出國報告（出國類別：國際會議）

2012 果樹生物技術國際研討會

服務機關：行政院農委會高雄區農業改良場
姓名職稱：蔡奇助副研究員
派赴國家：紐西蘭
出國期間：**101** 年 **3** 月 **23~30** 日
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摘要

2012 果樹生物技術國際研討會(Biotechfruit 2012)在紐西蘭舉行，由紐西蘭植物與食品研究所(Plant & Food Research Institute)所主辦，會議主席爲植物與食品研究所的所長 Peter Landon-Lane。研討會總計來自世界各國約 250 位學者參與此會，其中台灣僅筆者一位參加，中國大陸約有 50 位參加。主要的研討內容包含種原特性、基因體分析、基因選殖、基因轉殖、組織培養與分子標誌等領域。心得與建議包括：1. 次代定序技術(Next Generation Sequencing)的應用：次代定序已經大量的被應用於生物技術相關領域的研究，確實可以提升研究的廣度與深度。2. 應積極發展作物基因轉殖技術：目前世界各國都積極在發展作物基因轉移技術，台灣應該不能錯失發展基因轉移的機會。3. 熱帶果樹的發展機會：台灣目前在熱帶果樹的種原蒐集及育種與栽培技術都有一定的基礎，不過在生物技術方面的研究並不多，因此政府應當鼓勵與補助熱帶果樹生物技術方面的研究經費，這對產業的未來發展與永續發展具有積極、正面的影響。4. 鼓勵研發人員積極參與國際活動：台灣現今科學研發人才國際化的程度低，將會是未來的隱憂，政府應該採取積極鼓勵的態度。

目錄

一、目的

本次的 2012 果樹生物技術國際研討會(Biotechfruit 2012)主要針對果樹的生物技術相關領域進行研討，最重要的部分包含基因體分析、基因選殖、基因轉殖與分子標誌。自從台灣在 2006 年以來作物可以申請品種權，讓擁有品種權的業者或育種人員的品種得以受到保護，但由於外部形態判定不易，因此積極開發各種作物的品種 DNA 鑑定技術是刻不容緩的事。作物品種 DNA 鑑定技術主要以簡單序列重複(simple sequence repeat, SSR)爲主流，此技術不僅具有極高的鑑別率，其穩定性亦高，因此是目前國際間共同認可的品種 DNA 鑑定技術。藉由本次的研討得以瞭解目前果樹生物技術方面的最新發展，同時也發現次代定序(next generation sequencing)已經成爲研究基因體、轉錄體及分子標誌的重要技術，未來將次代定序應用於台灣熱帶果樹與蘭花 DNA 分子標誌及轉錄體方面的研究。

二、行程表及研習內容

「2012 果樹生物技術國際研討會」行程內容

BiotechFruit 2012

23nd-30th Mar 2012

時間		地點	行程內容
3 月 23 日	（五）	高雄、香港	高雄小港機場->香港機場->夜宿飛機上
3 月 24 日	（六）	紐西蘭(住宿：威靈頓)	抵達紐西蘭奧克蘭國際機場->尼爾遜機場->飯店
3 月 25 日	（日）		抵達會場及註冊 開幕式 專題演講
3 月 26 日	（一）		歡迎茶會 第一節：Genome sequence 第二節：Germplasm 第三節：Transcriptome James Giovannoni 教授專題演講 海報展示簡報
3 月 27 日	（二）		第四節：Biotic and biotic A 第五節：Plant development 技術參訪
3 月 28 日	（三）		第六節：Health 第七節：Flavour 第八節：Ripening Harry Klee 教授專題演講
3 月 29 日	（四）		ISHS Business Meeting 第九節：Molecular markers 第十節：Tissue culture and transformation Dennis Gonsalves 教授專題演講 閉幕 飯店->尼爾遜機場->奧克蘭國際機場->夜宿飛機上
3 月 30 日	（五）	紐西蘭、香港、高雄、屏東	飛抵香港機場->高雄小港機場->屏東

