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Genetic Relationships of the Big-fruited Wax Apple-lines Collected from Taiwan by Random Amplified Polymorphic DNA (RAPD)

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Abstract

Random amplified polymorphic DNA (RAPD) analysis was used to study the genetic relationships of the 13 big-fruited wax apple (*Syzygium samarangense* Merr. et Perry) lines whose morphological identification were difficult. Among the 100 primers for the RAPD analysis, 8 primers revealed reproducibly distinct RAPD profiles. The 13 wax apple lines in the 95% similarity can be classified into 7 groups, 'YingSheng 1', an independent group, is different from the other 12 wax apple lines with average similarity of only 72%. The other 6 groups are 'Pink'; 'SoonDer 1', 'SoonDer 2', 'SoonDer 3', and 'JanGee 1'; 'FengShe 2', 'FengShe 3', and 'FengShe 4'; 'FengShe 1', and 'FengShe 7'; 'FengShe 6'; 'YingSheng 2'.

Abbreviations: RAPD, random amplified polymorphic DNA; PCR, polymerase chain reaction; UPGMA, unweighted pair-group method using arithmetic averages

Key words: RAPD, Wax apple, Genetic relationship

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Ⅰ.Introduction

The wax apple, belonging to Myrtaceae, is a tropical fruit tree with its origin in the Malay archipelago (Shü, et al. 2007). It is now spreading all over the world with most of the commercial plantings concentrated in Taiwan, south China and Southeast Asia. The area, total production and production value for the wax apple industry in Taiwan were 7031 hectares, 69,234 metric tons and $189 million US dollars in 2006. 'Pink' has been the leading cultivar, representing 95% of the planted areas in Taiwan (Wang, 1991), although the percentage decreased to about 85% in 2007 (Wang, pers. comm.). There are many other wax apple cultivars in Taiwan other than 'Pink'. Some of the cultivars are divided according to their skin colors, such as 'Pink', 'Light Red ', 'Deep Red', 'White' and 'Green' (Wang, 1991). In recent years, there are some mutant lines with their origin being clamming from the 'Pink'. The fruits of these mutant lines are bigger than that of the 'Pink' and thus are sold with trade names as "Black King Kong" or "Big Fruit". Although these mutant lines have the same trade names, however, the fruit of them have different characteristics and local names. Beside, these mutant lines come from different geographic locations with their botanical traits varies.

The aim of the present study was to locate the genetic relationship among the 'Pink' cultivar and big-fruited wax apple mutant lines.

Ⅱ.Materials and methods

DNA preparation and purification

Method for genomic DNA extraction was made by modifying a CTAB method developed by Doyle and Doyle (1987). Fresh fruits were frozen immediately in liquid nitrogen. The frozen tissue were ground into powder and stored at -20℃ until used. The frozen powder was placed subsequently in 500 μL 2 × CTAB [cetyl trimethyl ammonium bromide, 100 mM Tris-HCI, 1.4 M NaCl, 30 mM EDTA, and 2 % (w/v) CTAB], incubated at 55℃ for 10 min, 500 μL CI (Chlorofrom：Isoamyl alcohol；24：1) solution was then added, and shaken at 40 rpm for 30 min. Sample was centrifuged at 13,000 rpm for 20 min and the supernatant was transferred into to a fresh tube. Later 70 μL 10% CTAB (at 55℃) and 500 μL ppt buffer [1 % (w/v) CTAB, 50 mM Tris-HCI (pH 8.0), 10 mM EDTA] was added, mixed, and centrifuged at 13,000 rpm for 20 min. The supernatant was discarded. Then 60 μL 1 M NaCl-TE was added and incubated in water bath at 55℃ for 5 min. Then 500 μL 2-propanol was added and centrifuged at 13,000 rpm for 20 min. The supernatant was discarded and the

precipitated double-stranded DNA was washed with 70% ethanol. After drying DNA and was redissolved in 200 μL 1 × TE (tris（hydroxymethyl）aminomethane-ethylenediaminetetraacetic acid) buffer and stored at -20℃.

Five μL DNA samples were diluted with 495 μL double distilled water to make a final volume of 500 μL. DNA concentration was determined based on 260 nm absorption using a Hitachi U-2000 spectrophotometer.

