出國報告（出國類別：出席國際會議）

出席『第六屆體外培養與園藝育種國際研討會』報告

Sixth International Symposium on In Vitro Culture and Horticultural Breeding

姓名職稱與服務機關：
蔡奇助 高雄區農業改良場
派赴國家：澳洲
出國期間：97 年 8 月 23 日～30 日
報告日期：97 年 11 月 4 日

報告名稱:

出席『第六屆體外培養與園藝育種國際研討會』

主辦機關:

高雄區農業改良場

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蔡奇助

出國類別: 參加國際研討會

出國地區: 澳洲

出國期間: 民國 97 年 08 月 23 日 -民國 97 年 08 月 30 日

報告日期: 民國 97 年 10 月 31 日

分類號/目:

關鍵詞: 體外培養、遺傳資源、園藝育種、國際研討會

內容摘要: 本次國際研討會由 ISHS 在澳洲布利斯班舉辦,由昆士蘭科技大學 Acram Taji 教授負責籌辦。來自世界各國的一百多位學者參與此盛會。研討會進行五天，分為七個主題，共發表 135 篇報告，其中台灣代表 3 篇，除了本人還有來自亞蔬的 Dr. Robert 及生技中心的劉博士。內容主要介紹世界各地組織培養、分子育種、體胚再生、基因轉殖、體外培養育種、種原保育等的最新研究，收穫甚多，也瞭解目前國際間體外培養及園藝育種的新方向。心得與建議包括：體外培養與園藝育種是農業領域中發展快速一項，此兩項技術也息息相關，體外培養除了可以大量繁殖外，應用在育種上，如遠緣雜交的胚拯救、試管受精克服不親和的雜交、染色體加倍等都能創造新的園藝品種，增加品種多樣化，提升產業競爭力。深深感受到國外的研究機構與學校最重視的是人才培育，重視團隊的合作與研發，如此往往能事倍功半。不過台灣在體外培養產業化的普及度並不比國外差，尤其是體外培養技術在蘭花大量繁殖應用的成績是有目共睹的。總之，台灣在蝴蝶蘭產業已經佔有很好的基礎，應該持續向前邁進。

一、目的

體外培養、園藝育種及遺傳資源在農業的發展上佔有重要角色。台灣地窄人稠適合發展高經濟價值的種苗業或設施園藝,藉此國際研討會可以瞭解目前國際間在體外培養、園藝育種與重要遺傳資源的發展趨勢並吸取技術新知,藉此可以釐清更適合台灣發展的園藝產業。

二、過程

「第六屆體外培養與園藝栽培國際研討會」內容

23nd-30th Aug 2008

時間		地點	行程內容
8 月 23 日	（六）	高雄、香港	高雄小港機場->香港機場->澳洲布利斯班國際機場
8 月 24 日	（日）	澳洲布利斯班	抵達及註冊 開幕式及專題演講
8 月 25 日	（一）		第一節植物組織培養最新發展 第二節植物組織培養最新發展(續) 第三節植物分子育種 第四節植物分子育種(續)
8 月 26 日	（二）		第五節新近發展技術 第六節體胚再生 布利斯班植物園參觀
8 月 27 日	（三）		第七節植物基因轉殖 第八節植物基因轉殖(續) 第九節體外培養育種 第十節體外培養育種(續)
8 月 28 日	（四）		第十一節種原保育 第十二節植物組織培養新近發展 第十三節微體繁殖

8 月 29 日	（五）	澳洲、香港、高雄	澳洲布利斯班國際機場->澳洲凱恩斯機場暫停->香港機場->高雄小港機場 (預計 23:35 抵達)
8 月 30 日	（六）	高雄、屏東	高雄小港機場出關->屏東