三、 研討會記要

第二屆果樹生物技術國際研討會(Biotechfruit 2012)是由紐西蘭植物與食品研究所(Plant & Food Research Institute)所主辦，本研討會是在紐西蘭的尼爾森舉行，此地是紐西蘭水果的重要產區，產值約 50 億紐西蘭幣，折合台幣約 1200 億元。尼爾森地區有超過 5000 公頃的果園，包括蘋果，漿果(berryfruit)，彌猴桃(kiwifruit)、橄欖(olive)和啤酒花(hop)，鄰近著名的馬爾堡 (Marlborough) 葡萄酒產區。本研討會吸引來自各界各國的果樹生物技術方面的專家，包含果樹的種原(germplasm)、基因體序列(genome sequence)、轉錄體分析(transcriptome)、生物性與非生物性逆境的基因轉移(biotic and abiotic gene transformation)、植物發育的基因調控(gene regulation for plant development)、果樹老化相關基因研究(gene regulation for fruit ripening)、分子標誌(molecular marker)、組織培養與基因轉殖(tissue culture and transformation)等。另外還利用半天的時間參訪紐西蘭植物與食品研究所 Motueka 分所。每天重要的行程如下：

3 月 25 日：報到，然後由 Mihi Whakatau 博士主持開幕式。藉著紐西蘭由植物與食品研究所的所長 Peter Landon-Lane 代表歡迎及開幕致詞。接著由英國約翰·英納斯中心 Cathie Martin 博士進行一場專題演講，題目爲「Biotechnological opportunities for Citrus improvement arising from the molecular study of Sicilian blood oranges」。專題演講結束後進行開幕酒會。

3 月 26 日：第一節爲基因體序列 (genome sequence)，由 Andy Allan 教授主持，研討的內容包含蘋果、彌猴桃新近基因體相關的研究，以及基因體序列目前的應用與未來將面對的討戰。第二節主題爲種原(germplasm) ，由 Harry Klee 教授主持，研討的內容包含藍莓(blueberry)、彌猴桃、番石榴(guava)的遺傳資源(genetic resource)與基因體資源(genomic resource)，以及阿爾巴尼亞(Albania)的亞熱帶樹種等。第一節爲轉錄體，由 Cameron Peace 教授主持，研討的內容包含蘋果質地(texture)的分子調控(molecular

control)、柑橘(citrus fruit)的轉錄體與蛋白質體的分析、蘋果果皮與果肉的差異性表現基因分析、番茄老化的遺傳與上位遺傳(epigenetic)的調控。

3月27日：第四節爲生物性與非生物性逆境的基因轉移(biotic and abiotic gene transformation)，由 Erik Rikkerink 教授主持，研討的內容包含抗赤霉病基因(crab resistant gene)之選殖、抗氧化酵素基因轉殖可增加番茄在缺水逆境下的產量、木瓜抗PRSV-P病毒病之基因轉殖、海棗水通道蛋白(aquaporin)的分離與特性分析等。第五節爲植物發育的基因調控(gene regulation for plant development)，由 Dennis Gonsalves 教授主持，研討的內容包含蘋果砧木誘導矮化rootstock-induced dwarfing)的遺傳基礎、蘋果SEPALLATA1/2相似基因調控其果肉發育與後熟、蘋果*Co*基因的特性分析等。中午休息一個半小時後參訪紐西蘭植物與食品研究所Motueka 分所，該分所主要的研究作物爲梨子、蘋果、彌猴桃與啤酒花，任務包含上述作物的種原蒐集、栽培技術改進、病蟲害防治、育種等。

3 月 28 日：第六節爲健康，由 Cathie Martin 教授主持，研討的內容包含藍莓的好處、花青素調控、L-galactose 代謝途徑中的 GDP-L-Galactose Phosphorylase 基因經過度表現後可抗壞血酸 ascorbate 能力、從雨生紅球藻(*Haematococcus pluvialis*)選殖的 BKT 與 CRTR-B 基因轉入蘋果，可以讓蘋果合成蝦青素(astaxanthin)等。第七節爲香味(flavour)，由 Kevin Davies 教授主持，研討的內容包含葡萄(*Vitis vinifera*)香氣形成的遺傳、不同品種葡萄的單萜類(monoterpene) 含量、蘋果的酯類香氣複合物(ester flavour compound)的遺傳與生化基礎。第八節果樹老化 (ripening)，由 Chris Watkins 教授主持，研討的內容包含番茄成熟後快速老化(fruit ripening booster)的角色、水果果皮顏色遺傳與環境因子的調控、梨子褐變的轉錄體分析、遺傳與環境因子對蘋果果肉影響的評估、水果香味的分子基礎等。接下來進行海報的簡報與討論。晚上進行晚宴。

