ORIGINAL ARTICLE

Relationships between some Thai cultivars of pineapple (*Ananas comosus*) revealed by RAPD analysis

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Abstract

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Relationships between some Thai cultivars of pineapple (*Ananas comosus*) revealed by RAPD analysis

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RAPD analysis of nine Thai pineapple cultivars, including 'Phulae', 'Sawee', 'Tradsithong', 'Phuket', 'Pattavia', 'Intrachitdang', 'Intrachitkhow', 'Petburi No.1', and 'Nanglae', showed that, of 40 arbitrary 10-mer primers, 17 primers gave 206 DNA fragments ranging from 510 to 4,700 bp. One hundred and forty-five (70.4%) of the amplified fragments were polymorphic. RAPD analysis using NTSYS-pc Version 2.01e also showed that the similarity coefficients among the cultivars were 0.643-0. 963. The dendrogram indicated that the cultivars were clustered into 3 groups, consistent with the morphological data. The first group, consisting of 'Phuket', 'Phulae', 'Tradsithong', 'Sawee', and 'Petburi No.1', had morphological characteristics of the Queen group, while those of the second ('Intrachitdang' and 'Intrachitkow') and the third ('Nanglae' and 'Pattavia') groups could be determined morphologically to be members of the Spanish and Cayenne groups, respectively. 'Intrachitdang' and 'Intrachitkow' have similarity coefficient of 0.963, while that of 'Phulae'

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and 'Phuket' is 0.950. These pairs of cultivars are probably the same cultivars. The morphological differences between them are probably caused by mutations, differences in environment and agricultural practices, or combinations of these factors.

Key words : pineapple, *Ananas comosus*, random amplified polymorphic DNA (RAPD) analysis

บทคัดย่อ

สยาม ภพลือชัย สมฤดี อ้นโต และ ประภัสสร ดำรงกุล อึ้งวณิชยพันธ์

ความสัมพันธ์ทางพันธุกรรมของสับปะรดพันธุ์ปลูกในประเทศไทย โดยวิธีอาร์เอพีดี

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การวิเคราะห์ความหลากหลายทางพันธุกรรมของสับปะรดพันธุ์ปลูกในประเทศไทยจำนวน 9 สายพันธุ์ ได้แก่ ภูแล สวี ตราดสีทอง ภูเก็ต ปัตตาเวีย อินทรชิตแดง อินทรชิตขาว เพชรบุรี 1 และนางแล โดยวิธีอาร์เอพีดีนั้น พบว่าไพร์เมอร์จำนวน 7 ไพรเมอร์จาก 40 ไพร์เมอร์ สามารถให้แถบดีเอ็นเอได้ โดยได้แถบดีเอ็นเอทั้งหมด 206 แถบ มีขนาด 510 ถึง 4,700 คู่เบส มีเปอร์เซ็นต์ polymorphism เท่ากับ 70.4% และเมื่อวิเคราะห์โดยใช้โปรแกรม NTSTS-pc version 2.01e พบว่ามีค่าความคล้ายคลึง (similarity coefficients) อยู่ในช่วง 0.643-0.963 เมื่อทำการจัดกลุ่มในรูปของ dendrogram พบว่าสามารถแยกความแตกต่างได้ 3 กลุ่ม กลุ่มที่ 1 ประกอบด้วย สับปะรดสายพันธุ์ ภูเก็ต ภูแล ตราดสีทอง สวี และเพชรบุรี 1 กลุ่มที่ 2 ประกอบด้วย อินทรชิตแดง และอินทรชิตขาว กลุ่มที่ 3 ประกอบด้วย ภูแล และปัตตาเวีย ซึ่งการจัดกลุ่มที่ได้สอดคล้องกับลักษณะทางสัณฐานวิทยาของสับปะรด โดยกลุ่มที่ 1 เป็น Queen group กลุ่มที่ 2 เป็น Spanish group กลุ่มที่ 3 เป็น Cayenne group ตามลำดับ สับปะรดพันธุ์อินทรชิตแดง และอินทรชิตขาว มีค่าความคล้ายคลึงเท่ากับ 0.963 สับปะรดพันธุ์ภูแล และภูเก็ต มีค่าความคล้ายคลึงอยู่ที่ 0.950 อาจจะเป็นไปได้ว่าสับปะรดทั้งสองพันธุ์ในแต่ละคู่เป็นพันธุ์เดียวกันแต่เกิดการกลายพันธุ์เกิดขึ้น จึงทำให้ลักษณะทางสัณฐานวิทยาแตกต่างกันออกไป หรืออาจเกิดจากความแตกต่างของสภาพแวดล้อม การเพาะปลูก และอิทธิพลอื่น ๆ

