Genetic diversity of local peaches in Thailand based on AFLP markers

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Abstract

In highland areas of northern Thailand, peach trees have been planted around the hill-tribe villages. Their fruits were mainly used for pickle as they possess small and soft flesh. Recent discovery of rust (*Tranzschelia pruni-spinosae*) resistance in some genotypes has prompted the use of these peaches in the ongoing breeding program to develop resistant cultivars. Due to lack of any genetic information about these local peaches, this study was carried out to estimate their genetic diversity and to determine their similarity to other standard low-chilling peach cultivars. Twenty-three local peach trees found near villages of Royal Project Development Centers (12 locations) were randomly selected for genetic analysis using Amplified Fragment Length Polymorphism (AFLP) markers. Three low-chilling peaches: 'TropicBeauty', 'Premier' and 'Okinawa' were included for the comparison. DNA was extracted from young leaves using a CTAB method. The AFLP analysis showed that 10 primer pairs produced 148 scorable bands in which 121 markers (81.8%) were polymorphic. Genetic diversity among local peaches and genetic similarity of local peaches to standard low-chilling peaches were calculated using Nei and Li coefficients. Cluster analysis was done by UPGMA method. Genetic relationships of 27 peach cultivars showed two main clusters and three sub-clusters within local peach based on AFLP markers. Genetic similarity ranged between 0.54 and 1.00. Local peaches in Thailand were very closely related genetically.

Introduction

PEACH [*Prunus persica* (L.) Batsch.] is one of the temperate fruits cultivated in Thailand. The local peach was brought by the hill tribe people who migrated to Thailand (Subhadrabandhu, 1987). Because the fruit are very small and soft, they are mainly used for pickling when they are young. Trees are well adapted, exhibiting vigorous growth, which makes them suitable for use as a rootstock. Recently, rust (*Tranzschelia pruni-spinosae*) resistance was observed in some local peach genotypes. Breeders have promoted the use of these peaches in the ongoing breeding program to develop rust-resistant cultivars. Due to a lack of any genetic information, genetic comparison to other peach germplasm would reveal relative and genetic diversity of these local peaches. This information will be useful for future breeding programs.

There are many techniques to evaluate genetic relationships, such as morphological markers, isozyme markers (Torres, 1983) and DNA markers, eg AFLP (Amplified Fragment Length Polymorphism) (Vos et al., 1995), RFLP (Restriction Fragment Length Polymorphism) (Botstein et al., 1980), RAPD (Randomly Amplified Polymorphic DNA) (Welsh and McClelland, 1990; Williams et al., 1990). DNA markers have become a popular choice due to their high selectivity and insensitivity to the environment. Among these the AFLP technique produces a large number of markers and requires no previous information about the markers or genomic sequences. It has been used successfully for estimating genetic relationships in fruit crop species, eg pears (Hayashi and Yamamoto, 2002), apples (Tignon, 2001), mango (Hautea et al., 2001), grapes (Narvaez and Andres, 1998) and peaches (Rajapakse et al., 1995; Dirlewanger et al., 1998; Lu et al., 1998; Aranzana et al., 2001; Arus et al., 2003).

The objectives of this study were to estimate genetic diversity of local peaches and to determine their similarity to other standard low-chilling peach cultivars using AFLP analysis.

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edited by Alan P. George and Unaroj Boonprakob
ACIAR Technical Reports No. 61
(printed version published in 2005)

Materials and methods

Plant materials

Twenty-seven peach genotypes were used. They were local cultivars (Angkhang and Inthanon groups) and three low-chilling commercial cultivars (Southern China group). One genotype 'ShenzHouMiTao' (Northern China cultivar) was included for comparison (Table 1). These local peach trees, found near villages or Royal Project Development Centers (Fig. 1), were randomly selected for AFLP analysis.

DNA extraction

The CTAB procedure was modified from Dellaporta et al. (1983) and Agrawal et al. (1992) for use with a 1.5 ml microcentrifuge tube with 50 mg of young fully expanded leaf tissue. The DNA concentration was quantified by visual comparison with SibEnzyme DNA marker 1 Kbp (SibEnzyme, USA) on 0.8% agarose gel (Promega, USA) in 0.5× TBE buffer. The DNA stock was then diluted to 50 ng/µl working stock with sterile reverse osmosis water.

