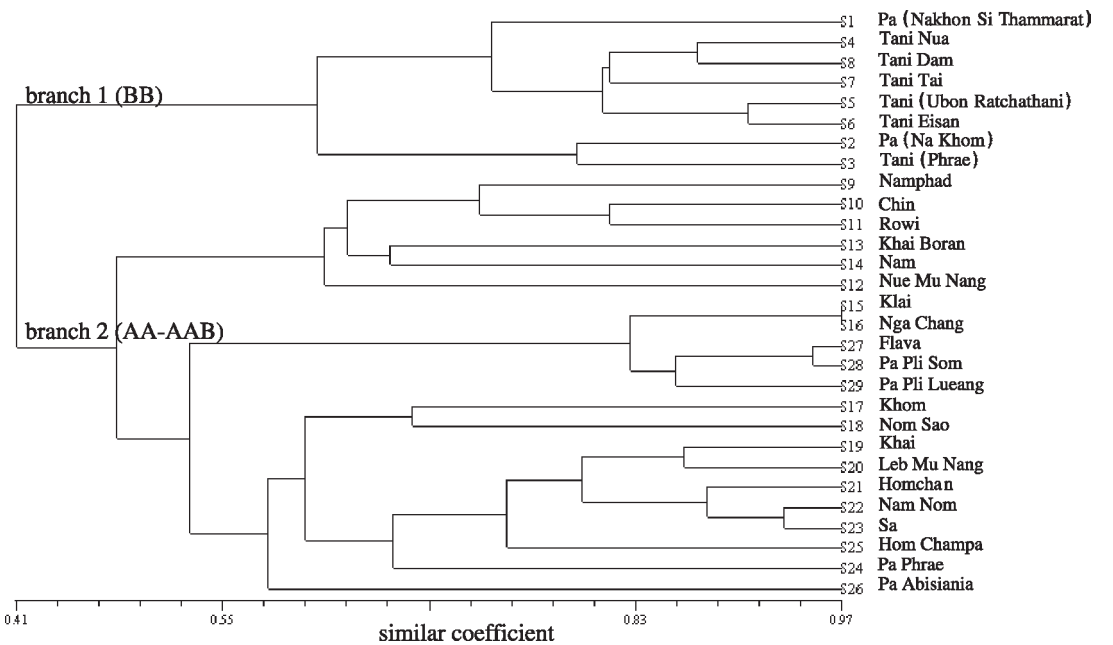

Figure 2 Phylogenetic tree of banana cultivars (RAPD data).

SRAP analysis

From 41 SRAP primer pairs, The eleven pairs were selected. They could generate 129 polymorphic bands ranged from 960 bp to 2,260 bp. The dendrogram from this SRAP was very similar to the RAPD, only a little difference. The cultivars originating from *M. balbisiana* (wild banana with *BB* genome) were on one branch and the cultivars originating from *M. acuminata* (wild banana with *AA* and *AAB* genome) were on the other branch. The first branch consisted of two subgroups with 8 cultivars. Within this group, some cultivars were closely related such as K. Pa from (Nakhon Si Thammarat) and K. Pa (Na Khom), K. Tani Eisan and K. Tani Dam. The second branch separated the 21 cultivars into three subgroups. The first subgroup was composed of accession with hybrid cultivars in AAB group except K. Klai, K. Nga Chang, K. Khom and K. Nom Sao, including the diploid wild banana with *AA* genome K. Pa Phrae. The second subgroup included some of diploid wild banana with *AA* genome, hybrid cultivars in AA group and some of hybrid cultivars in AAB group. Within this group, K. Klai and K. Nga Chang were closer related to K. Flava and K. Pa Pli Som than the others. The last subgroup was K. Pa Abisiania (Figure 3).

Amplification with the primer pair A4-B9 showed the difference between AA-AAB and BB bananas where the band at 340 bp. All cultivars had a 220 bp band except K. Nam (lane 14) and K. Homchan (lane 21) appeared (Figure 4). Primer A7-B6 revealed that K. Klai and K. Nom Sao could be distinguished from the others. Amplification with primer pair A10-B2 showed specific bands to be present only in hybrid cultivars in AA and AAB group at 260 bp band. K. Nam Phad, K. Chin, K. Rowi, K. Khai Boran, K. Nam, K. Klai, K. Khom, K. Nom Sao, K. Homchan, K. Sa, K. Hom Champa and K. Flava had a clear 190 bp band (Figure 5).

