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Original Article

Analysis based on AFLP markers of the genetic variations and their relationships for pummelo cultivars grown in the central region of Thailand

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Abstract

Using 15 selected AFLP primers, this investigation looks at the genetic diversity and relationships among pummelo (*Citrus maxima* (Burm.) Merrill) cultivars grown in the central region of Thailand, resulting in amplification of 133 reproducible polymorphic fragment products out of 295 band score. The percentages of polymorphic markers for AFLP ranged from 33.33% for E-CGT/M-TGA and E-AAG/M-AGA to 62.50% for E-TAC/M-TCG. The phylogenetic tree dendrogram showed that the 97 leaf samples from 15 pummelo cultivars grown in central region of Thailand could be classified into two groups. The first group consisted of two sub-groups. The first sub-group consisted of *Khaopuang, Takoi, Toolkaol,* and *Khaojook* cultivar. The second one consisted of *Khaopan, Khaohom, Khaonamphueng, Khaoyai, Phaiseethong, Khaotanggwa, Kkewmorakot,* and seedless cultivar. The second group consisted of *Thongdee, Bangkhunnon* and *Tabtim* cultivar. Moreover, the results indicate that the samples collected from the same cultivar in different locations were for the main part genetically similar.

Keywords: pummelo, AFLP, genetic diversity, *Citrus maxima* (Burm.) Merrill

1. Introduction

Pummelo (*Citrus maxima* (Burm.) Merrill) is the world's largest citrus fruit. There is a potential in Thailand for the development this fruit as an export crop because its thick rind makes it easy to handle and transport. Along the banks of the *Tha Chin* River in the central plains area west of Bangkok is a well established pummelo growing area and as a result of the excellent flavor of the fruit grown there the trees are used as a source for worldwide pummelo germplasm.

The original three recorded Thai pummelo cultivars were the necked *Khaopuang*, the flattened or rounded *Khaopan*, and the pink-fleshed *Thongdee*. Recently, a white fleshed variety called *Khaonamphueng* has been developed, which is considered to have a superior flavor and is so becoming a favorite with both fruit growers and domestic consumers alike (Sethpakdee, 2002). Nonetheless, *Thongdee* is still the preferred and favorite export cultivar.

Throughout Thailand new varieties of pummelo are being bred and given different names by the various growing areas resulting in a large number of names as well as confusion. The variability among pummelo cultivar over the names of a single cultivar are still unknown. Information on the genetic diversity of and relationships between pummelo cultivars would be useful to eliminate the confusion and help future pummelo breeding programs and germplasm collections.

Increasingly, molecular marker technologies are playing an important role in assessing genetic diversity, identifying genetic relationships, and aiding germplasm fingerprint-

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ing in plant collections. Over the last few decades a variety of different genetic analytical techniques have emerged in the field of molecular genetics along with several PCR-based genetic markers that have now been established and are used to provide information on genetic variations in plant species. Initially, RAPD was employed for genetic analyses but problems regarding reproducibility had been reported (Jones *et al.,* 1997), so the amplified fragment length polymorphism (AFLP) technique was then introduced because it has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques giving a reliable and reproducible marker system (Vos *et al.,* 1995).

AFLP, a relatively new DNA fingerprinting technique (Vos *et al.,* 1995) uses selective amplification of restriction fragments. It has a high multiplex ratio, does not require DNA probes or prior sequence information, and is now preferred over other DNA-based marker systems in instances where little is known about the genomic structure (Pejic *et al.,* 1998; Garcia-Mas *et al.,* 2000; Vuylsteke *et al.,* 2000; Yuan *et al.,* 2000). In addition, a larger number of loci are detected *per* reaction in comparison with RAPD and it is seen to give a higher precision than RAPD. For example, at the species level the technique is proficient at revealing diversity and effective in covering a wide area of the genome in a single assay (Zhu *et al.,* 1998). The procedure is simple, largely, requires only small amounts of DNA and can be performed without the use of radioactivity (Krap *et al.,* 1996). This PCR-based method generates complex banding patterns of DNA types amplifying up to at least 100 fragments in each reaction. However, despite a few drawbacks to the procedure, it is more intensive and expensive than other procedures, such as RAPD, and it has the potential to be very useful in genetic analysis (Hill *et al.,* 1996; Kardolus 1998; Hussein *et al.,* 2002).