AFLP reaction

The purified DNA was digested with *Eco*RI (BioLabs, England) and *Tru*9I (BioLabs, England) restriction enzymes. Digestion was carried out in the volume of 25 µl in 10 × NE buffer (50 mM NaCl, 100 mM Tris-HCl, 10 mM $MgCl_2$, 0.025% Triton X-100), 10 × SE bufferW [10 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT], 1 Unit *Eco*RI, 0.6 Unit *Tru*9I and 50 ng of genomic DNA for 16 h at 37°C. Two different adaptors, one for the *Eco*RI sticky end and another for the *Tru*9I sticky end were ligated to DNA by adding 0.2 µl of a mixture containing 5 pmol *Eco*RI adaptor (BioBasic, Thailand), 25 pmol *Mse*I adaptor (BioBasic, Thailand), 10 × ligase buffer [50 mm Tris-HCl (pH 7.5 at 25°C), 10 mm $MgCl_2$, 5 mM DTT, 1mM ATP, 25 µg/ml BSA] and 0.2 Unit T4 ligase (SibEnzyme, USA) to the digestion. The ligation was incubated for 12 h at 20°C.

Table 1. Peach genotypes used in genetic relationship study.

Order	Genotypes	Location*	Note
1	KaeNoi 1	CM, 950–1000 m	large fruit and tip, green ground
2	KaeNoi 2	CM, 950–1000 m	round, small, yellow ground
3	KaeNoi 3	CM, 950–1000 m	round, green-yellow ground
4	MaeHa 2	CM, 1200 m	ovate, large tip, green ground
5	MaeHa 3	CM, 1200 m	
6	MaeHa 1	CM, 1200 m	round, yellow ground, white flesh
7	InThaNon 1	CM, 1300 m	new clone
8	InThaNon 2	CM, 1400 m	old clone
9	KhunHuayHang 1	CM, 1300 m	
10	KhunHuayHang 2	CM, 1300 m	
11	WatChan 1	CM, 1000 m	imported from PangDa Royal Agricultural Station
12	WatChan 2	CM, 900-1200 m	large fruit, large tip, green ground
13	HuayNamKhun 1	CR, 1100 m	small fruit, cling, white flesh, imported from Angkhang Royal Agricultural Station
14	HuayNamKhun 2	CR, 1100 m	small fruit, cling, white flesh, imported from Angkhang Royal Agricultural Station
15	HuayNamRin	CM	
16	MaePunLuang	CM, 1100 m	imported from Banluang Angkhang
17	Local Angkhang	CM, 1400 m	green ground
18	Local Angkhang F10	CM, 1400 m	rust resistance
19	NohgHoi	CM, 1200 m	imported from Doi Pui
20	MongNgow	CM, 1200 m	
21	Local Khunwang	CM, 1300 m	
22	White Angkhang	CM, 1400 m	planted at Angkhang Royal Agricultural Station
23	Red Angkhang	CM, 1400 m	planted at Angkhang Royal Agricultural Station
24	TropicBeauty	CM, 1400 m	medium, yellow flesh round, semi-free, imported from Florida, USA
25	Okinawa	CM, 1200 m	small, white flesh, origin in Florida, USA
26	Premier	CM, 1400 m	white flesh, low acid, Southern China type
27	ShenzHouMiTao	China	fairly firm, sweet, juicy, white flesh, cling, Northern China type

Note * CM (Chiang Mai); CR (Chiang Rai)

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A first preselective Polymerase Chain Reaction (PCR) amplification was performed using *Eco*RI + A and *Mse*I + C primer pairs in 2.5 µl 10× Mg^{2+} free buffer [10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1 % Triton X-100], 50 mM $MgCl_2$, 2 mM of each dNTP, 6.25 pmol of each primer, and 1 Unit DyNAzyme II DNA Polymerase (Roche, USA). The reaction was carried out in a PTC-0225 Peltier Thermal Cyler (MJ Research Inc., USA) and the samples were subjected to 30 cycles of denaturing at 94°C for 30 s, annealing at 56°C for 60 s, and extension at 72°C for 60 s. The preamplification products were diluted make the starting material for the selective amplification by using 5 µl of preamplified material to 95 µl sterile reverse osmosis water.

