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GENETIC DIVERSITY ANALYSIS OF TAIWAN AVOCADO ACCESSIONS

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SUMMARY

Forty-two avocado accessions maintained at the Chiayi Agricultural Experimental Station (Chiayi, Taiwan) were evaluated for genetic diversity using random amplified polymorphic DNA (RAPD) markers. A total of 107 polymorphic bands were detected for genetic diversity analysis upon polymerase chain reaction (PCR) amplification of 21 octamer primers, an average of five scorable bands per primer. These primers were considered highly informative because they amplified at least one polymorphic band that distinguished between accessions. Jaccard's coefficient was applied to calculate genetic similarity, and UPGMA cluster analysis to generate the dendrogram. The forty-two accessions were first divided into two main groups (I and II), at 0.27 of Jaccard's coefficient similarity and the second main group was further separated into two subgroups(II-1 and II-2), at 0.33. It suggested that there was high degree of genetic diversity between the first main groups of cultivated avocadoes as well as the two subgroups of the second main group. The first main group can be further divided into four subgroups (I-1, I-2, I-3 and I-4). Major local selected accessions were assigned to the first two subgroups of the first main group (I-1 and I-2) showed either Guatemalan or West Indian origin. Except Nabel, a cultivar introduced from Guatemala, most cultivars of the third subgroup (I-3) are hybrids with Guatemalan origin. The accessions of the first subgroup of the second main group (II-1) are either hybrids of Mexican and Guatemalan varieties or Guatemalan cultivars. Mexican cultivars are assigned to the second subgroup of the second main group (II-2). In most cases, dendrogram constructed from UPGMA cluster analysis of 42 avocado cultivars, based on similarity index, correlated well with their respective recorded origin.

Key Words:.RAPD, local selection, genetic diversity, identification

INTRODUCTION

The commercial avocados, which belong to *Lauraceae*, are perennial evergreen fruit trees. The species *Persea americana* Mill. is polymorphic and can be categorized into Mexican, Guatemalan, and West Indian 'horticultural' races which regarded as geographical ecotypes (Scora et al., 2002). Horticultural traits such as new flush color, exocarp thickness, seed coat, cotyledon, and tightness in seed cavity have been traditionally employed for classification. However, a lot of traits used for classification are fruits traits and can't be utilized before fruits are mature. Plenty of hybrids have been bred with parents of different origins since there is no hybridization barrier or incompatibility between groups. Molecular markers, which can detect DNA polymorphism, have been utilized for taxonomy study and genetic diversity research in recent years (Clegg et al., 1999). A high level of polymorphism was detected in avocado by random amplified polymorphic DNA (RAPD) markers (Bufler and Ben-Ya'acov, 1992). These methods provide unique tools to understand the genetic relationship between avocado varieties as well as for early selection to utilize resources better and acceleration of breeding process. The purpose of this study is to evaluate the genetic diversity and phylogenetic relationship of the forty-two avocado accessions maintained at the Chiayi Agricultural Experimental Station (Chiayi, Taiwan) with RAPD markers.

MATERIAL AND METHODS

Material and DNA extraction:

Fresh leaf samples of the 42 avocado cultivars were collected from Chiayi Agricultural Experimental Station of the Taiwan Agriculture Research Institute. CTAB protocol of Kidwell and Osborn (1992) was followed for total DNA extraction.

RAPD assay:

A total of 100 UBC (University of British Columbia) primers (set 1: No.1 to 100) were used. RAPD reactions were carried out in 25μl volume containing 1X buffer (10mM Tris-HCl pH8.3; 50mM KCl), 100μM dNTPs, 3mM MgCl2, 0.5μM primer, 1.5 unit Taq DNA polymerase and 25ng template DNA. Amplifications were performed in PERKIN ELMER GenAmp 2400. The thermal cycle program was five min at 94μ, followed by 30 sec at 94μ, 30 sec at 38μ, and one min at 72μ for 45 cycles, and then further extened at 72μ for 10 minutes. PCR amplification products were seperated in 1.5% agarose gel by electrophoresis at 100V, 0.5X TBE buffer, then stained with ethidium bromide (0.5ug/ml).

Data analysis:

The scorable and polymorphic RAPD bands were scored with 0/1 scoring method (0 as absent and 1 as present). The band size was decided by Bio-Gene (Copyright c1999 Vilber-Lourmat) software. Cluster analysis was done with NTSYSpc (version 2.01b) using Jaccard's coefficient and unweighted paired grouped method using arithmetic average (UPGMA).