Within perennial fruit cultivars Lopes-Valenzuela *et al*. (1977) have detected substantial genetic variations and also demonstrated that cultivars can be discriminated on the basis of their genetic characteristics. Recent reports have focused on using DNA based markers, particularly AFLP markers, to measure the genetic diversity and relationships in fruit species; such as, cherry (*Prunus avium*) (Gerlach and Stosser, 1997), lemon (*Citrus lemon* L.) (Machado *et al.,* 1996), mango (*Mangifera indica* L.), peach (*Prunus persica* L.), pear (*Pyrus* sp.) (Monte-Corvo *et al.,* 2000), litchi (*Litchi chinensis*) (Panie *et al.,* 2002), citrus (Hussein *et al.,* 2003), Chinese pomegranate (*Punica granatum* L.) (Yuan *et al.,* 2007), and pummelo (Kongsri and Boonprakob, 2008). However, in this study we will use the AFLP technique, which is different to Kongsri and Boonprakob (2008), who used the simple sequence repeat (SSR) to estimate the genetic diversity. Moreover, more pummelo leaf samples per cultivar than previous report will be taken for precise estimation the genetic diversity of pummelo and focusing on pummelo cultivars especially grown in the central region of Thailand. Therefore, the objective of this study was to estimate the genetic diversity and relationships among pummelo cultivars grown in the central region of Thailand using AFLP analyses.

2. Materials and Methods

2.1 Plant material

Fresh young leaf samples were taken from 15 pummelo cultivars at various locations of the central region of Thailand. Leaves were collected from 2-3 plants *per* cultivar *per* collection site; a total of 97 samples (see Table 1).

2.2 DNA extraction

DNA was extracted using a modified CTAB protocol (Doyle and Doyle, 1990). From the leaves collected 20 mg were ground and placed in a 1.5 ml microfuge tube over liquid nitrogen. Next, 700 µl of preheated extraction buffer containing 2% CTAB, 100 mM Tris-Cl, 1.4 M NaCl, 20 mM EDTA, 0.625% 2-Mercaptoethanol and 3% PVP was added and the mixture incubated for 30 min at 65°C and then placed on ice for 10 min. Following this, a further mixture was made by adding 300 µl of 5 M potassium acetate and placed back on the ice for a further one hour. The resulting cooled mixture was then placed in a centrifuge and spun for 10 min at 14,000 rpm. The supernatant was poured into a new microfuge tube and 700 µl of chloroform:isoamylalcohol at 24:1 was added. This mixture was then centrifuged for 20 min at 14,000 rpm and the supernatant poured into a new microfuge tube and mixed with an equal amount of ice-cold 95% ethylalcohol (EtOH) for 5 min. Using the centrifuge again the mixture was centrifuged for 5 min at 14,000 rpm and the supernatant poured into a new microfuge tube and mixed with 500 µl of 70% EtOH and centrifuged for 5 min at 14,000 rpm after which the supernatant was reined out. The subsequent produced DNA pellet was dried at 65°C and then 200 µl of 1X TE buffer was added to the pellet and incubated at 65°C for 1 hr. Finally, the DNA was kept at -20°C in freezer waiting for AFLP analysis.

2.3 Amplified fragment length polymorphism analysis

AFLP analysis was conducted as described by Vos (1995) with some modifications. Initially/first Genomic DNA (100 ng) was digested for 3 hrs at 37°C to a final volume of 25 µl with 10 units of *Eco*RI and 10 units of *Mse*I in 1X R/L restriction/ligation buffer (33 mM Tris-HCl, pH 7.5, 10 mM potassium chloride, 0.5 mM DTT). To this mixture was added 10 µl of ligation mix containing 7.5 pmol adapter for *Eco*RI and 75 pmol adapter for *Mse*I, 1.2 units T4-DNA ligase, 1.2 mM ATP and 1x ligation buffer. Next the ligation reaction was performed at 37°C for 3 hrs after which a DNA template was prepared by diluting DNA with 10 X dH_2O and 3 µl of the resulting digestion-ligation mixture (DNA template) was used for PCR pre-amplification by adding 0.25 mM of primer, 1X *Taq* buffer, 1.5 mM $MgCl_2$, 200 mM dNTPs, and 0.3 units of *Taq* DNA polymerase, in a final volume of 10 µl. The thermal conditions for PCR were: 24 cycles of 30 s at 94°C, 1 min at 56°C and 1 min at 72°C. A GeneAmp^R PCR System 9700