For selective amplification *Eco*RI and *Mse*I primers with two selective nucleotides were used (Table 2). The PCR reaction was performed in a 20 µl volume of 2 µl 10 × Mg^{2+} free buffer, 50 mM $MgCl_2$, 2 mM of each dNTP, 5 pmole *Eco*RI primer,

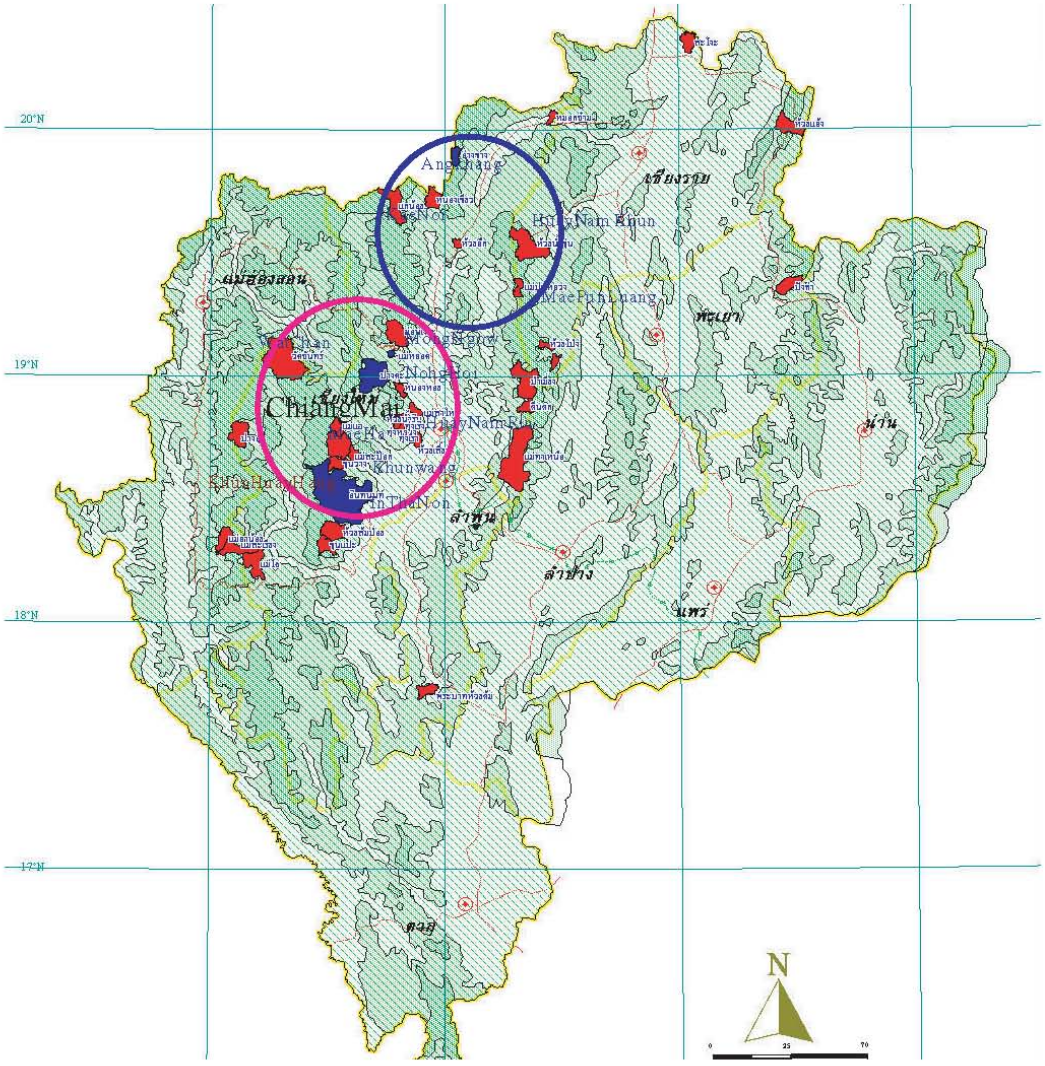

Figure 1. Map of 36 Royal Project Development Centers location; O, local Inthanon group, O, local Angkhang group.