RESULTS AND DISCUSSION

100 UBC octamer primers were screened for polymorphism and 21 of them were able to generate polymorphic bands. 107 polymorphic bands were detected and employed for genetic diversity analysis upon polymerase chain reaction (PCR) amplification of the 21 polymorphic primers, an average five scorable bands per primer. These primers were considered highly informative because they amplified at least one polymorphic band that distinguished between accessions. Jaccard's coefficient was applied to calculate genetic similarity, and UPGMA cluster analysis to generate the

dendrogram. The forty-two cultivars were separated into two main groups (I and II) by cluster analysis (Figure 1). They were first divided at 0.27 of Jaccard's coefficient similarity and then the second main group was further separated at 0.33 (II-1 and II-2), suggesting that there was high degree of genetic diversity between them. The first main group can be further divided into four subgroups (I-1, I-2, I-3 and I-4). Accessions assigned to the first two subgroups of the first main group are of either Guatemalan or West Indian origin. Almost all of the local selections belong to subgroups I-1 and I-2, with exception of 'CAES11' in subgroup I-3 and 'Hung Chi Chao' in I-4 (the only accession in the subgroup). Except Nabel, a cultivar introduced from Guatemala, most cultivars of the third subgroup are hybrids between Mexican and Guatemalan varieties. The accessions of the first subgroup of the second main group (II-1a) are either hybrids of Mexican and Guatemalan varieties or Guatemalan cultivars. Subgroup II-1b contains only one accession 'G755', which is a hybrid of Guatemalan race and *Persea schiedeana*. Mexican races are assigned to the second subgroup of the second main group (II-2).

Some accessions showed unique banding pattern could be used to identify horticultural races. For example, UBC 1-800 only be found in 'Mexicola' and UBC 39-750 could be found in G755. This is an easy way to identify specific cultivar. The genetic diversity information revealed by this study also provided a way to identify the local avocado selections in Taiwan. In the case of 'Tou Wei Chi' and 'Yang Hui Huang -1', it's hard to distinguish them by traditionally morphological differences. After this study, we can confirm the situation by their highly similarity of 0.97. The genetic diversity analysis of the avocado cultivars from Chiayi Agricultural Experimental Station of the Taiwan Agriculture Research Institute confirms that there is high degree of heterzygosity preserved at the avocado germplasm orchard. All of the banding pattern are useful to estimate the genetic basis for selection of parents to explore the heterosis for avocado improvement in the future.

CONCLUSIONS

RAPD analysis of the avocado cultivars preserved at the avocado germplasm ochard of the Chiayi Agricultural Experimental Station (Chiayi, Taiwan) revealed there is high degree of genetic diversity among there accessions and provided a unique tool to differentiate avocado accessions of different origins with more concrete molecular evidence. RAPD analysis were able to provide accurate and reliable genetic diversity analysis in this study under an economic and time-saving manner.

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Table 1. Plant material used in the study and their reported origins.

No.	Accession	Reported OriginZ	No.	Accession	Reported Origin
v1	CAES1	LS	v29	Hall	W x G
v2	CAES2	LS	v30	Pollock	W
v3	CAES3	LS	v33	Hawaii #5	G
v4	CAES4	LS	v34	Lula	G x M
v6	CAES6	LS	v35	Ettinger	M
v11	CAES11	LS	v38	Tou Wei Chi	LS
v13	Lin Der Tsung	LS	v39	Mexican	U
v14	Topa Topa	M	v40	Choquette	G x W
v15	Fuerte	M x G	v44	Reed	G
v16	Hass	G x M	v45	Hung Hsin Hsi Yeh	LS
v17	Hung Chi Chao	LS	v47	Horshim	M
v18	Nabal	G	v54	Yang Hui Huang -1	LS
v19	Bacon	M	v55	79-6-5-3	LS
v20	Zutano	M	v56	G Hass	U
v21	Susan	M	v58	Yang Hui Huang -2	LS
v22	Chuang Nan Shan	LS	v59	Chang An	LS
v23	Stewart	M	v60	G755	P. sch x G
v24	Sweetgarut	U	v61	Toro canyon	U
v26	Duke7	M	v62	Borchard	U
v27	Halemana	G	v63	Mexicola	M
v28	Anaheim	G	v64	Jim	M

ZM:Mexican race, G:Guaremalan race, W:West Indian race, LS:Local Selection,

U:no confirmed record, x: indicated hybridization, *P. sch*:*Persea schiedeana*.

