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甜瓜對白粉菌 *Sphaerotheca fuliginea* race 1 抗性之遺傳研究與抗病篩選[1]

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摘　要

王毓華、黃晉興、林子凱、王三太、蕭吉雄。2004。甜瓜對白粉菌 *Sphaerotheca fuliginea* race 1 抗性之遺傳研究與抗病篩選。中華農業研究 53:1-8。

利用人工接種白粉菌(*Sphaerotheca fuliginea* race 1)於葉片圓盤上，檢定台灣地區19個甜瓜(*Cucumis melo* L.)栽培品種之抗感性，其中僅有秋華二號、橙芳及蜜華等3個栽培品種對白粉菌 race 1 具有抗性表現，其他16個品種皆為感病性。為進行抗病性研究，篩選本所已純化之 36 個甜瓜自交系(S_8～S_{12})，結果顯示 02053 等 21 個自交系對白粉菌 race 1 具有抗病性，而其他 15 個自交系皆為感病。為了有效利用其抗病性，進行自交系 02080 之抗病性遺傳分析，抗病自交系分別與感病自交系 02077 雜交及回交，獲得 F_1、F_2、BC_R、BC_S等後裔族群，經人工接種白粉菌 race 1 後調查發病情形，結果顯示抗病自交系 02080 對白粉菌 race 1 之抗病性是由一對顯性基因所控制。

關鍵詞：甜瓜、白粉病、抗病篩選、抗病育種、遺傳分析。

前　言

甜瓜(*Cucumis melo* L.)為葫蘆科(Cucurbitaceae)一年生蔓性草本作物，根據2002年農業統計年報，目前台灣栽培面積約 9200 公頃，主要生產地於台南縣、台南市、嘉義縣、雲林縣及宜蘭縣等地。由於甜瓜香甜多汁，為極受消費者歡迎的蔬果，屬高經濟價值作物。根據本所於甜瓜生產區調查，目前於台灣栽培的甜瓜主要為東方(薄皮)甜瓜及洋香瓜二種。東方甜瓜依外觀型態及果皮顏色可分為梨形綠皮的梨甜瓜，俗稱為梨仔瓜、美濃瓜，肉色淡黃綠；另一種為圓柱狀黃皮的黃香瓜，肉色乳白，但較少見。洋香瓜屬厚皮甜瓜，果實較大，果皮外觀有網紋與光滑兩種，果皮色有綠色、淺黃色、金黃色等，果肉色有綠色、橙色及白色等不同顏色，幾乎一年四季皆有栽培。

甜瓜自民國46年即有引進栽培記載，隨著甜瓜育種受到重視，民間種苗業者及種苗代理商不斷推出新品種，無論品質或產量均有卓越表現，栽培面積也逐年增加。然而台灣地處亞熱帶及熱帶地區，氣候高溫多濕，因此甜瓜栽培過程中，從苗期到採收時期，經常面臨各種病蟲害威脅，其中甜瓜白粉

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病(powdery mildew)之發生極為普遍，無論田間露地栽培或設施內栽培，幾乎一年四季皆會發生，它可危害作物的葉片、莖及果實，使得品質及產量低下，造成農民嚴重損失。由於目前栽培品種大部分對白粉病無抗病性，農民多以化學藥劑防治之，此法不但提高生產成本，還有農藥殘毒之虞，因此抗病育種為今後甜瓜育種工作重點之一(蔡 & 童 1995； Kyle 1995； Sitterly 1978)。白粉病之病原菌可藉由風力將無性孢子傳播出去，在乾燥及光線不足的情況下危害最烈(Sitterly 1978)。依據報導，有兩種病菌可造成甜瓜白粉病，*Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.和 *Erysiphe cichoracearum* DC ex Merat，其中 *S. fuliginea* 分佈較廣(Kenigsbuch & Cohen 1992； Pitrat *et al.* 1998； Sitterly 1978； Takada 1982；Vakalounakis & Klironomou 1995)，而台灣的甜瓜白粉病主要也是 *S. fuliginea* 引起(黃等 2002)。根據報告指出，*S. fuliginea* 目前至少有 7 個生理小種存在，包含 race 0、1、U.S.-2、France-2、3、4、5(Pitrat *et al.* 1998)，而在台灣至今僅發現一個生理小種 race 1(黃等 2002)。研究人員針對目前已發表之抗病品種(系)，進行遺傳分析，綜合研究結果，大多數抗病品種(系)對白粉病單一生理小種之抗病性是由單一顯性基因所控制(Bohn & Whitaker 1964； Epinet 1993； Floris & Alvaeez 1995； Kenigsbuch & Cohen 1992； McCreight *et al.* 1987； Pitrat *et al.* 1998)。

本研究目的為：(1)檢定目前在台灣地區栽培的甜瓜品種對白粉菌(*S. fuliginea* race 1)之抗感性；(2)從本所已育成之自交系(S_8～S_{12})中篩選抗病自交系；(3)進行自交系之抗病性遺傳分析，以了解自交系對白粉病抗性之遺傳作用，並有效應用於甜瓜抗白粉病育種之改良。

材料與方法

甜瓜栽培品種對白粉菌(*S. fuliginea* race 1)之抗感性檢定

包含翠蜜、香華、秀蜜、藍寶石、秋蜜、金后、紅寶石、秋華二號、橙芳、蜜華等 10 個網紋甜瓜品種；蜜世界、狀元、金姑娘、金蜜等 4 個光皮甜瓜(冬甜瓜)品種；新世紀 1 個哈密瓜品種；以及金輝、銀輝、嘉玉、嘉輝等 4 個東方甜瓜(脆瓜類)品種，共計 19 個栽培品種，採完全逢機設計(CRD)，三重複，每重複 5 株。

甜瓜自交系對白粉菌(*S. fuliginea* race 1)之抗感性檢定

包含 02053、02057、02062、02066、02067、02068、02069、02070、02071、02072、02073、02077、02078、02079、02080、02081、02082、02083、02084、02086、02089、02091、02092、02093、02095、02096、02097、02098、02101、02102、02103、02104、02105、02106、02107 及 02108 等 36 個 S_8～S_{12} 自交系，每個自交系各 10 株。

自交系 02080 對白粉菌(*S. fuliginea* race 1)抗病性之遺傳分析試驗

將本所育成之抗病自交系 02080(S_{12})與感病自交系 02077(S_9)於 89 年秋作利用人工授粉方式生產一代雜交種(F_1)， 90 年春作自交生產 F_2 後裔族群，並將 F_1 分別與雜交親本的抗病自交系(02080)和感病自交系(02077)進行回交以生產 BC_R 和 BC_S 後裔族群。分別將前述之親本、雜交及回交等後裔族群栽培於隔離生長箱中，包含抗病自交系(02080)、感病自交系(02077)兩個親本和 F_1 各 20 株、F_2 後裔族群 114 株、回交抗病親本之後裔族群(BC_R)105 株以及回交感病親本之後裔族群(BC_S)104 株。

白粉菌(*S. fuliginea* race 1)菌株接種源之備製

從台中縣霧峰鄉農業試驗所甜瓜栽培田取回感染白粉病菌之葉片，將孢子吹落於附有水瓊脂塊之玻片上，再將玻片放入培養皿濕室內，24 小時後，於解剖顯微鏡下挑取已發芽之單一白粉病菌孢子，移植於葉片圓盤上(IRAN-H 品種)，於 16℃ 光照 12 小時之定溫箱中培養 12 16 天，使白粉病菌長滿整個葉片圓盤作為接種源。本試驗以分離自金輝品種之白粉病菌分離株 9912-WF-A1 為供試菌株，經黃等(2002)鑑定確定為白粉菌 *S. fuliginea* race 1。

接種方法及病害調查

將前述試驗材料之種子消毒浸種後播種於直徑 10cm 之塑膠盆，栽培於 28℃/22℃(日/夜溫)光照 12 小時之隔離生長箱中，以苗齡 4 星期之第 3 或第 4 片本葉為供試材料備用。依黃等(2002)研發之方法進行白粉菌接種，每植株切取 2 片展開葉，以自來水洗淨，再經消毒水(1%次氯酸鈉加入數滴 Tween-20 介面活性劑攪拌)表面消毒 2～3mins，無菌水漂洗三次，吸乾游離水，再以經高溫高壓滅菌之金屬圓柱器具切取直徑 1.5cm 之葉片圓盤，置於盛有二層濾紙之培養盒(20×15×5cm)，添加含有 50 ppm tetracyclin 之 M-solution(mannitol 10,000ppm 及 benzimidazole 30ppm 之水溶液)，使濾紙完全濕潤並有游離水產生，以維持葉片圓盤之活力。再以人工接種方式將白粉菌孢子(*S. fuliginea* race 1)均勻地吹入培養盒中，孢子量約 300 spores/cm^2，接種後將含有葉片圓盤之培養盒置於 24℃/18℃(日/夜溫)光照 12 小時之定溫箱中，10 天後調查其發病指數(黃等 2002; Cohen 1993; Cohen *et al.* 1995; Kenigsbuch & Cohen 1992)。發病指數分為 0~9 共 10 級：0 級-無病斑；1 級-病斑佔葉面積 10%；3 級-病斑佔葉面積 50%；5 級-病斑佔葉面積 80%；7 級-病斑佔葉面積 100%但孢子量稀薄；9 級-病斑佔葉面積 100%但孢子量厚實且葉片圓盤褐化(Epinet *et al.* 1993; Mohamed *et al.* 1995)。依據 Floris & Alvaeez(1995)，發病指數＜1 為抗病，發病指數 1～3 為中感，發病指數＞3 則為感病。

統計分析

F_2 後裔族群及回交後裔族群之抗感病性分離比以卡方檢定(χ^2 test)進行適合度檢定(goodness of fit)，顯著水準 p 值小於 0.05(Mead *et al.* 1993)。

結　果

甜瓜栽培品種對白粉菌(*S. fuliginea*) race 1 之抗感病性

本試驗以目前在台灣地區栽培之甜瓜品種為材料，利用葉片圓盤法進行對白粉菌(*S. fuliginea* race 1)抗感病反應之檢定，經人工接種 10 天後，調查發病指數，其結果如表 1 所示，其中 5 個白(綠)肉品種之網紋甜瓜中，以香華發病指數最高，平均為 8.8；翠蜜發病指數最低，平均為 7.9。5 個橙肉品種之網紋甜瓜中，以金后發病指數最高，平均為 7.7；秋華二號及橙芳發病指數最低，平均為 0。而 4 個光皮甜瓜品種中，以金蜜發病指數最高，平均為 8.8；金姑娘發病指數最低，平均為 8.1。哈密瓜品種新世紀平均發病指數為 8.0。而東方甜瓜 4 個品種中，以嘉玉發病指數最高，平均為 8.6，嘉輝發病指數最低，平均為 7.2。由試驗結果得知，本試驗所篩選台灣地區甜瓜栽培品種中，白(綠)肉之網紋甜瓜品種對白粉菌(*S. fuliginea*) race 1 皆為感病；橙肉之網紋甜瓜品種僅秋華二號、橙芳及蜜華等 3 個品種對白粉菌(*S. fuliginea*) race 1 為抗病，其他皆為感病；光皮甜瓜栽培品種、哈密瓜栽培品種新世紀及東方甜瓜栽培品種對白粉菌(*S. fuliginea*) race 1 均為感病。

甜瓜自交系對白粉菌(*S. fuliginea*) race 1 之抗感病性

利用人工接種白粉菌(*S. fuliginea*) race 1，檢定本所育成之 02053 等 36 個高世代自交系(S_8～S_{12})之抗感性表現，結果列於表 2，包含 02053、02067、02068、02069、02070、02072、02078、02079、02080、02081、02082、02083、02089、02091、02095、02101、02104、02105、02106、02107 及 02108 等 21 個自交系對白粉菌(*S. fuliginea*) race 1 表現抗病，其他包含 02057、02062、02066、02071、02073、02077、02084、02086、02092、02093、02096、02097、02098、02102 及 02103 等 15 個自交系皆表現感病。

表 **1.** 台灣甜瓜栽培品種對白粉菌（*Sphaerotheca fuliginea*） race 1 之抗感病檢測
Table 1. Evaluation of the resistance of the cultivars of melon (*Cucumis melo*) in Taiwan to race 1 of *Sphaerotheca fuliginea* [z]

Cultivar	Average of disease index[y]	Disease reaction[x]
Tsuimi	7.90	S
Hsianghua	8.82	S
Hsiumi	7.97	S
Lanpaoshih	8.01	S
Chiumi	8.19	S
Hungpaoshih	6.14	S
Chinhou	7.68	S
Chinmi	8.83	S
Autumn Waltz No.2	0.00	R
Chengfang	0.00	R
Mihua	0.75	R
Honey World	8.54	S
Golden Prize	8.51	S
Golden Lady	8.12	S
New Century	8.03	S
Golden Light	8.30	S
Silver Light	7.56	S
Chiayu	8.64	S
Chiahui	7.18	S

[z] The leaf discs were inoculated with conidia (300 spores/cm^2) of the pathogen race 1 of *S. fuliginea*, and then were incubated at 24℃/18℃ (day/night) with 12 hours photoperiod for 10 days.
[y] Disease index was evaluated on a scale from 0 to 9 depend on the leaf disc from health to severe.
[x] R＝Resistant, disease index＜1； S＝Susceptible, disease index＞3.

表 **2.** 利用葉片圓盤法檢測甜瓜自交系對白粉菌（*Sphaerotheca fuliginea*） race 1 之抗感病性反應
Table 2. Evaluation of the resistance of the melon inbred lines to race 1 of *Sphaerotheca fuliginea*

Inbred line	Disease reaction[z]	Inbred line	Disease reaction	Inbred line	Disease reaction
02053	R	02078	R	02095	R
02057	S	02079	R	02096	S
02062	S	02080	R	02097	S
02066	S	02081	R	02098	S
02067	R	02082	R	02101	R
02068	R	02083	R	02102	S
02069	R	02084	S	02103	S
02070	R	02086	S	02104	R
02071	S	02089	R	02105	R
02072	R	02091	R	02106	R
02073	S	02092	S	02107	R
02077	S	02093	S	02108	R

[z] R＝Resistant, disease index＜1； S＝Susceptible, disease index＞3.

自交系 02080 對白粉菌(*S. fuliginea*) race 1 抗病性之遺傳分析

本試驗以人工接種白粉菌(*S. fuliginea* race 1)，接種後 10 天調查白粉病發病情形，由表 3 結果得知，抗病自交系 02080 接種 20 株，其表現皆為抗病；感病自交系 02077 接種 20 株，其表現皆為感病；F_1(02080×02077)接種 20 株，皆為抗病，F_2(02080×02077)後裔族群接種 114 株，抗病株數與感病株數的比例為 83：31；F_1 與抗病自交系 02080 之回交後裔族群 BC_R(F_1×02080)接種 105 株，均為抗病；F_1 與感病自交系 02077 之回交後裔族群 BC_S(F_1×02077)接種 104 株，抗病株數與感病株數比例為 53：51，根據 χ^2 檢定結果顯示，F_2 抗病株數與感病株數之比例符合 3：1，F_1 與感病自交系之回交後裔族群 BC_S 之抗病株數與感病株數比例符合 1:1，由此可推測抗病自交系 02080 對白粉菌(*S. fuliginea*) race 1 之抗性是由一對顯性基因所控制。

表 3. 抗病自交系 02080 與感病自交系 02077 雜交及回交後裔族群對白粉菌（*Sphaerotheca fuliginea* race 1）抗病性之分離比

Table 3. Segregation of resistance of the progenies between cross and back cross of the resistant melon inbred line 02080 and the susceptible inbred line 02077 to race 1 of *Sphaerotheca fuliginea*

Pedigree	Generation	Number of plants			Expected ratio	χ^2	P
		R[z]	S[z]	Total			
02080	P_1	20	0	20	-	-	-
02077	P_2	0	20	20	-	-	-
02080×02077	F_1	20	0	20	-	-	-
02080×02077	F_2	83	31	114	3：1	0.292	0.589
(02080×02077)×02080	BC_R	105	0	105	-	-	-
(02080×02077)×02077	BC_S	53	51	104	1：1	0.038	0.845

[z] R＝Resistant, disease index＜1； S＝Susceptible, disease index＞3.

討　論

白粉病為甜瓜栽培之重要病害，若在發病適期無論設施或露地栽培幾乎都會受到白粉病侵襲，勢必對甜瓜栽培造成很大的影響。雖然目前農民利用農藥防治白粉病之效果良好，但是考量為維護消費者健康及避免污染環境，再加上近年來發生抗藥性問題(蔡 & 童 1995； Khadar & Abdou 1972； Sitterly 1978； Zitter *et al.* 1996)，因此育成抗病品種為解決當前甜瓜白粉病之最佳途徑。

自從抗白粉病 *S. fuliginea* race 1 品種 PMR45 育成(Jagger & Scott 1937)與抗白粉病 *S. fuliginea* race 2 品種 PMR5 育成(van Haltern *et al.* 1943)後，許多育種人員也開始從甜瓜種原中篩選抗病材料，以作為抗病育種使用。根據 Pitrat 等人(1998)之研究報告指出，許多抗病種原都來自印度。隨著抗病品種廣泛地被育成釋出及栽培後，白粉菌(*S. fuliginea*)之其他生理小種在世界各地續出現(Cohen *et al.* 1996； Hosoya *et al.* 1999； Kaur & Jhooty 1986；Pitrat *et al.* 1998；Reifschneider *et al.* 1985； van Haltern *et al.* 1943)，包含 race 0、1、U.S.-2、France-2、3、4、5，所以進行抗病育種工作時，需特別注意生理小種之變異問題。黃等(2002)針對台中農試所試驗田所採集之菌株進行分析鑑定，確定僅發現 *S. fuliginea* race 1，但是台灣地區在不同地方或不同季節是否有其他生理小種存在，則須在蒐集病菌後，進一步利用葉片圓盤法檢定。

本試驗首先以台灣地區目前栽培之 19 個栽培品種為材料，以葉片圓盤法進行抗感病性檢測，根

據檢定結果顯示，幾乎所有受檢栽培品種，包含網紋洋香瓜、光皮洋香瓜、哈密瓜及東方甜瓜等，對 *S. fuliginea* race 1 皆為感病性，其中僅網紋洋香瓜秋華二號、橙芳及蜜華等 3 品種具有抗病性，因此台灣地區甜瓜抗病育種仍有許多值得育種人員努力的空間。其次進行本所育成之 36 個自交系（S_8～S_{12}）對白粉菌 *S. fuliginea* race 1 抗感性檢定，其結果顯示，其中有 21 個自交系具有抗病性，可進一步調查抗病自交系之園藝性狀，評估其生長勢及果實特性，並進行組合力檢定，選拔優良抗病自交系，並與組合力高之自交系相互雜交，以期育成抗白粉病（*S. fuliginea*）race 1 之雜交新品系。

有關抗病性之遺傳分析方面，已有許多研究人員進行抗病品種（系）對白粉菌 *S. fuliginea* race 1 和 race 2 以及 *Erysiphe cichoracearum* race 1 之抗病性遺傳分析（Bohn & Whitaker 1964； Epinet 1993； Floris & Alvaeez 1995；Harwood & Markarian 1968a；Harwood & Markarian 1968b； Kenigsbuch & Cohen 1992； McCreight *et al.* 1987； Pitrat *et al.* 1998），由於每個抗病品種之抗病性分別表現於不同生理小種上，因此其抗病基因也不盡相同，綜合研究結果，大多數抗病品種（系）對白粉病單一生理小種之抗病性是由單一顯性基因所控制。為了瞭解本所甜瓜抗病自交系之遺傳行為，乃進一步以抗病自交系 02080 與感病自交系 02077 為材料進行遺傳分析，根據雜交及回交後裔族群對 *S. fuliginea* race 1 之抗感病性分離比，顯示抗病自交系 02080 之抗病性應由一對顯性基因所控制，與前人研究報告中所提之對 *S. fuliginea* race 1 具有抗性的品種，其抗病基因也大多為單一顯性基因所控制之結果相符合（Epinet *et al.* 1993； Floris & Alvaeez 1995； Kenigsbuch & Cohen 1992； McCreight *et al.* 1987； Pitrat *et al.* 1998），而此抗病自交系 02080 對於其他生理小種之抗感病性反應，則須進一步測定才能得之結果。

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本研究係行政院農委會補助經費，法國 INRA 機構 Dr. Pitrat 提供甜瓜抗白粉病生理小種之鑑別品種，本所劉邦基博士、許華欣小姐提供相關資訊，一併誌謝。

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Genetic Studies and screening for Resistance to Powdery Mildew (*Sphaerotheca fuliginea* race 1) in Melon (*Cucumis melo* L.)[1]

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Summary

Wang, Y. H., J. H. Huang, T. K. Lin, S. T. Wang, and C. H. Hsiao. 2004. Genetic studies and screening for resistance to powdery mildew (S*phaerotheca fuliginea* race 1) in melon (*Cucumis melo* L.). J. Agric. Res. China 53:1-8.