Table 1. Accession of pummelo used in the variation study

No.	Name	Collection site*	No.	Name	Collection site*
1	Khaophuang	Nakhon Chai Si	51	Takoi	Pichit
2	Khaophuang	Nakhon Chai Si	52	Khaojook	Pichit
3	Khaophuang	Nakhon Chai Si	53	Toolkaol	Pichit
4	Khaophuang	Sam Phran	54	Toolkaol	Pichit
5	Khaophuang	Sam Phran	55	Toolkaol	Pichit
6	Khaopan	Sam Phran	56	Seedless	Plant nursery
7	Khaopan	Sam Phran	57	Seedless	Plant nursery
8	Khaopan	Sam Phran	58	Seedless	Plant nursery
9	Khaopan	Sam Phran	59	Seedless	Plant nursery
10	Khaopan	Sam Phran	60	Seedless	Plant nursery
11	Khaohom	Sam Phran	61	Seedless	Plant nursery
12	Khaohom	Sam Phran	62	Seedless	Plant nursery
13	Khaohom	Sam Phran	63	Kaonamphueng	Nakhon Chai Si 2-5
14	Khaohom	Pichit	64	Kaonamphueng	Nakhon Chai Si 2-6
15	Khaonamphueng	Nakhon Chai Si 1-1	65	Khewmorakot	Kamphaeng Saen
16	Khaonamphueng	Nakhon Chai Si 1-2	66	Khewmorakot	Kamphaeng Saen
17	Khaonamphueng	Nakhon Chai Si 1-3	67	Khaoyai	Samut Songkhram2-4
18	Khaonamphueng	Nakhon Chai Si 2-1	68	Khaoyai	Samut Songkhram2-5
19	Khaonamphueng	Nakhon Chai Si 2-2	69	Khaoyai	Samut Songkhram3-5
20	Khaonamphueng	Nakhon Chai Si 2-3	70	Khaoyai	Samut Songkhram3-6
21	Khaonamphueng	Nakhon Chai Si 2-4	71	Khaoyai	Samut Songkhram4-1
22	Khaoyai	Samut Songkhram1-1	72	Khaoyai	Samut Songkhram4-2
23	Khaoyai	Samut Songkhram1-2	73	Khaonamphueng	Nakhon Chai Si 1-4
24	Khaoyai	Samut Songkhram1-3	74	Khaonamphueng	Nakhon Chai Si 1-5
25	Khaoyai	Samut Songkhram2-1	75	Khaonamphueng	Nakhon Chai Si 1-6
26	Khaoyai	Samut Songkhram2-2	76	Khaonamphueng	Nakhon Chai Si 2-7
27	Khaoyai	Samut Songkhram2-3	77	Khaonamphueng	Nakhon Chai Si 2-8
28	Khaoyai	Samut Songkhram3-1	78	Khaonamphueng	Nakhon Chai Si 2-9
29	Khaoyai	Samut Songkhram3-2	79	Bangkhunnon	Nakhon Nayok
30	Khaoyai	Samut Songkhram3-3	80	Bangkhunnon	Nakhon Nayok
31	Khaoyai	Samut Songkhram3-4	81	Bangkhunnon	Nakhon Nayok
32	Phaiseethong	Suphan Buri	82	Bangkhunnon	Nakhon Nayok
33	Phaiseethong	Suphan Buri	83	Bangkhunnon	Nakhon Nayok
34	Thongdee	Nakhon Chai Si 1-1	84	Bangkhunnon	Nakhon Nayok
35	Thongdee	Nakhon Chai Si 1-2	85	Tabtim	Prachin Buri
36	Thongdee	Nakhon Chai Si 1-3	86	Tabtim	Prachin Buri
37	Thongdee	Nakhon Chai Si 2-1	87	Khaotanggwa	Chai Nat
38	Thongdee	Nakhon Chai Si 2-2	88	Khaotanggwa	Chai Nat
39	Thongdee	Nakhon Chai Si 2-3	89	Khaotanggwa	Chai Nat
40	Thongdee	Sam Phran 1-1	90	Thongdee	Pichit
41	Thongdee	Sam Phran 1-2	91	Thongdee	Pichit
42	Thongdee	Nakhon Chai Si 3	92	Thongdee	Pichit
43	Thongdee	Pichit	93	Takoi	Pichit
44	Thongdee	Pichit	94	Takoi	Pichit
45	Thongdee	Pichit	95	Takoi	Pichit
46	Khaotanggwa	Pichit	96	Toolklao	Pichit
47	Khaotanggwa	Pichit	97	Toolklao	Pichit
48	Khaotanggwa	Pichit			
49	Takoi	Pichit			
50	Takoi	Pichit			

*Nakhon Chai Si 1-1 means pummelo leaf collected from the 1st pummelo trees from the 1st orchard in Nakhon Chai Si district

(Applied Biosystem) was used.

