出國報告（出國類別：出席國際會議發表論文）

參加基因轉殖技術和植物遺傳育種技術國際研討會報告

服務機關：國立嘉義大學
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出國期間：102 年 2 月 12-23 日
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摘要

奧地利維也納國際植物會議協會(VIPCA, Vienna International Plant Conference Association)是一個非商品和營利的組織於 2009 成立，位於維也納大學內，其目的在主辦專業和高品質的生物和農業研討會。本次出國目的是參加「Genetic Transformation Technology」和「Plant Genetica and Breeding Technology」研討會，會議會期分別爲 2 月 14-15 日的「基因轉殖技術研討會」和 18-20 日的「植物遺傳育種研討會」。研討會在中歐的奧地利維也納大學舉行，參加的研究學者大部分來自歐洲國家，所以論文發表內容與國內研究不太一樣，許多的論文都是有關數量遺傳和應用分子生學工具於作物輔助育種研究，研究內容與我們強調轉殖幾個基因的生物技術有很不一樣的思考，很值得國內育種研究方向參考。參加本次研討會成果除於各於兩個研討會發表論文 1 篇共 2 篇報告和交換研究成果外，學習到國際上動物轉殖基因技術主要集中於動物基因轉殖用於疾病治療。分子農場技術不再轉殖 DNA 而是 RNA，目前國內和本系都有分子農場的研究也相當成功，但因都在 DNA 層次因此無法克服基因污染環境的問題，可能將來須要轉爲轉殖 RNA 才能有商業應用價值。

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壹、目的：

自從 VIPCA 公告舉辦本次研討會和將研討會議程經由電郵到我的信箱後，就引起我很大興趣，因爲會議地點維也納大學創始於 1365 年是德語系最古老的大學(圖 1)，卻有新的生命科學院和分子生物中心，而且 VIPCA 專於主辦專業和高品質的生物和農業研討會，與我的教學研究領域相符合因此就決定申請前往開會，會議在 2 月 14 日於古老的校園內舉行(圖 1)

圖 1：維也納大學(左)和會場位於古老的校園內舉行國際研討會(右)

貳、過程：

奧地利維也納國際植物會議協會(VIPCA, Vienna International Plant Conference Association)是一個非商品和營利的組織於 2009 成立，位於維也納大學內，其目的在主辦專業和高品質的生物和農業研討會，研討會設定為國際水準的平臺提供研究學者和產業界發表未來的創新、合作和應用科學。本次參加的「Genetic Transformation Technology」和「Plant Genetica and Breeding Technology」研討會是由 2 月 11-22 日四個系列研討會之中的兩個，會期分別為 2 月 14-15 日和 18-20 日，因為一趟飛往維也納可以參加兩個國際研討會費用較節省。

2 月 14-15 日的「Genetic Transformation Technology」研討會：

該研討會所有的論文有 2/3 都是研究動物基因轉殖用疾病治療，雖然我的專長領域在植物基因轉殖，但感覺上與國內研究大部分仍是傳統動物轉基因研究方向有很大不同。另外由德國 Icon Genetics GmbH and Nomad Bioscience GmbH 公司創辦人 Dr. Yuri Glebau 演講的題目「general aspects amd future perspectives of plant expression technologies for plants」，提出植物分子農場已不再考慮轉殖 DNA，而只轉殖 RNA，雖然只是短暫表現但產生的蛋白質量已過，因為 RNA 短時間內就會被分解，不會如同轉殖 DNA 會造成基因逃逸，所以商業化不須經過傳統的 GM 生物的申請程式，可能成為未來分子農場發展方向。

本次研討會張貼的本人碩士班學生的碩士論文海報「藉由高效率和快速的花粉基因轉殖法將 *DhPEX11*-like 基因轉殖到甜瓜以增加耐逆境能力」(摘要如附件 1、海報如附件 2)，其中有美國 Altria Client Services 公司的 Dr. Xu Dongmei 對我們發展的花粉基因轉殖技術很有興趣(圖 2)，並詢問是否已申請專利，我告訴她沒有經費申請專利，她就不再問了，我想她要根據我的研究報告自行開發技術。另外美國喬治亞大學的 Zhang Gruisheng 研究團隊也建議開發 DhPEX11 蛋白質用於治療人類疾病，因為我們選殖到這個基因與細胞胞器過氧化體分裂有關，這個基因缺陷會引起遺傳疾病，我們已將這個基因大量表現於甜瓜值得利用。本次會議巧遇我大學的黃弼臣教授兒子黃力夫教授(美國北卡羅納大學)倍感親切(圖 2)。