三、心得及建議

1. 國際研討會的價值：

本次國際研討會在澳洲布利斯班市中心舉辦，借用一家飯店的大廳舉行，沒有氣派的場地與豪華設備，不過研討會內容精彩且行程流暢，給予本人深深的體悟，學術研究重要的不是硬體，而是軟體，也就是人才。如何啟發、教育年輕學子積極投入研究是未來大家需要思考的地方。

2. 台灣的體外培養的研究：

台灣在蘭花方面組織培養的研究與實際的應用是全世界有目共睹的，尤其是蝴蝶蘭的育種與種苗大量繁殖，我們無須妄自菲薄，應該更積極投入相關的研究，搭配基因工程相關研究，使台灣蘭花的研究更上一層。

3. 遠緣雜交與胚拯救技術的應用：

育種是園藝產業能否持續發展的重要基石，若能擴大遺傳資源的應用，則能比別的國家佔有更有利的位置，而遠緣雜交與胚拯救技術能夠擴大育種的親本來源。本次研討會也有不少遠緣雜交與胚拯救技術方面的研究報導，筆者也發表蘭花的遠緣雜交與胚拯救技術，在蘭花方面居於領先的地位，建議政府能持續投入研究經費。

4. 提升台灣體外培養與基因轉殖之研發能量：

台灣在體外培養及園藝育種與基因轉殖方面都有不錯的成果，不過在論文的國際能見度上遠遠不及實質的成果，主要礙於科學性

的英文論文寫作方面，若能有一個專門為研究人員修飾英文論文寫作的專責機構應該對我國的科學論文的研發與發表甚有幫助。另外，文獻在台灣的流通也相當不方便，在資源分享方面應該有多元的管道，尤其是電子期刊的時代來臨，若能善加應用，應該更能提升台灣的研發能量。

四、 研討會記要

本次研討會由國際園藝學會(International Society for Horticultural Science, ISHS)主辦，昆士蘭科技大學 Acram Taji 教授負責籌辦。主題是『第六屆體外培養與園藝育種』國際研討會。來自 41 個國家，總計 168 人參加，其中台灣有三位參加，分別是亞洲蔬菜中心的 Dr, Robert、財團法人生物技術開發中心的劉博士及本人。值得一提的是大陸有 11 人參加，可見中國大陸學者現今也相當積極參與國際研討會。

研討會進行五天，可以歸納為七個主題，共發表 152 篇報告。內容涵蓋植物組織培養新近發展、分子育種、體胚形成、植物基因轉殖、體外育種、遺傳資源保育、微體繁殖等。

第一主題「植物組織培養新近發展」(Advances in Plant Tissue Culture)：主要在闡述組培褐化的成因、植物生長調節劑的偕同利用、屬間雜交及胚拯救在園藝上的應用，組織培養在植物上位遺傳的調控等。

第二主題「分子育種」(Molecular Plant Breeding)：敘述分子標誌在品種鑑別之應用與組培變異的偵測，玫瑰的育種、草皮草的水分及養分的利用、人造四倍體百香果的上位遺傳效應、改造園藝植物品質的 GMO 平台、利用基因轉殖縮短蘋果幼年期、利用 ISSR 分析印度芒果的品系內變異、利用基因工程擴大柑橘種原。

第三主題為「體胚形成」(Embryogenesis)：含香蕉的體胚形成、秋海棠的體胚形成與植株再生、椰子的體胚形成、原生質體培養等。

第四主題為「植物基因轉殖」(Transgenics)，敘述香蕉轉殖提早開花基因、蘋果轉殖抗病基因、文心蘭轉殖突變的乙烯接受器、基因改造中國結球白菜的基因流傳測定等。

第五主題為「體外育種」(In Vitro Breeding)，遠緣雜交的早期胚拯救、體外育種在傳統育種之應用、甜椒與辣椒之花藥及花粉培養、體外培養輔助杜鵑育種、體外培養輔助核果育種、喜樹的組織培養與轉殖。