Leaf disc inoculation method was used to evaluate the resistance of 19 commercial cultivars of *Cucumis melo* L. to *Sphaerotheca fuliginea* race 1, the causal agent of powdery mildew in Taiwan. The results showed that 3 of the entries, *i.e.* Autumn waltz No.2, Chengfang, and Mihua, were resistant, while the rest 16 varieties were susceptible. This indicated that most of the melon cultivars in Taiwan are susceptible to *S. fuliginea* race 1. The disease reactions of a total of 36 melon inbred lines (S_8～S_{12}), developed in our institute, to the same pathogen were also tested by using the same screen method. Among them, 21 inbred lines showed resistant and the other 15 lines were susceptible. In order to understand the inheritance of resistance to *S. fuliginea* race 1 in the resistant inbred line 02080(S_{12}) cross hybridization between inbred line 02080 and a susceptible inbred line 02077(S_9) was conducted. The parents (P_1 and P_2) and their progenies, including F_1, F_2, BC_R, and BC_S, were inoculated and the disease response in each individual plant was recorded and analyzed. The segregation ratios of resistance vs. susceptibility obtained from this experiment indicated that the resistance trait seems being under the control of a single dominant gene.

Key words: Melon, Powdery mildew, Resistance screening, Breeding for resistance, Genetic analysis.

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基因轉殖作物品種檢測技術之開發－利用 35S 啟動子分子標誌檢測轉基因玉米及大豆[1]

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摘　要

王昭月、范明仁、林俊義。2004。基因轉殖作物品種檢測技術之開發－利用 35S 啟動子分子標誌檢測轉基因玉米及大豆。中華農業研究 53:9-17。

本研究係分別以轉抗殺草劑基因之大豆以及轉抗蟲基因之玉米作為模式植物，建立基因轉殖植物在 DNA 層次之初步檢測分析。研究中基因轉殖與非基因轉殖之玉米及大豆共十七個樣品，經比較不同 DNA 萃取成本、DNA 純度以及聚合酵素連鎖反應結果，以 Wizard™ Genomic DNA Purification Kit 萃取結果較佳。另比較利用巢式聚合酵素連鎖反應(Nested Polymerase Chain Reaction)以及一般聚合酵素連鎖反應，進行基因轉殖大豆及玉米之鑑定，另以歐盟認可之基因轉植產品偵測試劑組(Biosmart Allin 1.0 GMO Screening System)為對照，經依作物調整其聚合酵素連鎖反應條件，結果可正確測得基因轉殖玉米及大豆樣品中的 CAMV 35S 啟動子特有之 150bp DNA 片段 ；此外，依 CAMV 35S 啟動子序列，所合成之 35S 1/35S 2 引子以及 35S-F/ 35S-R 引子，亦可分別用以測得基因轉殖樣品中 CAMV 35S 啟動子特有之 195bp DNA 片段與 207bp 之 DNA 片段。

關鍵詞：基因轉殖作物、檢測。

前　言

二十世紀是分子生物科技邁向基因工程實際應用的時代，在農作物改良上，已利用分子生物技術開發出高產量、高品質、健康、環保以及具有抵抗不良環境衝擊之基因轉殖植物(Transgenic plant)或稱之為基因改造作物(Genetically modified crops；GM crops)(潘 2000)。首宗基因改造作物 “Flavr Savr™ tomato” ，於 1994 年獲准於美國及英國上市，統計至目前(2003 年)全球已通過 78 個基因改造作物之安全評估並上市，而依據 ISAAA(International Service for the Acquisition of Agri-biotech Applications)統計，2002 年全球商業性種植之基因改造作物已達五千八百萬公頃(James 2002)。唯基

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因轉殖作物雖然為人類帶來高農業生產力、高品質以及高附加利用價值，但卻也可能產生一些潛在對人類及農業生態的負面影響。包括：基因轉殖作物可能加速全世界作物品種的單純化，以其強勢競爭力可能造成對其他作物生長之威脅，因而衝擊生態及物種(基因)的平衡與多樣性；以及，基因轉殖作物可能藉著花粉將所攜帶的新基因傳給其他地方種或野生種，因而造成基因汙染，並有衍生「超級野草」的危機，進而有破壞地球生態之虞(Losey *et al.*1999)。

在種原多樣性保育與永續利用前提下，國內的種原收集、保存與利用工作者，除繼續對傳統育種改良之種原材料詳實掌控其基因背景與利用外；對於新推出的基因轉殖作物，在進行生態及生物體影響安全評估同時，仍亟需建立快速且正確的基因轉殖植物之分子鑑定技術或篩檢方法，以提供與傳統非基因轉殖種原之區隔，另方面亦可協助作為基因轉殖作物在生態環境中傳播監控之指標。

材料與方法

供試轉基因植物

自中華民國穀類研究所進口之大豆及玉米材料中，取得標誌為基因改造之大豆、玉米之種子樣品，包含 5 批轉抗殺草劑基因大豆(含 Roundup Ready soybean)種子及 7 批轉抗蟲基因玉米(含 BT-176)種子；另各取二個本省選育之玉米與大豆品種種子樣品，以及一個購自市場的大豆種子樣品，計 5 個對照樣品。綜合以上共計 17 個試驗用種子樣品(表 1)。

表 1. 基因轉殖作物檢測用之玉米及大豆種子樣品
Table1. Samples of maize and soybean seeds prepared for transgenic crops detection

Sample no.	Crop	Import/ Local origin	Source	Remark
1	Soybean	Local origin #1	Supermarket	Non-GMO labeling
2	Soybean	Local origin #2	Supermarket	Non-GMO labeling
3	Soybean	Local origin #3	Supermarket	—
4	Soybean	Import #1	CGPRDI[z]	GMO[z]
5	Soybean	Import #2	CGPRDI	GMO
6	Maize	Import #3	CGPRDI	GMO
7	Maize	Import #4	CGPRDI	GMO
8	Maize	Import #5	CGPRDI	GMO
9	Maize	Tainung no.2	TARI[z] inbreed	Non-GMO
10	Maize	Tainung no.3	TARI inbreed	Non-GMO
11	Maize	Import #6	CGPRDI	GMO
12	Maize	Import #7	CGPRDI	GMO
13	Maize	Import #8	CGPRDI	GMO
14	Maize	Import #9	CGPRDI	GMO
15	Soybean	Import #10	CGPRDI	GMO
16	Soybean	Import #11	CGPRDI	GMO
17	Soybean	Import #12	CGPRDI	GMO

[z] abbreviation, GMO:genetically modified oganism；CGPRDI:China Grain Products Research and Development Institute；TARI:Taiwan Agricultural Research Institute.

轉基因植物之分析與檢測

以聚合酵素連鎖反應(Polymerase Chain Reaction, PCR)以及巢式聚合酵素連鎖反應(Nested Polymerase Chain Reaction, Nested PCR)分別建立偵測基因轉殖作物種子樣品中所含之 CAMV 35S 啟動子的 DNA 片段(Meyer 1999；Lipp *et al.* 1999)。Nested PCR 反應中，並以歐盟認可之基因轉殖產品 PCR 偵測用試劑組作為對照(Kuiper 1999)；而 PCR 分析則參考 CAMV 35S 啟動子的序列(Wolf *et al.* 2000)，合成專屬之引子進行反應，並比較其鑑別結果。

DNA **之萃取與純化：**將待檢測之玉米與大豆種子磨粉，並分別取 50~80mg 之樣品，利用 Wizard™ Genomic DNA Purification System(Promega, Madison, WI)、Wizard® Magnetic DNA Purification System(Promega)，以及 Premier Excell Pure™ Plant/Fungi Genomic DNA Kit 等，三種不同的 DNA 的萃取試劑組，進行 DNA 萃取。萃取得之 DNA 再以 Spectrophotometer 以 OD_{260} 測定萃取物濃度，最後並分別稀釋其濃度為 80ng/ μl，提供作為 PCR 反應的模版，以進行 PCR 分析並比較其結果。

引子設計：35S l/35S 2 以及 35S-F/35S-R 二組引子，係參考 Wolf 等人(2000)檢測 CAMV 35S 啟動子所設計之專屬引子。35S l 序列為 5'GCT CCT ACA AAT GCC ATC A3'；35S 2 序列為 5'GAT AGT GGG ATT GTG GGT CA5'。另 35S-F 引子其序列為 5' CCT ACA AAT GCC ATC ATT GCG A3'；35S-R 序列為 5'GGG TCT TGC GAA GGA TAG TG 5'。

CAMV 35S **啟動子之** PCR **分析：**PCR 反應總體積為 25 μl，內含 1 unit Taq polymerase (Roche-Fast Start)、1×PCR buffer (10 mM KCl；50 mM Tris-HCl，pH 8.3；5 mM $(NH_4)_2SO_4$)、1.5 mM $MgCl_2$ 、200 μM dNTP、0.5 μM primer 及 100 ng template DNA。PCR 反應儀器為 Perkin Elmer Cetus Thermal Cycler 9600，反應溫度條件依 CAMV 35S 啟動子合成的引子序列之 Tm 值分別設定如下：(一)35S 1/ 35S 2 引子 【95℃ 5 分鐘一次；95℃ 20 秒， 57℃ 40 秒，72℃ 1 分鐘，進行 40 次；接續 72℃ 3 分鐘；反應完成後貯藏在 4℃。】(二)35S-F/ 35S-R 引子【94℃ 10 分鐘一次；95℃ 25 秒， 62℃ 30 秒，72℃ 45 秒，進行 50 次；接續 72℃ 7 分鐘；反應完成後貯藏在 4℃。】

CAMV 35S **啟動子之** Nested PCR **分析：**Nested PCR 係用於增進反應的專一性，此方法包含兩個反應步驟，首先利用外側的引子組進行第一次增殖作用；隨後，以第一次 PCR 產物作為模板，設計一個內部的新引子組，再進行第二次 PCR 反應。由於以整體複雜性較低的模版 DNA 進行第二次 PCR 反應，可確保產生較多特定的同質終產物。

Nested PCR 分析係以基因轉殖產品檢測用之試劑組(Biosmart Allin 1.0 GMO Screening System)進行之，其中第一次反應總體積為 50 μl，內含 1 unit Taq polymerase (Roch- Fast Start、Promega Taq-buffer B)、 40 μl Allin Mix 1 (內含 35S 啟動子 篩檢之引子、 dNTP 與 PCR buffer) ，及加入 400 ng template DNA；第二次反應係以第一次 PCR 產物，取 1 μl 當作反應模版，再加入 49 μl Allin Mix 2 再進行二次反應， 反應混合液中特別帶有 internal control(Zein 基因)，可用以監測 PCR 反應，是否存在抑制產物生成之因子。兩次反應溫度條件分別設定如下：(一) Nested PCR 第一次反應【95℃ 3 分鐘一次；95℃ 30 秒， 55℃ 40 秒，進行 40 次；接續 72℃ 3 分鐘；反應完成後貯藏在 4℃。】(二) Nested PCR 第二次反應【95℃ 1 分鐘一次；95℃ 45 秒， 50℃ 40 秒，72℃ 30 秒，進行 45 次；接續 72℃ 3 分鐘；反應完成後貯藏在 4℃。】

DNA **電泳：**經不同聚合酵素連鎖反應而增殖之 DNA ， 取 12 μl 加入 6 倍的電泳緩衝液 (6×loading buffer：0.25% bromophenol blue 及 xylene cyanol，40% w/v sucrose)，以含 2.0% agarose 與 1.25 %Neu-Sev 3：1 agarose 之膠體，在 0.5X TBE buffer 中進行電泳，電泳槽均為 Cosmo, Mupid-2，電壓 100 伏特，電泳時間約 25～30 分鐘，結束後置於 0.5 mg/ml 的 ethidium bromide 中染色 20-30

分鐘，再於紫外燈光箱中，檢視膠體上 DNA 多型性片段，並照相及貯存影像於 IS 2000 Digital Imaging System (Alpha Innotech Corporation)，接續進行基因轉殖作物之 CAMV 35S 啟動子 之 DNA 片段檢測。

結 果

DNA 快速萃取及比較

以包含基因轉殖之大豆及玉米等十七個樣品 50~80mg 進行 DNA 之萃取，並比較三種 DNA 萃取試劑(Promega / Wizard® Genomic DNA Purification Kit；Promega /Wizard® Magnetic DNA Purification System for Food；Premier Excel Pure™ Plant/Fungi Genomic DNA Kit)之 DNA 純度與產量，結果三種 DNA 萃取試劑組均可成功萃取得玉米與大豆種子樣品之 DNA。萃取得之 DNA 再以 Spectrophotometer 測定其 OD_{260} 數值，推算其 DNA 量結果為 2μg ~4 μg。另由電泳檢測 DNA 品質結果，三種萃取試劑均可獲得品質良好之 DNA，但以 Wizard® Genomic DNA Purification Kit 之品質最好。就萃取所需之工作時間則分別為 1~4 小時，但以 Wizard® Magnetic DNA Purification System for Food 僅需 1 小時，為最省時(表 2)。萃取費用則為 25 元至 100 元之間，但其中則以 Wizard® Genomic DNA Purification Kit 僅需 25 元之費用最低(表 2)。

表 2. DNA 快速萃取試劑組及萃取 DNA 品質之比較
Table 2. Different DNA extraction kits and the comparisons of their quality

DNA Extraction Kit	Quality	Time (Hours)	Cost (NT dollars/sample)
Promega / Wizard® Genomic DNA Purification Kit	Good	2	25
Promega /Wizard® Magnetic DNA Purification System (food)	Fine	1	150
Premier ExcelPure™ Plant/Fungi Genomic DNA Kit	Fine	4	60

CAMV 35S 啟動子之 PCR 分析

依 CAMV 35S 啟動子基因的 5’與 3’兩端之序列，合成 35S 1/35S 2 引子組(35S 1 序列為 5’GCT CCT ACA AAT GCC ATC A3’；35S 2 序列為 5’GAT AGT GGG ATT GTG GGT CA5’)；以及 35S-F/ 35S-R 引子組 (35S-F 引子其序列為 5’ CCT ACA AAT GCC ATC ATT GCG A3’；35S-R 序列為 5’ GGG TCT TGC GAA GGA TAG TG 5’)。合成之 35S 1/35S 2 引子以及 35S-F/ 35S-R 引子經 PCR 反應結果，可分別測得基因轉殖大豆及玉米樣品中 CAMV 35S 啟動子基因之 195bp DNA 片段(圖 1)以及 207bp 之 DNA 片段(圖 2)，其中 P 樣品為 positive control 係以 Biosmart Allin 1.0 GMO Screening System 所附之對照組(含 80ng DNA /μl；0.5% each, Round up Ready® soybean and BT-176 maize)；編號 1,2 為非基因轉殖大豆，編號 3,4,14,15,16 是基因轉殖大豆樣品；編號 9,10 則為非基因轉殖玉米，編號 6,7,8,11,12,13 則是基因轉殖玉米樣品。據 35S 1/35S 2 引子之 PCR 電泳圖譜結果顯示，所有基因轉殖大豆及玉米種子樣品所抽得之 DNA(圖譜樣品編號：3,4,14,15,16,6,7,8,11,12,13)均可分別出現 195bp DNA 片段(圖 1)；而 35S-F/ 35S-R 引子之 PCR 電泳圖譜結果，所有基因轉殖樣品則具有 207bp 之 DNA 片段(圖 2)；另外屬於非基因轉殖之樣品(1,2, 9,10)則均不具此二個片段。

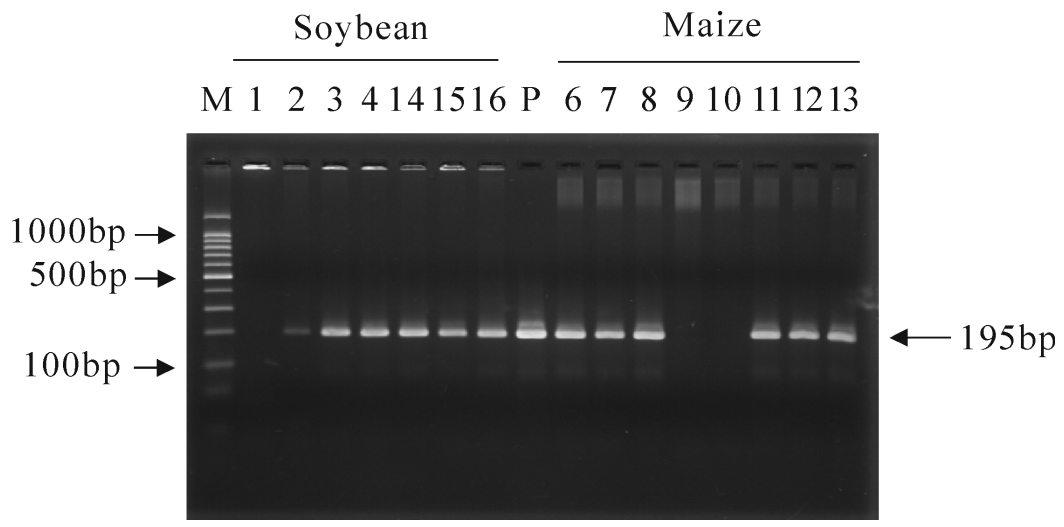


圖 1. 以 CAMV 35S 啟動子之特定序列 (35S 1/35S 2) 為引子，進行玉米及大豆基因轉殖樣品之檢測。

Fig. 1. Surveyed GMO samples by using PCR with CAMV 35S promoter specific sequence as primers (35S 1/35S 2).
Sample no. 1, 2 are non-GM soybean；Sample no. 3, 4, 14, 15, 16 are GM soybean； Sample no. 9, 10 are non-GM maize；Sample no.6, 7, 8, 11, 12, 13 are GM maize. Lanes M: size marker (100 ladder of promega). P: Positive control .

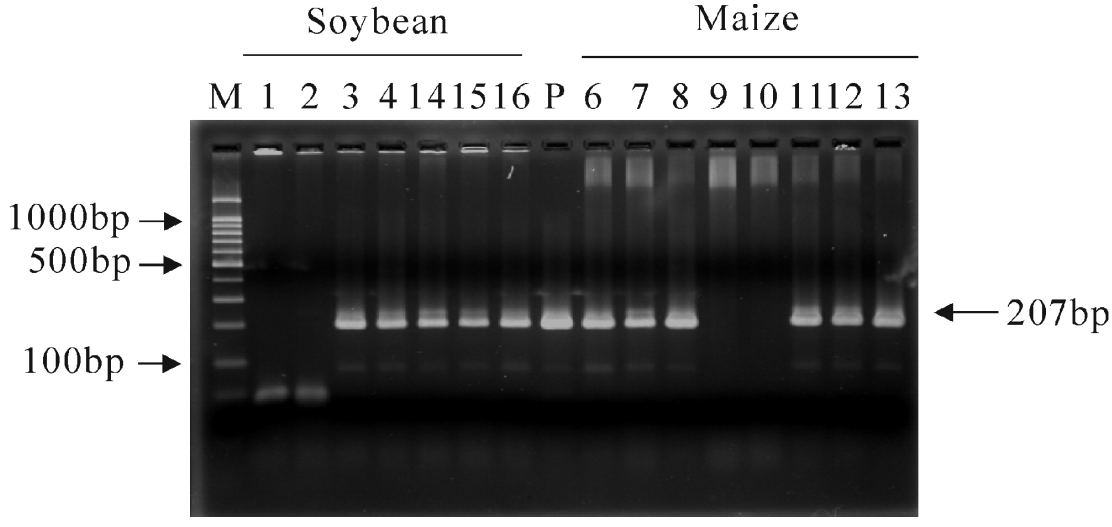


圖 2. 以 CAMV 35S 啟動子之特定序列 (35S-F / 35S-R) 為引子，進行玉米及大豆基因轉殖樣品之檢測。

Fig. 2. Surveyed GMO samples by using PCR with CAMV 35S promoter specific sequence as primers (35S-F/35S-R).
Sample no. 1,2 are non-GM soybean；Sample no. 3, 4, 14, 15, 16 are GM soybean； Sample no. 9, 10 are non-GM maize；Sample no.6, 7, 8, 11, 12, 13 are GM maize. Lanes M: size marker (100 ladder of promega). P: Positive control.