A template for selective amplification was made from 2 µl of pre-amplification product and a mixture of 0.25 µM of primer *Mse*I, 0.25 µM primer *Eco*RI, 1X *Taq* buffer, 1.5 mM $MgCl_2$, 200 mM dNTP, and 0.3 units *Taq* DNA polymerase (Euroclone) to a final volume of 10 µl. The following PCR conditions were observed and the annealing temperature was reduced every cycle by 1°C: nine cycles of 30 s starting at 94°C down to 65°C and a further 1 min at 72°C. The next stage involved a further 30 cycles for 30 s at 94°C, 30 s at 56°C, 1 min at 72°C and hold at 4°C until the reaction was complete. It was stopped with the addition of 5 µl of loading buffer (10 mM EDTA pH 8.0, 98% formamide, Bromophenol Blue & Xylenecyanol). Selective PCR was performed in A GeneAmp® PCR System 9700 (Applied Biosystem). Amplified fragments were separated by 4.5% (w/v) polyacrylamide gel electrophoresis: silver staining. The DNA bands were visualized by autoradiography and manually scored for their presence or absence.

2.4 Data analysis

The NTSYS program was used for cluster analysis and based on a similarity matrix. The matrix was analyzed by the unweighted pair-group method with arithmetic mean (UPGMA) (Rohlf, 1990) and relationships between the cultivars were illustrated as a dendrogram. AFLP polymorphic bands were scored as either present (1) or absent (0) to process a binary matrix. The Jaccard similarity index was computed for each pair of cultivars, (Jaccard, 1908) and the program Winboot was used for the bootstrap analysis with 100 resampled datasets. Bootstrap value in range of 85-100% means high reliability, 71-84% means medium reliability, and 50-70% means low reliability (Richardson *et al.,* 2000).

3. Results and Discussion

3.1 Polymorphism as detected by AFLPs

The pairs of primers generated a total of 295 bands of which 133 bands (45.08%) were polymorphic. The mean number of band per assay was 19.67. Part of a typical gel is shown in Figure 1. Fifteen primer pairs were selected from 64 pairs of *Eco*RI/*Mse*I primers based on their sharp electropherogram and specific polymorphism. Genomic DNA of the sample was tested using AFLP analysis with the primer pairs. Hussein *et al.* (2003) have stated that for citrus the AFLP amplified fragments ranged from 50 to 650 bp and the number of bands produced by the different primer combinations ranged from 35 to 79. The size of AFLP fragments generated by the different primer combinations in this study ranged from 100 to 726 bp (Figure 1) and the number of bands produced by the different primer combinations ranged from 8 to 30.

Table 2 shows the obvious differences in the total bands amplified by various primers. The maximum number of polymorphic bands was amplified with the E- TAC/M-TCG primer pair identifying 62.50% polymorphism and the minimum number of polymorphic bands was amplified with the E-CGT/M-TGA and E-AAG/M-AGA primer pairs identifying 33.33% polymorphism. A total of 295 AFLP bands were identified with 15 primer pair combinations. Similar to the results seen by Cervera *et al.*(1998), who also applied the AFLP technique, with grapevine accessions and obtained a 49% polymorphism level this study has found a total of 133 (45.08%) polymorphic bands with a range from 33.33% to 62.50% and an average number of polymorphic bands of 8.87 *per* AFLP primer combination (see Table 2). In citrus fruit, Pang *et al.*(2007) have reported from their experiment that six primer combinations generated, 571 of 599 fragments were polymorphic with the range of polymorphic bands per primer combination being 63 to 119 (mean of 95.2) with the average polymorphic rate of AFLP markers was 95.3%. Previous reports have shown a high level of polymorphism but they were done for different species of a single plant. The lower level of polymorphism in our study is possibly due to the samples being obtained from the same specie but of different cultivars so it is likely there will not be a great genetic difference. Similar finding to this study were reported by Goulao (2001) in a study of apple cultivars that reported 208 (57.5%) polymorphic bands from 362 bands were observed. El-Khishin *et al.* (2003) studied AFLP fingerprinting of Egyptian date palm cultivars and found that the number of polymorphic amplicons was 233 representing a level of polymorphism of 53.81%. Han *et al.* (2000) studying tea species, they found an average of 10.5 polymorphic bands per primer combination. The polymorphic bands amplified by any AFLP primer in our study were sufficient to discriminate all pummelo accessions. An example of the pattern of amplified products obtained with one AFLP primer pair is shown in Figure 1.