PCR and electrophoresis

Purified DNAs diluted at 50 ng/μL concentration were used for PCR template. PCR reactions containing 1 μL DNA (50 ng) was carried out for 35 cycles at 94℃ for 5 min, 94℃ for 1 min and 37℃ for 2 min. Temperature at 72℃ for 10 min was used in the last cycle. PCR products were loaded onto 1.5% (w/v) agarose horizontal slab gels and electrophoresis was carried out in 0.5 × TBE (tris-borate) buffer. Gels were stained using ethidium bromide and photographed at 260 nm.

RAPD profiling

One hundred 10-mer primers (90 primers came from University of British Columbia, Canada (UBC) and 10 primers came from Operon) were used to produce amplicons using PCR machines. Among the 100 primers only 8 primes were found to be able to differentiate polymorphism among the tested 13 wax apple lines.

Analysis of polymorphic bands

An analysis of DNA fragmentation procedure was implemented to calculate a formula for comparing the genetic similarities of different wax apple variety and lines according to Jaccard's coefficient of similarity. In comparison to the genetic similarities of different lines, cluster analysis was done by UPGMA followed by NTSYSpc (version 2.0) analyses.

Ⅲ.Results

Among the 100 primers for RAPD analysis, 8 primers revealed reproducibly distinct RAPD profiles. The 8 primers randomly generated 84 DNA fragments. Among all the primers, the UBC-412 primer generated the most abundant, 14, random DNA polymorphic bands; OPA-04 and OPA-07 generated 12 random DNA fragments; OPAO-03 primer had 11 polymorphic bands; UBC-427, UBC-431 and OPA-09 primers had 9 polymorphic fragments; OPA-08 prime generated the least, only 8, random DNA fragments (Table 1).

Genetic similarities of different lines obtained using Jaccard's coefficient of

similarity are shown in Table 2. The genetic relationship among the 13 wax apple lines are shown in Fig. 1 using genetic similarity of matrix by UPGMA. The 13 wax apple lines in the 96% similarity can be classified into 7 groups, ‘YingSheng 1’, an independent group, is different from the other 12 wax apple lines with average similarity of only 72%. The other 6 groups are ‘Pink’; ‘SoonDer 1’, ‘SoonDer 2’, ‘SoonDer 3’, and ‘JanGee 1’; ‘FengShe 2’, ‘FengShe 3’, and ‘FengShe 4’; ‘FengShe 1’, and ‘FengShe 7’; ‘FengShe 6’; ‘YingSheng 2’.

Table 1. Sequence of random primers and number of polymorphic bands produced by wax apple RAPD analysis.

primer ID	Sequence（5’→3’）	polymorphic bands
UBC-412	TGCGCCGGTG	14
UBC-427	GTAATCGACG	9
UBC-431	CTGCGGGTCA	9
OPAO-03	AGTCGGCCCA	11
OPA-04	AATCGGGCTG	12
OPA-07	GAAACGGGTG	12
OPA-08	GTGACGTAGG	8
OPA-09	GGGTAACGCC	9

Table 2. Genetic similarity of RAPD markers among 13 wax apple lines according to Jaccard's coefficient of similarity.

	'Pink'	SoonDer 1	SoonDer 2	SoonDer 3	JanGee1	YingSheng 1	YingSheng 2	FengShe1	FengShe2	FengShe3	FengShe4	FengShe6	FengShe7
'Pink'	1.00												
SoonDer 1	0.85	1.00											
SoonDer 2	0.84	0.98	1.00										
SoonDer 3	0.84	0.98	1.00	1.00									
JanGee1	0.82	0.97	0.98	0.98	1.00								
YingSheng 1	0.64	0.76	0.77	0.77	0.76	1.00							
YingSheng 2	0.74	0.88	0.89	0.89	0.91	0.68	1.00						
FengShe1	0.79	0.94	0.95	0.95	0.94	0.73	0.85	1.00					
FengShe2	0.79	0.94	0.95	0.95	0.94	0.73	0.85	0.94	1.00				
FengShe3	0.81	0.95	0.97	0.97	0.95	0.74	0.86	0.95	0.98	1.00			
FengShe4	0.79	0.94	0.95	0.95	0.94	0.73	0.85	0.94	0.97	0.98	1.00		
FengShe6	0.74	0.88	0.90	0.90	0.91	0.71	0.82	0.91	0.91	0.92	0.94	1.00	
FengShe7	0.76	0.91	0.92	0.92	0.91	0.71	0.82	0.97	0.94	0.95	0.97	0.94	1.00