Nest-PCR 在基因改造大豆及玉米之鑑定

以歐盟認證之基因轉殖檢測用試劑組(Biosmart Allin 1.0 GMO Screening System)，配合利用 Nest-PCR 分析進行基因轉殖大豆及玉米之鑑定。初步經修正反應之模板 DNA 濃度為 400ng(PCR 反

應總體積 50μl)，或 200ng(PCR 反應總體積 25μl)，均可獲得穩定之鑑定結果，並正確的偵測得基因轉殖玉米及大豆樣品中之 CAMV 35S 啟動子基因專屬的 150bp DNA 片段(圖 3)。

Nest PCR 分析結果與基因轉殖樣品之判讀，按 Biosmart Allin 1.0 GMO Screening System 所採用之條件及結果說明書，判讀本試驗中所獲得之四個條帶，分別為 278bp (Zein 之基因，其來源為 Maize)，217bp (Zein 之基因， DNA 來源為 Internal Control)，150bp (CAMV35 啟動子基因) ，118bp (Lectin 之基因)。Nest PCR 圖譜中編號 1,2, 為非基因轉殖大豆，編號 10 則為非基因轉殖玉米樣品，均不具 150bp 之 DNA 片段；其餘編號 3,4,5 為基因轉殖大豆樣品，6,7,8,12 為基因轉殖玉米樣品，在 150bp 位置皆可得一清淅之條帶。

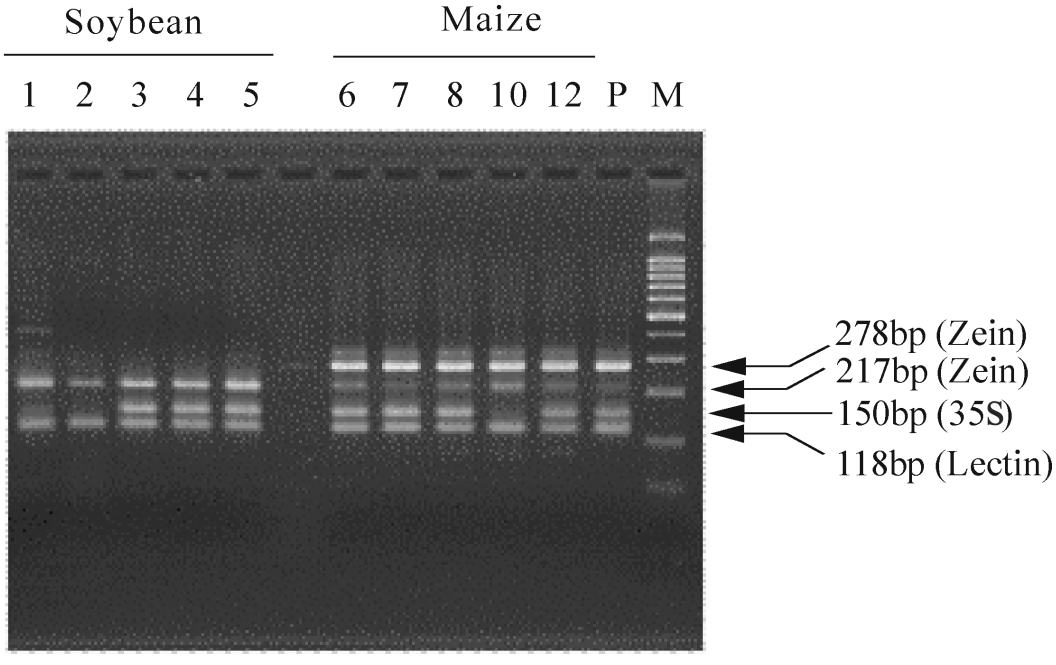


圖 3. 以 Biosmart Allin 1.0 試劑系統進行基因轉殖玉米及大豆樣品 CAMV 35S 啟動子之篩檢。

Fig. 3. Surveying maize and soybean GMO sample by Biosmart Allin 1.0 GMO Screen system for CAMV 35S promoter with nested PCR. Sample no. 1, 2 are non-GM soybean ；Samples no. 3, 4, 5 are GM soybean；Sample no. 10 is non-GM maize；Samples no. 6, 7, 8, 12 are GM Maize； Lanes P: positive control； M: size marker (100 ladder of promega).

討 論

基因轉殖作物的偵測，是生物科技與作物管理上重要之研究主題；完備之檢測方法也成為基因轉殖作物管制之基礎，唯基因轉殖作物偵測首要了解其轉殖方法及其基因構築，才能設計合適之方法以方便進行偵測。目前國際間鑑別基因轉殖生物的方法，主要分為兩大類，第一類以核酸為基礎之偵測方法：如聚合酵素連鎖反應(Polymerase Chain Reaction, PCR)，包括傳統 PCR(Gachet 1999)、Nested PCR (Meyer 1999)與 Real-Time PCR 等；第二類以蛋白質分析為基礎之方法(Rogan *et al.* 1999)：如酵素連結免疫吸附分析法(Enzyme-linked immunosorbent assay, ELISA)，或西方墨點分析法(Western blot

analysis)等。唯目前歐洲官方研究機構所發表之基因轉殖作物檢測，多以第一類的核酸為主(Schreiber 1999)，可藉 CAMV 35S 啟動子設計之引子進行初步 PCR 之篩選；接續則作多基因鑑定之 PCR 分析(Multiplex PCR)，或根據基因轉殖構築中，插入之啟動子(promoter)、終結子(terminator)、篩選基因(selection gene)以及欲表現之目標基因(target gene)等，設計專屬之引子，以進行 Nested PCR 或特定基因轉殖作物之確認。此外，PCR 反應產物，將可再藉核酸定序、核酸內切酵素圖譜、探針雜交等分子技術，進行後續之確認。以上由特定單一基因的 PCR 分析乃至 Nested PCR 或多基因的 PCR 分析，將是本研究後續會開發以及建立的 GMO 鑑定系統。

唯不論是傳統 PCR、Nested PCR 與 Real-Time PCR，檢測基因轉殖作物之準確性以及再現性，皆與樣品 DNA 之品質息息相關，好的 DNA 品質為鑑定成功之基礎。由於本試驗使用之材料為種子，而糧食作物的種子尤其富含澱粉及其他二次代謝物等物質，DNA 含量普遍偏低，要獲得良好 DNA 品質是一種挑戰，故本試驗初步採用了三種 DNA 萃取試驗組，其所得之 DNA 經 PCR 結果可具穩定之再現性，且 PCR 產物清晰可判讀，唯兼顧 DNA 萃取時間與成品之考量，則以 Wizard®Genomic DNA 萃取試劑組表現較佳，可兼具快速萃取(二小時)及低萃取成本(25 元/樣品)等優點，以 50~80mg 之微量樣品，約可獲得 2μg 以上之 DNA，按一般 PCR 或 Nested PCR 反應條件，已可提供進行 20 次以上之 PCR 反應使用。

由於 Nest PCR 分析圖譜中，用以判讀 CAMV35 啟動子基因的 150bp 片段以及豆科種子必然存在的 Lectin 基因之 118bp 片段，兩者間分子大小相近且均為小於 150bp 之片段，以一般 DNA 電泳用的 2% Agrose 很難正確判讀及區隔，故本試驗採用了改良式 Agarose 膠體，以適當比率之 Agarose 及 Neu-Sev3:1 之膠體，電泳結果已可以將樣品中非基因轉殖大豆與玉米(樣品編號 1,2 與 10)，以及基因轉殖大豆與玉米(編號 3,4,5 與 6,7,8,12)，藉 150bp 之有無，明顯區隔出是否為基因轉殖產品。

Wolf 等研究人員(2000)曾針對基因轉殖產品中 CAMV 35S 啟動子之偵測，設計並提出一系列 PCR 反應之專屬引子，其中包含 35S 1/S 2、35S-F/35S-R，本試驗即參考其序列合成引子，以進行玉米大豆基因轉殖樣品之檢測，而試驗結果也與 Wolf 研究結果相符，分別可產生用以偵測 CAMV 35S 啟動子之 195bp 與 207bp 之片段。Wolf 研究結果，曾表示使用 35S 1/S 2 引子之 PCR 產物除 CAMV 35S 啟動子之專屬之 195bp 外，尚產生許多非專一性條帶，不若 35S-F/35S-R 僅產生唯一之 207bp 片段；而本試驗 PCR 反應之聚合酵素採用 Fast start polymerase ，結果不論是 35S 1/S 2 或 35S-F/35S-R 引子組，均僅產生唯一條帶，具有較佳之專一性及判讀性；唯 35S 1/S 2 引子的 PCR 結果在 non-GM 的大豆(樣品 2)，呈現不確定的 35S 啟動子 之 195bp 條帶；而在 35S-F/35S-R 引子對 non-GM 樣品偵測則無此現象。另外，針對 35S 啟動子的 Nested PCR 分析，由於反應液內含的 internal control(Zein-217bp 之 DNA)，故全數樣品之 PCR 產物均有 Zein- 217bp 條帶外；然所有樣品中之 Nested PCR 產物，均產生的 Lectin 基因之 118bp 片段，對玉米樣品而言值得進一步確認是否為人為之污染所致，唯對目前針對 35S 啟動子的偵測不致影響其判讀，故將於未來做為其他品系或作物進行 GMO 偵測時，詳加探討。

目前歐盟認可之 PCR 檢測鑑定方法可分成三個層級(Kuiper 1999)。一為檢測(Detection)：乃檢測樣品內是否含基因轉殖成分之材料，其方法一般以 PCR 為主，檢測對象為是否具有 CAMV35 啟動子或以其 selector marker 為檢測基因；而層級二為鑑定(Identification)：鑑定係以基因轉殖“品種”為對象，瞭解基因轉殖之樣品是否為歐盟所認可之“品種”，此階段亦以 PCR 分析為主，但需設計特殊引子以檢測此基因轉殖之樣品內之 structure gene 為何，並與對照品種比對(此層級之分析，於本報告內雖尚未實施，但已列為將進行之工作)；第三層級為定量(Quantification)：為檢測樣品材料內基因轉殖成分之含量，一般係使用 Real-Time PCR。綜合以上歐盟對基因轉殖材料鑑定的方法，本研究在未來

在基因轉殖作物鑑定上，除考量開發快速有效率的鑑定方法外，為兼顧偵測的正確性，將配合以上多種篩檢方式，逐年作成綜合性之探討。

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Developing the Techniques for Transgenic Crops Detection -Detection of Genetically Modified Soybeans and Maize by 35S Promoter Molecular Marker[1]

Jau-Yeuh Wang [2,4], Ming-Jen Fan[2] and Chien-Yih Lin[3]

Summary

Wang, J. Y., M. J. Fan, and C. Y. Lin. 2004. Developing the techniques for transgenic crops detection- detection of genetically modified soybeans and maize by 35S promoter molecular marker. J. Agric. Res. China 53:9-17.

Biotechnology has enabled the modification of crops in a very precise way thereby improving productivity and yield. Till 2003 about 78 transgenic crops were through safety evaluated and already approved. Since that, the problem for discriminating between transgenic crops and non- transgenic crops in the market was posed. In this study, we used herbicide resistance transgenic soybean and *Bacilnus tumufacisos* (Bt) corn as model plants for setup the DNA level detection of transgenic crops. Three commercial DNA extraction kits were evaluated for DNA quality and PCR efficiency. The results showed that the Wizard® Genomic DNA Purification Kit can be manipulated easily to obtain DNA with good quality. The results of transgenic crops detection revealed that Nested PCR （Nest Polymerase Chain Reaction） analysis based on Biosmart Allin 1.0 GMO Screening System allowed the detection of transgenic soybean and transgenic corn by CaMV 35S promoter specific gene fragment of 150bp. Furthermore, the synthesized primers of 35S 1/35S 2 and 35S-F/35S-R also showed very efficient in detected the CaMV 35S promoter and produced the fragments of 195bp and 207bp respectively.

Key words: Transgenic crops, Detection.

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澱粉糖化酵素活性簡易測定法於甘藷加工特性之應用[1]

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摘 要

利幸貞、陳一心。2004。澱粉糖化酵素活性簡易測定法於甘藷加工特性之應用。中華農業研究 53:18-26。

本研究測定四種甘藷品種之澱粉糖化酵素活性及澱粉、可溶性糖、質地等加工特性，並探討兩者之間的關係。生藷狀態下進行之澱粉糖化酵素活性檢測，台農 66 號、台農 57 號於採收當日澱粉糖化酵素即呈(+)的活性，而台農 68 號採收當日澱粉糖化酵素活性呈(−)，採收後貯藏 14 天才呈(+)的活性，顯示此品種採收後貯藏一段時間有利於甜度提高；而對照品種 Satsumahikari 為低糖特性品種其澱粉糖化酵素活性在試驗期間均呈(−)。烤藷可溶性糖含量的增加與澱粉減少的程度呈相對應的趨勢，烤藷所含澱粉殘餘量最高為對照品種 Satsumahikari，其次為台農 68 號；而台農 66 號與台農 57 號烤藷所含可溶性糖含量則較高。供試品種質地表現差異，粉質程度明顯依序為極粉質之對照品種 Satsumahikari，粉質之台農 68 號，偏粉質之台農 57 號，此粉質品種在組織質地分析儀之物理特性檢測時探針需要施加較大的壓力才能穿透塊根周皮組織，因此在探針壓力走勢圖上會呈現明顯突出曲線，不同於泥質質地之台農 66 號。本試驗結果顯示甘藷品種烘烤加工處理後澱粉含量的變化與澱粉轉化成可溶性糖含量之比例高低並非由生藷本身所含之澱粉及可溶性糖含量多寡決定，而是因生藷內澱粉糖化酵素活性高低之差異影響烘烤過程中澱粉的水解量；而澱粉糖化酵素活性的高低亦會影響蒸藷質地。利用品種特性能改善甘藷加工產品之品質及協助選育加工適性的品種，而快速、簡易的測定方法有其必要性。

關鍵詞：甘藷、澱粉、可溶性糖、澱粉糖化酵素、質地。

前 言

甘藷含豐富的營養，由於收穫後應用的目的不同，品種選育指標亦不同，各品種間品質特性也有很大的差別(李等 1992；Takahata *et al.* 1994；山村穎 1981)。早期甘藷品種的選育偏重於鮮食品種，因此，就食用方面以甜度高的甘藷品種較受歡迎，食品加工上要求製品需具一定規格，但因作為原料

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的甘藷所需添加糖量的多寡是件複雜的作業，而以不含糖的原料來處理較為容易，日本也因此目的選育出 Satsumahikari 作為加工甘藷品種(Yamakawa *et al.* 1994；山村穎 1981；佃和民 1996)；為使甘藷生產利用朝向多元化發展，台灣近年已積極開發適合加工用品種，隨著作為加工原料的用途日益擴大，含糖度極少之低糖甘藷品種需求也隨之增加。目前甘藷改良著重於食用品質、加工品質及營養價值的改進，以提高產品利用之經濟價值(李等 1994；江等 1989；Picha 1985)。本研究希望藉由探討不同甘藷品種之澱粉、可溶性糖及質地等重要加工特性及其影響的原因，並發展簡易、快速且準確的甘藷品質理化測定方法， 使甘藷能製造成更多樣化的商品，並期能建立甘藷品質評估之技術進而提高育種選育時效。

材料與方法

供試甘藷品種與田間設計

本試驗以甘藷台農 57 號、台農 66 號、台農 68 號品種及對照品種 Satsumahikari(薩摩光)為試驗材料，田間試驗設計採逢機完全區集設計，試區畦長 6 公尺，行株距 1×0.25 公尺，四行區三重複。各甘藷品種分別於採收當日、採收後貯藏 7 天及採收後貯藏 14 天進行取樣分析調查，貯藏溫度為 15℃。

化學成分分析

樣品調製：甘藷塊根削去表皮製簽再於 100℃烘箱中烘乾至水分含量 7~8%左右，所得材料用高速磨粉機研磨成粉末並以 100 mesh 篩網過篩。精秤樣品 0.1g 加入 80%酒精 10ml 充分混和置於 80℃水浴 30 分鐘，以 3000r.p.m.離心 15 分鐘，取澄清液備用，沈澱部份進行澱粉含量分析。

可溶性糖含量測定：澄清液定量至 100ml，取 1ml 定量至 5 ml，於冰水浴中加 10ml Anthrone 試液充分攪拌，靜置 20 分鐘再於 100℃水浴中加熱 10 分鐘，冷卻後以光電比色計波長 630nm 測定。

澱粉含量測定：沈澱部份以 50℃烘乾，加入 3ml 蒸餾水混和均勻，以 100℃水浴加熱 30 分鐘，冷卻後加入 2 ml 9.2N 之過氯酸靜置 15 分鐘，定量至 10 ml，經 3000 r.p.m.離心 15 分鐘，取澄清液定量至 100ml。取 0.5ml 定量至 5ml，於冷水浴中加入 10ml Anthrone 試液充分攪拌，靜置 20 分鐘再於 100℃水浴中加熱 10 分鐘，冷卻後以光電比色計測定。

澱粉糖化酵素活性簡易測定(Kumagai & Umemura 1989)

取生藷汁液上層澄清液 0.05ml，分別加入 (A) 0.5ml 2%可溶性澱粉 (B) 0.5ml 純水之兩試管中，置於 37℃水浴下 10 分鐘，加入還原糖定量用試劑 Resuter(柴田科學器械工業株式會社 日本)反應完畢，A、B 兩試管進行色差(Resuter 色見本)比較，色差越大，顯示澱粉糖化酵素(β-amylase)活性越大。

甘藷質地物理特性測定

甘藷塊根洗淨以 100℃蒸氣蒸煮 20 分鐘後冷卻，利用組織質地分析儀(stevens-lfra texture analyzer, made in Germany)檢測甘藷品種之質地物理特性，設定值為 Speed：0.5mm/sec；Model：normal；Probe：diameter 6mm；記錄器(stevens l 250E made in Germany)設定值為 speed：2.0mm/sec。

結 果

烘烤前後甘藷塊根澱粉含量之變化

四個甘藷供試品種中其生藷狀態下之澱粉含量在採收當日以台農 57 號之 64.4%最高，台農 68 號與 Satsumahikari 兩品種無顯著差異，其值分別為 61.0%及 61.2%，而台農 66 號最低為 58.7%；採收後貯藏 7 天依序為台農 68 號之 61.7%、台農 57 號之 61.3%及 Satsumahikari 之 60.5%及台農 66 號

53.4%，四品種之間彼此皆差異顯著；採收後貯藏 14 天以台農 57 號 69.1%最高、其次依序為 Satsumahikari 之 63.0%、台農 66 號之 61.9%，而以台農 68 號 57.9%較低。比較三個貯藏期，品種間生藷澱粉含量的差異各有高低並無特定的變化趨勢(表 1)。經過烘烤處理後，除了 Satsumahikari 外，其餘 3 個品種之澱粉含量皆明顯下降，且品種間差異的趨勢在三個貯藏期間非常一致，但不論是採收當日立即烘烤或貯藏 7 天、14 天後烘烤，烤藷中澱粉含量均以 Satsumahikari 最高，三個貯藏期分別為 61.1%、59.1%、62.4%；其次是台農 68 號，三個貯藏期分別為 33.8%、35.9%、32.8%；台農 57 號三個貯藏期則分別為 32.9%、28.3%、26.9%，台農 66 號烤藷澱粉含量最低，三個貯藏期分別為 27.1%、21.3%、20.8%(表 1)。

表 1. 貯藏期間不同品種甘藷塊根烘烤前後澱粉含量變化比較
Table 1. The starch content (% dry wt) between raw and baked roots of sweet potato cultivars on three storage durations[z]

Cultivar	Starch content of raw root after storage for different days (% dry wt)			Starch content after baking and storage for different days (% dry wt)		
	0	7	14	0	7	14
Tainung 57	64.4 a[y]	61.34 b	69.13 a	32.9 b	28.3 c	26.9 c
Tainung 66	58.7 c	53.38 d	61.88 c	27.1 c	21.3 d	20.8 d
Tainung 68	61.0 b	61.69 a	57.94 d	33.8 b	35.9 b	32.8 b
Satsumahikari	61.2 b	60.54 c	63.00 b	61.1 a	59.1 a	62.4 a

[z] Storage temperature was 15℃.
[y] Means followed by the same letter within each column are not significantly different at 5% level by DMRT.