3 月 29 日：第九節爲分子標誌(molecular marker)，由 Cesare Gessler 教授主持，研討

的內容包含蘋果育種複雜性狀篩選、發展可以應用石榴(Pomegranate)的育種的基因體工具、判定彌猴桃幼苗性別的分子標誌、提升蘋果維他命 C 含量的分子育種、分子標誌輔助薔薇科(Rosaceae)植物育種、覆盆子(raspberry)多酚混合物的基因座定位等。第十節爲組織培養與基因轉移(tissue culture and transformation)，由 Fred Gmitter 教授主持，研討的內容包利用秋水仙素(colchicine)處理育成四倍體(tetraploids)柑橘、彌猴桃與蘋果的基因轉殖、蘋果薔薇 (*Rosa pomifera*)的組織培養等。最後由 Dennis Gonsalves 進行一場專題演講，題目爲「Hawaii's transgenic papaya: development, deregulation and commercialization」。然後進行閉幕式。

四、心得及建議

1. <u>次代核酸定序的應用：</u>

次代核酸定序是最新發展的研究分子生物學的工具，其效能遠百萬倍於傳統核酸序列分析，因此可以有效的提升分子生物各領域的研究效能。此技術不僅可以進行物種基因體的讀序，也可以釣取作物的 SSR 基因座，以及進行轉錄體差異性表現分析，以釣取有用的基因，可供應用於未來作物的基因轉殖。

2. <u>應積極發展作物基因轉殖技術：</u>

雖然目前基因轉殖在國際間仍分兩派，一派主張積極開放，另一派剛好相反，雖然如此，但消極保守的國家也一樣積極發展基因轉殖技術，如日本、歐洲等國家無不積極發展基因體、基因轉殖科技，唯有台灣目前對於基因轉移技術的發展，不僅採取消極保守政策，連研究發展的研究經費也少的可憐，當有朝一日基因科技被全球各國接受後，台灣這方面的技術將遠遠的落後，這是值得深思與重視的問題。

3. <u>熱帶果樹的發展機會：</u>

目前世界西方先前國家從事的研究主要以溫帶果樹爲主，還有柑橘與彌猴桃，因

此熱帶果樹是台灣的強項，台灣目前在熱帶果樹的種原蒐集及育種與栽培技術都有一定的基礎，不過在生物技術方面的研究並不多，因此政府應當鼓勵與補助熱帶果樹生物技術方面的研究經費，以站穩台灣熱帶果樹，如蓮霧、芒果、印度棗、鳳梨、番石榴等的研究地位，這對產業的未來發展與永續發展具有積極、正面的影響。

4. 鼓勵研發人員積極參與國際活動：

中國大陸現在參與國際研討會的相當踴躍，本次國際研討會參與的人數約 250 人，中國大陸大約占 1/5，可見中國大陸現在不僅具有很強的經濟實力，在科學研究方面的軟實力也在積極培育，不僅鼓勵出國進修，也鼓勵積極參與國際活動，未來的科學研發將無可限量。反觀台灣參與的人數僅筆者一名，由於經費的限制，根本住不起研討會會議室所在的飯店，這是會令人對台灣未來的競爭力感到不安。建議應該利用積極正面的角度去鼓勵研究人員參與國際活動，不僅能提昇研究的水平，也能增進研發人員的國際視野。

五、附件

1. 發表論文內容

Isolation and characterization of 16 polymorphic microsatellite markers from *Mangifera indica* L. (Anacardiaceae)

Chi-Chu Tsai
Crops Improvement Division
Kaohsiung District Agricultural Research and Extension Station
Pingtung County
Taiwan.

Keywords: cultivar identification, genetic diversity, microsatellite DNA

Abstract

Sixteen polymorphic microsatellite loci for mango (*Mangifera indica* L.), an important commercial fruit tree in East Asia, were developed to evaluate the genetic diversity and identification of cultivars. The 16 polymorphic microsatellite markers were isolated from mango using a magnetic bead-enrichment method and polymorphisms were identified in 22 mango cultivars. The number of alleles ranged from 2 to 8, with an expected heterozygosity ranging from 0.304 to 0.826. The polymorphism information content (PIC) ranged from 0.253 to 0.756 (average = 0.543). These polymorphic loci should be useful and convenient for further studies of the genetic diversity and identification of cultivars in mango.