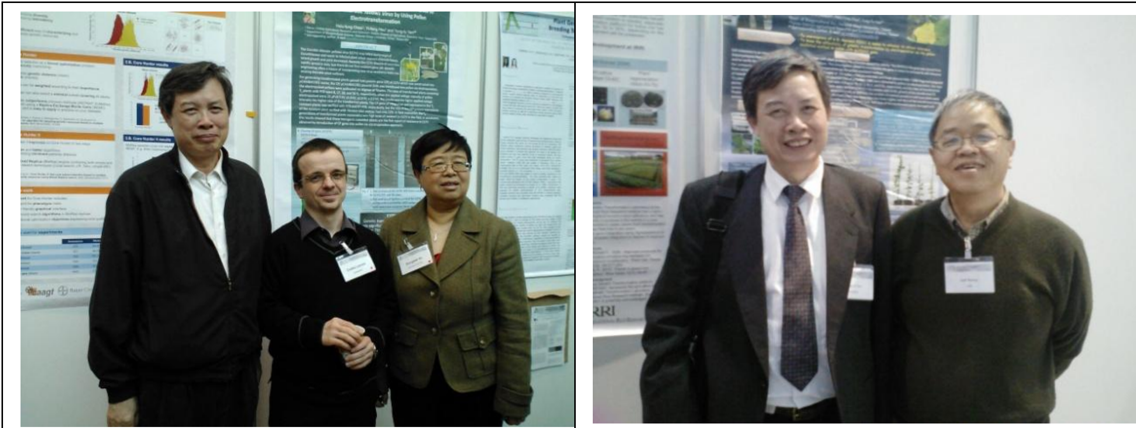

圖 2：本人與美國 Altria Client Services 公司的 Dr. Xu Dongmei(右)於海報前合影(左)。

本次會議巧遇我大學的老師黃弼臣教授兒子黃力夫教授(美國北卡羅納大學)(右)

2 月 18-20 日的「Plant Genetica and Breeding Technology」研討會

經過星期假日的休息後，2 月 18 日繼續「Plant Genetica and Breeding Technology」研討會，這一個研討會參加的研究學者更多，幾乎做滿整個會場(圖 3)，中場休息時可以看到大部份是來自歐洲國家，因爲奧地利位於中歐所以不少來自東歐(圖 3)。本次研討會論文集中於基因體、蛋白質體學、分子標示輔助育種和數量遺 傳等於育種上之應用(圖 4)，而且集中於溫帶地區小麥、大麥等糧食作物研究。水稻有來自國際稻米研究中心(IRRI)和日本有論文宣讀 4 篇；我們海報發表「轉殖 CCYV 病毒 coat protein 基因獲得胡瓜抗 CCYV 病毒植株(摘要如附件 3、海報如附件 4)。日本學者也發表水稻的耐寒和光合作用的數量遺傳研究。國際稻米研究中心發表 SNP 應用在水稻屬植物的柱頭性狀的基因圖譜研究，另一篇發表開發 MAGIC 組群(mu lti-parent advanced generation intercross (MAGIC) population)應用於分子輔助育種研究，未來可用於數量遺傳研究，也可以與 SNP 分子標示技術結合應用於輔助育種，澳洲昆士蘭大學也發表第二代基因體學應用於作物育種，其實整個研討會許多的論文都是有關數量遺傳和應用分子生學工具於作物輔

助育種研究(圖 4 右)，這個領域國內也有研究，但仍應用較早期的 PCR 技術，無法應用在提高產量和數量遺傳研究。國內雖然也有第二代基因定序儀儀器分佈在不同研究機構，但在農業機關或大學仍缺乏，本系有應用第二代基因定序儀研究經驗的教授 2 位，也曾有過申請補助購買但因爲經費較多仍未實現，未來仍找尋機會申請補助。

圖 3. 研討會參加的研究學者更多，幾乎做滿整個會場(左)，因爲奧地利位於中歐所以不少來自東歐(右)