สาขาวิชาเทคโนโลยีชีวภาพ สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง จังหวัดเชียงราย 57100

Pineapple (*Ananas comosus*) is one of the most important fruits cultivated in tropical and sub-tropical countries. It is a member of the Bromeliaceae. Pineapple can be divided into 5 groups according to morphological characteristics, including spination, length and shape of the leaves, and weight, shape, texture and taste of the fruits. These 5 groups are Abacaxi, Cayenne, Maipure or Perolera, Queen, and Spanish (Leal and Soule, 1977; Py *et al.*, 1987). In 1994, Bartholomew and Malezieux grouped some of Thai pineapple cultivars according to their morphologies. They reported that 'Tradsithong', 'Phuket' and 'Sawee' were in the Queen group, 'Pattavia', 'Nanglae' and 'Petburi' in the Cayenne, and 'Intrachitdang' in the Spanish. The pineapples in the Queen group have small and very spiny leaves. The fruits are small and oblong with full yellow shells, small prominent eyes and sweet and crispy golden-yellow flesh. The Cayenne has leaves with the spines confined to the tips. The fruits are ovoid and medium-sized. They ripen progressively, turning yellow from the base to the top of the fruits. The flesh is pale-yellow, soft and juicy. The cultivars in the Spanish group have small, oval to cylindrical-shaped, and dark purple fruits that will turn copper-orange when ripening. The flesh is golden-yellow, low sugar and acidity, and poor in taste. The leaf spines are varied from clone to clone.

Sripaoraya *et al.* (2001) studied pineapples cultivated in Thailand using the RAPD technique and found that, of 8 economically important

cultivars, 'Tradsithong', 'Phuket', 'Sawee', and 'Thainan' belonged to the Queen group, while 'Pattavia' and 'Petburi' were in the Cayenne and 'Intrachitdang' in the Spanish group. However, since then, there have been many new and improved cultivars in Thailand that still require to be studied genetically. In this study, we were interested in pineapples that are grown commercially in Thailand. These included 3 new cultivars; 'Phulae', 'Petburi No.1' and 'Intrachitkow'.

Random amplified polymorphic DNA (RAPD) markers were used to investigate genetic relationships among these Thai pineapple cultivars. The RAPD technique is simple, rapid, and does not require DNA sequence data (Williams *et al.*, 1990). There have been many studies that successfully revealed genetic relationships using this technique for example in tea (Wachira *et al.*, 1995), sea grass (Angel, 2002), and sea buckthorn (Ruan *et al.*, 2003). Therefore, in this study, RAPD data were used for cluster analysis and these may be useful for improving Thai pineapple cultivars in the near future.

Materials and Methods

Nine commercial cultivars of pineapple in Thailand were investigated (Table 1). The cultivar 'Phulae', was collected from Chiang Rai province, while the rest, i.e. 'Sawee', 'Tradsithong', 'Phuket', 'Pattavia', 'Intrachitdang', 'Intrachitkhow', 'Petburi no.1', and 'Nanglae', were obtained from the Petburi Horticultural Research Station, Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand.

Young leaves from intact shoots were harvested. Genomic DNA was extracted using DNA EziKit (Sunolin, Thailand) and dissolved in 1xTE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). The concentration and purity of each DNA sample were estimated by 0.8% agarose gel electrophoresis in comparison to *Hind*III digested λ DNA marker.

The RAPD method was adapted from Williams *et al.* (1990). The RAPD reaction mixture of 15 µl contained 50 ng DNA template, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.1% Triton-X100, 0.3 µM 10-mer primer (Operon Technologies, Alameda), 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, and 0.75 unit *Taq* DNA polymerase (Invitrogen, San Diego). Amplifications were performed in a Thermal Cycler (Bio-Rad, Hercules) programmed for an initial incubation at 95°C for 10 min, followed by 35 cycles of 1 min at 93°C, 1 min at 37°C and 1 min 20 s at 72°C, using programmed transition times of 2.06 min. The samples were incubated at 72°C for 5 min and held at 4°C prior to analysis. Amplified products were analyzed by 2% (w/v) agarose gel electrophoresis. Gels were stained with 0.5 µg/ml ethidium bromide solution and visualized on a UV transilluminator (Bio-Rad, Hercules). *Pst*I digested λ DNA was used as a size

Table 1. Pineapple cultivars used for RAPD analysis

Source of material	No.	Cultivar	Morphological Group*
Chiang Rai province	2	Phulae	Queen
Petburi Horticultural Research Station, Petburi province	1	Phuket	Queen
	3	Nanglae	Cayenne
	4	Intrachitdang	Spanish
	5	Intrachitkow	Spanish
	6	Pattavia	Cayenne
	7	Tradsithong	Queen
	8	Sawee	Queen
	9	Petburi No.1	Queen

*** Information from Petburi Horticultural Research Station (unpublished data)**

marker.