3.2 Cluster analysis of pummelo cultivars

From the AFLP cluster analysis, performed with a similar coefficient as illustrated in the dendrogram of the phylogenetic tree (Figure 2), the similarity coefficients ranged from 0.75-1.00. The dendrogram, constructed from 15 AFLP markers, indicates that the pummelo cultivars grown in the central region of Thailand can be clearly divided into two groups at 0.80 of similarity coefficients with a bootstrap value of 100%. This grouping observation is consistent with existing morphological classifications of pummelo pulp color and are also similar to previous reports that indicate the pulp varies from greenish-yellow or pale-yellow to pink or red (Morton, 1987).

The first group in this study contains two sub-groups, the first of which is the *Khaopuang*, *Takoi*, *Toolkaol*, and *Khaojook* cultivar, while the second sub-group is *Khaopan*, *Khaohom*, *Khaonamphueng*, *Khaoyai*, *Phaiseethong*, *Khaotanggwa*, *Khewmorakot*, and other seedless cultivars. Kongsri and Boonprakob (2008) also showed that *Khaopan, Khaoyai*, and *Khaotanggwa* were in the same group. With

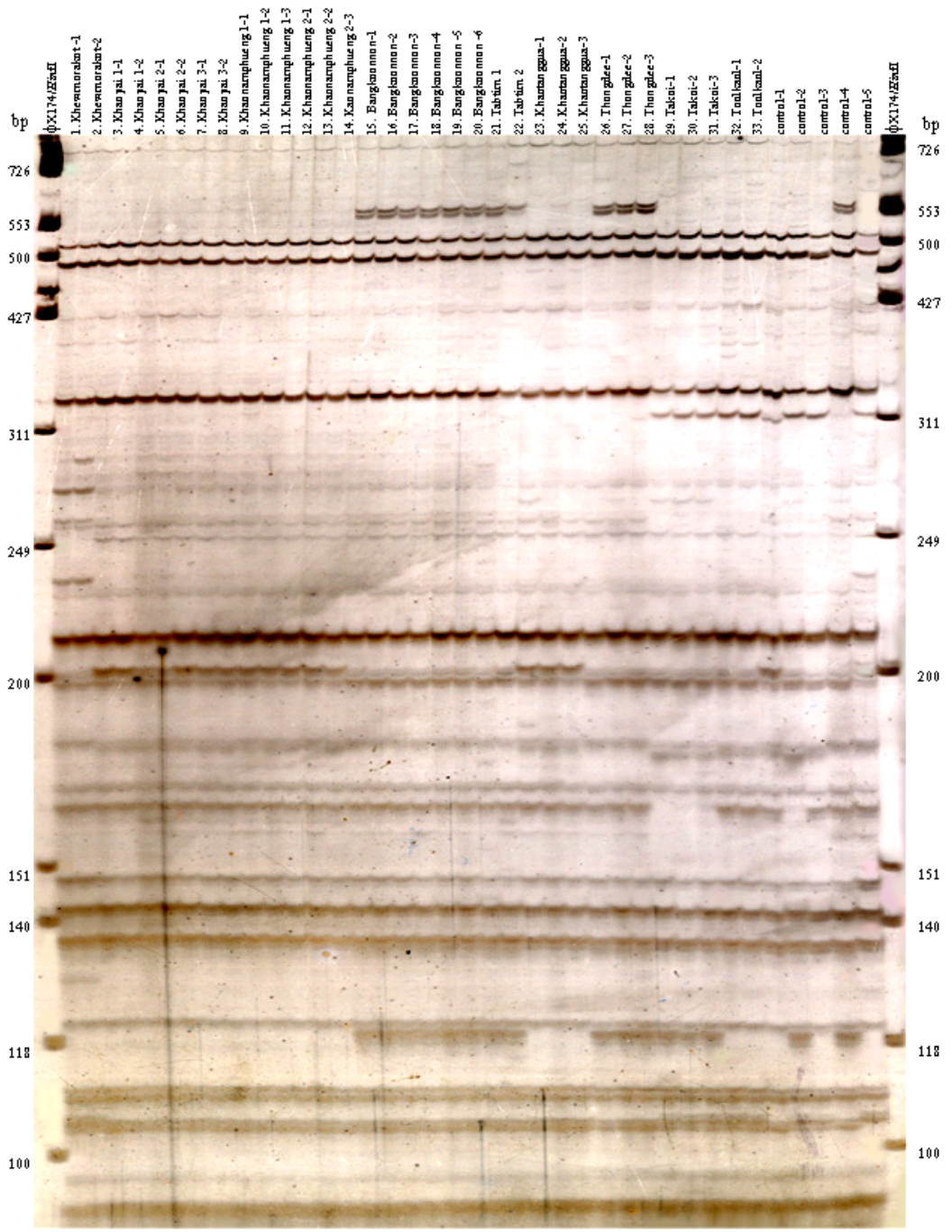

Figure 1. An AFLP profile of pummelo genomic DNA using primer combination E-CAG/M-TGA

the exception of the *Takoi* cultivar, other members of the first group had pulps in the white to yellow color range, while the second group consisting of *Thongdee*, *Bangkhunnon*, and *Tabtim* cultivars, had pulp in the pink to red color range. According to a work by Davies and Albrigo (1994) the many cultivars of pummelo are generally divided into three country groups: Thai, Chinese, and Indonesian. Major pummelo cultivars found in the Thai group fall into the white and red fleshed varieties. However, in this current study the *Takoi* cultivar became clustered into a different group to *Thongdee* even though they are both well known red fleshed Thai cultivars. Similarity coefficient showed 0.76 between these cultivars.

The cultivar differences at the DNA level were determined by comparing their genetic similarity. Tests in this study showed that *Khaonamphueng*, *Khaoyai*, *Phaiseethong*, and