圖 4. 大會海報許多基因體、蛋白質體學、分子標示輔助育種和數量遺 傳等於育種上之應用(左)，數量遺傳資料庫論文宣讀(右)。

參、心得及建議：

本次研討會在中歐的奧地利維也納舉行參加的國家大部分來自歐洲國家，所以論文發表內容與國內研究不太一樣，許多的論文都是有關數量遺傳和應用分子生學工具於作物輔助育種研究，這個領域國內也有研究，但仍應用較早期的 PCR 技術，無法應用在提高產量和數量遺傳研究。作物集中於溫帶地區小麥、大麥等糧食作物研究，雖然作物不一樣但研究工具和方法是相同的，研究內容與我們強調轉殖幾個基因的生物技術有很不一樣的思考，很值得國內育種研究方向參考。

轉殖基因技術已不再是困難技術，本次研究會主要集中於動物基因轉殖用於疾病治療。分子農場技術不再轉殖 DNA 而是 RNA，甚至已有 RNA 轉殖機器開發出來，目前國內和本系都有分子農場的研究也相當成功，但因都在 DNA 層次因此無法克服基因污染環境的問題，可能將來須要轉爲轉殖 RNA 才能有商業應用價值。

肆、附件

Overexpressing *DhPEX11-like* gene for enhancing stress tolerance of melon with high efficient gene transfer method of electroporation via the pollen-mediated

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Abstract

Salt tolerance is an important trait that is required to cope with plant productivity reduction caused by salinity. The *DhPEX11-like* gene was cloned from *Debaryomyces hansenii* which was induced in high salt medium. This investigation aimed to overexpress the gene in melon to enhance its salt tolerance and to verify the efficiency of genetic transformation by electroporation via the pollen-mediated method.

For comparing efficiences of transformed plants with four DNA types of the supercoiled plasmid DNA and linear plasmid DNA, T-DNA fragment prepared from constructed pC1380-35S-DhPEX1 vector and positive control plasmid DNA of pCAMBIA 1380 were delivered to pollens by electroporation, the rates of transformed T1 plants with *DhPEX11-like* and *HPTII* genes verified with PCR were 70, 67.3, 65.5, and 0 %, respectively, and the rates of transforemed T2 plants were 57.5 and 50 %, respectively. The transformed T3 plants were verified *DhPEX11-like*

protein with ELISA, growth of the transformed T3 plants under 300 mM NaCl stress were positively correlated with expression levels of DhPEX11-like protein, in contrast growth of wild-type plants were severely inhibited. Under salt stress, biomass production of the transformed plants was more than two times higher, leaf discs of the transformed plants floated in 300 mM NaCl displayed a 6-fold higher chlorophyll content compared to the wild type, leaf discs of the transformed plants damaged with paraquat and hydrogen peroxide also showed much high retention of chlorophyll.

In conclusion, overexpression of *DhPEX11-like* gene in higher plants holds considerable potential for crop improvement toward enhancing stress tolerance, the *DhPEX11-like* gene should make an important contribution to better understanding about its function.

Overexpressing DhPEX11-like Gene for Enhancing Stress Tolerance of Melon with High Efficient Gene Transfer Method of Electroporation via the Pollen-mediated

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The aim of the study

- To overexpress of a D. hansenii PEX11 in melon to enhance its stress tolerance.
- To know the effectivity of genetic transformation by electroporation via the pollen-mediated method in melon with DhPEX11 gene.

Abstract

Salt tolerance is an important trait that is required to cope with plant productivity reduction caused by salinity. The *DhPEX11-like* gene was cloned from *Debaryomyces hansenii* which was induced in high salt medium. This investigation aimed to overexpress the gene in melon to enhance its salt tolerance and to verify the efficiency of genetic transformation by electroporation via the pollen-mediated method.

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Transgenic plant methods

Is what PEX11-like?

1. PEROXIN11 (PEX11) is a peroxisomal membrane protein in fungi and mammals, and was proposed to play a major role in peroxisome proliferation (Marshal *et al*., 1995; Gurvitz *et al*., 2001; Orth, 2007).
2. *DhPEX11-like* gene cloned from extremely halophilic yeast *Debaryomyces hansenii* is induced-salt gene and its overexpression enhanced salt tolerance in yeast (Hue, 2009).
3. The function of the *DhPEX11-like* gene in higher plants has not been reported so far.