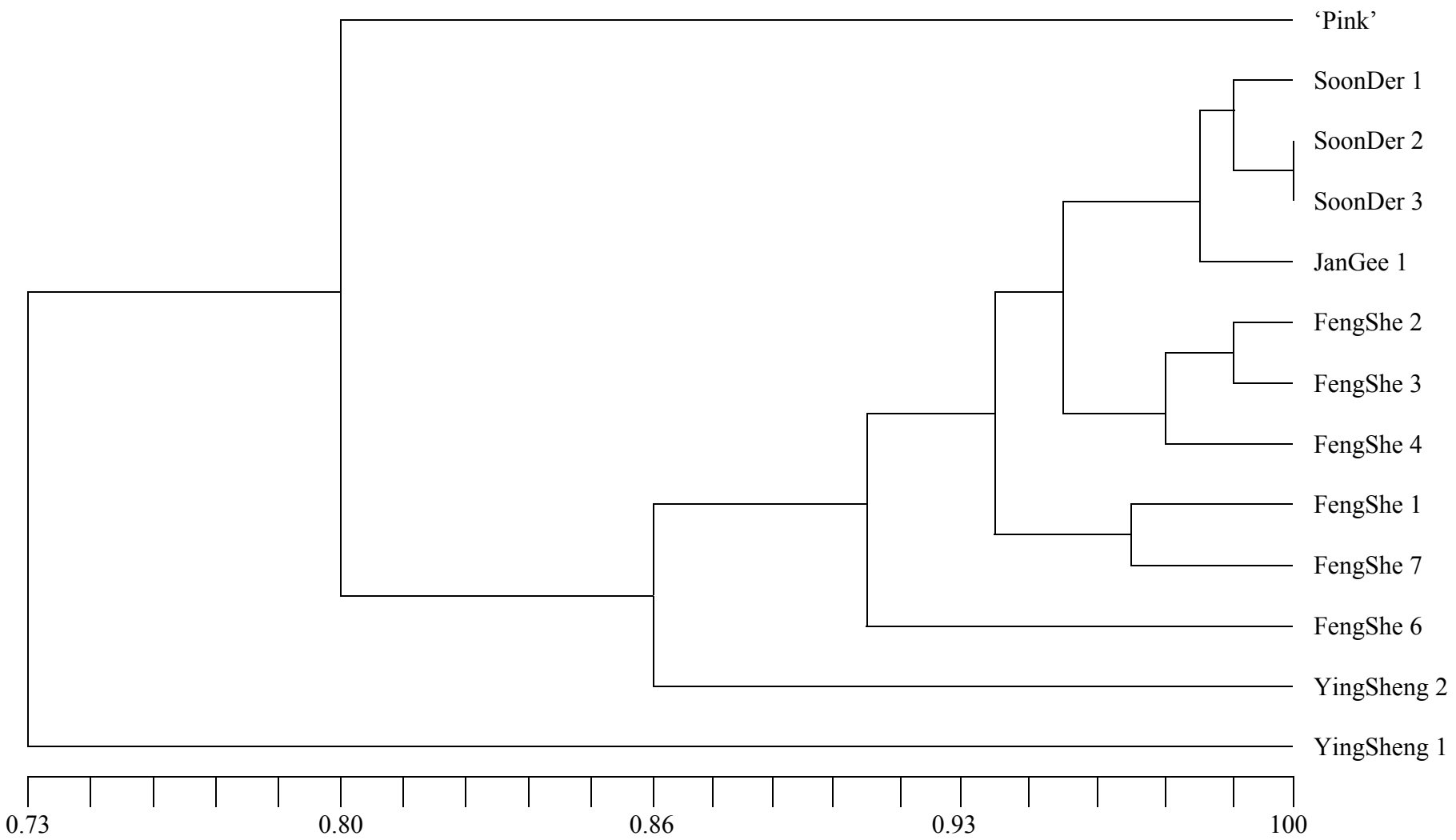

Fig 1. Genetic relationships among the 13 wax apple lines.

Ⅳ.Discussion

The mutant wax apple lines used in the present study are not officially named. The local names of these mutant lines were named following the discoverer's names or locations although they have the same trade names as "Black King Kong" or "Big Fruit". Sine the morphologies of the wax apple trees and organs are easily affected by environmental and cultural operations, it is not easy to discriminate cultivars or lines by morphological traits. The present study thus used RAPD markers to study the phylogenetic relationships of wax apple varieties and lines in Taiwan.