5 pmole *Mse*I primer, 1 Unit DyNAzyme II DNA Polymerase (Roche, USA), and 5 µl of diluted preamplified DNA. The selective amplification was carried out using the following cycling parameters; 1 cycle of 60 s at 94°C, 60 s at 65°C, 90 s at 72°C followed by 10 cycles in which the annealing temperature was lowered by 1°C per cycle, followed by 22 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C.

Table 2. Primer pairs and number of AFLP markers used for estimation of genetic relationships.

Primer	AFLP markers		
	Monomorphic	Polymorphic	Total
E-AAC/M-CAC	2	14	16
E-AAC/M-CAT	2	9	11
E-AAC/M-CTC	4	8	12
E-AAC/M-CTT	4	12	16
E-ACC/M-CAT	3	11	14
E-ACC/M-CTA	2	9	11
E-ACC/M-CTC	0	13	13
E-AGC/M-CAC	4	12	16
E-AGC/M-CTT	3	17	20
E-ACG/M-CAG	3	16	19

After completion of a selective PCR, the samples were denatured to single strand by adding formamide buffer containing 99% formamide, 10 mM EDTA, 0.05% bromo-phenol blue, and 0.05% xylene cyanol. The samples were heated for 5 min at 100°C and immediately placed in ice. Six µl of each sample was loaded on a 6% acrylamide/bisacrylamide (19:1), 7.5 M urea and 5 × TBE buffer, 10% APS, Temed gel (14 × 24.5 cm). Electrophoresis was carried out at a constant voltage of 295 V for 210 min in a Dual Slab Gel Unit (DSG-200, C.B.S. Scientific Co., USA). The polyacrylamide gel was visualised by silver staining method (Piyachoknakul, 2002). Gels were dried at room temperature for 2 days.

AFLP analysis

At least two replications of each sample and primer combinations were successfully done to correctly score the observation. Scoring of data was based on consistent or major AFLP markers. All amplifications were scored as either a present or absent marker. The genetic similarity matrix among genotypes was calculated in SIMQUAL program (Sokal and Sneath, 1963) using Dice (Sneath and Sokal, 1973) similarity coefficients [$(C_{jk}) = 2a/(2a+b+c)$; where a is the number of AFLP markers that were present in both j and k genotypes, b is the AFLP marker that was present only in j genotype and c is the AFLP marker that was present only in k genotype].

This similarity coefficient is equivalent to the Nei and Li coefficient (1979). The dendrogram representing genetic relationship based on the similarity coefficient from AFLP markers was constructed by using the Unweighted Pair Group Method with Arithematic Average (UPGMA) (Sokal and Michener, 1958) in the SAHN program (Sneath and Sokal, 1973). The SIMQUAL and SAHN programs are the packaging of NTSYS-pc 2.00 program (Rohlf, 1997).

Results and discussion

Ten primer pairs which produced intense and scorable bands were selected. They were tested and PCR amplification yielded approximately 11–20 AFLP markers per primer pair which ranged in size from 100 to 700 bp (Table 2 and Fig.2). Out of 148 reproducible AFLP markers, 121 were polymorphic (81.8%) and 27 monomorphic (18.2%).

Cluster analysis of 27 peach cultivars

Based on the cluster analysis (UPGMA method) we constructed a dendrogram which showed that the varieties could be divided into two main clusters (Fig. 3).

Cluster 1 included 26 peach genotypes with genetic similarity coefficients between 0.79–1.00. This cluster could be divided further into three sub clusters.

Sub-cluster A included clones of KaeNoi group, MaeHa group, InThaNon group, WatChan group, KhunHuayHang group, MongNgow, 'White Angkhang', 'Okinawa' and 'TropicBeauty' with genetic similarity coefficients between 0.88–1.00. Almost all the peach genotypes in this cluster are local peaches, except 'Okinawa' which originated in Florida, USA (Okie, 1998) and 'TropicBeauty' that was bred in Florida, USA (Rouse and Sherman, 1989). Both cultivars shared a southern China ancestor.

Sub-cluster B included 'MaePunLuang', 'NohgHoi' and 'Local Khunwang' with genetic similarity coefficients between 0.91–0.93.

Sub-cluster C included 'Local Angkhang F10' and 'Premier' with genetic similarity coefficients of 0.9. Both genotypes were observed as rust (*Tranzschelia pruni-spinosae*) resistant.