INTRODUCTION

Mango belongs to the genus *Mangifera* of the family Anacardiaceae. The genus *Mangifera* includes several fruit trees. We are commonly known as mangos belonging to the species *Mangifera indica* L. (Singh, 1968). The plant originates in tropical Asia, including the north-eastern region of India, the western region of Myanmar, and Bangladesh (Mukherjee, 1972). Mango is a popular commercial fruit tree and has been cultivated in India for more than 4,000 yr (Mukherjee, 1972).

Usually, mango cultivars have obtained through open pollination and seedlings selection. Commercialized mango cultivars have been propagated and cultivated all over the world (Ravishankar et al., 2000). In general, mango cultivars belong to two distinct groups, i.e. polyembryonic types and monoembryonic types (Mukherjee, 1949). Traditionally, morphological and agronomical characters have been employed as criteria for mango cultivars identification (Mukherjee, 1948; Singh, 1968). Recently, DNA markers have been widely used for genetic relationship and cultivar identification. In Mango, several types of DNA

markers has been applied for the cultivar identification, such as amplified fragment length polymorphism (AFLP) (Kashkush *et al.*, 2001), random amplified polymorphic DNA (RAPD) (Adato *et al.*, 1995; Schnell *et al.*, 1995; Ravishankar *et al.*, 2000), inter-simple sequence repeat (ISSR) (Eiadthong *et al.*, 1999). Recently, Viruel *et al.* (2005), Schnell *et al.* (2005), and Ravishankar *et al.* (2011) have developed 16, 15, and 30 microsatellite markers for mango, respectively.

In the study, we have developed an additional set of 16 microsatellite loci and tested these using 22 mango cultivars/lines.

MATERALS AND METHODS

Plant materials

22 mango samples were collected and cultivated at KDARES, Taiwan and farmers' field (Table 1).

Methods

Mango samples were collected and cultivated at Kaohsiung District Agricultural Improvement and Research Station (KDARES), Ping-Tung County, Taiwan. Variety Tsar-Swain was chosen for further SSR loci isolation. Genomic DNA was extracted from adult leaf powder using the CTAB procedure (Murray and Thompson, 1980).

Microsatellite loci were isolated following the method of Liao *et al.* (2009), modified from the method proposed by Zane *et al.* (2002). Genomic DNA was digested using the restriction enzyme *Mse*I (Promega, USA) and DNA fragments from 400 to 1,000 bp were isolated from agarose gels using the MinElute Gel Extraction Kit (Qiagen). The purified partial genomic library was ligated to adaptors (complementary oligo A: 5'-TACTCAGGACTCAT-3' and 5' phosphorylated oligo B:5'-GACGATGAGTCCTGAG-3'). The partial genomic library was enriched using 15 cycles of prehybridization polymerase chain reaction (PCR) using adaptor specific primers (5'-GATGAGTCCTGAGTAAN-3', hereafter referred to as *Mse*I-N). The enriched partial genomic library was denatured and hybridized to two different biotinylated probes [B-$(AG)_{15}$, B-$(AC)_{15}$] at 68℃ for 1 h for enrichment. The DNA hybridized to probes was incubated and captured using Streptavidin MagneSphere Paramagnetic Particles (Promega, USA). The enriched DNA fragments were then used as templates for 25 cycles of PCR amplification using *Mse*I-N. Purified PCR products were cloned directly into the pGEM-T Easy Vector System (Promega, USA).

Plasmids containing the PCR product were isolated using an alkaline lysis protocol (Birnboim and Doly, 1979), screened using PCR [primer pairs: $(AG)_{10}$ or $(AC)_{10}$ and SP6 or T7), and purified with a QiaGen kit (Qiagen, Germany)]. The selected plasmids were subsequently sequenced in both directions using an ABI BigDye3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, USA) with the ABI PRISM 3700 DNA Automated Sequencer.