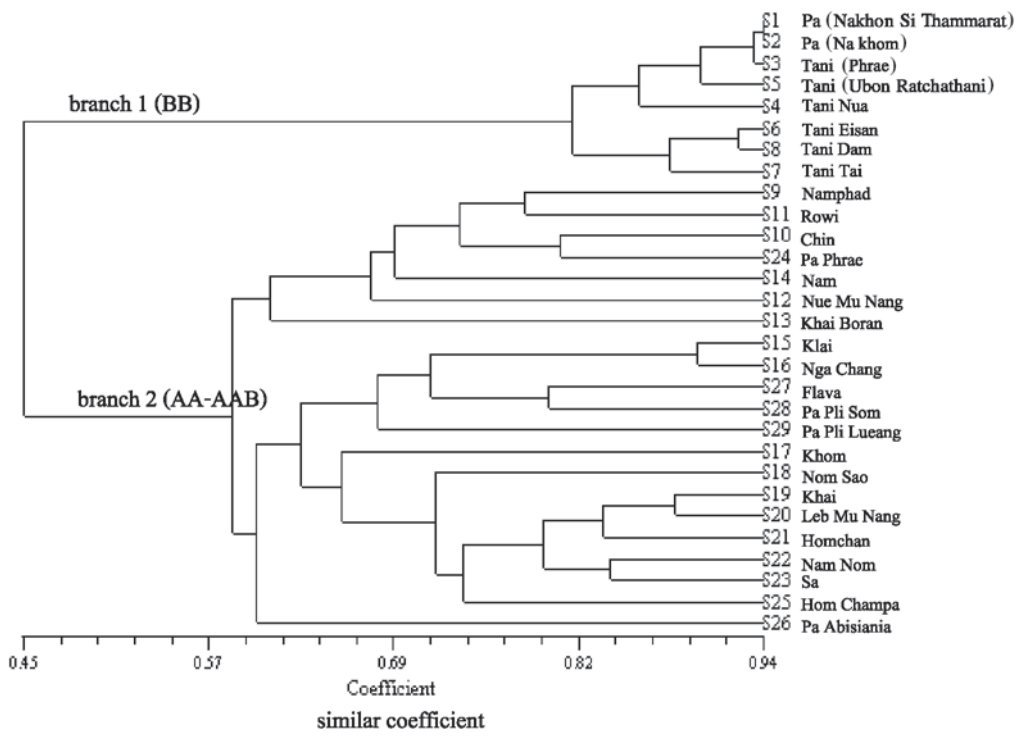

Figure 3 Phylogenetic tree of banana cultivars (SRAP data).

Phylogenetic tree of RAPD and SRAP data

From the RAPD and SRAP data, another phylogenetic tree could be constructed. It was the same as tree from SRAP. The difference was similar coefficients only (Figure 6).

DISCUSSION

Although RAPD-PCR has been successfully used for identification of varieties or cultivars in various plant species, including *Musa* (Crouch *et al.*, 2000), problems have been reported concerning the reproducibility of results. Most of these problems seem to be due to mis-priming of the short decamers at the relatively low temperatures common in RAPDs and competition between different DNA fragments for amplification (Halld'en *et al.*, 1996). In this study

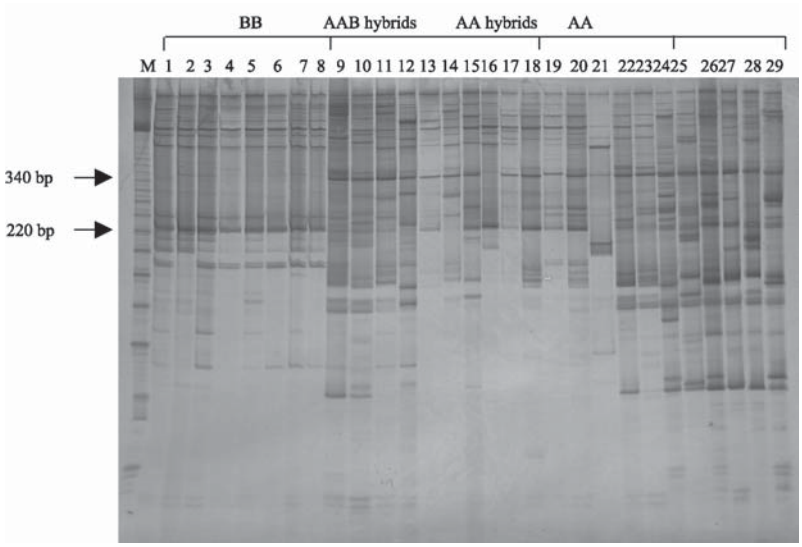

Figure 4 SRAPs amplified by primer A4-B9 in banana cultivars.