Cluster II consisted of one genotype, 'ShenzHouMiTao' that originated in northern China (Zailong, 1984). Genetic similarity coefficients between both clusters is 0.54. These results indicated all local peaches in Thailand have a southern China ancestor.

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edited by Alan P. George and Unaroj Boonprakob
ACIAR Technical Reports No. 61
(printed version published in 2005)

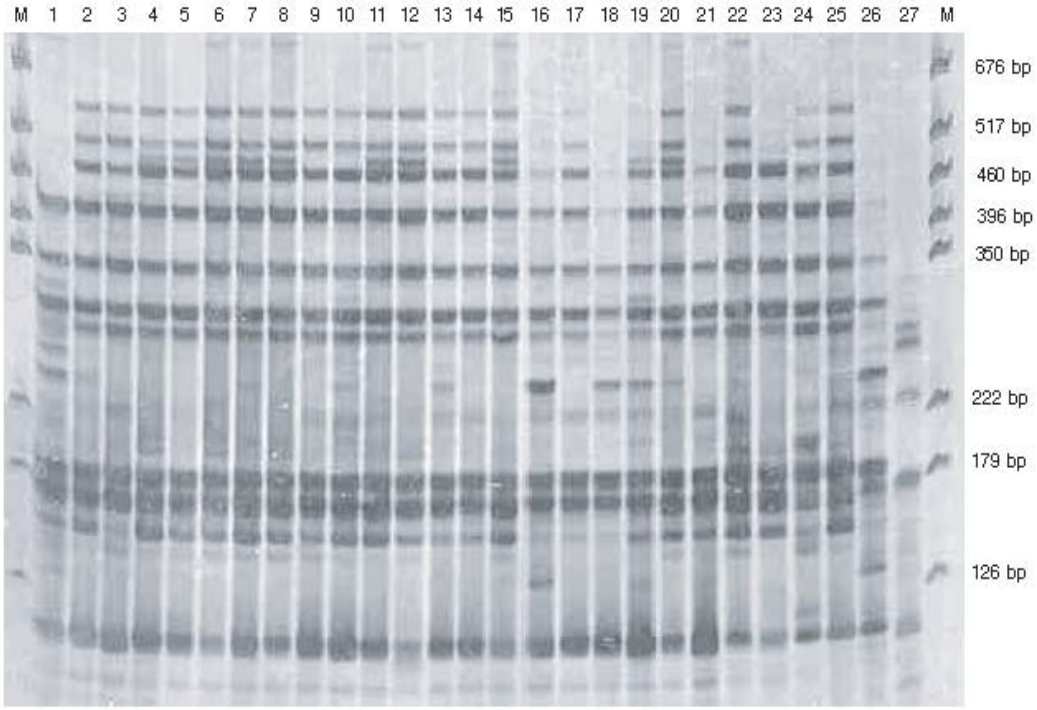

Figure 2. Fingerprint of AFLP markers from E-AAC /M-CAC primer pair; m, standard marker; No. 1–27, label of peach genotype in Table 1.

Genetic relationship between local Angkhang group, local Inthanon group, southern China group and northern China cultivar

Peaches in China could be divided into three groups: a northern group, a southern group and a European or Persian group (Zai-long, 1984). Two groups — the northern and southern groups — were included in this study. Genetic relationship showed a broad genetic diversity of 0.78–0.89 (Table 3) between the local Angkhang group, local Inthanon group, southern China group and the northern China cultivars Three groups, except the northern China cultivars, being grown successfully in Thailand had genetic similarity between 0.87–0.89. Of these, genetic diversity within the local Inthanon group was lowest (0.9). The result also indicated that the genetic relationships between the local Angkhang and local Inthanon groups is the closest (0.9). Local peaches from the southern China group were more closely related than those from northern China. This indicated that local peaches in Thailand were introduced from southern China. These results agree with the suggestions of Punsri et al. (1995).

Table 3. Average of similarity coefficience of peach groups; local Angkhang group (LAK), local Inthanon group (LITN), southern China group (SC) and northern China cultivar (NC).