烘烤前後甘藷塊根可溶性糖含量之變化

生藷可溶性糖含量在採收當日以台農 57 號、台農 66 號最高，分別是 16.9%和 16.7%；其次是台農 68 號之 14.7%，而 Satsumahikari 為 14.2%；採收後貯藏 7 天以台農 66 號 19.1%最高，台農 68 號之 16.3%、台農 57 號之 15.1%次之，而以 Satsumahikari 之 14.8%最低；採收後貯藏 14 天以台農 68 號之 23.1%最高、其次為台農 66 號之 17.2%，而台農 57 號與 Satsumahikari 均為 14.4%，兩品種無顯著差異；比較三個貯藏期，生藷可溶性糖含量除 Satsumahikari 都是最低外，其餘三個品種間互有高低，沒有特定的變化趨勢。經過烘烤處理後除了 Satsumahikari 外，其餘三品種之可溶性糖含量皆明顯上升。品種間差異的趨勢在三個貯藏期間頗為一致，不論是採收當日即烘烤或貯藏 7 天、14 天後烘烤，烤藷中可溶糖含量均以 Satsumahikari 最低，三個貯藏期分別為 19.7%、15.3%、16.2%；而以台農 66 號之含量最高，三個貯藏期分別為 48.6%、56.3%、56.5%；其次為台農 57 號，三個貯藏期分別為 47.7%、47.5%、51.3%，以台農 68 號較低，三個貯藏期分別為 45.4%、47.5%、47.5% (表 2)。

表 2. 貯藏期間不同品種甘藷塊根烘烤前後可溶性糖含量變化比較
Table 2. The soluble sugar content (% dry wt) between raw and baked roots of sweet potato cultivars on three storage durations[z]

Cultivar	Soluble sugar content of raw root after storage for different days (% dry wt)			Soluble sugar content after baking and storage for different days (% dry wt)		
	0	7	14	0	7	14
Tainung 57	16.9 a[y]	15.13 c	14.41 c	47.7 b	47.5 b	51.3 b
Tainung 66	16.7 a	19.11 a	17.21 b	48.6 a	56.3 a	56.5 a
Tainung 68	14.7 c	16.30 b	23.06 a	45.4 c	47.5 b	47.5 c
Satsumahikari	14.2 d	14.80 d	14.41 c	19.7 d	15.3 c	16.2 d

[z] Storage temperature was 15℃.
[y] Means followed by the same letter within each column are not significantly different at 5% level by DMRT.

採收後甘藷塊根澱粉糖化酵素(β-amylase)之反應

利用甘藷澱粉糖化酵素活性簡易測定法於四個供試品種之呈色反應顯示，台農 66 號、台農 57 號、台農 68 號其 A 與 B 兩試管色差差異明顯(圖 1)，即澱粉糖化酵素活性表現均呈(＋)的反應，對照品種 Satsumahikari 呈色反應則無差異(圖 1)。然澱粉糖化酵素活性的高低因品種而異，台農 57 號及台農 66 號品種在採收當日、採收後貯藏 7 天及採收後貯藏 14 天其澱粉糖化酵素活性表現均呈(＋)的反應，而台農 68 號於採收當日及採收後貯藏 7 天其澱粉糖化酵素活性表現為(－)，在採收後貯藏 14 天澱粉糖化酵素活性才呈(＋)反應(表 3)。對照品種 Satsumahikari 其澱粉糖化酵素活性測定結果則不論採收當日、採收後貯藏 7 天及採收後貯藏 14 天均為(－)的表現，顯示其澱粉糖化酵素活性極低。

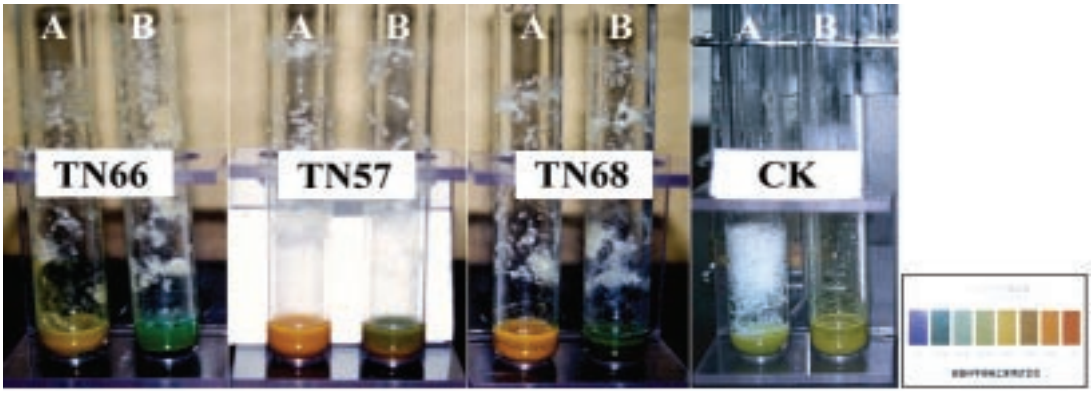


圖 1. 甘藷澱粉糖化酵素活性簡易測定法於四個供試品種生藷之呈色反應(由左至右分別為台農 66 號、台農 57 號、台農 68 號、對照品種 Satsumahikari)。

Fig.1. The rapid test method for β-amylase activity in fresh root of sweet potato cultivars (from left to right: Tainung 66, Tainung 57, Tainung 68, Satsumahikari (CK)).

表 3. 貯藏期間不同甘藷品種澱粉糖化酵素(β-amylase)活性簡易測定結果比較

Table 3. The β-amylase activity in raw roots of sweet potato cultivars on three storage durations[z]

Cultivar	Storage days								
	0			7			14		
	β-amylase activity[y]	Resuter color index[x]		β-amylase Activity	Resuter color index		β-amylase activity	Resuter color index	
		A	B[x]		A	B[x]		A	B[x]
Tainung 57	＋	0.4	0.03	＋	0.6	0.05	＋	0.6	0.05
	＋	0.6	0.05	＋	0.6	0.05	＋	0.6	0.08
Tainung 66	＋	0.4	0.05	＋	0.6	0.08	＋	0.6	0.05
	＋	0.4	0.05	＋	0.6	0.08	＋	0.6	0.08
Tainung 68	－	0.2	0.08	－	0.6	0.4	＋	0.6	0.05
	－	0.6	0.4	－	0.2	0.08	＋	0.6	0.05
Satsumahikari	－	0.6	0.4	－	0.2	0.2	－	0.2	0.2
	－	0.2	0.2	－	0.2	0.2	－	0.2	0.2

[z] Storage temperature was 15℃.

[y] ＋: With β-amylase activity；－: Without β-amylase activity.

[x] Resuter color index: A:The color index of '0.05 ml raw root juice + 0.5 ml 2% soluble starch + Resuter'. B:The color index of '0.05 ml raw root juice + 0.5 ml H_2O + Resuter'.

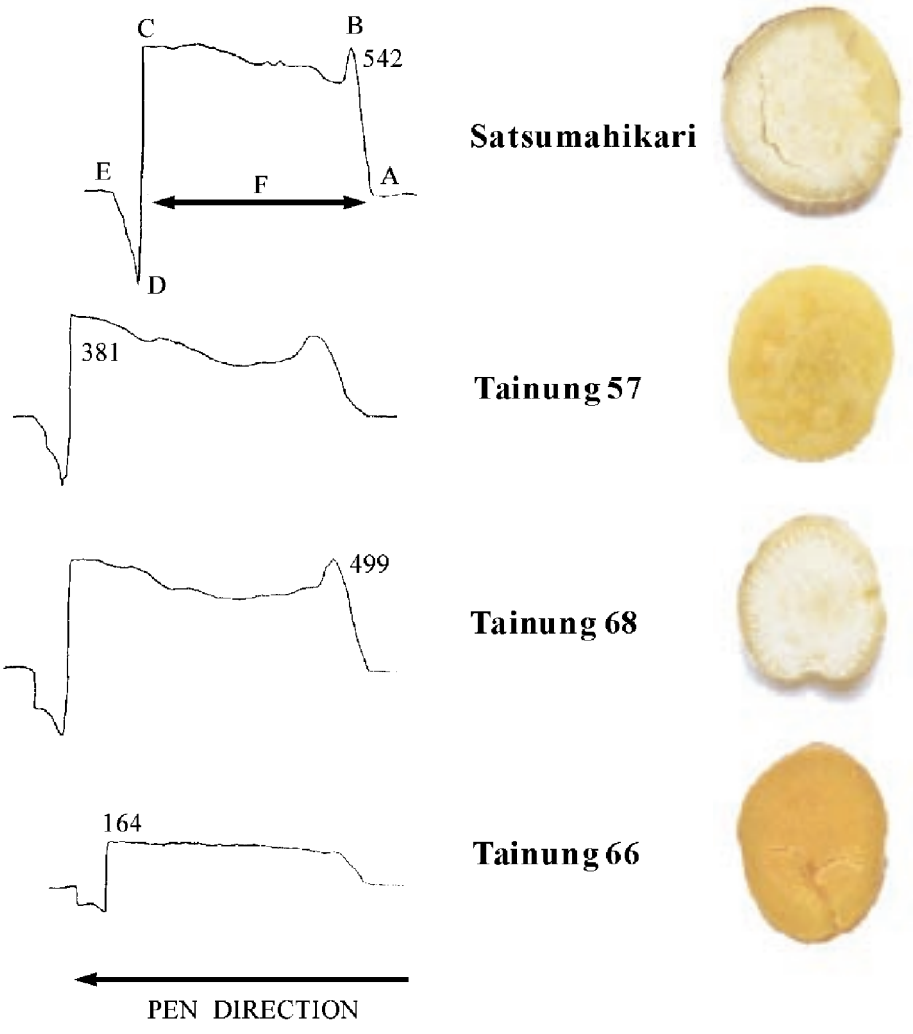


圖 2. 組織質地分析儀於四個甘藷供試品種蒸藷質地特性之檢測。

Fig.2. The probe pressure curve on different cultivars of sweet potato measured by the texture analyzer. A: The point of probe attack the root; B: The point of probe attack the periderm tissue of root; C: The point of the root center; D: Viscosity; E: The probe leaves from the root; F: Radius of root.

蒸熟後甘藷塊根之質地比較

利用組織質地分析儀測試四個甘藷供試品種塊根蒸熟後之質地，其結果如圖 2 所示，Satsumahikari、台農 68 號及台農 57 號品種在探針穿過塊根周皮組織時呈現明顯之突出壓力曲線，其壓力最高值大小依序為 Satsumahikari、台農 68 號、台農 57 號品種；而台農 66 號品種在探針穿過塊根周皮組織時無此突出壓力曲線；三個貯藏期各品種的質地特性有相同的結果。相對於口感質地品評，其粉泥質程度亦依序為極粉質之 Satsumahikari，粉質之台農 68 號，偏粉質之台農 57 號，而台農 66 號在口感上則呈泥質質地。

討　論

甘藷塊根中因為含多量的澱粉，所以在烹調過程中風味及質地的改變與澱粉有密不可分的關係(康 1987)。探討甘藷甜味的來源除了塊根本身所含的還原糖和蔗糖外，主要是由蒸煮、烘烤的加熱過程中澱粉轉變成麥芽糖而來。Balls *et al.*(1948) 首先分離出澱粉糖化酵素，並證明此酵素能將澱粉分解成麥芽糖，由於酵素作用，使甘藷中還原糖迅速增加。Picha(1985)測定鮮藷中可溶性糖成分主要是蔗糖，而果糖及葡萄糖較低，不含麥芽糖；經過烘烤加工後蔗糖、果糖及葡萄糖僅少量增加，麥芽糖顯著大量產生；而麥芽糖的生成量主要是受澱粉糖化酵素活性高低影響(Takahata *et al.* 1992，1994)。Walter *et al.*(1975)報告指出甘藷依品種不同，鮮藷內澱粉經烘烤處理後約 42%至 95%被轉化，而水解之產物 70%以上為麥芽糖，其餘為糊精(dextrins)。本試驗比較 4 個供試品種烘烤加工前後澱粉及可溶性糖含量變化顯示，4 個供試品種其生藷之澱粉含量均很高，品種間在各貯藏期間雖有顯著差異，但在三個貯藏期間品種間並無一致的差異趨勢，然經烘烤後，除了 Satsumahikari 外，其餘 3 品種之澱粉含量皆明顯下降，且在三個貯藏期間由低而高依序都是台農 66 號、台農 57 號、台農 68 號，若進一步計算比較此四個供試品種經烘烤加工後相較於生藷狀態下其澱粉含量減少比率，則在品種間亦有顯著差異，以台農 66 號差異最大，三個貯藏試期分別達 53.8%、60.1%、66.3%；其次為台農 57 號，分別是 48.9%、53.7%、61.0%，且兩品種隨著貯藏日數延長其澱粉減少量隨著提高；台農 68 號澱粉減少比率較低，介於 41.3%至 44.6%；對照品種 Satsumahikari 生藷經烘烤後其澱粉含量減少比率在三個貯藏試期僅有 0.1%、2.36%、0.9%，並無顯著變化。至於可溶性糖含量方面，在生藷狀態下於各個貯藏期 Satsumahikari 之含量都是最低，其餘 3 個品種之間雖有顯著差異，但綜觀於三個貯藏期品種間的差異並沒有特定的趨勢；然經烘烤後除了 Satsumahikari 外，其餘三品種之可溶性糖含量皆明顯上升，且品種間差異的趨勢在三個貯藏期間相當一致，都是台農 66 號最高，台農 57 號其次，台農 68 號最低；當進一步計算烘烤前後各品種可溶性糖的增加量亦顯示以台農 66 號烤藷內可溶性糖增加量最多，三個貯藏試期分別達 31.91%、37.18%、39.25%；其次為台農 57 號，分別是 30.78%、32.34%、36.87%；台農 68 號較低，三個貯藏試期分別是 30.69%、31.23%、24.41%；對照品種 Satsumahikari 經烘烤後其可溶性糖含量與生藷兩者間並無顯著增加，三個貯藏試期僅有 5.51%、0.49%、1.81%。若將上述烘烤前後澱粉和可溶性糖含量變化對照澱粉糖化酵素活性的測定結果，則可清楚顯示甘藷品種烘烤加工處理後澱粉含量的減少與澱粉轉化成可溶性糖含量之比率高低並非由生藷本身所含之澱粉及可溶性糖含量多寡決定，而是生藷內澱粉糖化酵素活性高低之差異影響烘烤過程中澱粉的水解，進而影響風味與口感質地。

本試驗亦初步探討生藷經過貯藏後，烘烤前後澱粉與可溶性糖含量的變化與生藷澱粉糖化酵素活性反應。各品種在三個貯藏期間生藷的澱粉含量並沒有特定的變化趨勢，但烘烤後台農 57 號及台農 66 號隨著貯藏期間的增長而逐漸下降，其中降幅較大的階段為採收當日與貯藏 7 日之間，台農 68 號及 Satsumahikari 則在三個期間變化的幅度不大且沒有特定的趨勢。至於生藷各品種在三個貯藏期間可溶性糖含量變化趨勢，Satsumahikari 幾乎沒有變化，台農 57 號及 66 號雖有變化但幅度不大僅在 2.5％之內，唯台農 68 號隨著貯藏期間的增長而上升，其中貯藏 7 日至 14 日之間增加近 7％，呈現明顯的變化；烤藷的可溶性糖於台農 66 號及 57 號隨著生藷貯藏日數的增長而提升，其中台農 66 號在貯藏 7 日即有進一步提升而台農 57 號在貯藏 14 日才提高，台農 68 號則呈穩定的狀態，而 Satsumahikari 則貯藏 7 日較低，若對照澱粉糖化酵素的測定結果，台農 57 號、66 號及 68 號均具澱粉糖化酵素測定活性，所不同的是台農 57 號及 66 號在採收當日即表現，而台農 68 號要在採收後儲藏 14 日才表現，顯示似乎具有澱粉糖化酵素活性的品種，在活性顯現後持續將生藷貯藏有利於烘烤過程澱粉的轉換，

因此台農 68 號若在採收 14 日後持續貯藏則在烘烤後澱粉轉換率或可提高，至於台農 68 號在生藷貯藏過程中可溶性糖大幅提高的原因有待進一步的探討。

消費者對甘藷的接受性受感官反應的影響極大，而質地是重要的因素之一；依口感可將甘藷質地分為 2 類：乾性 (dry mouth feel) 為粉質，濕性 (moist mouth feel)為泥質(康 1987)。粉質口感的甘藷品種在蒸煮過程中會因周皮組織及形成層環的纖維較明顯，在利用組織質地分析儀進行質地物理特性檢測時探針需要施加較大的壓力才能穿透周皮組織，因此在探針壓力走勢圖上會呈現明顯突出曲線，且壓力值較高。Sisturnk *et al.*(1954)認為原料中澱粉含量不同，是造成甘藷加工後形成粉、泥質地差異的主因，陳等(1987)研究則指出當甘藷糊細胞中仍存有大量未被水解之澱粉時，細胞易維持形狀且水分被澱粉吸收而呈粉質，反之，當甘藷糊內之澱粉被酵素大量水解產生單寡糖類而溶解於水中，使得甘藷糊產生許多空細胞且流動性增大則呈泥質。李等(1991)研究甘藷食用品質特性與理化性質之關係指出甘藷蒸煮後之質地與適口性和還原糖有顯著之正相關，和澱粉含量則成顯著負相關。Walter *et al.*(1975)的研究指出甘藷的泥質程度與烤後殘留的澱粉含量成反比。本試驗顯示生藷澱粉糖化酵素會影響蒸烘烤等加工甘藷質地，若生藷的澱粉糖化酵素活性愈高，蒸烘烤等加工後澱粉含量愈低而可溶性糖含量愈高，導致粘度下降(viscosity)下降，而質地愈趨於泥質，供試品種台農 66 號與台農 57 號其生藷澱粉糖化酵素活性高於台農 68 號，因此相對的加工後風味甜且較泥質，而對照品種 Satsumahikari 澱粉糖化酵素活性極低，加工後不具甜味且質地為極粉質。利用組織質地分析儀可明顯測定出其間的差異，蒸藷粉質程度明顯依序為極粉質之對照品種 Satsumahikari，粉質之台農 68 號，偏粉質之台農 57 號，而台農 66 號在口感上則呈泥質。

目前甘藷的利用及消費者對產品的需求已日趨多樣化，藉著不同甘藷品種之加工特性的了解慎選適當的品種製成合宜的產品，將可大幅提高產品利用之經濟價值，本試驗結果，台農 66、57 號甘藷都屬於澱粉糖化酵素活性高，加工後澱粉轉化成可溶性糖含量亦較高的品種，因此口感上甜味高，加工應用上較適合做烤藷或蒸煮，但不適合作低糖甘藷產品，台農 68 號採收後貯藏一段時間應可增加其食用甜味。由於澱粉糖化酵素活性高的品種在進行加工時雖可增加蒸烤時的甜度風味，但也影響產品調製之規格化，因此慎選澱粉糖化酵素活性極低的品種如 Satsumahikari 可有改善效果(Yamakawa *et al.* 1994；久木村久等 1986)。

基於食用品質優良或及適合加工用品種是目前甘藷育種的主要目標，如能利用現代化簡易測定理化性儀器和方法以尋求甘藷食用品質特性與塊根中重要之理化性質間的關係，使能以較省時省力和客觀化之理化性質作為甘藷食用品質特性評定方法之依據，以獲得評定和選拔之實際效果(李等 1991)。然試驗分析甘藷加工調理後之甜度與質地的改變無法由其生藷狀態下所含的澱粉及可溶性糖的含量判斷，且在烤藷狀態下，以傳統的化學分析法雖可精確的定量成分，但曠日廢時延遲育種時效；本試驗顯示檢測生藷中澱粉糖化酵素活性，可應用於加工過程中澱粉的水解、烤藷甜度風味及質地的判斷。藉由對選育品系之生藷汁液簡易色差測定澱粉糖化酵素活性進行篩選，必要時再進一步佐以組織質地分析儀檢測物理特性，將可快速有效的提高甘藷育種效率及作為加工品質評定之依據。

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Application of the β-amylase Activity Rapid Test Method on Processing Character of Sweet Potato[1]

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Summary

Lee, H. C. and Y. S. Chen. 2004. Application of the β-amylase activity rapid test method on processing character of sweet potato. J. Agric. Res. China 53:18-26.