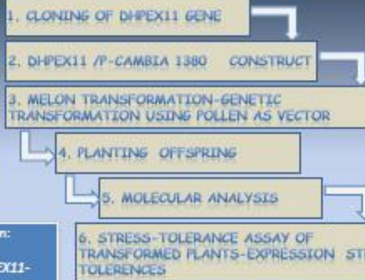

Table 1 The efficiencies of pollen electroporation:
1. Screening T1 offspring by PCR.
2. The % of co-integration of *HPTII* and/or *DhPEX11-like* genes in T1 offspring plants.

Types of DNA	No. of Fruits	No. of Seeds	Total tested plants	% of HPTII	% of DhPEX11-like	% of transformant with 2 genes
Positive plasmid	2	396	20	13	0	0
Supercoiled (9912 bp)	4	633	40	28	28	70
Linear (9912 bp)	2	353	52	35	36	67.3
T-DNA (3682 bp)	3	428	29	19	19	65.5
Wild Type	2	468	14	0	0	0

Fig. 3 Salt tolerance of transformed melon plants overexpressing *DhPEX11-like* grown in presence of 300 mM NaCl.

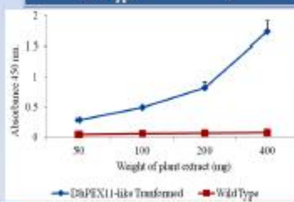

Fig. 1 Relationship between absorbance at 450 nm in ELISA and weight of sampled T2 transformed plant s under salty stress.

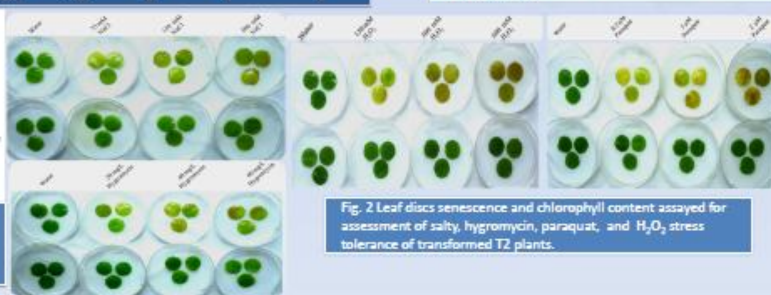

Fig. 2 Leaf discs senescence and chlorophyll content assayed for assessment of salty, hygromycin, paraquat, and H_2O_2 stress tolerance of transformed T2 plants.

Conclusion: **1.** Transgenic melon plants could be transferred by electroporation using pollen as vector. **2.** there was no significant difference between DNA types of supercoiled plasmid DNA, linear plasmid DNA, and T-DNA on genetic transformation of melon. **3.** Genetic transformed Melon plants overexpressed *DhPEX11-like* increasing tolerance to salinity and oxidative stresses. **4.** overexpressing of DhPEX11-like gene in melon holds considerable potential for crop improvement toward enhanced stress tolerance. **5.** further work is needed to study the subcellular localization of DhPEX11-like and mechanism of DhPEX11-like gene to enhancing tolerance to stresses in plants.

Generation of Transgenic Cucumber with resistance to *Cucurbit chlorotic yellows virus* by using Pollen electrotransformation

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The Cucurbit chlorotic yellows virus (CCYV) may infect many crops of *Cucurbitaceae*, the infected plants appear chlorosis leaves and result to decrease yield and low quality of fruits. Recently the CCYV disease rapidly spread in Asia including Japan, China and Taiwan, then has intruded into Sudan, Middle East. The virus has become to a serious disease, but there do not find resistant varieties yet. The goal of this experiment was to generate resistant plants by transforming the coat protein gene of CCYV. Due to gene transformation mediating *Agrobacterium* requires tedious and time-consuming tissue culture procedures for regenerating intact plant from transformed cells, alternately, generation of transgenic plant with pollen electrotransformation which is high efficiency and rapid. In transformational procedures, the CCYV coat protein (CP) gene will be firstly constructed into the pCAMBIA 1302, its plasmid DNA was introduced into pollen via electropotation, the electropulsed pollens were pollinated on stigmas of flowers. The rates of T1 transformed plants screening with PCR were 8, 17, 28, and 58 %, respectively, when the applied voltage intensity of pollen electropulsed were 25 μF×0.5 kV, ×1.0 kV, ×1.5 kV, × 2.0 kV, the results was the higher applied voltage intensity the higher rate of the transformed plants. The CP gene of integration and expression in the T2 resistant plants based on field test was verified with PCR and RT-PCR, respectively, but the rate of the GFP protein expressions of the resistant plant verified with Western blot analysis had only 22%. In field tests the CP-transgenic T3 and T4 transformed plants appeared resistant to CCYV too. In conclusion, the results showed that these transgenic plants are the first report of resistance to CCYV obtained by introduction of CP gene into pollen via electroporation approach.