Table 2. List of AFLP primers, their sequence, number of bands, polymorphism (%) of AFLP analysis

No. of primer	Sequence	Total number of band	AFLP fragment score		Polymorphism %
			Monomorphic	Polymorphic	
1	GCA/AAT	14	8	6	42.86
2	AAC/GTG	15	8	7	46.67
3	AAG/CAA	29	15	14	48.28
4	ACT/CAC	13	6	7	53.85
5	CAG/TGA	17	7	10	58.82
6	TAC/TAG	21	11	10	47.62
7	AAG/CAC	14	8	6	42.86
8	TAC/TCG	8	3	5	62.50
9	CGT/TGA	24	16	8	33.33
10	TAC/TGA	23	14	9	39.13
11	AAG/AAT	30	19	11	36.67
12	AAG/ACC	16	8	8	50.00
13	AAG/AGA	30	20	10	33.33
14	AAG/CTG	22	9	13	59.09
15	TAC/TAC	19	10	9	47.37
Total	15	295	162	133	45.08
Average		19.67	10.8	8.87	

Khaotanggwa were closest with a range of similarity coefficient of 0.99–1.00 (Figure 2) with a bootstrap value of 95 %, indicating how narrow the genetic diversity is within these cultivars. This result is according to the similarity of fruit and leaf morphology and pulp color among these cultivars given information by the grower. This result is also similar to the report of Kongsri and Boonprakob (2008) who indicated the narrow genetic diversity between *Khaoyai* and *Khaotanggwa* cultivar tested by simple sequence repeat (SSR) marker. Therefore, it is likely that they are from the same progenitor material or alternatively are the same cultivar but growing in a different location and so have been given a different name. Similar phenomena have been reported by Steiger *et al*. (2002, 2003) of the high degree of genetic similarity between coffee and macadamia cultivars. However, Kongsri and Boonprakob (2008) classified *Khaonamphueng* in the different group to *Khaoyai* and *Khaotanggwa* and reported that *Khaonamphueng* and *Khaotanggwa* are the different cultivar (Kongsri and Boonprakob, 2006). This difference in results may be due to a different technique used here and in the earlier report. However, there might be also a problem with the samples used in the previous report because in this study we used the SSR technique to test the cultivar differences between *Khaoyai* and *Khaonamphueng* with the same markers mentioned in the previous report, but no differences between these cultivars were found in our study (data not showed).