The objectives of this research are to study β-amylase activity in raw root status, and changes of starch and soluble sugar content and texture after processing in sweet potato (*Ipomoea batatas* (L.) Lam.). Tainung 66 and Tainung 57 showed (+) β-amylase activity immediately after harvesting. Tainung 68 did not show (+) activity until stored two weeks. Satsumahikari did not show (+) activity through the experiment period. After baking, the starch content decreased in Tainung 66, Tainung 57 and Tainung 68, and the ratio of decreasing were larger in Tainung 66 and Tainung 57. The soluble sugar content was higher in Tainung 66 and Tainung 57 than in Tainung 68. The texture of steamed sweet potato could be measure by the texture analyzer by judging the probe pressure curve. The probe pressure curve jutted trend from high to low were Satsumahikari, Tainung 68,Tainung 57, and Tainung 66, in that order. The probe pressure curve jutted more; the texture was more dry mouth feel. The results indicated that after baking the soluble sugar content influenced by β-amylase activity in raw root status. The β-amylase activity influenced the texture character of the steamed sweet potato, also. It's necessary to use the β-amylase activity rapid test method to detect β-amylases activity in raw status to select the appropriate variety to improve the product quality of processing in sweet potato.

Key words: Sweet potato, Starch, Soluble sugar, β-amylase, Texture.

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臺灣原生藥用植物－高氏柴胡腋芽培養之大量繁殖研究[1]

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摘　要

陳威臣、葉茂生、蔡新聲。2004。臺灣原生藥用植物－高氏柴胡腋芽培養之大量繁殖研究。中華農業研究 53:27-38。

本研究目的在探討臺灣原生藥用植物-高氏柴胡(*Bupleurum kaoi* Liu, Chao et Chuang)經由組織培養大量繁殖種苗的可行性。試驗結果顯示，將腋芽培養於含有 1 mg/l Benzyladenine (BA)配合 0.1 mg/l α-Naphthaleneacetic acid (NAA)之 1/2MS 基本鹽類培養基，可建立高氏柴胡之無菌培養。將此無菌苗培養於添加 0.25 mg/l BA 之 1/2MS 基本鹽類培養基可達到大量繁殖的目的，然而部份芽體卻形成玻璃質化現象；組織培養苗培養於含有 0-0.8 mg/l kinetin 之 1/2MS 培養基則可避免玻璃質化苗產生，但繁殖倍率較 BA (0.25-2 mg/l)處理低。基本鹽類濃度以 1/2MS 培養基對於高氏柴胡芽體增值較佳；蔗糖則以 3%最適合。液態培養下正常苗繁殖率低於固態培養，且培養 6 週後產生嚴重的玻璃質化問題；以液態培養基預培養 2-14 天後再移至固態培養基，繁殖倍率仍無法高於固態培養，因此液態培養方式並不利於高氏柴胡組培苗之增殖。

關鍵詞：高氏柴胡、藥用植物、大量繁殖、玻璃質化。

前　言

柴胡(Chai-Hu)列為神農本草經、草部上品，具有去腸胃中結氣、寒熱邪氣，更有推陳致新、久服輕身、明目益精等功能，廣泛應用於中國大陸、日本、韓國及台灣等地區；中國藥典正品柴胡為繖形科(Umbelliferae)、柴胡屬(*Bupleurum* L.)植物之北柴胡(*Bupleurum chinense* DC.)的乾燥根(甘 1985；Hiraoka 1989；Tang & Eisenbrand 1992)。本省特有之原生種藥用植物-高氏柴胡(*Bupleurum kaoi* Liu, Chao et Chuang)之保肝效果優於日本三島柴胡(*Bupleurum falcatum* L. var. *komarowi* Koso-Polj)及中國

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大陸北柴胡，發展並推廣高氏柴胡有其必要性(甘 1985；林＆顏 1999；Yen *et al.* 1991)。柴胡栽培歷史不長，屬常異交植物，並無經過長時間的人為選拔，栽培品種間遺傳變異相當大，且柴胡種子具有低溫需求性，其種子發芽率低且發芽勢不佳，造成栽培上的困擾(黃＆郭 1995)。組織培養技術係利用植物體細胞具有分化全能性(totipotency)，在人為的環境中培養植物體之器官、組織或細胞，得到大量個體以進行栽培、育種或生理學等領域之研究(蔡 1999)；因此，利用組織培養技術生產柴胡種苗遂成為最佳的途徑。藥用植物已有不少被大量繁殖成功的例子，如：人參、川芎、半夏、白朮、附子、貝母、百合、黃耆、地黃、柴胡、紫草、大黃、山藥、毛地黃、防風、當歸、金線連等重要的藥用植物(蔡 1999；Nishioka 1988；Sagare *et al.* 2000)。台灣常用之三島柴胡可利用組織培養技術大量生產組培苗(許等 1993；Kohda *et al.* 1990)；然而台灣本土特有種高氏柴胡之大量繁殖研究則未見報導。因此利用組織培養大量繁殖技術，生產品質均一的高氏柴胡種苗，乃成為最佳的育苗與推廣之途徑。本研究嘗試建立高氏柴胡組織培養大量繁殖種苗之方法，期能提供優良種苗供農民種植，如此應有助於省產藥用保健植物之產業發展。

材料與方法

供試高氏柴胡與培植體來源

本研究材料係由農委會農業試驗所特作研究室提供之高氏柴胡(*Bupleurum kaoi* Liu, Chao et Chuang)植株，依據黃＆郭(1995)所指之育種選拔性狀指標於田間選取優良植株。切取田間栽培之高氏柴胡基部新生分枝上(圖 1a)的腋芽作為培植體，每新生分枝取 10 個腋芽，並區分為上部位與下部位各 5 個腋芽，進行腋芽誘導生長與增殖試驗，所得無菌組培苗可作為後續大量繁殖試驗之培植體來源。

培養基配製、培養環境與處理方法

本研究主要以 MS (Murashige & Skoog 1962)無機鹽類及有機成分為基本配方且內含 3%蔗糖及 0.9%洋菜(Difco Bacto-agar)，並添加各類植物生長調節劑，培養基加入洋菜前先用 1N NaOH 或配合 HCl 將 pH 值調至 5.7±0.1，然後以 121℃、1.05 kg/cm^2 (15 lb/in^2)高壓滅菌 15 分鐘後，放冷備用。液態培養基則不加洋菜，且 pH 值調至 5.2±0.1。固態培養是以試管或 125ml 三角瓶為培養容器，分別注入 10 與 25ml 培養基進行試驗，而液態培養則利用含有 10ml 液態培養基之 125ml 三角瓶進行振盪培養。培植體於接種後，置於 25±1℃之恆溫與照光 16 小時(約 2,800 lux)條件下進行固態培養，液態培養則在相同溫度與光照條件，置於水平迴轉式振盪器以 100 rpm 速度進行振盪培養。

高氏柴胡腋芽誘導無菌培養之建立：切取田間獲選高氏柴胡植株分枝(圖 1a)，將除去葉片之不同部位的莖段以 0.5% NaOCl (每 100ml 溶液含有 2-3 滴 Tween 20)進行 15 分鐘殺菌處理，經無菌水清洗 3-4 次後，切取莖段上之腋芽培養於含有 1 mg/l BA 及 0.1 mg/l NAA 之 1/2MS 基本鹽類培養基，測試不同部位腋芽對誘導組培苗之差異。經培養 2 週後腋芽即逐漸發育形成小苗(圖 1b)，將生長達 6 週(圖 1c)之 1.5cm 無菌苗繼代培養於相同配方之新鮮培養基，可建立高氏柴胡瓶苗無菌培養(圖 1d)，待繁殖足量瓶苗後進行後續試驗。

BA 與 kinetin 對高氏柴胡組培苗增殖之影響：利用除去部分葉片且高約 1.5cm 的高氏柴胡無菌苗作為培植體，培養於添加不同濃度 BA (0.25、0.5、1.0 及 2.0 mg/l)與 kinetin (0.1、0.2、0.4 及 0.8 mg/l)之 1/2MS 基本鹽類固態培養基，經培養 6 及 8 週後，調查組培苗總數及玻璃質化苗數，探討 BA 與 kinetin 對高氏柴胡組培苗增殖之影響。

MS 鹽類濃度對高氏柴胡組培苗增殖之影響：利用除去部分葉片且高約 1.5cm 的高氏柴胡無菌苗作為培植體，培養於含有 0.25 mg/l BA 之不同 MS 基本鹽類(1/4MS、1/2MS、MS 與 2MS)固態培養基，經培養 6 週後，調查正常苗及玻璃質化苗數，探討 MS 鹽類對高氏柴胡組培苗增殖之影響。

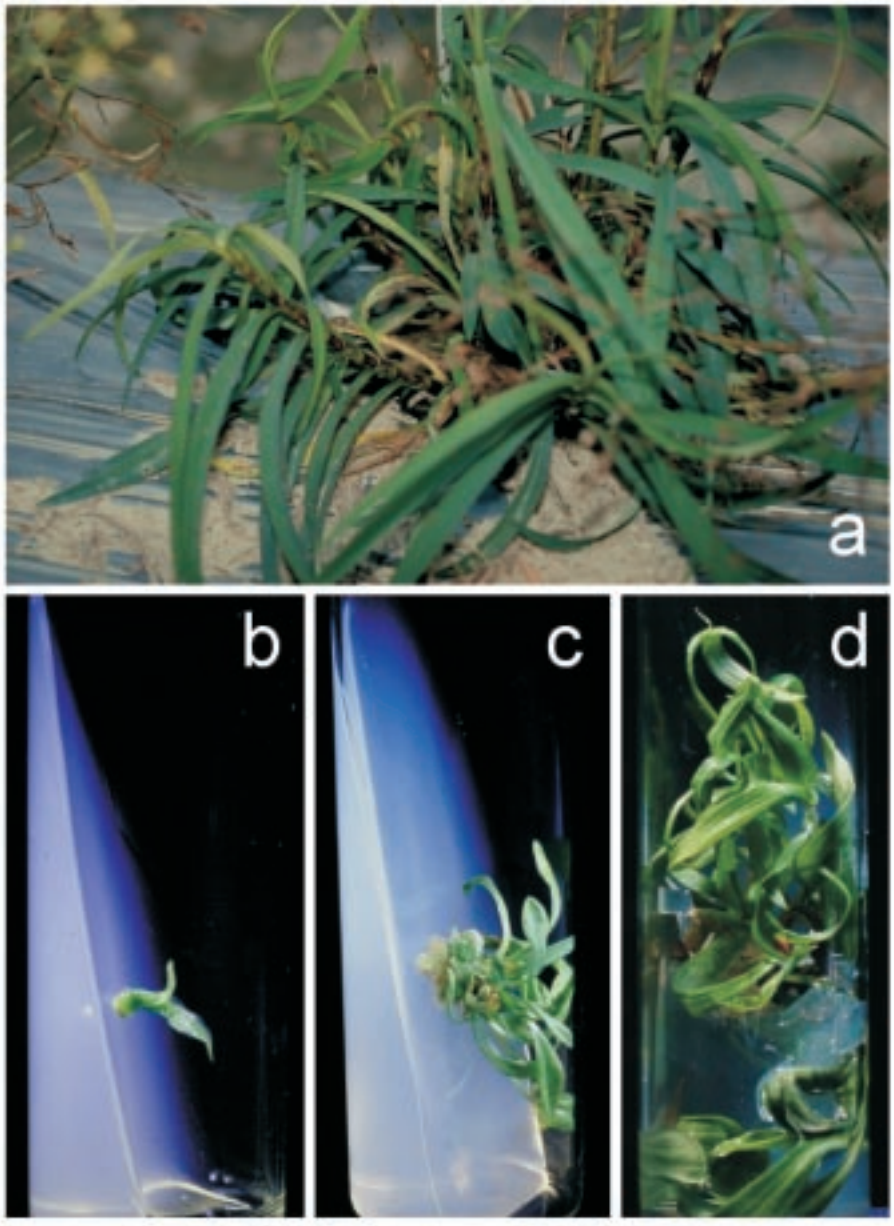


圖 1. 高氏柴胡培植體來源與無菌組培苗培養之建立
農業試驗所農場栽種達一年之高氏柴胡獲選植株基部的新生分枝(a)；腋芽培養於含有 1 mg/l BA 及 0.1 mg/l NAA 之 1/2MS 基本鹽類培養基，經培養 2 週(b)與 6 週(c)後之組培苗生長情形；無菌苗(1.5cm)繼代培養於相同配方之新鮮培養基達 6 週(d)之組培苗增殖情形。

Fig. 1. Plant material and establishment of shoot cultures of *Bupleurum kaoi*. Growth of new shoots in one-year-old *Bupleurum kaoi* plant maintained at Agricultural Research Institute's farm in Taichung (a); Sprouted shoot in the axillary bud after two weeks (b) and proliferated shoots after six weeks (c) of culture on half-strength MS basal medium supplemented with 1 mg/l BA and 0.1 mg/l NAA; Established shoot culture using axenic explant (*in vitro* grown shoot, 1.5cm in length) on the same medium after 6 weeks of culture (d).

蔗糖濃度對高氏柴胡組培苗增殖之影響：利用除去部分葉片且高約 1.5cm 的高氏柴胡無菌苗作為培植體，培養於含有 0.25 mg/l BA 與不同蔗糖濃度(1.5、3、6 與 12%)之 1/2MS 基本鹽類固態培養基，經培養 6 週後，調查正常苗及玻璃質化苗數，探討蔗糖對高氏柴胡組培苗增殖之影響。

固態與液態培養對高氏柴胡組培苗增殖之影響：利用除去部分葉片且高約 1.5cm 的高氏柴胡無菌苗作為培植體，培養於內含 25ml 固態或 10ml 液態培養基之 125ml 三角瓶，培養基配方為添加 0.25 mg/l BA 之 1/2MS 基本鹽類培養基，經培養 2、4 與 6 週後，調查正常苗及玻璃質化苗數，探討固態與液態培養對高氏柴胡組培苗增殖之影響。

液態預處理時間對高氏柴胡組培苗增殖之影響：利用除去部分葉片且高約 1.5cm 的高氏柴胡無菌苗作為培植體，培養於內含 10ml 液態培養基之 125ml 三角瓶，培養基配方為添加 0.25 mg/l BA 之 1/2MS 基本鹽類液態培養基，經 100 rpm 振盪速度液態預培養 2、6、10 與 14 天後，移植於內含 10ml 固態培養基之 125ml 三角瓶，培養基配方與液態培養基相同，經培養達 6 週後(包括預處理時間)，調查正常苗及玻璃質化苗數，探討液態預處理時間對高氏柴胡組培苗增殖之影響。

資料分析

本研究除腋芽誘導無菌培養試驗中，每處理培養 25 個培植體(每處理含 5 個重複，每重複含 5 個培植體)外，其餘試驗每處理均培養 20 個培植體(每處理含 4 個重複，每重複含 5 個培植體)；試驗所得資料經 SAS 8.02 (SAS Institute Inc. 2001)套裝統計分析軟體進行變方分析，若處理間差異顯著，則利用 Student's *t* test 或 Least significant difference test 比較各處理平均值間之差異；或以 Sigmplot 8.0 軟體繪圖表現其差異。

結　果

高氏柴胡腋芽誘導無菌培養之建立

高氏柴胡上部位與下部位腋芽經培養於誘導培養基後之結果如表 1 所示，上部位與下部位之污染率分別為 64%與 56%；無菌苗形成率分別為 80%與 66.7%；上部位之每個有效培植體平均可形成 3.3 苗，而下部位平均僅 2.6 苗；然而上述試驗資料經 Student's *t* test 分析結果均顯示，上部位與下部位腋芽處理對於污染率、芽體誘導率與誘導芽體數之差異均未達顯著水準。

表 1. 腋芽位置對高氏柴胡組培苗誘導之影響
Table 1. Influence of axillary bud explant position on shoot induction of *Bupleurum kaoi* [z]

Explant position [y]	No. of explants cultured	Percentage of explants contaminated	Percentage of explants responded	Average no. of shoots per responding explant
Upper	25	64	80.0	3.3
Lower	25	56	66.7	2.6
t value		0.49	0.43	0.58
Pr > |t|		0.64	0.68	0.59

[z] Explants were cultured on half-strength MS basal medium supplemented with 1 mg/l BA, 0.1 mg/l NAA, and 3% sucrose for 4 weeks. Each treatment contained 5 replications with 5 explants in each replication.

[y] Explants were collected from new shoots from the field grown plants. Each new shoot had approximately 10 axillary buds. The axillary bud explants were categorized as upper (near to apical meristem) and lower position.

BA 與 kinetin 對高氏柴胡組培苗增殖之影響

試驗結果如表 2 所示，0.25 mg/l BA 處理於培養 6 與 8 週後，每個培植體分別可產生 6 及 7.3 苗，其次為 0.5 mg/l BA，分別為 5 與 6.2 苗，再其次為 1 mg/l BA，分別為 4.1 與 6 苗，而 2 mg/l BA 效

果最差，分別僅 3 與 3.9 苗。kinetin 處理對於組培苗增殖效果較低，培養 6 與 8 週後，每個培植體約形成 2.5-4.1 苗，不含生長調節劑之對照組組培苗增殖效果最低，僅 1.3 與 1.9 苗。此外，不含生長調節劑之對照組與 kinetin 處理均無玻璃質化苗產生，而 0.25 mg/l BA 處理於培養 6 與 8 週後分別形成 0.6 及 1.2 個玻璃質化苗(各約 10 與 16.4%)，0.5 與 1 mg/l BA 處理在培養 8 週後，其玻璃質化苗比率已高達 46.8%，雖然 2 mg/l BA 處理在培養 8 週後之玻璃質化苗比率較低(41%)，但每個培植體僅增殖成 3.9 苗。

表 2. BA 與 kinetin 對高氏柴胡組培苗增殖之影響
Table 2. Influence of BA and kinetin on shoots multiplication of *Bupleurum kaoi* [z]

Plant growth regulators		No. of shoots produced per explant [y]		No. of hyperhydric shoots developed per explant		Percentage of hyperhydric shoots	
Kinetin(mg/l)	BA(mg/l)	6 weeks	8 weeks	6 weeks	8 weeks	6 weeks	8 weeks
0	0	1.3 g [x]	1.9 e	0 c	0 c	0	0
0.1	0	2.5 f	2.6 de	0 c	0 c	0	0
0.2	0	2.6 ef	3.0 d	0 c	0 c	0	0
0.4	0	2.9 de	4.1 c	0 c	0 c	0	0
0.8	0	2.7 def	2.7 d	0 c	0 c	0	0
0	0.25	6.0 a	7.3 a	0.6 b	1.2 c	10.0	16.4
0	0.5	5.0 b	6.2 b	1.1 a	2.9 a	22.0	46.8
0	1.0	4.1 c	6.0 b	1.1 a	2.8 a	26.8	46.7
0	2.0	3.0 d	3.9 c	1.0 a	1.6 b	33.3	41.0

[z] *In vitro* grown shoots about 1.5 cm in length were cultured on half-strength MS basal medium supplemented with various concentration of BA and kinetin.
[y] Shoots smaller than 2 cm in length were not scored.
[x] Twenty explants were tested per treatment and the data was collected after 6 and 8 weeks of culture. Means within a column followed by the same letter/s are not significantly different from each other at the 5% level by LSD test.

MS 鹽類濃度對高氏柴胡組培苗增殖之影響

試驗結果如表 3 所示，1/2MS 處理增殖效果最佳，每個培植體可產生 3.8 個正常苗，其次為 MS 處理之 3.2 個正常苗，而 1/4MS 與 2MS 處理之結果較低，每個培植體分別產生 2 與 1.3 個正常苗。同時，1/4MS 處理並無玻璃質化苗形成，其餘處理均產生玻璃質化苗，分別為 1/2MS、MS 與 2 MS 處理之 3.8、8.6 及 3.7%之玻璃質化苗，雖然 2 MS 處理之玻璃質化苗比率較低，但是每個培植體僅形成 1.3 個正常苗。

表 3. MS 鹽類濃度對高氏柴胡組培苗增殖之影響
Table 3. Influence of MS basal salts on shoots multiplication of *Bupleurum kaoi* [z]

Strength of MS basal medium	Avg. no. of normal shoots produced per explant [y]	Avg. no. of hyperhydric shoots produced per explant [y]	Percentage of hyperhydric shoots
1/4×	2.0 c [x]	0 b	0
1/2×	3.8 a	0.15 ab	3.8
1×	3.2 b	0.30 a	8.6
2×	1.3 d	0.05 b	3.7

[z] *In vitro* grown shoots about 1.5 cm in length were cultured on half-strength MS basal medium supplemented with 0.25 mg/l BA and 3% sucrose.
[y] Shoots smaller than 2 cm in length were not scored.
[x] Twenty explants were tested per treatment and the data was collected after 6 weeks of culture. Means within a column followed by the same letter/s are not significantly different from each other at the 5% level by LSD test.