Generation of Transgenic Cucumber with Resistance to *Cucurbit Chlorotic Yellows Virus* by Using Pollen Electrotransformation

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ABSTRACT

The *Cucurbit chlorotic yellows virus* (CCYV) may infect many crops of *Cucurbitaceae* and result to infected plant which appears chlorosis leaves, retard growth and yield decreased. Recently the CCYV disease of cucumber rapidly spread in Asia, but there do not find resistant gene yet. Genetic engineering offers a means of incorporating new virus resistance traits into existing desirable plant cultivars.

For generating transformated plants gained coat protein gene (CP) of CCYV which was constructed into *pCAMBIA*1302 vector, the CP/ *pCAMBIA*1302 plasmid DNA was introduced into pollen via electropotation, the electropulsed pollens were pollinated on stigmas of flowers. The rates of transformed plants screening T_1 plants with PCR were 8, 17, 28, and 58 %, respectively, when the applied voltage intensity of pollen electropulsed were 25 μFx0.5 kV, x1.0 kV, x1.5 kV, x 2.0 kV, the results was the higher applied voltage intensity the higher rate of the transformed plants. The CP gene of integration and expression in the T_1 resistant plants was verified with PCR and RT-PCR, respectively, but the rate of the GFP protein expressions of the resistant plant verified with Western blot analysis had only 22%. In field evaluation, the T_3 generations of transformed plants appeared a very high level of resistant to CCYV in the field. In conclusion, the results showed that these transgenic cucumber plants are the first report of resistance to CCYV obtained by introduction of CP gene into pollen via electroporation approach.

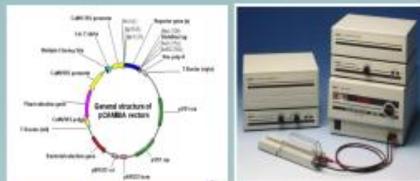
A. Cloning CP gene of CCYV:

CCYV-F-NcoI :
CCCCATGGATGGAGAAGACTGACAATAAACAAAA

CCYV-R-BglII :
AAAGATCTTTTACTACAACCTCCCGGTGCCAACTG

B. Constructing the expression vector of CP/pCAMBIA1302

C. Pollen electropulsed



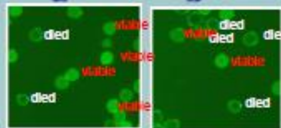


Fig. 1 Viability as stained by FDA before (a) and after (b) electroporation of cucumber pollen.

Fig.3 Anti-CCYV expression of transgenic cucumber. a: negative b: positive

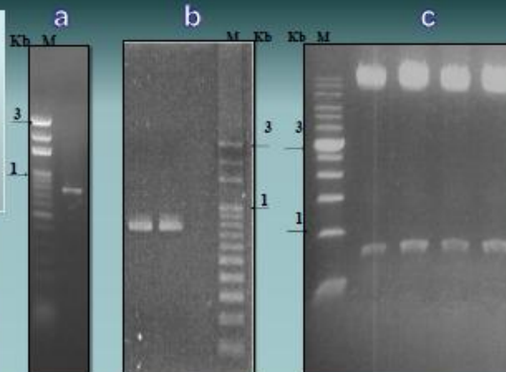


Fig. 2 a. Gel analysis of the CCYV (CP) from cucumber.
b. CCYV (CP) add RE sites.
c. Gel analysis of ligation product for CCYV (CP) constructd in *pCAMBIA* 1302 vector and digested with restriction enzymes (*Nco*I and *Bgl* II).

CONCLUSIONS

Genetic transformation can improve agricultural traits significantly faster than conventional breeding practice. This experiment demonstrated transferring CP gene of CCYV which might result in plant resistance to CCYV disease. In addition to, the results of generated transgenic cucumber plants indicated that CP gene could be successfully transferred by electroporation using pollen as vector. The genetic transformation method that used pollen as vector is without tissue culture steps and regeneration steps, so relatively simple and easy.