第六主題為「遺傳資源保育」(Germplasm Conservation)，敘述香蕉轉殖提早開花基因、蘋果轉殖抗病基因、文心蘭轉殖突變的乙烯接受器、基

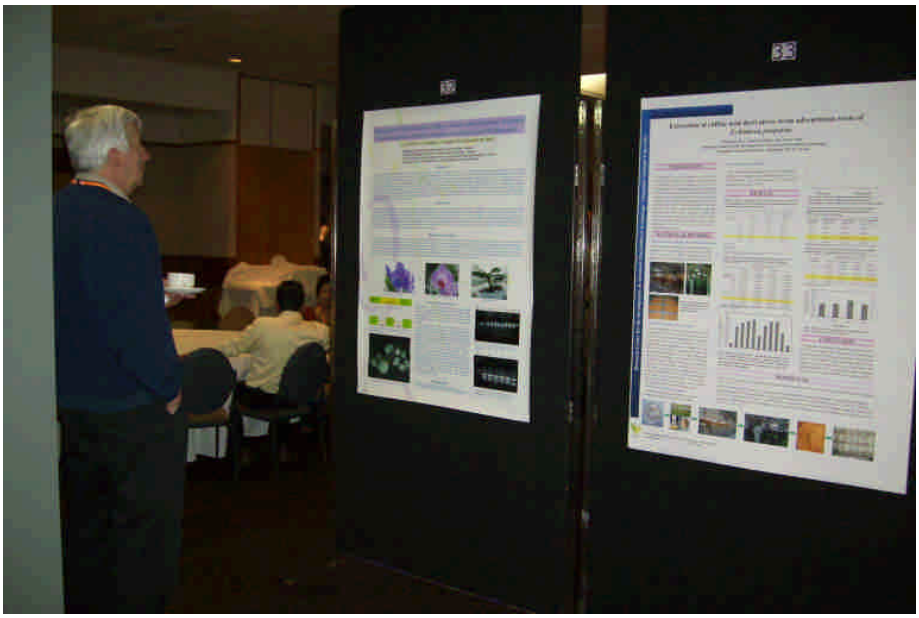
因改造中國結球白菜的基因流傳測定等。

第七主題為「微體繁殖」(Micropropagation)，敘述體外發根、園藝植物半自動培養系統、仙客來的組培的組織切騙、組培提供健康種苗使更適合商業生產、莎草科植物的組織培養、菊花的組織培養等。

本次國際研討會相關照片

研討會場地

會議籌辦人昆士蘭科技大學 Acram Taji 教授的演講



海報展示場景

筆者和與會學者合照

8 月 26 下午參觀布利斯班植物園

8 月 27 晚上舉辦研討會歡迎晚宴，澳洲當地原住民歌舞**表演**

附錄一：議程

Sixth IVCHB symposium

Time schedule and agenda

Sunday, 24 August, 2008

16:00-16:30 **Official Opening**

Monday, 25 August, 2008

09:00-10:35 **Session 1: Advances in Plant Tissue Culture**
10:35-11:00 MORNING TEA
11:00-12:00 **Session 2:Advances in Plant Tissue Culture（Continued）**
12:00-13:00 **POSTER SESSION ONE**
13:00-13:45 LUNCH
13:45-15:20 **Session 3: Molecular Plant Breeding**
15:20-15:40 AFTERNOON TEA
15:40-16:55 **Session 4: Molecular Plant Breeding（Continued）**
16:55-17:25 Biotechnology Commission Meeting

Tuesday, 26 August, 2008

09:00-10:35 **Session 5: Emerging techniques**
10:35-11:00 MORNING TEA
11:00-12:15 **Session 6: Embryogenesis**
12:15-13:15 LUNCH
13:30-17:00 **Brisbane Botanic Gardens** Mt Coot-tha Tour（coach transport provided,departing Chifley）