蔗糖對高氏柴胡組培苗增殖之影響

試驗結果如表 4 顯示，3%蔗糖處理下的每個培植體可產生 3.1 個正常苗，組培苗增殖效果最佳，其次為 1.5%與 6%蔗糖處理，分別為 2.5 與 2.4 個正常苗，而 12%蔗糖處理之結果最低，每個培植體僅形成 1.8 個正常苗。此外，1.5%蔗糖處理並無玻璃質化苗形成，而其餘處理均產生玻璃質化苗，其比率在 3、6 與 12%蔗糖處理下分別為 13.9、5.9 及 2.7%。

表 4. 蔗糖對高氏柴胡組培苗增殖之影響
Table 4. Influence of sucrose on shoots multiplication of *Bupleurum kaoi* [z]

Sucrose conc. in the medium (%)	Avg. no. of normal shoots produced per explant [y]	Avg. no of hyperhydric shoots produced per explant [y]	Percentage of hyperhydric shoots
1.5	2.5 b [x]	0 b	0
3.0	3.1 a	0.50 a	13.9
6.0	2.4 b	0.15 ab	5.9
12.0	1.8 c	0.05 b	2.7

[z] *In vitro* grown shoots about 1.5cm in length were cultured on half-strength MS basal medium supplemented with 0.25 mg/l BA and various sucrose concentration for 6 weeks.
[y] Shoots smaller than 2cm in length were not scored.
[x] Twenty explants were tested per treatment and the data was collected after 6 weeks of culture. Means within a column followed by the same letter/s are not significantly different from each other at the 5% level by LSD test.

固態與液態培養對高氏柴胡組培苗增殖之影響

固態與液態培養 2 週後的結果如圖 2 與圖 3 顯示，每個培植體約可產生 1.6 個正常苗，兩個處理間並無顯著差異，而後於培養 4 週時則固態顯然較液態處理佳，每個培植體分別產生 2.7 及 1.9 個正常苗，而培養達 6 週時的差異更為明顯，每個培植體分別形成 4.2 與 1.3 個正常苗。若以玻璃質化苗而言，固態培養在培養 2 週後並無玻璃質化苗產生，而後於培養 4 與 6 週時亦僅產生 0.6 與 0.9 個玻璃質化苗；反觀液態培養下，培養 2、4 與 6 週後均可發現玻璃質化苗，分別為 0.1、1.7 與 2.7 個玻璃質化苗，約各佔 6、47 及 68%。

液態預處理時間對高氏柴胡組培苗增殖之影響

試驗結果如表 5 所示，液態預培養 2 天處理之組培苗增殖效果最佳，每個培植體可產生 3.6 個正常苗，其次為液態預培養 6 天處理之 3.2 個正常苗，而液態預培養 10 天與 14 天處理之結果較低，每個培植體所形成之苗數均未達 3 個正常苗。若以玻璃質化苗而言，液態預培養 2 天與 6 天的處理僅產生 0.3 及 0.4 個玻璃質化苗，液態預培養 10 天處理則形成較多玻璃質化苗，比率已達 29.9%，而液態預培養 14 天處理之玻璃質化苗比率更高達 60%。

表 5. 液態培養基預處理方式對高氏柴胡組培苗增殖之影響
Table 5. Influence of liquid medium pretreatment on shoots multiplication of *Bupleurum kaoi* [z]

Liquid pre-treatment (days)	Avg. no. of normal shoots produced per explant [y]	Avg. no of hyperhydric shoots produced per explant [y]	Percentage of hyperhydric shoots
2	3.6 a [x]	0.30 c	7.7
6	3.2 b	0.40 c	11.1
10	2.7 c	1.15 b	29.9
14	1.6 d	2.40 a	60.0

[z] *In vitro* grown shoots about 1.5cm in length cultured on liquid half-strength MS basal medium supplemented with 0.25 mg/l BA for 2, 6, 10 and 14 days and then transfer to solid medium of similar composition in 125ml Erlenmeyer flask.
[y] Shoots smaller than 2cm in length were not scored.
[x] Twenty explants were tested per treatment and the data was collected after 6 weeks of culture. Means within a column followed by the same letter/s are not significantly different from each other at the 5% level by LSD test.

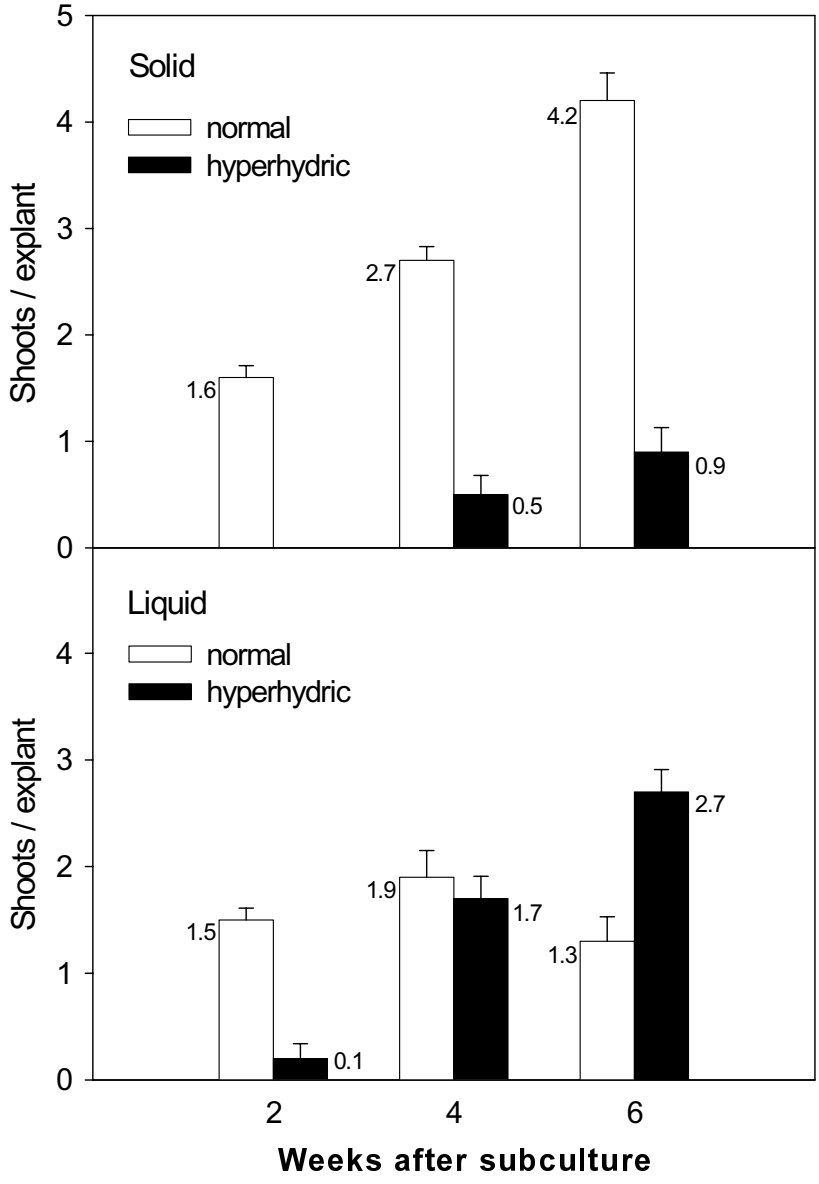


圖 2. 固態(Solid)與液態(Liquid)培養對高氏柴胡組培苗增殖之影響
高氏柴胡組培苗培養於添加 0.25 mg/l BA 之 1/2MS 基本鹽類固態(Solid)或液態(Liquid)培養基，經培養 2、4 與 6 週後所形成之正常苗(normal；□)與玻璃質化苗(hyperhydric；■)數。

Fig. 2. Effect of solid or liquid medium on *in vitro* shoot multiplication of *Bupleurum kaoi*. Normal (□) and hyperhydric (■) shoots of *Bupleurum kaoi* produced, when explants cultured on half-strength MS basal medium supplemented with 0.25 mg/l BA, and with (Solid) or without (Liquid) 0.9% Difco Bacto-agar for 2, 4 and 6 weeks.

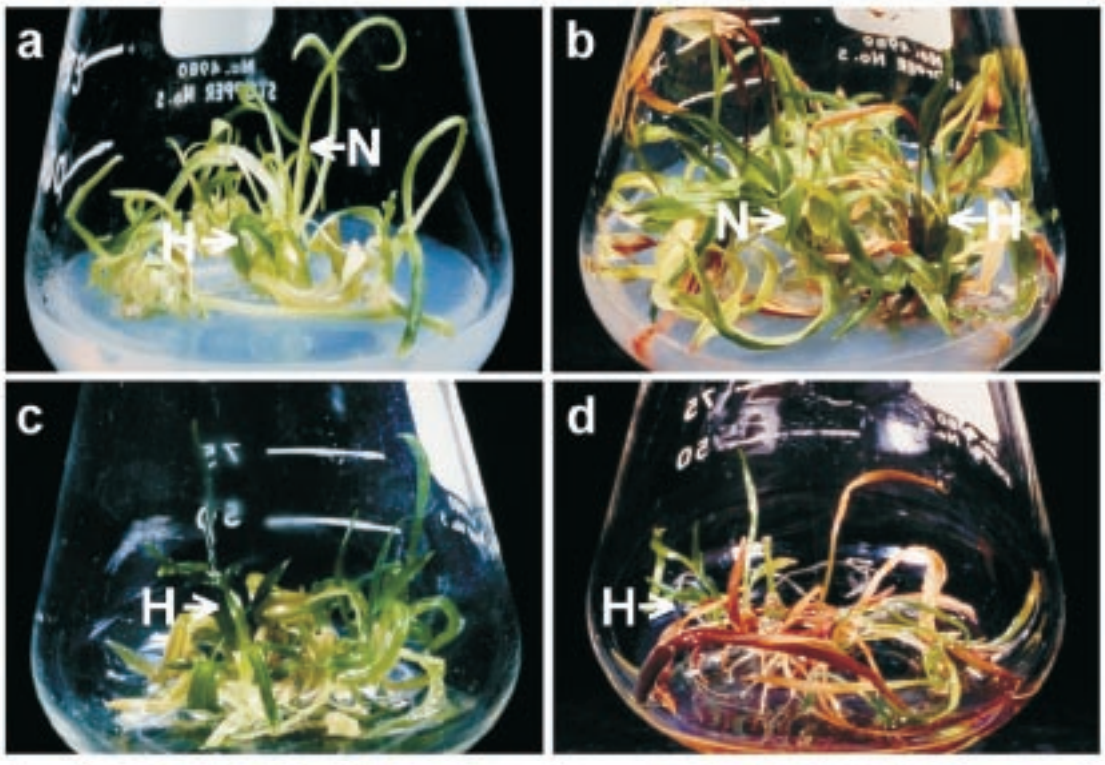


圖 3. 高氏柴胡組培苗於固態與液態培養基增殖之情形
高氏柴胡組培苗培養於添加 0.25 mg/l BA 之 1/2MS 基本鹽類之固態(a, b)或液態(c, d)培養基，經培養 4 週(a, c)與 6 週(b, d)後之正常苗(N)及玻璃質化苗(H)形成之情形。

Fig. 3. Effect of solid or liquid medium on *in vitro* shoot multiplication of *Bupleurum kaoi*. Normal (N) and hyperhydric (H) shoots produced on half-strength MS basal medium supplemented with 0.25 mg/l BA and 0.9% Difco agar and cultured for 4 weeks (a) and 6 weeks (b). Hyperhydric (H) shoots produced in liquid half-strength MS basal medium supplemented with 0.25 mg/l BA and cultured for 4 weeks (c) and 6 weeks (d).

討　論

自 1902 年 Haberlandt 首次提出植物單細胞具有分化全能性(totipotency)理論後，組織培養技術廣泛應用於農藝、園藝及森林等領域；此外，利用組織培養技術亦可於短期內大量繁殖人參、半夏、黃耆、柴胡、紫草、山藥、毛地黃、當歸、紅豆杉、銀杏、白芷、金線連等重要的藥用植物的報告不勝枚舉(蔡 1999；Nishioka 1988；Sagare *et al*. 2000)。許多研究結果顯示，同一品種不同部位之反應不盡相同，此因不同部位培植體具有不同之分化能力所致，故選擇適當培植體乃組織培養成功之重要關鍵(Chueh *et al*. 2000；Huang *et al*. 2000；Kohda *et al*. 1990；Komalavalli & Rao 2000；Miller *et al*. 1991；Shoyama *et al*. 1986，1987；Tang & Eisenbrand 1992)。Hiraoka (1989)於三島柴胡(*B. falcatum*)大量繁殖研究指出，利用近頂芽之嫩葉作為培植體，較近主軸莖部葉片之效果為佳。Alen & Jain (1997)則認為側芽為長春花(*Catharanthus roseus*)組織培養大量繁殖之最佳培植體。由臺灣龍膽(*Gentiana davidii* var. *formosana*)微體大量繁殖的研究顯示，以葉片、莖段、莖節與根部作為培植體，其中以莖節之芽體誘導能力最強，全部培植體均可形成芽體，且平均每個培植體可產生 6.3 個組培苗，反觀其他培植體，在相同培養條件下則完全無法誘導芽體形成(Chueh *et al*. 2000)。本研究利用臺灣特有之高

氏柴胡腋芽作為培植體，培養於添加 1 mg/l BA 配合 0.1 mg/l NAA 之 1/2MS 基本培養基，可誘導腋芽生長而建立無菌苗培養方式，而後利用含有 0.25mg/l BA 之 1/2MS 培養基可進行組培苗大量增殖(圖 1)；此外，表 1 之結果則顯示，上半部與下半部腋芽之組培苗誘導率並無顯著差異。

Cytokinin 類植物生長調節劑具有促進細胞分裂、分化與生長之生理作用，亦有促進癒合組織生長及幼芽誘導與生長之功效，對於打破頂芽優勢而促進側芽生長則具有絕佳的效果，但過量會抑制側根與不定根之形成；常用之 cytokinins 有 BA、kinetin、zeatin 和 2ip 等四種，其中以 BA 的生理作用較強(Gaspar *et al.* 1996)。Cytokinins 濃度愈高則株高愈矮，且不定芽的誘導能力愈強，但其玻璃質苗比率亦較高，常常造成組培苗的大量損失(Paques 1991)。Tsay (1998)於康乃馨(*D. caryophyllus*)莖頂培養時，發現 BA 易使芽體產生玻璃質化，且濃度愈高情形愈嚴重。三島柴胡(*B. falcatum*)頂芽培養於含有 1~2 mg/l BA 與 0.1 mg/l NAA 之 1/4MS 基本鹽類培養基，可建立三導柴胡臺農 1 號之無菌培養(許等 1993)。本研究結果則顯示，利用含有 1 mg/l BA 與 0.1 mg/l NAA 之 1/2MS 基本鹽類培養基，可誘導腋芽生長而建立高氏柴胡無菌培養，添加 0.25 mg/l BA 之 1/2MS 培養基雖可大量繁殖高氏柴胡組培苗，然部份組培苗卻形成玻璃質化現象；組織培養苗培養於含有 0-0.8 mg/l kinetin 之 1/2MS 基本鹽類培養基雖完全無玻璃質化苗形成，但其繁殖倍率則較 BA (0.25-2 mg/l)處理低(表 2)。因此，如何控制玻璃質化現象且又不影響增殖效率，則是另一個值得研究的重點；此外，有關高氏柴胡組培苗玻璃質化現象的檢討，將於後續報導中進行討論。

海芙蓉(*Limonium wrightii*)芽體大量繁殖結果顯示，其芽體較適宜培養於 MS 或 2MS 之高鹽類濃度培養基，約 80 與 87.5%培植體均可形成芽體，且平均每個培植體可產生 7.4 及 8.2 個組培苗(Huang *et al.* 2000)。此外，適合於金線連(*Anoectochilus formosanus*)芽體增殖的是 1/4MS (何等 1987)、當歸(*Angelica sinesis*)體胚增殖適用 MS 與 1/2MS (陳等 1998)，三島柴胡芽體增殖適用 1/4MS (許等 1993)，這些研究顯示因作物或培養目的之不同，而有其適當的基本鹽類濃度。醣類是組織培養上另一個重要的因子，是為培養基中含量最多之有機物質，主要功能為提供植物生長所需碳源、供給能量及調節滲透壓，依其培養目的差異而分別扮演不同的角色。張等(2000)研究結果顯示，0-5%的蔗糖濃度均可誘導甘薯(sweet potato)組培苗的形成，但以 2-3%蔗糖濃度具有最佳的效果，而且在參試的四個品種間的誘導效率並不相同。本試驗結果則顯示，以 1/2MS 鹽類濃度與 3%蔗糖處理較有利於高氏柴胡組培苗之增殖(表 3、表 4)。

許多研究結果顯示，液態培養對於組培苗增殖是一個相當有效率的培養方式。Shoyama *et al.* (1986)利用浙貝母(*Flitillaria verticillata*)鱗莖培養於 1 mg/l BA 與 0.5 mg/l NAA 之 MS 液體培養基，9 週後可得到 26 個小鱗莖，而固體培養 13 週只獲得 8.2 個鱗莖。三島柴胡頂芽培養研究結果顯示，液態培養之組培苗繁殖率高於固態培養，添加 1 mg/l BA 之液態培養基在震盪條件下培養頂芽一個月，可有 13 倍的繁殖效果(許等 1993)。此外，利用液態培養方式進行金線連芽體或莖段培養，不但可縮短芽體成苗之時間，且老化之培植體於液態震盪培養下有逐漸恢復生機之趨勢，其誘導側芽生長的效果最佳(何等 1987)。湖北貝母(*Fritillaria hupehensis*)研究結果亦指出，液態培養效果優於固態培養，芽球相似體與小鱗片經 30 天培養後，分別增重可達 19.6 及 23.7 倍(楊等 2001)。Shoyama *et al.* (1983)於半夏(*Pinellia ternata*)液體培養研究結果則顯示，培養一個珠芽之組織團塊，理論上一年後可繁殖出 4×10^{23} 以上之植株，但此液體培養方式的強盛繁殖力往往僅能維持數代。Chen *et al.* (2001)於輪葉沙參(*Adenophora triphylla*)研究亦顯示，液態培養對於芽體增殖的效率遠高於固態培養，但卻發現許多玻璃質化苗形成。因此，液態培養增殖效率的確較固態培養高，然而卻使得玻璃質化苗比率也相對提高，此結果對於組培苗大量繁殖造成相當大的困擾(Paques 1991)。本研究結果則顯示，固態培養達 4 與 6 週時僅產生少數玻璃質化苗，分別約為 18.5%與 21.4%；反觀液態培養達 2、4 與 6 週時均可發現玻璃質化苗，甚至於培養達 6 週時形成高達 2.7 個玻璃質化芽體，約佔組培苗總數的 67.5% (圖 2、圖 3)。

由上述結果得知，液態培養方式非但無法增進高氏柴胡組培苗增殖效率，而且造成相當嚴重的玻璃質化問題，顯然液態培養方式並不適合於高氏柴胡組培苗之增殖。

Tsay *et al*. (1989)與 Nishioka (1988)有效利用固態與液態交替培養的方式，使得半夏(*P. ternata*)培植體維持旺盛的繁殖力，獲得不錯的微體大量繁殖效果，一個珠芽經一年之培養繁殖，估計可獲得 2×10^{27} 株幼苗。若將百合屬植物 *Lilium japonicum* 先於固體培養基誘導鱗片形成，再移至液體培養基中，亦可得到令人驚訝的生長速率(Shoyama *et al*. 1987)。因此，利用固態與液態交互培養是否有利於高氏柴胡組培苗的大量繁殖，同時也可減輕玻璃質化苗的問題呢？然而本試驗結果卻顯示，即使利用液態預培養方式，對於高氏柴胡組培苗增殖或玻璃質化的控制仍無法獲得令人滿意的結果(表 5)，顯然液態培養基預培養方式也不適合於高氏柴胡組培苗之增殖。由上述高氏柴胡組培苗增殖試驗結果發現，其正常苗繁殖倍率似乎隨著繼代培養的次數而有下降的趨勢(資料未列)。康乃馨微體繁殖研究指出，繼代培養次數過多是造成玻璃質化苗的原因之一(Tsay 1998)。因此，高氏柴胡正常苗繁殖倍率的逐漸下降，是受到玻璃質化現象日趨嚴重的影響，抑或是因繼代培養次數過多而造成組培苗生長弱勢所導致，值得進一步深入探討。

誌　　謝

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Studies on *in vitro* Shoot Multiplication of Native Medicinal Herbs - *Bupleurum kaoi* by Axillary Bud Culture [1]

Uei - Chin Chen [2], Mau-Shing Yeh [3] and Hsin-Sheng Tsay [4, 5]

Summary

Chen, U. C., M. S. Yeh, and H. S. Tsay. 2004. Studies on *in vitro* shoot multiplication of native medicinal herbs - *Bupleurum kaoi* by axillary bud culture. J. Agri. Res. China 53: 27-38.