Peach group*	LAK	LITN	SC	NC
LAK	0.87 ± 0.03			
LITN	0.89 ± 0.03	0.90 ± 0.02		
SC	0.87 ± 0.04	0.89 ± 0.03	0.87 ± 0.05	
NC	0.80 ± 0.15	0.86 ± 0.12	0.78 ± 0.16	0.55

* LAK included 'KaeNoi 1', 'KaeNoi 2', KaeNoi 3', 'HuayNamKhun 1', 'HuayNamKhun 2', 'MaePunLuang', 'local Angkhang' and 'local Angkhang F10'
LITN included 'MaeHa 1', 'MaeHa 2', 'MaeHa 3', 'InThaNon 1', 'InThaNon 2', 'KhunHuayHang 1', 'KhunHuayHang 2', 'WatChan 1', 'WatChan 2', 'HuayNamRin', 'NohgHoi', 'MongNgow' and 'Local Khunwang'
SC included 'White Angkhang', 'Red Angkhang', 'TropicBeauty', 'Okinawa' and 'Premier'
NC, 'ShenzHouMiTao'

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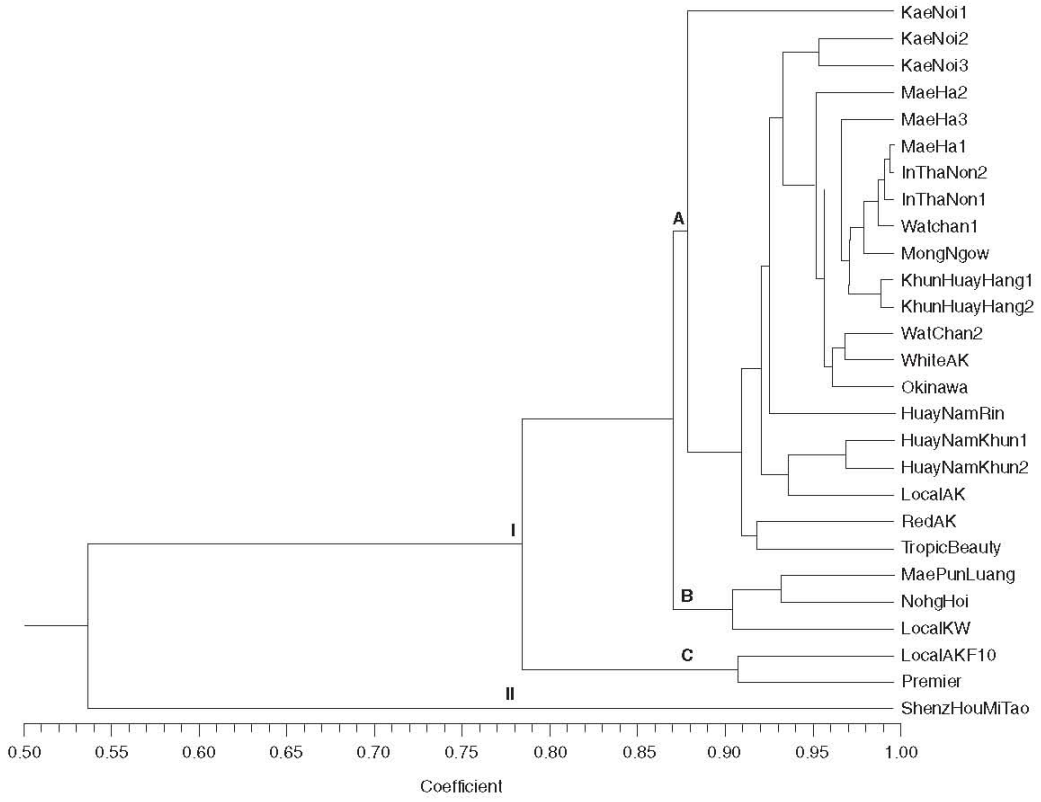

Figure 3. Dendrogram of 27 peach genotypes based on cluster analysis (UPGMA method) on 148 AFLP markers.

Conclusions

Using 10 primer pairs we were able to score 148 AFLP markers indicating that one primer could reveal 14 markers. Out of these markers, 121 markers (81.8%) were polymorphic.

Genetic relationships of 27 peach cultivars that consisted of local cultivars (Angkhang and Inthanon groups), commercial cultivars (southern China group) and 'ShenzHouMiTao' (northern China cultivar) showed two main clusters and three sub-clusters within local peach. Genetic similarity ranged from 0.54 to 1.00 with local peaches in Thailand very closely related genetically.

Acknowledgment

The authors wish to express their appreciation to the Royal Project Foundation for financial support of this research work.

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