The RAPD bands were recorded according to the presence (1) or absence (0) of a DNA band at the same location on the gel. Data were statistically analyzed by the software program NTSYS-pc 2.01e (Numerical Taxonomy and Multivariate Analysis System) (Rohlf, 1997). The genetic relationship among cultivars was calculated using the similarity coefficient of Nei and Li (1979). Dendrogram was constructed by Unweighted Pair Group Method with Arithmetic Averages (UPGMA) cluster analysis using Dice similarity coefficient.

This experiment was done in triplicate.

Results and Discussion

Seventeen primers (Table 2) were selected from 40 arbitrary RAPD primers as they amplified and gave polymorphic bands in all DNA samples. The rest of the primers were able to amplify most, but not all, of the samples. From 9 pineapple genomic DNA samples of different cultivars, 206 RAPD fragments, ranging from 510 bp to 4700 bp in length, were amplified. One hundred and forty-five (70.4%) of the amplicons were polymorphic. The average of polymorphic fragments per primers was 8.5. The similarity coefficients between each pair of the pineapple cultivars ranged from 0.643 to 0.963 (Table 3). The lowest was the similarity coefficient between the cultivars 'Phuket' and 'Nanglae', while the highest was between 'Intrachitdang' and 'Intrachitkow'.

From the dendrogram (Figure 1), it was shown that the 9 cultivars were clustered into 3 groups. The first group consisted of 5 cultivars, 'Phuket', 'Phulae', 'Tradsithong', 'Sawee', and 'Petburi No.1'. In the second group, there were 2 cultivars, 'Intrachitdang' and 'Intrachitkow'. 'Nanglae' and 'Pattavia' were clustered to form the third group. These results indicated that the pineapple cultivars with similar morphologies

Table 2. Primers assessed in RAPD analysis and numbers of polymorphic bands generated from nine pineapple cultivars.

Primer	Sequence 5′→3′	Number of amplified bands	Number of monomorphic bands	Number of polymorphic bands	Percentage of polymorphic bands (%)
OPA 2	TGCCGAGCTG	13	3	10	76.9
OPA 3	AGTCAGCCAC	18	7	11	61.1
OPA 4	AATCGGGCTG	21	9	12	57.1
OPA 9	GGGTAACGCC	8	4	4	50.0
OPA 13	CAGCACCCAC	15	6	9	60.0
OPC 2	GTGAGGCGTC	10	4	6	60.0
OPC 5	GATGACCGCC	14	3	11	78.6
OPC 8	TGGACCGGTG	14	4	10	71.4
OPC 9	CTCACCGTCC	7	1	6	85.7
OPC 10	TGTCTGGGTG	12	2	10	83.3
OPC 11	AAAGCTGCGG	8	3	5	62.2
OPC 12	TGTCATCCCC	12	1	11	91.7
OPC 14	TGTCATCCCC	8	1	7	87.5
OPC 15	GACGGATCAG	13	8	5	38.5
OPC 16	CACACTCCAG	12	2	10	83.3
OPC 19	TTCCCCCCAG	11	2	9	81.8
OPC 20	ACTTCGCCAC	10	1	1	90.0
Total		**206**	**61**	**145**	**70.4**