An efficient method for *in vitro* shoot multiplication of *Bupleurum kaoi* Liu, Chao et Chuang using the axillary bud explants has been developed. The axillary buds dissected from nodal segments of the field grown plants induced shoots after culturing for 4 weeks on half-strength MS medium supplemented with 1 mg/l benzyladenine (BA) in combination with 0.1 mg/l α-naphthaleneacetic acid (NAA). Among the various concentrations of BA (0 - 2 mg/l) or kinetin (0 - 0.8 mg/l) tested, maximum shoot multiplication (7.3 shoots per explant) was achieved in the *in vitro* grown shoots by culturing on half-strength MS medium supplemented with 0.25 mg/l BA for 8 weeks. Although BA was more effective than kinetin in shoot multiplication, BA stimulated hyperhydric shoots (10 - 33%) after 6 weeks of culture. Prolonged culture period from 6 weeks to 8 weeks resulted in higher number of hyperhydric shoots (16 - 47%). Half-strength of MS basal medium and 3% sucrose as carbohydrate source was found optimum for shoot multiplication. Pretreatment of the explants by culturing them on liquid half-strength MS basal medium containing 0.25 mg/l BA for 2-14 days did not support shoot multiplication.

Key words: *Bupleurum kaoi*, Medicinal plant, Shoot multiplication, Hyperhydricity.

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A Synopsis of the *Syntretus* Foerster Species of Taiwan (Hymenoptera: Braconidae: Euphorinae)[1]

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ABSTRACT

Papp J. 2004. A synopsis of the *Syntretus* Foerster species of Taiwan (Hymenoptera: Braconidae: Euphorinae). J. Agric. Res. China 53:39-62.

The present synopsis is the first review of the *Syntretus* Foerster species distributed in Taiwan. Twelve species are revised of which seven proved to be new to science: *S. choui* sp. n., *S. extensus* sp. n., *S. secutensus* sp. n., *S. subglaber* sp. n., *S. temporalis* sp. n., *S. transitus* sp. n. and *S. varus* sp. n. The rear five species are new to the fauna of Taiwan. Detailed descriptions are presented for the new species; taxonomic remarks are added to the known species. A key is provided for the twelve *Syntretus* species recorded in Taiwan and completed with 84 original figures of diagnostic significance. A checklist was compiled for the *Syntretus* species of the East Palaearctic and Oriental Regions.

Key words: Braconidae, *Syntretus*, New species, New record, Key to species, Taiwan, Checklist.

Introduction

The genus *Syntretus* Foerster, 1862 is a middle-sized taxon comprising 31 species in the Holarctic Region (28 species are listed in the Palaearctic Region, three species in the Nearctic Region) and three species in the Ethiopian Region (Shenefelt 1969; Tobias 1986; Belokobylskij 1993b, 1996; Chen & van Achterberg 1997). In the Oriental Region the first *Syntretus* species has been described by Belokobylskij (1993a). This paper is the second one reporting *Syntretus* species from the Oriental Region. By this contribution the number of the *Syntretus* species increased to 42 world-wide.

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In the present paper a total of 12 *Syntretus* species are reviewed: 7 are new to the science (their enumeration is given in the abstract) and 5 have been previously listed but hitherto not from Taiwan (*S. bulbus* Chen et van Achterberg, *S. glaber* Chen et van Achterberg, *S. lyctaea* Cole, *S. parvicornis* (Ruthe), *S. testaceus* (Capron)).

Concerning the hosts of the *Syntretus* species two Palaearctic ones have been recorded as the parasitoids of adult ichneumonid wasp (*S. lyctaea*) and adult bumble-bee (*S. splendidus*), respectively.

In the terminology Huber & Sharkey (1993) as well as Wahl & Sharkey (1993) were considered. In the material examined only "in BP" (= in the Hungarian Natural History Museum, Budapest) is indicated as the depository institution; otherwise the depository is the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan.

Key to the *Syntretus* Foerster species of Taiwan

The 12 species in Taiwan represent the nominate subgenus *Syntretus*. Belokobylskij (1993a) has set up the subgenus *Parasyntretus* within the genus *Syntretus* on the basis of the species *S. amoenus* Belokobylskij described from Vietnam and deviating from all other *Syntretus* species by its conspicuously shortened radial cell (Fig. 106 in Belokobylskij 1993a).

1 (14) Propodeum entirely polished, at least close along its hind margin rugulose (Figs. 73, 76, 79).

2 (3) Temple in dorsal view longer than eye; head in dorsal view subcubic, 1.5 times as broad as long (Fig. 15). Petiole three times as long as broad behind (Fig. 60). Antenna with 17 antenomeres, first flagellomere a bit longer than second flagellomere, further flagellomeres gradually shortening so that penultimate flagellomere twice as long as broad. Hind femur 3.8 times as long as broad medially (Fig. 67). R1 approaching tip of wing (Fig. 44). Head dark rusty brown, meso- and metasoma blackish brown, legs reddish yellow, wings subhyaline. Female: 2.3 mm ---------- *S. varus* sp.n.

3 (2) Temple in dorsal view either as long as eye (Figs. 6, 9, 12) or shorter than eye (Figs. 7, 10, 11).

4 (9) Temple in dorsal view as long as eye (Figs. 6, 9, 12).

5 (6) Pterostigma unusually long, four times as long as wide, r–rs distinctly longer than half width of pterostigma (Fig. 35). Hind femur long, five times as long as broad, dorso-medially slightly indented (Fig. 63). Antenna with 26–32 antennomeres. Head in dorsal view 1.5–1.6 times as broad as long (Fig. 6). R1 reaching tip of wing (Fig. 35). Basal half of petiole somewhat flattened, pair of spiracles at middle or slightly beyond middle of petiole. Body reddish yellow, vertex and frons brown, mesosoma dorsally and tergites black. Legs yellow. Female: 5 mm... ---------- *S. extensus* sp. n.

6 (5) Pterostigma less long, at most 3–3.3 times as long as wide, r–rs at most half as long as width of pterostigma (Figs. 38, 41). Hind femur less long, 3.8–4 times as long as broad. Antenna with 18–23(–25) antennomeres.

7 (8) Pterostigma issuing r-rs less distally from its middle, r-rs half as long as width of pterostigma (Fig. 41). Petiole 2.8 times as long as broad behind, posteriorly more broadening, i.e. twice broader behind than basally (Fig. 57). Flagellomeres 2–10 relatively short and thick, 1.6–1.8 times as long as broad (Fig. 81–82). Dark coloured species: head reddish yellow, vertex dark brownish; mesosoma and metasoma black; legs reddish yellow. Female: 3.2 mm ---------- *S. temporalis* sp. n.

8 (7) Pterostigma issuing r-rs more distally from its middle, r-rs clearly less than half as long as width of pterostigma (Fig. 38). Petiole 3–3.3 times as long as broad behind, posteriorly less broadening, i.e. only somewhat broader behind than basally (Fig. 54). Flagellomeres 2–10 relatively less short and less thick, 2.1–2.2 times as long as broad (Fig. 83). Light coloured species: head and mesosoma yellow to brownish yellow; vertex, mesonotum, scutellum and propodeum light brown to brown; metasoma brown to dark brown; legs yellow to pale yellow. Female: 1.9–3 mm, male: 1.5–2.9 mm. See also couplet-lead 13 (12) ---------- *S. parvicornis* (Ruthe)

9 (4) Temple in dorsal view shorter than eye, head in dorsal view 1–1.7 times as broad as long (Figs. 7, 10).

10(11) Pterostigma long, four times as long as wide (Fig. 39). Hind femur five times as long as broad medially (Fig. 64). Antenna with 37 antennomeres, first flagellomere 1.6 times as long as second flagellomere. Petiole three times as long as broad behind, broadening at its hind third (Fig. 55). Nervellus (or Cu & cu-a) of hind wing antefurcal (Fig. 48, see horizontal arrow). Body yellow; vertex, mesonotum and propodeum brown to blackish brown; tergites brown, petiole black; tegula pale yellow; legs yellow. Female: 5.5 mm---------- *S. secutensus* sp. n.

11(10) Pterostigma less long, at most 3.5 times as long as wide (Figs. 36, 38). Hind femur 4–4.5 times as long as broad medially. Petiole broadening at its hind fourth (Fig. 54).

12(13) Pterostigma long, 3.3–3.4 times (female, Fig. 36) and 2.9 times (male) as long as wide. Antenna with 26–28 (female) and 23 (male) antennomeres, first flagellomere 3–3.2 times as long as broad apically (Fig. 1). Nervellus (or Cu & cu-a) of hind wing interstitial (Fig. 47, see horizontal arrow). Head in dorsal view transverse, 1.6–1.7 times as broad as long, temple shorter than eye and receded close beyond eye (Fig. 7). Reddish yellow; vertex, mesosoma dorsally and tergites brown to blackish. Female: 3–4 mm, male: 3–3.2 mm ---------- *S. glaber* Chen et van Achterberg

13(12) Pterostigma less long, 2.8–3.1 times as long as wide (Fig. 38). Antenna with (17–)18–25 (female) and 16–21 (male) antennomeres, first flagellomere 2.5–3 times as long as broad apically (Fig. 2). Nervellus (or Cu & cu-a) of hind wing antefurcal (cf. Fig. 46, see horizontal arrow). Head in dorsal view somewhat less transverse, 1.5–1.6 times as broad as long, temple usually as long as (sometimes slightly shorter than) eye and receded not close beyond eye (Fig. 9). Reddish yellow or yellow, dorsally brownish to dark brown. Female: 1.9–3 mm, male: 1.5–2.9 mm. See also couplet-lead 8 (7)---------- *S. parvicornis* (Ruthe)

14 (1) Propodeum at least medially rugulo-subrugulose (Figs. 71, 72, 74), usually with carinae or areolation and at least along carinae rugo-rugulose (Figs. 68–72, 74–75, 77–78).

15(16) Propodeum polished, at least medially and transversely rugulose-subrugulose (Figs. 71, 74). For further details see couplets 11 (10) — 13(12) ---------- *S. glaber* Chen et van Achterberg + *S. parvicornis* (Ruthe)

16(15) Propodeum with carinae (Figs. 72, 75) or areolation (Figs. 68–70, 77–78).

17(20) Propodeum with a pair of posterior carinae, between carinae propodeum more (*S. lyctaea*) or less (*S. subglaber*) rugo-rugulose (Figs. 72, 75).

18(19) Eye in dorsal view 1.3 times as long as temple, i.e. temple somewhat shorter than eye and moderately receded (Fig. 8). Petiole 2.9 times as long as broad behind, relatively thick, i.e. its hind breadth 1.7 times as broad as its thinnest section at laterope (Fig. 53). Antenna with (25-)29 antennomeres, first

flagellomere 1.3 times as long as second flagellomere (cf. Fig. 1) (cf. Fig. 1C in Cole 1959). Nervellus (or Cu & cu-a) of hind wing interstitial (cf. Fig. 47, see horizontal arrow). Propodeum smooth and shiny, medially rugose and laterally rugulose (Fig. 72). Ground colour of body reddish yellow, dorsally (mesonotum, scutellum, propodeum and tergites) brown to blackish. Female: 3.2–3.5 mm ---------- *S. lyctaea* Cole

19(18) Eye in dorsal view 1.9 times as long as temple, i.e. temple about half as long as eye and rounded (Fig. 11). Petiole 3.6 times as long as broad behind, relatively thin, i.e. its hind breadth twice as broad as its thinnest section before its middle (Fig. 56). Antenna with 22 antennomeres, first flagellomere just longer than second flagellomere (Fig. 3). Nervellus (or Cu & cu-a) of hind wing antefurcal (Fig. 49, see horizontal arrow). Propodeum rugulose between carinae and laterally (Fig. 75). Corporal colour similar to previous species. Female: 3–3.1 mm ---------- *S. subglaber* sp. n.

20(17) Propodeum areolated, along carinae more or less rugo-rugulose (Figs. 68–70, 77–78).

21(22) Marginal cell of fore wing short, as long as length of pterostigma along metacarpal vein (or R1), i.e. R1 ending clearly before tip of wing; pterostigma 2.8–3 times as long as wide (Fig. 42). Antenna short, about half as long as body and with 19 antennomeres, flagellomeres short, penultimate flagellomere cubic. Propodeum with areola petiolaris, above areola propodeum striolate, areola itself at most uneven (Fig. 77). Petiole 3.3 times as long as broad behind, its hind half evenly broad. Eye in dorsal view somewhat longer than temple (Fig. 13). Body reddish yellow, three maculae of mesonotum and petiole brownish. Female and male: 4.5–5 mm ---------- *S. testaceus* (Capron)

22(21) Marginal cell of fore wing long, at least one-fifth longer along metacarpal vein (or R1) than pterostigma, i.e. R1 approaching (Fig. 43: *S. transitus*) or reaching (Fig. 34, *S. choui*) tip of wing. Antenna long, about 0.7–0.8 times as long as body, penultimate flagellomere always longer than broad.

23(24) Temple in dorsal view half as long as eye and rounded, eye somewhat protruding, head in dorsal view 1.5 times as broad as long (Fig. 4). Eye in lateral view as wide as temple, eye anteriorly clearly broadening (Fig. 16, see arrows). Hind femur thin, five times as long as broad medially (Fig. 61). Along carinae of propodeum with rugae (Fig. 68). Antenna with 22–28 antennomeres. Body brownish yellow to rusty brown with more (Taiwanese specimens) or less (Chinese specimens) brown to blackish brown pattern. Female: 3–3.2 mm ---------- *S. bulbus* Chen et van Achterberg

24(23) Temple in dorsal view as long as eye (Figs. 5, 14). Eye in lateral view at most just wider than temple (Figs. 17–18, see arrows). Hind femur 3.5–4 times as long as broad (Figs. 62, 66). Along carinae of propodeum at most with rugulae (Figs. 69, 70, 78).

25(26) Marginal cell of fore wing long, along metacarpal vein (or R1) 1.7 times as long as pterostigma and reaching at margin close to tip of wing (Fig. 34). Areola petiolaris extending to half of propodeum (Figs. 69, 70). Petiole 3.5 (female, Fig. 51) and four times (male) as long as broad behind. Body chestnut brown, legs, yellow. Female and male: 2–2.5mm ---------- *S. choui* sp. n.

26(25) Marginal cell of fore wing less long, along metacarpal vein (or R1) at most one-fifth longer than pterostigma and reaching at margin in a distance from tip of wing (Fig. 43). Areola petiolaris extending to two-thirds of propodeum (Fig. 78). Petiole 3–3.2 times as long as broad behind (Fig. 59). Body yellow, dorsally brownish to brown. Female: 1.8–1.9 mm ---------- *S. transitus* sp. n.

Syntretus bulbus Chen et van Achterberg (Figs. 4, 16, 61, 68)

Syntretus bulbus Chen et van Achterberg, 1997: Zool. Verh. Leiden 313: 127 (in key) and 127 (description) ♀♂.

Material examined (1 ♀). — 1 ♀: Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 18–21 October 1982, leg. K. C. Chou et S. C. Lin.

Female: Body 3.1 mm long. Antenna with 22 antennomeres, first flagellomere 1.3 times as long as second flagellomere. Head in dorsal view (Fig. 4) 1.5 times as broad as long, eye also 1.5 times as long as temple. Propodeum areolated, carinae strong (Fig. 68). Hind femur thin, five times as long as broad medially (Fig. 61). Pterostigma 3.3 times as long as wide. Petiole 3.6 times as long as broad behind. Body dark coloured: head rusty brown, face, ocellar field and occiput dark brownish. Mesonotum brownish black, mesopleuron dark rusty. Metasoma dark brown, petiole blackish. Legs yellow, hind coxa brownish.

Distribution: People's Republic of China, Taiwan (new to her fauna).

Syntretus choui sp. n. (Figs. 5, 17, 23, 29, 34, 45, 51, 62, 69, 70)

Material examined (1 ♀ + 3 ♂♂). — Female holotype + one male paratype: Central Taiwan, Nantou Hsien, Tsuifeng, 2300 m, 3 June 1980 and leg. L. Y. Chou et C. C. Chen (holotype), 9 May 1984 and leg. K. C. Chou et C. C. Pan. — One male paratype: Central Taiwan, Nantou Hsien, Meifeng, 2150 m, 7–9 May 1981, leg. K. S. Lin et S. C. Lin. — One male (not paratype owing to its poor condition, in BP): Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 23–27 July 1894, leg. K. C. Chou et C. H. Yang.

Holotype and two paratypes are deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan; one male specimen in the Hungarian Natural History Museum (Department of Zoology), Budapest.

Etymology. — The new species is dedicated to the late DR. LIANG-YIH CHOU (1949–2000), braconid specialist and the collector of the holotype specimen, who has arranged (before his unexpected decease) the Taiwanese *Syntretus* material for elaboration.

Description of the female holotype. — Body 2.3 mm long. Antenna damaged, both right and left antenna with 17 antennomeres. First and second flagellomeres equal in length, i.e. three times as long as broad, further flagellomeres progressively shortening. — Head in dorsal view (Fig. 5) 1.6 times as broad as long, eye as long as temple, temple rounded, occiput weakly excavated. Ocelli small and elliptic, POL = OOL. Eye in lateral view 1.7 times as high as wide, temple beyond eye just less wide than eye and ventrally faintly narrowing (Fig. 17, see arrows). Face almost twice as wide as high, inner margin of eyes parallel. Clypeus 2.5 times as wide as high, its lower margin just convex (Fig. 23). Malar space shorter than basal width of mandible. Head polished.

Mesosoma in lateral view 1.3 times as long as high. Notaulix missing. Mesosoma polished. Propodeum areolated, areola petiolaris relatively wide, pentagonal and extending to posterior half of propodeum, close along antero-medio-longitudinal carina with rugulae, otherwise polished (Fig. 69). — Hind femur four times as long as broad medially (Fig. 62). Inner spur of hind tibia half as long as basitarsus. Hind claw long downcurved (Fig. 29).

Fore wing: Pterostigma (Fig. 34) 2.8 times as long as wide and issuing r-rs distally from its middle, r-rs shorter than half width of pterostigma, Rs3–4 reaching tip of wing; marginal cell long, along metacarpal vein 1.7 times as long as pterostigma. Basal and subbasal cells evenly setose. — Hind wing: Nervellus (or Cu & cu-a) antefurcal (Fig. 45, see arrows).

Metasoma somewhat longer than mesosoma and shorter than head and mesosoma combined. Petiole 3.5 times as long as broad behind, pair of lateropes beyond middle of petiole, petiole itself parallel-sided and broadening beyond lateropes (Fig. 51). Petiole anteriorly and along medio-longitudinal line uneven, otherwise together with further tergites polished. Ovipositor sheath somewhat shorter than hind tarsomeres 1–2 combined.

Body castaneous brown. Scape and flagellum brown; pedicel straw yellow, basally brownish. Palpi straw yellow. Tegula yellow. Legs brownish yellow, coxae and trochanters yellow. Wings feebly fumous or clearly subhyaline, pterostigma and veins brownish.

Description of two male paratypes + one male specimen. — Similar to the holotype. Body 2 (1 ♂), 2.2 (1 ♂) and 2.5 (1 ♂) mm long. Antenna somewhat shorter than body and with 19 antennomeres (1 ♂; flagellum of further two males damaged). First flagellomere a bit longer than second flagellomere, penultimate flagellomere twice as long as broad. Areola superomedia (anteriorly of areola petiolaris) missing (Fig. 70). Pterostigma 2.6 times as long as wide; marginal cell rather approaching tip of wing (2 ♂♂). Pterostigma brownish to yellow.