RESULTS AND DISCUSSION

We confirmed 58 microsatellite loci from 171 selected sequences using Tandem Repeats Finder version 4 (Benson, 1999). Microsatellite primer pairs were designed based on the flanking sequences of microsatellite loci using FastPCR software version 5.4 (Kalendar, 2009). We then synthesized 30 primer pairs and screened them using a gradient PCR protocol with a Labnet MultiGeneTM 96-well Gradient Thermal Cycler to find the best annealing temperature. The thermocycling profiles were: initial denaturation at 94℃ for 5 min, followed by 30 cycles of 40 s denaturation at 94℃, 1 min annealing at 50~63℃, 1 min extension at 72℃, and a final extension for 10 min at 72℃. PCR products were separated on 1% agarose gels to evaluate the optimal annealing temperature (Table 2). To screen for polymorphisms in Indian jujube, 21 samples were examined using the designed primers. PCR was performed using the GeneAmp PCR System 9700 (Applied Biosystems, Singapore) and products were separated using 10% polyacrylamide gel electrophoresis. In total, 16 polymorphic microsatellite loci were obtained.

The degree of polymorphism—including the number of alleles (N_A), the number of effective alleles (Ne), and the expected heterozygosity (H_E)—and polymorphism information content (PIC) were computed using GenAlEX version 6 (Peakall and Smouse, 2006). Results for the polymorphisms are summarized in Table 3. The number of alleles per locus ranged from 2 to 8. As shown in Table 2, Ne, H_E, and PIC ranged from 1.423 to 5.177 (average = 2.719), 0.304 to 0.826 (average = 0.614), and 0.253 to 0.756 (average = 0.543), respectively. In final, we conclude that all microsatellite loci reported in the study have the potential to be used to estimate gene diversity and cultivar identification of mango.

ACKNOWLEDGEMENTS

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Tables

Table 1. Mango cultivars used in this study.

No.	Cultivars	Voucher
1	Tsar-Swain	C. C. Tsai 3001
2	Haden	C. C. Tsai 3002
3	Jin-Mi	C. C. Tsai 3003
4	Tainung No. 1	C. C. Tsai 3004
5	Jin-Huang	C. C. Tsai 3005
6	Irwin	C. C. Tsai 3006
7	Keitt	C. C. Tsai 3007
8	Red Keitt	C. C. Tsai 3008
9	Sensation	C. C. Tsai 3009
10	Yu-Un No. 6	C. C. Tsai 3010
11	Jin-Xing	C. C. Tsai 3011
12	Shan-Lin No. 1	C. C. Tsai 3012
13	Kaohsiung No. 3	C. C. Tsai 3013
14	Line 8302-1	C. C. Tsai 3014
15	unknown	C. C. Tsai 3015
16	unknown	C. C. Tsai 3016
17	unknown	C. C. Tsai 3017
18	ChokAnan	C. C. Tsai 3018
19	Line 8302-2	C. C. Tsai 3019
20	Line 8302-3	C. C. Tsai 3020
21	Line 8302-4	C. C. Tsai 3021
22	Tsar-Swain	C. C. Tsai 3022

Voucher specimens were deposited at the herbarium of National Museum of Natural Science, Taiwan (TNM)

Table 2. Characteristics of 16 microsatellite loci isolated from *Mango* (*Ta*, optimized annealing temperature)