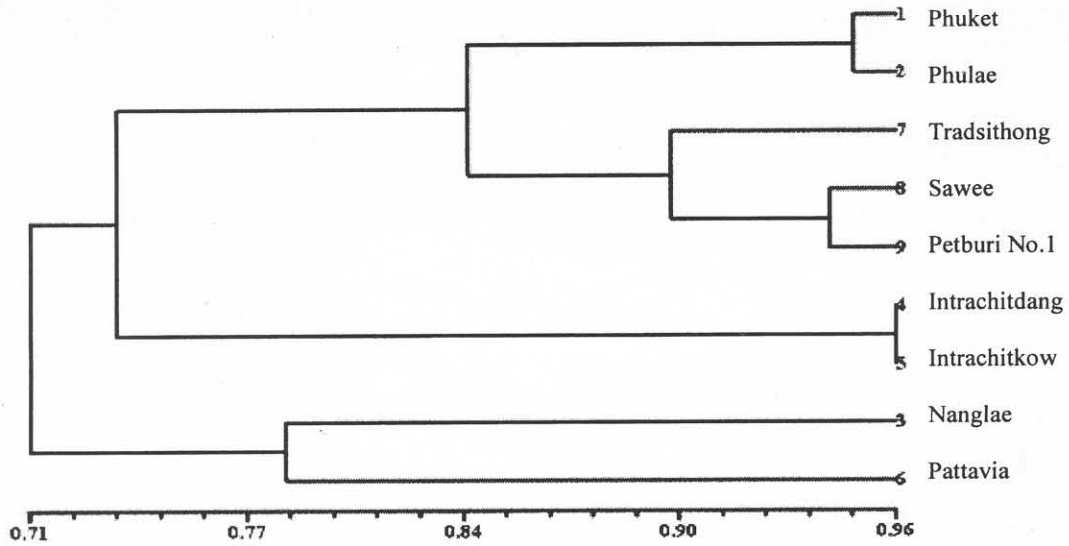

Figure 1. A UPGMA dendrogram of genetic relationships between 9 pineapple cultivars based on Dice similarity coefficient values

Table 3. Similarity coefficients between the pineapple cultivars

Cultivars*	1	2	3	4	5	6	7	8	9
1	***								
2	0.950	***							
3	0.643	0.678	***						
4	0.705	0.727	0.683	***					
5	0.696	0.717	0.667	0.963	***				
6	0.693	0.722	0.784	0.723	0.735	***			
7	0.835	0.846	0.653	0.710	0.729	0.756	***		
8	0.846	0.872	0.659	0.753	0.777	0.799	0.926	***	
9	0.799	0.825	0.695	0.751	0.775	0.817	0.867	0.943	***

*** 1 = Phuket, 2 = Phulae, 3 = Nanglae, 4 = Intrachitdang, 5 = Intrachitkow, 6 = Pattavia, 7 = Tradsithong, 8 = Sawee, and 9 = Petburi No.1**

were clustered together when analyzed genetically by RAPD technique. The cultivars in the first group ('Phuket', 'Phulae', 'Tradsithong', 'Sawee', and 'Petburi No.1') had morphological characteristics of the Queen group, while those of the second ('Intrachitdang' and 'Intrachitkow') and the third ('Nanglae' and 'Pattavia') groups could be morphologically determined as members of the Spanish and Cayenne groups, respectively.

When the clusters in the dendrogram (Figure 1) were considered, it was cleared that, within the first group, 'Phuket' and 'Phulae' were more closely related to each other than to the rest of the group members. To the same extent, 'Sawee' and 'Petburi No.1' were more closely related. 'Intrachitdang' and 'Intrachitkow' of the second group appeared to be the most closely related pairs of all cultivars.

'Intrachitdang' and 'Intrachitkow' have very similar morphologies including the shape of the plants and leaves and the fruits' size, shape and taste. The only difference among them is the color

of the leaves. 'Intrachitdang' has green leaves with red pigments scattered, while 'Intrachitkow' has no red pigments in the leaves. The Petburi Horticultural Research Station reported that they have developed 'Intrachitkow' from 'Intrachitdang' cultivar (personal communication). This report supports our results that they are very closely related (similarity coefficient = 0.963). It is possible that they are of the same cultivars.

In Thailand, it is believed that 'Phulae' (Queen), Chiang Rai's most popular and widely cultivated pineapple, resulted from crosses between 'Phuket' (Queen), a cultivar originated from the south of Thailand, and 'Nanglae' (Cayenne), a Chiang Rai's cultivar introduced from China. The RAPD analysis results indicated that the similarity coefficient between 'Phulae' and 'Phuket' was 0.950 and that those between 'Phulae' and 'Nanglae' and between 'Phuket' and 'Nanglae' were 0.678 and 0.643, respectively. These numbers showed that 'Phulae' and 'Phuket' were more closely related to each other than to 'Nanglae'. Moreover, the cluster analysis and dendrogram placed 'Phulae' and 'Phuket' in the same cluster while placing 'Nanglae' in another group. Based on results from this study, it should be possible to say that 'Phulae' and 'Phuket' were of the same cultivars. Moreover, the National Research Council of Thailand (2004) has reported that 'Phulae' is 'Phuket' cultivated in Chiang Rai. Therefore, the morphological differences between them were probably caused by mutations, environmental differences, different agricultural practices, or combinations of these factors.

Conclusions

The data from the RAPD analysis showed that, of 9 cultivars, the pineapples studied were clustered into 3 groups. These groups could be identified as Queen, Spanish, and Cayenne according to their morphologies. RAPD technique is also useful for identifying the new cultivars, which included 'Phulae', 'Petburi No.1' and 'Intrachitkow'. The results indicated that 'Intrachitkow' was closely related to 'Intrachitdang', 'Phulae' to 'Phuket', and 'Petburi No.1' to 'Sawee'. Each pair had very high similarity coefficients.

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