Wednesday, 27 August, 2008

09:00-10:20 **Session 7: Transgenics**
10:20-10:45 MORNING TEA
10:45-11:30 **Session 8: Transgenics（Continued）**
11:30-12:30 **POSTER SESSION TWO**
12:30-13:30 LUNCH
13:30-15:05 **Session 9: In Vitro Breeding**
15:05-15:30 AFTERNOON TEA

15:30-17:00 **Session 10: In Vitro Breeding（Continued）**

18:30-22:30 **Symposium Dinner-Customs House**
399 Queen Street （Walk to Customs House down Queen Street or along the river boardwalk）

Thursday, 28 August, 2008

09:00-10:35 **Session 11: Germplasm Conservation**

10:35-11:00 MORNING TEA

11:00-12:30 **Session 12: Recent Advances in Plant Tissue Culture**

12:30-13:30 LUNCH

13:30-15:15 **Session 13: Micropropagation**

15:15-15:45 CLOSE OF SYMPOSIUM and afternoon tea

附錄二：論文內容

Intergeneric hybridization, embryo rescue and molecular detection for intergeneric hybrids between *Ascocenda* and *Phalaenopsis*

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Keywords: moth orchid, internal transcribed spacer, chloroplast DNA, PCR-RFLP

Abstract

Intergeneric hybridization and embryo rescue were conducted between *Ascocenda* John De Biase "Blue" (♀) and *Phalaenopsis* Chih Shang's Stripes (♂) in this study. Capsules of intergeneric hybrids were harvested after four months pollination. The immature embryos with placenta from those capsules were transplanted into artificial tissue culture medium. About 200-300 intergeneric embryos for each capsule were rescued. After one-year subculture and two-week hardiness, intergeneric hybrids were cultivated in greenhouse. Different morphologies not only stem high bout also leaf shape were found in those intergeneric hybrids. For confirming their genetic background among intergeneric hybrids, polymerase chain reaction (PCR) amplified restriction fragment length polymorphism (RFLP) [PCR-RFLP] of internal transcribed spacer (ITS) of ribosomal DNA were used to analyze the inheritance of hybrids (F_1). For the ITS of PCR-RFLP, 60 hybrids were analyzed and the banding pattern of 59 hybrids were biparental inheritance and one hybrid was maternal inheritance. Unequal crossover or gene conversion was obviously turned on to homogenize the repeat sequence of ITS during the intergeneric hybridization on the hybrid with maternal inheritance. In this study, based on the molecular data, intergeneric hybridization and embryo rescue is successful.

INTRODUCTION

Orchids belong Orchidaceae and are the largest family within angiosperms. It includes approximately 900 genera and 35000 species (Arditti, 1992). One of popular, commercial and beautiful orchid is *Phalaenopsis* (moth orchid). The native species for the genus are approximately 66 species, mainly distribute in Southeast Asia (Christenson, 2001). Up to the present, orchid breeding has been conducted for over 150 years (Lenz and Wimber, 1959). Intrageneric or intergeneric hybrids are useful for the breeding of new cultivars. However, in some cases, successful intrageneric or intergeneric crosses are difficult to finish. Post-zygotic barriers are commonly observed, and can be overcome through embryo rescue (Palmer et al., 2002). The rescue of hybrid embryos resulted from intra- and inter-generic crosses is commonly applied in *Phalaenopsis* breeding programs. From past few decades, thousands of *Phalaenopsis* varieties have been bred and commercialized based on Intrageneric hybridization. In the study, in order to introduce unique blue color gene from *Ascocenda* into *Phalaenopsis*, intergeneric hybridization and embryo rescue were conducted between *Ascocenda* John De Biase "Blue" (♀) and *Phalaenopsis* Chih

Shang's Stripes (♂). Furthermore, the genetic analysis of those intergeneric hybrids was conducted based on molecular data.