SSR Locus	Primers (5'→3')	Allelic size (bp)	Repeat Motif	*Ta*(℃)
Mango-2	F: ATACCCGCAAGAGAGTATGC R: CAAGACTTCAACTTTGTGTCAG	165-179	$(CT)_{15}$	58
Mango-13	F: TTCTGGCAATGAAATACTACG R: ATCTACACTCACGGCAGAAG	143-177	$(AT)_7(GT)_{15}(TA)_7(GA)_{11}$	58
Mango-103	F: AGAGTGGTGATGGGAGCGTA R: GTCGGATCTGCTAAAACCCG	146-160	$(AG)_{15}$	58
Mango-154	F: AGTAAACCAGTTTGACGTCC R: TGTTTAGCAGCTCAGTCCTC	164-184	$(CT)_{16}$	58
Mango-160	F: AATGCCAAAACTCTAGAGTC R: TCAAAACCCACTCATACGAC	251-259	$(GA)_{21}$	58
Mango-175	F: ACTCAAATCAGCTTTAGGTC R: AGCGTAAATAGAAGAGAGCAG	189-205	$(TC)_{14}$	58
Mango-182	F: AAGTGCACAAGCATGTGCTG R: TGTTGATATGCGTCCTCAAC	139-163	$(GA)_{13}$	58
Mango-195	F: ACGGGATAAGCATCAGAGTCG R: TTCTCCTCGACCTACTGCT	214-238	$(GGAGAA)_{13}$	58
Mango-221	F: TGCCATGTACCATATTGCTG R: TTCAACCACAATAATCTGCC	187-207	$(CA)_{15}$	58
Mango-265	F: ACTAGCAATCAGCATGAACC R: TTGTCCTCCTATTTTATCCG	129-133	$(TC)_{17}$	58
Mango-267	F: ACTGGTATTGCGGTTTTGAG R: AGGAGGGTGGTTCTAATAGC	211-225	$(TGA)_{12}$	58
Mango-325	F: TGTACTTCTGTTTGCTTTTG R: AGTATCCTCTTGAACATAGG	171-193	$(CT)_{15}$	58
Mango-326	F: AGAGGTAGGCCATCGATTCC R: ATACTGTACGCTACCTCCTC	144-146	$(CT)_{15}$	58
Mango-336	F: TTGCAGAGTAATTACGGCAG R: GTCTTGAGTAAACACAATGC	161-169	$(TC)_{23}(TG)_6$	58
Mango-343	F: AGGAGTTCAGTTGTGGACAG R: TTTTGGACCACCTCATCTCG	148-180	$(AG)_{14}$	58
Mango-364	F: CAAGCAATCAGTCAGTAAGC R: CTTTTGAGTGGCAAGTACTC	172-196	$(TC)_{28}$	58

Table 3. Genetic diversity of mango

SSR Locus	N_A	N_e	H_E	PIC
Mango-2	4	2.890	0.669	0.591
Mango-13	7	5.177	0.826	0.756
Mango-103	6	2.338	0.586	0.503
Mango-154	8	2.727	0.648	0.581
Mango-160	4	3.470	0.728	0.666
Mango-175	4	2.300	0.578	0.482
Mango-182	5	3.102	0.693	0.667
Mango-195	2	1.423	0.304	0.253
Mango-221	4	3.000	0.682	0.613
Mango-265	3	2.498	0.604	0.519
Mango-267	2	1.996	0.511	0.375
Mango-325	4	2.839	0.663	0.563
Mango-326	4	1.940	0.496	0.437
Mango-336	3	2.469	0.609	0.535
Mango-343	5	2.004	0.513	0.469
Mango-364	6	3.338	0.717	0.672

N_A number of alleles, N_e number of effective alleles, H_E expected heterozygosity, PIC polymorphism information content

Figures

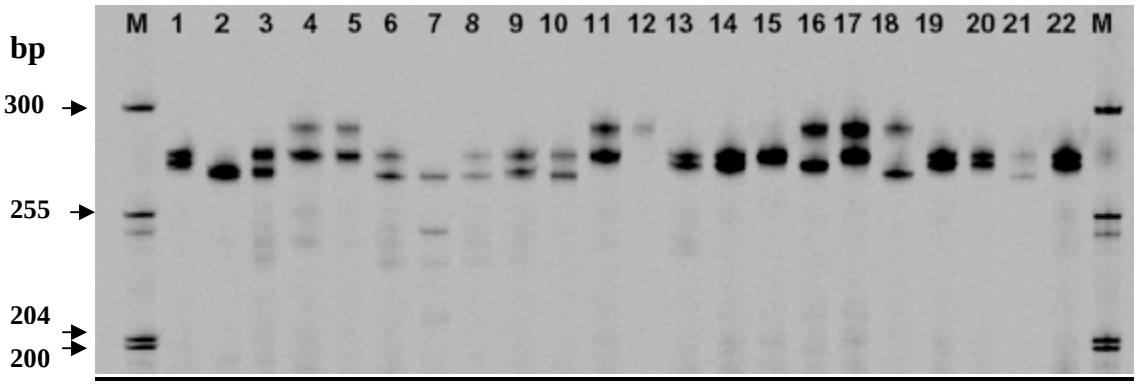

Figure 1. Mango-160 SSR locus analysis of polymorphism in 22 mango cultivars/lines. M: DNA marker. Lanes 1~22 represent different cultivars (M1~M22, see Table 1).

2. 本次國際研討會相關照片

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