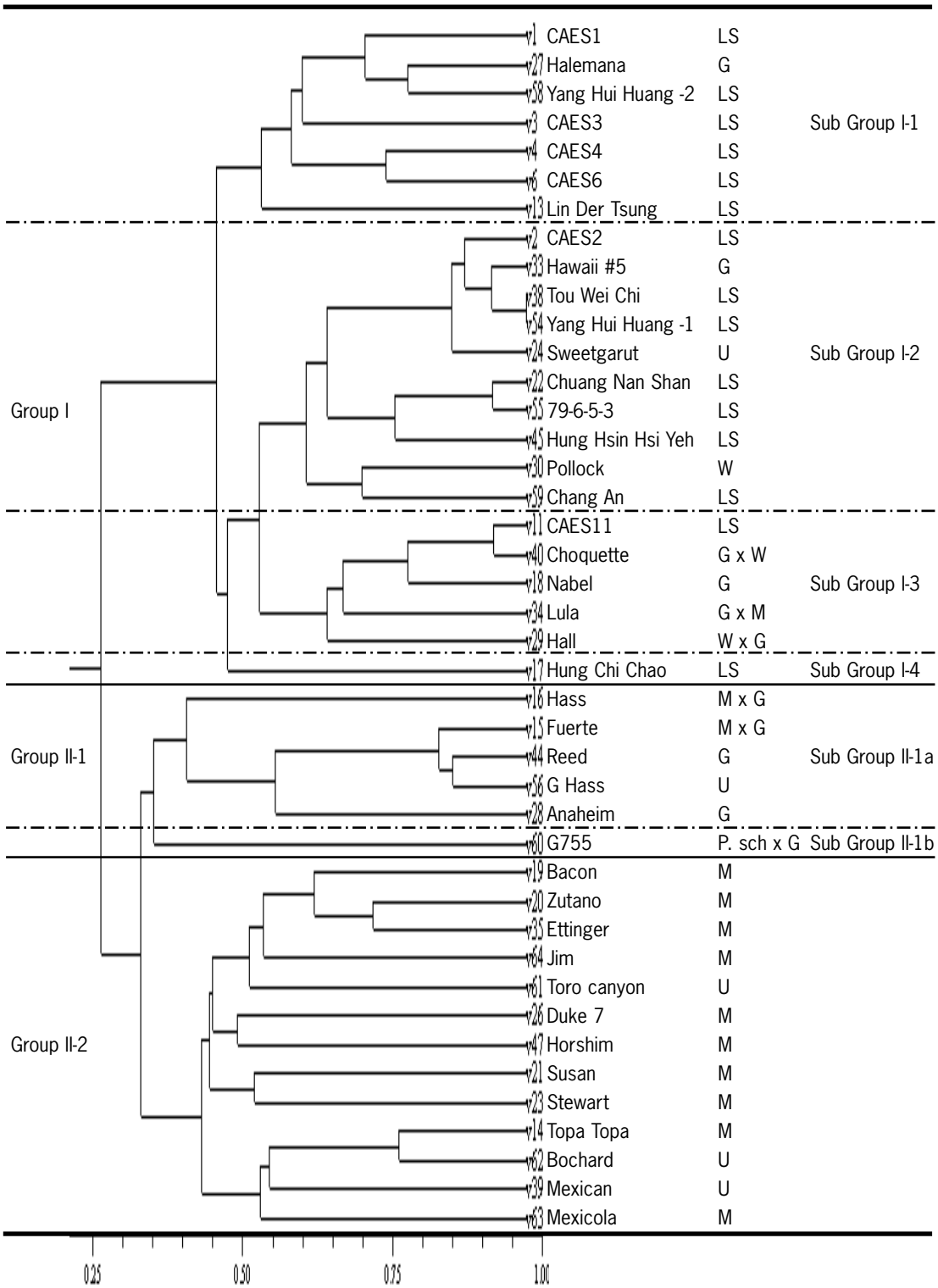

Fig. 1. Dendrogram of avocado accessions revealed by UPGMA cluster analysis on the basis of 107 polymorphic bands. Similarity calculated by Jaccard's coefficient. Description of plant material showed in Table 1.