Conflicting information given about the two cultivars *Khaonamphueng* (No.15-21, 63-64 and 73-78 in Table 1) and *Khaoyai* (No. 22-31 and 67-72 in Table 1), although they are very close in fruit and leaf morphology, has lead some pummelo growers to suggest that they are the same cultivar, while others suggest they are different cultivars. From our results these two cultivars are genetically similar when viewed in the phylogenetic tree dendrogram. Genetic similarity testing between 13 *Khaonamphueng* trees and 10 *Khaoyai* trees showed 0.99–1.00 similarity. The indications are that these two pummelo varieties may in fact be the same cultivar but because they were growing in different locations had been given different name. This, however, contradicts a previous report (Kongsri and Boonprakob, 2008) which found a genetic difference between *Khaonamphueng* and *Khaoyai*, tested by simple sequence repeat (SSR) marker. The differences between this current study results and those previously reported in the earlier report might be explained by the different techniques used or it might be that the DNA primers in this study were not capable to detect any of the genetic differences between the two cultivars. Consequently it is still unclear whether or not *Khaonamphueng* and *Khaoyai* are the same cultivar and more studies employing genetic markers will be required to distinguish between the two cultivars.

The similarity coefficient and a bootstrap value within pummelo cultivars were very high (Figure 2). This indicates that the genetic variation within pummelo cultivars, grown in different locations, is very narrow. Our results found that the leaf samples collected from the same cultivar, but in different locations (Table 1) were genetically similar. For example, *Thongdee* (No. 34-45 in Table 1) collected from Nakhon Pathom and Pichit Province, *Khaophuang* (No. 1-5 in Table