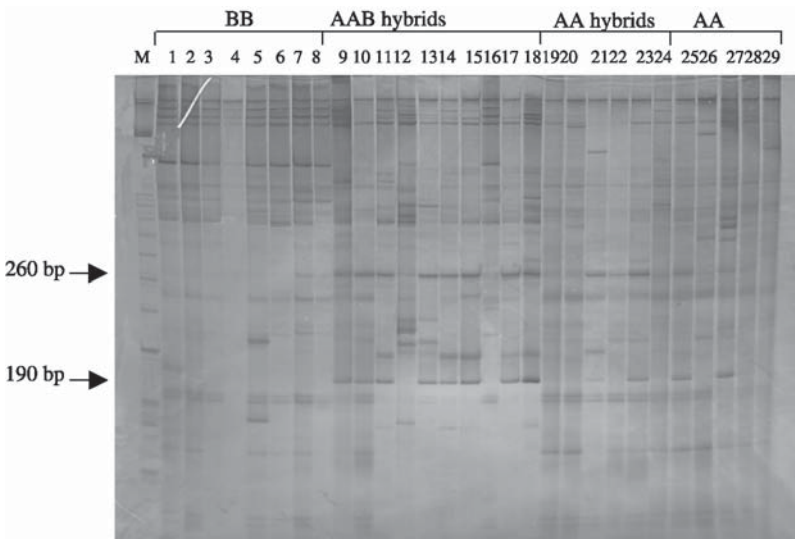

Figure 5 SRAPs amplified by primer A10-B2 in banana cultivars.

an annealing temperature was employed and the optimal temperature was found to be 35°C to 45°C for used primers.

Several studies have compared the effectiveness of different DNA-based marker system to determine relationships in crop plants. SRAP generally reveals more polymorphisms than RAPD and is more reliable for genetic molecular-marker assay (Li and Quiros, 2001).

This study, the combination of RAPD and SRAP data showed similar of genetic clustering and some RAPD or SRAP primer were very good for detecting the very closed cultivars in the same group (Figure 2, 3 and 6). The interesting result was the BB cultivated bananas which was a separate group. The same was found in six accessions of AAB banana. This showed the efficiency of selected RAPD and SRAP markers. The rest of AAB bananas in the AA group was still in doubt and it should be clarified in the future study.

CONCLUSION

Genetic relationship among 29 bananas in AA, AAB and BB groups could be revealed using RAPD and SRAP techniques and NTSYS-pc 2.01 software program for data analysis. The BB banana accessions were separately clustered into one group and also the AA banana accessions. Six from ten accessions of AAB banana were more closely related to Kluai Pa Phare. The rest of AAB bananas: Kluai Klai and Kluai Nga Chang were closer to Kluai Flava and Kluai Pa Pli Som while Kluai Khom and Kluai Nom Sao were closer to acuminata cultivars than the others. This result showed the genetics of cultivated bananas in Thailand and also the efficiency of both RAPD and SRAP techniques.

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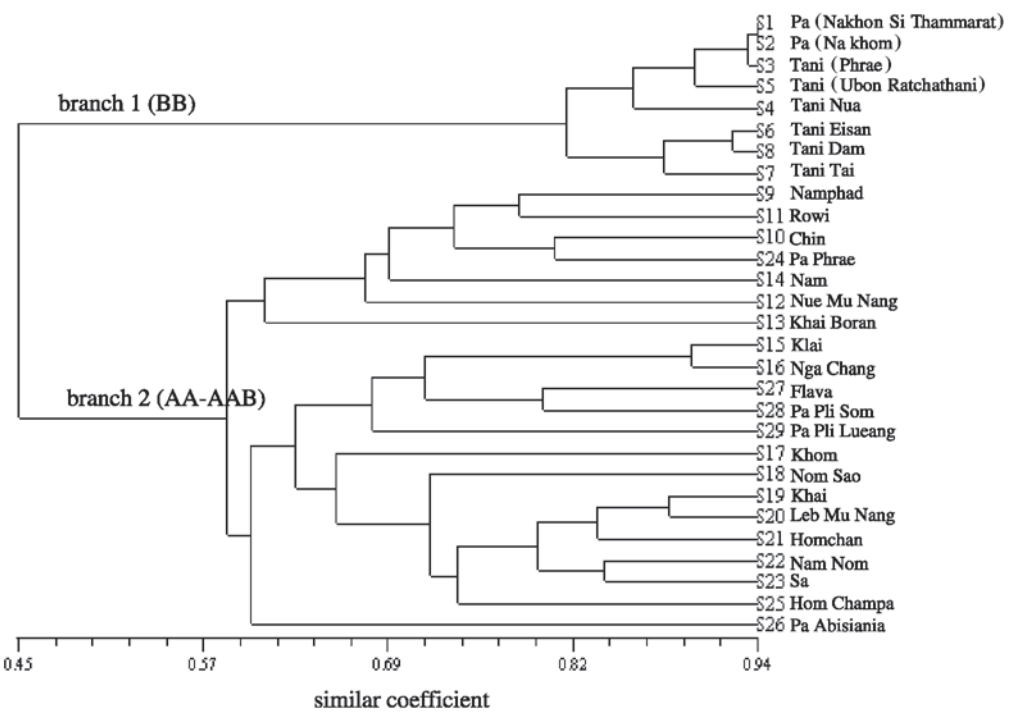

Figure 6 Phylogenetic tree of banana cultivars (combination of RAPD and SRAP data).

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