MATERIALS AND METHODS

Ascocenda John De Biase "Blue" and *Phalaenopsis* Chih Shang's Stripes are maternal and paternal parents, respectively. The immatured embryos were rescued after about 90 days of artificial pollination between *Ascocenda* John De Biase "Blue" (♀) and *Phalaenopsis* Chih Shang's Stripes (♂). The capsule was harvested and immature embryos with placenta were transplanted and cultured in medium of 3 g/L Hyponex No. 1, supplemented with 30 g/L peptone, 8 g/L agar, 150 ml/L coconut water, 30 g/L sucrose and 1 g/L of activated charcoal. The pH of the medium was adjusted to 5.8 before autoclaving. Total DNA was extracted from fresh etiolated leaves by use of cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). The *trn*L intron of cpDNA and internal transcribed spacer (ITS) of nrDNA were amplified by using universal primer pairs (Taberlet et al.,1991; Tsai and Huang, 2001). *Alu*I and *Taq*I restriction enzymes were used to detect polymorphism of PCR products of ITS and *trn*L intron, respectively.

RESULTS AND DISCUSSION

Capsules and seeds of intergeneric hybrids were harvested and immature embryos were rescued by tissue culture. After two-month culture, the embryos derived from intergeneric hybridization between *Ascocenda* John De Biase "Blue" and *Phalaenopsis* Chih Shang's Stripes grew up called protocorms (Fig. 1). Approximately 200-300 embryos in each capsule were successfully rescued. The protocorms were sub-cultured once per month for forming plantlet. After one year, plantlets were transplanted into greenhouse. The morphological characters of intergenric hybrids revealed intermediate phenotype of *Ascocenda* and *Phalaenopsis* parents.

Based on the nuclear ITS of PCR-RFLP, PCR products for ITSs of 60 F1 hybrids, maternal and paternal parents were all shown up about 746 bp (data not shown). Under *Alu*I restriction enzyme analysis, one cutting site (604 and 142 bp) was found in *Ascocenda* plant, and no cutting site was found in *Phalaenopsis* plant. Most of hybrids (59/60) are biparental inheritance and only one hybrid is maternal inheritance (Fig. 2). ITS repeat sequences are found to be biparental inheritance in natural hybrid of tetraploidy *Paeonia* (Sang et al., 1995), polyploid cotton (Wendel et al., 1995b). The homogeneity of ITS repeat sequences also can be found in *Gossypium* (Wendel et al., 1995a). The maternal inheritance of hybrid might be resulting from rapid concerted evolution based on unequal crossing over (Schlotterer and Tautz, 1994) and biased gene conversion (Hillis et al., 1991). Furthermore, recombination within ITS region between maternal and paternal plants was found existing in natural hybrid derived from *Phalaenopsis x intermedia* (Tsai et al., 2006). However, it was not found in these intergeneric hybrids in the study.

Based on chloroplast *trn*L intron of PCR-RFLP, the PCR products for ITSs of 60 F1 hybrids, maternal and paternal parents were all revealed about 785 bp (data not shown). From *Taq*I restriction enzyme analysis, the DNA band patterns between maternal, paternal parents and all hybrids are same (Fig. 3). It showed that maternal inheritance of chloroplast DNA is existing in the combination between *Ascocenda* and *Phalaenopsis*. The result is also in agreement with that uniparental inheritance introduced for chloroplast DNA (cpDNA) (Derepas and Dulieu, 1992). The maternal inheritance of chloroplast DNA in intergeneric hybrids also showed in intrageneric

hybrids of *Phalaenopsis* (Chang et al., 2000). Based on cpDNA analysis, intergeneric hybrids between *Ascocenda* and *Phalaenopsis* showed the maternal inheritance. Therefore, this technique is not suitable to identify intergeneric hybrids in advance. However, it indicated that cpDNA of intergeneric hybridization are maternal inheritance as most of intraspecific and interspecific hybridization in angiosperms.

In conclusion, intergenerc hybridization of *Ascocenda* and *Phalaenopsis* can result in increasing the cultivar diversity. New genes of certain horticultural characters can be introduced from *Ascocenda* into *Phalaenosis*, such as floral color. Molecular detection of ITS of PCR-RFLP can offer an efficient identification way for intergeneric hybrids in advance.