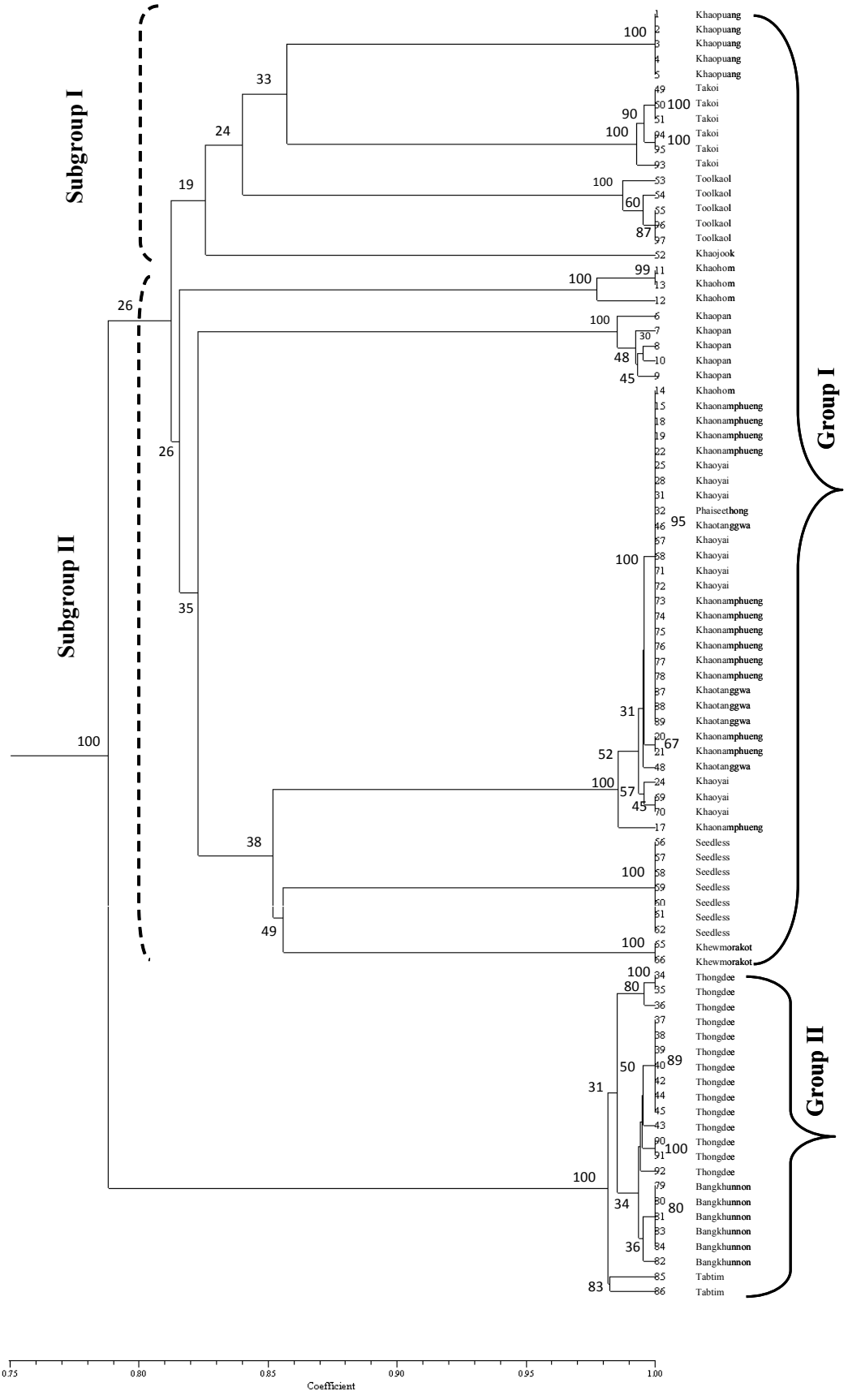

Figure 2. Dendrogram of phylogenetic tree with the bootstrap value showing genetic relationship among pummelo accessions.

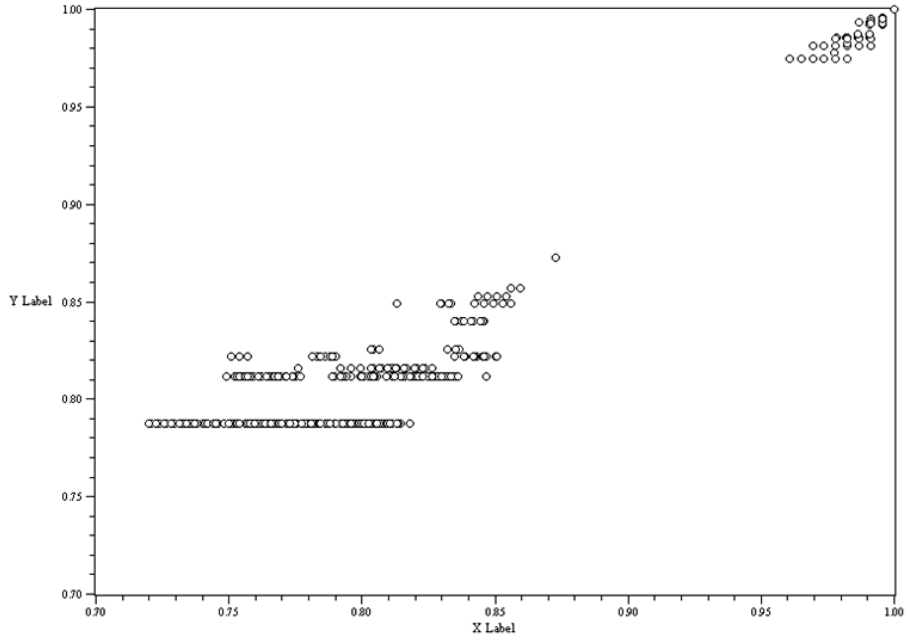

Figure 3. Data distribution of pummelo grouping from 97 samples, cophenetic correlation (r) =0.966

1) collected from Nakhon Pathom Province (Nakhon Chai Si and Sam Phran) and *Khaotanggwa* (No. 46-48, 87-89 in Table 1) collected from Pichit and Chai Nat Province showed a 0.99–1.00 similarity coefficient with a bootstrap value range 50-100 %. This is probably due to asexual propagation in the pummelo. This indicates that the difference in flavor or some morphological characteristics of the same pummelo cultivars grown in different locations may be an environmental effect.

As the cophenetic correlation was very high (r=0.966) (Figure 3) the results from this study clearly demonstrate the efficiency of the AFLP marker system for pummelo cultivar fingerprinting identification and typing using only a small number of primer combinations. These results are inconsistent with the findings of different authors on different plant species.

In conclusion, the phylogenetic tree dendrogram showed that the 97 leaf samples from 15 pummelo cultivars grown in central region of Thailand could be classified into two groups. The first group consisted of two sub-groups. The first sub-group consisted of *Khaopuang, Takoi, Toolkaol* and *Khaojook* cultivar. The second one consisted of *Khaopan*, *Khaohom*, *Khaonamphueng*, *Khaoyai*, *Phaiseethong*, *Khaotanggwa*, *Kkewmorakot* and seedless cultivar. The second group consisted of *Thongdee*, *Bangkhunnon* and *Tabtim* cultivar. Moreover, the results indicated that the samples collected from the same cultivar in different locations were for the main part genetically similar.

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