Among the 100 primers for RAPD analysis, 8 primers were identified to present polymorphic bands. According to Jaccard's coefficient of similarity, genetic similarities were calculated among different varieties and lines, and genetic relationships were reconstructed by UPGMA. 'YingSheng 1' has the farthest genetic distance as compared with 'Pink' and the other 11 lines, 'Pink', 'SoonDer 1', 'SoonDer 2', 'SoonDer 3', 'JanGee 1', 'FengShe 2', 'FengShe 3', 'FengShe 4', 'FengShe 1', 'FengShe 7', 'FengSh e6', and 'YingSheng 2', those were classified as a big group. The big group can be classed into two sub-groups. 'Pink' alone is a sub-group and the other 10 lines are in the other sub-group. With the exception of 'YingSheng', wax apple lines coming with the same name, such as 'SoonDer' and 'FengShe', are basically sitting closely with each other within the sub-group. 'YingSheng 2', coming from the same orchard as 'YingSheng 1', is the closest line next to 'YingSheng 1' although 'YingSheng 1' is grouped outside of the big group. 'JanGee1' has a very close relationship with 'SoonDer'.

References

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, *19*, 11-15.

Fielder, J., Bufler, G., & Bamgerth, F. (1998). Genetic relationships of avocado （*Persea Americana* Mill.）using RAPD markers. *Euphytica*, *101*, 249-255.

Jan, C. H., & Byrne, D. H. (1999). Rose germplasm analysis with RAPD markers. *HortScience*, *34*, 41-345.

Kresovich, S., Williams, J. G. K., McFerson, J. R., Routman, E. J., & Schaal, B. A. (1992). Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. *Theor. Appl. Genet.*, *85*, 190-196.

Oard, J. H., & Dronavalli, S. (1992). Rapid isolation of rice and maize DNA for analysis by random-primer PCR. *Plant Mol. Bio. Rep.*, *10*, 236-241.

Scovel, G. H. Ben-Meir, Ocadis, M., Itzhaki, H., & Vainsein, A. (1998). RAPD and RFLP markers tightly linked to the locus controlling carnation（*Dianthus caryophyllus*）flower type. Theor. *Appl. Genet.*, *96*, 117-122.

Shü, Z. H., Lin, T. S., Lai, J. M., Huang, C. C., Wang, D. N., & Pan, H. H. (2007). The Industry and Progress Review on the Cultivation and Physiology of Wax Apple – with Special Reference to 'Pink' Variety. *The Asian and Australasian Journal of Plant Science and Biotechnology*, *1*(2), 48-53.

Sun, Q. Z. Ni, Liu, Z., Gao, J., & Huang, T. (1998). Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. *Euphytica*, *99*, 205-211.

Wang, D. N. (1991). Past, present and future of wax apple production in Taiwan (in Chinese). In C. R. Yang (Ed.), *Proceedings of the Symposium Fruit Production, Research and Development in Taiwan* (pp. 339-355). Chia-Yi: Chia-Yi Agricultural Experiment Station, Taiwan Agricultural Research Institute.

Welsh, J., & McClelland, M. (1991). Genomic fingerprinting using arbitrarily primer PCR and a matrix of pairwise combinations of primers. *Nucleic Acids Res.*, *19*, 5275-5279.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, *18*, 6531-6535.

Wollf, K., Zeitkiewicz, E. & Hofstra, H. (1995). Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. *Theor. Appl. Genet.*, *91*, 439-447.

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以逢機複製多態性DNA (RAPD)技術探討蓮霧大果品系間的親源關係

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摘要

蓮霧大果品系是由‘粉紅’品種突變而來，因此以外部型態來分辨大果品系並不容易。本研究以逢機複製多態性 DNA (RAPD)技術來探討蓮霧大果品系間的親源關係。在以 100 條逢機引子來分析‘粉紅’和 12 個大果品系後，只有 8 條引子具有多態性。13 個品系在遺傳相似度係數 95% 下可以分成 7 群。‘英盛 1’，自己獨立成 1 群。其它 12 個品系可以分成 6 群，分別是‘粉紅’；‘順德 1’、‘順德 2’、‘順德 3’和‘建智 1’；‘鳳試 2’、‘鳳試 3’和‘鳳試 4’；‘鳳試 1’和‘鳳試 7’；‘鳳試 6’；‘英盛 2’。

關鍵字：逢機複製多態性 DNA 技術、蓮霧、親源關係

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