ACKNOWLEDGEMENTS

This research was supported by funding from the National Science Council, Executive Yuan, R.O.C.

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Figures

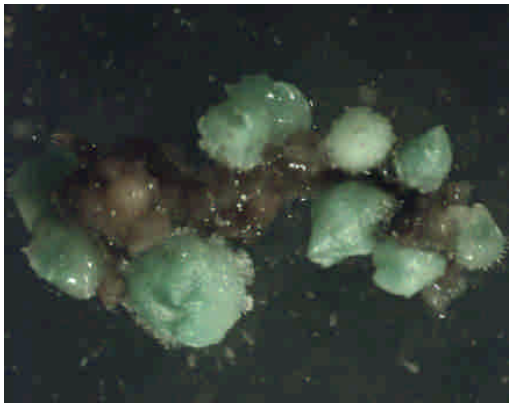

Fig. 1. Rescued embryos were showed after two-month culture.

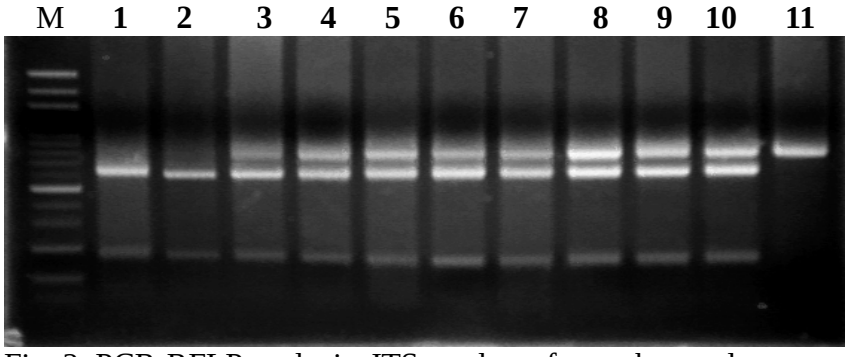

Fig. 2. PCR-RFLP analysis. ITS products for each sample were cut by *Alu*I restriction enzyme. Lanes 1 and 11 represent maternal (*Ascocenda* John De Biase "Blue") and paternal parent (*Phalaenopsis* Chih Shang's Stripes), respectively. Lanes 2-10 represent nice hybrids. M: 100 bp DNA ladder marker.

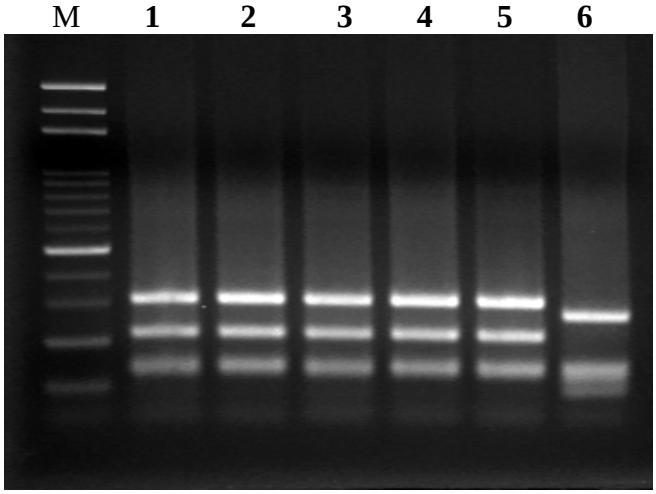

Fig. 3. PCR-RFLP analysis. The *trn*L introns for each sample were cut by *Taq*I restriction enzyme. Lanes 1 and 6 represent maternal (*Ascocenda* John De Biase

"Blue") and paternal parent (*Phalaenopsis* Chih Shang's Stripes), respectively. Lanes 2-5 represent four hybrids. M: 100 bp DNA ladder marker.