Host unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus choui*, is nearest to *S. varus* sp. n. and *S. bulbus* Chen et van Achterberg, 1997 (China), the specific distinction between *S. choui* and *S. varus* see at *S. varus* p.52; the distinction between *S. choui* and *S. bulbus* is disclosed below:

1 (2) Eye in dorsal view 1.4–1.6 times as long as temple (Fig. 553 in Chen & van Achterberg 1997). Hind femur 5–6 times as long as broad (Fig. 550 l.c.). Carinae of propodeum relatively strong and along them uneven to rugulose(-rugose) (Fig. 557 l.c. and Fig. 68). Female antenna with 22–28 antennomeres. first flagelomere 1.2 times as long as second flagellomere. Dark coloured, hind coxa brownish. Female: 2.8–3 mm----------*S. bulbus* Chen et Van Achterberg

2 (1) Eye in dorsal view as long as temple (♀, Fig. 5) or just shorter (♂). Hind femur 4.5 times as long as broad. Carinae of propodeum relatively less strong and along them surface smooth except medio-longitudinal fore carina, along it rugulose (Figs. 69–70). Male antenna with 19 antennomeres, flagellomeres 1–2 equal in length (female antenna damaged: with 17 antennomeres), female first flagellomere a bit longer than her second flagellomere. Dark coloured, hind coxa yellow, basally brownish. Female and male: 2–2.5 mm.---------- *S. choui* sp. n.

With the help of Belokobylskij's key (2000b) *S. choui* runs to *S. signatus* Belokobylskij and *S. areolatus* Belokobylskij, the three species are distinguished as follows:

1 (4) Mandible with pointed tooth.

2 (3) Pronotum with pronope. Metacarp 1–1.2 times as long as pterostigma (Fig. 237: 18 in Belokobylskij 2000b). Hind femur 4.5–4.6 times as long as broad (Fig. 237: 16 l.c.). Pterostigma brown, basally yellow. Female: 2.1–2.6 mm---------- *S. areolatus* Belokobylskij

3 (2) Pronotum without pronope. Metacarp 1.7 times asd long as pterostigma (Fig. 34). Hind femur four times as long as broad (Fig. 62). Pterostigma brownish to yellow. Female: 2.3 mm, male: 2–2.5 mm ----------
---------- *S. choui* sp. n.

4 (1) Mandible with obtuse small tooth. Pronotum without pronope. Metacarp as long as pterostigma (Fig. 237: 5 l.c.). Hind femur 4.8 times as long as broad (Fig. 237: 17 l.c.). Female: 2 mm----------
---------- *S. signatus* Belokobylskij

Syntretus extensus sp. n. (Figs. 6, 19, 24, 30, 35, 46, 52, 63, 80)

Material examined (1 ♀). — Female holotype: Central Taiwan, Nantou Hsien, Tsuifeng, taken with Malaise trap, 2300 m, September 1984, leg. T. Lin et K. C. Chou.

Holotype is deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan.

Etymology. — The species name "extensus" refers to the unusually long pterostigma.

Description of the female holotype. — Body 5 mm long. Antenna about as long as body and with 32 antennomeres. First flagellomere 1.3 times as long as second flagellomere and three times as long as broad apically (Fig. 80), further flagellomeres progressively attenuating so that penultimate flagellomere 2.2 times as long as broad. — Head in dorsal view (Fig. 6) subcubic, 1.5 times as broad as long, eye as long as temple, temple hardly rounded, occiput weakly excavated. Ocelli middle-sized and elliptic, POL = OOL. Eye in lateral view 1.7 times as high as wide, temple somewhat wider than eye and evenly wide beyond eye (Fig. 19, see arrows). Face somewhat wider than high. Clypeus hardly twice as wide as high, its lower margin convex (Fig. 24). Malar space as long as basal width of mandible. Head polished, face dispersely and very finely hairpunctured.

Mesosoma in lateral view 1.5 times as long as high. Notaulix missing. Propodeum smooth and polished. — Hind femur long, five times as long as broad, dorso-medially with a faint indentation (Fig. 63, see arrow). Inner spur of hind tibia just half as long as basitarsus. Hind claw less downcurved, basal lobe less large (Fig. 30).

Fore wing (left pair of wings missing): Pterostigma (Fig. 35) long, four times as long as wide and issuing r-rs clearly distally from its middle, r-rs distinctly longer than half width of pterostigma, Rs3–4 reaching tip of wing. Basal and subbasal cells evenly setose. — Hind wing: Nervellus (or Cu & cu-a) antefurcal (Fig. 46, see arrows).

Metasoma somewhat longer than mesosoma. Petiole 2.8 times as long as broad behind, fore part of petiole dorsally somewhat flattened, pair of lateropes slightly beyond middle of petiole, beyond laterope petiole broadening (Fig. 52). Petiole and tergites polished. Ovipositor sheath as long as middle basitarsus.

Ground colour of body reddish yellow. Scape and pedicel reddish yellow, flagellomeres 1–2 darkening reddish yellow to brownish, rest of flagellum brown. Frons, vertex and pronotum medially brown; mesonotum, scutellum, propodeum and tergites black. Palpi and tegula straw yellow. Legs reddish yellow, coxae rather yellowish. Wings hyaline, pterostigma and veins yellow.

Male and **host** unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus extensus*, is nearest to *S. secutensus* sp. n. and *S. makarovi* Belokobylskij, 1996 (Russian Far East), the three species are distinguished by the following features keyed:

1 (2) Flagellomeres 3—10–12 cubic, i.e. as long as broad (Fig. 77 in Belokobylskij 1996), antenna with 29 antennomeres. Petiole twice as long as broad behind (Fig. 83 l.c.). Hind femur four times as long as broad (Fig. 80 l.c.). In dorsal view eye somewhat longer than temple (Fig. 76 l.c.). Head and mesosoma yellow with much light brown or reddish yellow pattern, petiole and tergites black, legs yellow. Female: 3.4 mm ---------- *S. makarovi* Belokobylskij

2 (1) Flagellomeres 3—10–12 at least 1.5–1.6 times as long as broad, antenna with more than 30 antennomeres (Figs. 80, 84). Petiole 2.8–3 times as long as broad behind (Figs. 52, 55). Hind femur five times as long as broad.

3 (4) Head in dorsal view subcubic, 1.5 times as broad as long, eye as long as temple, temple hardly rounded (Fig. 6). Hind claw less downcurved (Fig. 30). Petiole broadening beyond its middle (Fig. 52). Antenna with 32 antennomeres. Mesosoma dorsally and tergites black. Female: 5 mm ----------*S. extensus* sp. n.

4 (3) Head in dorsal view transverse, 1.7 times as broad as long, eye 1.2 times as long as temple, temple rounded (Fig. 10). Hind claw more downcurved (Fig. 31). Petiole broadening from its middle (Fig. 55). Antenna with 37 antennomeres. Mesosoma dorsally and tergites brownish to blackish brown. Female: 5.5 mm ----------*S. secutensus* sp. n.

Syntretus glaber Chen et van Achterberg, male new (Figs. 1, 7, 36, 47, 71)

Syntretus glaber Chen et van Achterberg, 1997: Zool. Verh. Leiden 313: 126 (in key) and 128 (description) ♀.

Material examined (3 ♀ + 1 ♂). — Two females and one male: Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 1 ♀: 19–23 July 1982 leg. L. Y. Chou et T. Lin and 1 ♀ (in BP) + 1 ♂: 18–21 October 1982 leg. K. C. Chou et S. C. Lin. — One female: Central Taiwan, Nantou Hsien, Meifeng, 2150 m, 19–21 April 1983, leg. K. C. Chou et S. P. Huang.

Female: Body 3–4 mm long. Antenna with 26–28 antennomeres. Head in dorsal view (Fig. 7) 1.55–1.65 times as broad as long. Pterostigma (Fig. 36) 3.1–3.4 times as long as wide, Rs3–4 feebly curved. Petiole 2.7–2.8 times as long as broad behind.

Male: Similar to the female. Body 3–3.2 mm long. Antenna with 23 antennomeres, first flagellomere 1.25 times as long as second flagellomere and 3–3.2 times as long as broad apically (Fig. 1). Head in dorsal view 1.6 times as broad as long. Pterostigma 2.85 times as long as wide and issuing r-rs less distally from its middle. Petiole somewhat thick, 2.6 times as long as broad behind. Colour of body darker than that of female: Head reddish yellow, vertex and occiput with faint brownish tint. Mesosoma black, pronotum and prosternum reddish yellow. Metasoma blackish to black, second tergite yellowish brown. Legs yellow.

Distribution: People's Republic of China, Taiwan (new to her fauna).

Syntretus lyctaea Cole (Figs. 8, 37, 53, 72)

Syntretus lyctaea Cole, 1959: Ent. m. Mag. 95: l8 (description) ♀.

Material examined (1 ♀). — One female: Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 28 April – 2 May 1981, leg. T. Lin et C. J. Lee.

Female: Body 3.2–3.5 mm long. Antenna with 29 antennomeres, first flagellomere 1.3 times as long as second flagellomere. Head in dorsal view (Fig. 8) 1.6 times as broad as long, eye 1.3 times as long as temple. Propodeum on its hind third with a pair of carinae somewhat diverging anteriorly, medially and laterally rugo-rugulose, otherwise polished (Fig. 72). Pterostigma three times as long as wide and issuing r-rs distally from its middle (Fig. 37). Petiole 2.9 times as long as broad behind (Fig. 53).

Male: unknown.

Distribution: England, Hungary, Lithuania, European part of Russia (Ural Mts), Taiwan (new to her fauna).

Syntretus parvicornis (Ruthe) (Figs. 2, 9, 38, 54, 73, 74, 83)

Microctonus parvicornis Ruthe, 1862: in H. Reinhard 1862: Berl. ent. Zschr. 6: 323 ♂, type locality: "bei Berlin"

(Germany), male holotype in The Natural History Museum, London (3.c.73l); not examined. — Shenefelt 1969: 131 (literature up to 1946). Tobias 1986: 246 (in key).

Material examined (27 ♀ + 8 ♂). — Five females and one male: Central Taiwan, Nantou Hsien, Meifeng, 2150 m, 1 ♀: 2–4 June 1980 leg. L. Y. Chou et C. C. Chen, 1 ♀: 5–9 October 1980 leg. C. C. Chen et C. C. Chien, 1 ♀: 15 July 1982 leg. S. C. Lin et C. N. Lin, 2 ♀: 19–21 April 1983 leg. K. C. Chou et S. P. Huang, 1 ♂: 22–26 June 1983 leg. K. S. Lin et S. C. Lin. — Two females and one male: Central Taiwan, Hwalien Hsien, Tayuling, 2560 m, 6–9 September 1983 leg. L. Y. Chou et K. C. Chou. — Six females and four males: Central Taiwan, Nantou Hsien, Tsuifeng, 2300 m, 1 ♂: 3 June 1980 leg. L. Y. Chou et C. C. Chen, 1 ♀: 8 May 1981 leg. K. S. Lin et S. C. Lin, 1 ♀: 25–27 June 1981 leg. K. S. Lin et W. S. Tang, 1 ♀: 1–3 September 1982 taken with Malaise trap leg. L. Y. Chou et K. C. Chou, 1 ♀: July 1984 leg. K. S. Lin et K. C. Chou, 1 ♂: August 1984 leg. K. S. Lin et K. C. Chou, 1 ♀: 15–16 August 1984 leg. K. C. Chou, 1 ♀: September 1984 leg. K. S. Lin et K. C. Chou, 2 ♂ (1 ♂ in BP): 12–14 September 1984 leg. K. S. Lin et S. C. Lin. — Twelve females and one male (six females in BP): Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 1 ♀: 28 April – 2 May 1981 leg. T. Lin et C. J. Lee, 6 ♀ (4 ♀ in BP): 18–23 November 1981 leg. T. Lin et W. S. Tang, 2 ♀: 18–21 October 1982 leg. K. C. Chou et S. C. Lin, 2 ♀ (1 ♀ in BP): 22–25 November 1982 leg. K. C. Chou et S. P. Huang, 1 ♀ (in BP): 13–16 December 1982 leg. K. C. Chou et C. C. Pan, 1 ♀: 10–14 January 1983 leg. K. C. Chou et S. P. Huang. — One male: Central Taiwan, Taichung Hsien, Kukuan, 730 m, 14–17 October 1980, leg. K. S. Lin et C. H. Wang. — One male: Central Taiwan, Nantou Hsien, Wushe, 1150 m, 7 May 1984, leg. K. C. Chou et C. C. Pan.

Female: Body 1.9–3 mm long (1.9: 5 ♀, 2: 1 ♀, 2.1: 2 ♀, 2.2: 2 ♀ , 2.3: 3 ♀, 2.4: 2 ♀, 2.5: 3 ♀, 2.6: 3 ♀, 2.8: 1 ♀, 2.9: 1 ♀, 3: 4 ♀). Antenna somewhat shorter than to as long as body and with 17–25 antennomeres (17: 2 ♀, 18: 7 ♀, 20: 1 ♀, 22: 4 ♀, 23: 5 ♀, 24: 7 ♀, 25: 1 ♀). First flagellomere 2.5–3 times as long as broad (Figs. 2, 83). Head in dorsal view (Fig. 9) 1.5–1.6 times as broad as long, eye as long as temple (exceptionally slightly longer). Propodeum polished (Fig. 73), exceptionally medio-anteriorly uneven to rugulose (Fig. 74). Hind femur 4–4.2 times as long as broad. Pterostigma of fore wing 2.8–3.1 (exceptionally 3.3–3.4) times as long as wide, issuing r-rs clearly distally from its middle (Fig. 38). Nervellus (or Cu & cu-a) of hind wing antefurcal. Petiole 3–3.3 times as long as broad behind, broadening from its middle and relatively less broadening posteriorly (Fig. 54). Ground colour of body reddish yellow or yellow, dorsally dark colour variable brownish to dark brown.

Male: Similar to the female. Body 1.5–2.9 mm long (1.5: 1 ♂, 1.8: 1 ♂, 2: 1 ♂, 2.1: 1 ♂, 2.2: 2 ♂, 2.9: 2 ♂). Antenna shorter than body and with 15–21 antennomeres (15: 1 ♂, 16: 1 ♂, 17: 2 ♂, 18: 2 ♂, 19: 1 ♂, 21: 1 ♂). Pterostigma 2.8–3 times as long as wide. Dark colour of body more to much more extended.

Distribution: Palaearctic Region, Taiwan (new to her fauna).

Syntretus secutensus sp. n. ♀ (Figs. 10, 20, 31, 39, 48, 55, 64, 84)

Material examined (1 ♀). — Female holotype: Central Taiwan, Nantou Hsien, Meifeng, 2130 m, 2–4 June 1980, leg. L. Y. Chou et C. C. Chen. — Holotype is deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan.

Etymology. — The species name "secutensus" is the abbreviated form of secundoextensus indicating its close relation to *S. extensus* sp. n.

Description of the female holotype. — Body 5.5 mm long. Antenna about as long as body and with 37 antennomeres; first flagellomere 1.6 times as long as second flagellomere and 2.7 times as long as broad apically, its apical end obliquely truncate (Fig. 84); further flagellomeres progressively shortening and slightly attenuating

so that penultimate flagellomere 2.2 times as long as broad. — Head in dorsal view (Fig. 9) transverse, 1.7 times as broad as long, eye 1.2 times as long as temple, temple rounded, occiput weakly excavated. Ocelli relatively large, elliptic, POL = OOL. Eye in lateral view 1.8 times as high as wide, temple a bit wider than eye and ventrally indistinctly narrowing (Fig. 20, see arrows). Face 1.4 times as wide ad high. Intertentorial line three times as long as tentorio-ocular line. Clypeus 2.5 times as wide as high, its lower margin just convex (cf. Fig. 23). Malar space somwhat longer than basal width of mandible. Maxillar palp longer than height of head. Head polished, face finely hairpunctured.

Mesosoma in lateral view 1.4 times as long as high. Notaulix missing. Mesosoma polished. — Hind femur five times as long as broad medially (Fig. 64). Inner spur of hind tibia shorter than half basitarsus. Hind claw downcurved as in Fig. 31.

Fore wing: Pterostigma long (Fig. 39), 4.1 times as long as wide and issuing r-rs distally from its middle, r-rs somewhat oblique and longer than half width of pterostigma, Rs3–4 reaching tip of wing and very faintly sinuate. Basal and subbasal cells evenly setose. — Hind wing: Nervellus (or Cu & cu-a) antefurcal (Fig. 48, see arrows).

Metasoma about as long as head and mesosoma combined. Petiole (Fig. 55) 3.1 times as long as broad behind, fore part of petiole dorsally somewhat flattened, pair of lateropes at middle of petiole, beyond lateropes petiole progressively broadening. Petiole and tergites polished. Ovipositor sheath short, one-fourth shorter than hind basitarsus.

Ground colour of body yellow; vertex, mesosoma, propodeum brown to blackish brown, scutellum brownish yellow; petiole black, tergites dark brown. Palpi and tegula straw yellow. Scape and pedicel reddish yellow, flagellum dark brown. Wings hyaline, pterostigma and veins brownish yellow.

Male and **host** unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus secutensus*, is nearest to *S. extensus* sp. n. and *S. makarovi* Belokobylskij, their distinction see at *S. extensus* s. n., p.45 and in key-couplets 4 (9) — 10 (11) p.40 – 41.

Syntretus subglaber sp. n. ♀ (Figs. 3, 11, 21, 25, 32, 40, 49, 56, 65, 75)

Material examined (2 ♀). — Female holotype and one female paratype: Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 13–16 December 1982 leg. K. C. Chou et C. C. Pan (holotype) and 18–23 November 1981 leg. T. Lin et W. S. Tang (paratype). — Holotype and paratype are deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan.

Etymology. — The species name "subglaber" indicates that the new species is nearest to *S. glaber* Chen et Van Achterberg.

Description of the female holotype. — Body 3.1 mm long. Antenna about as long as body and with 22 antennomeres. First flagellomere somewhat longer than second flagellomere and three times as long as broad apically (Fig. 3), further flagellomeres progressively attenuating so that penultimate flagellomere 3.2 times as long as broad. — Head in dorsal view (Fig. 11) less transverse, 1.6 times as broad as long, eye 1.9 times as long as temple, temple rounded, occiput weakly excavated. Ocelli middle-sized, elliptic, OOL 1.5 times as long as POL. Eye in lateral view 1.4 times as high as wide and 1.35 times as wide as temple, temple ventrally slightly narrowing (Fig. 21). Face 1.4 times as wide as high, inner margin of eyes diverging ventrally. Clypeus 1.9 times as wide as high, its lower margin truncate (Fig. 25). Intertentorial line 3.3 times as long as tentorio-ocular line. Malar space as long as basal width of mandible. Head polished.

Mesosoma in lateral view 1.35 times as long as high. Notaulix missing. Mesosoma polished. Propodeum posteriorly with a pair of carinae, its hind declivous part rugulose-subrugulose, its fore horizontal part polished (Fig. 75). — Hind femur 3.8 times as long as broad medially (Fig. 65). Inner spur of hind tibia shorter than half of hind basitarsus. Hind claw downcurved as in Fig. 32.

Fore wing: Pterostigma (Fig. 40) 2.8 times as long as wide and issuing r-rs somewhat distally from its middle, r-rs shorter than half width of pterostigma, Rs3–4 arched and reaching tip of wing. Basal and subbasal cells evenly setose. — Hind wing: Nervellus (Cu & cu-a) antefurcal (Fig. 49, see arrows).

Metasoma about as long as head and mesosoma combined. Petiole 3.6 times as long as broad behind, relatively thin, i.e. its hind width twice as broad as its thinnest section before its middle (Fig. 56). Pair of laterope beyond middle of petiole (right laterope or spiracle on a spineform tubercule: a teratorologic product?). Petiole and tergite polished. Ovipositor sheath as long as hind tarsomeres 1–2 + half of third tarsomere.

Ground colour of body reddish yellow. Ocellar field, mesonotum, scutellum, propodeum, metapleuron, petiole dark brown; tergites 2–3 brown, tergite 3 blackish. Scape, pedicel and flagellomeres 1–3 reddish yellow, flagellomeres 4–5 darkening and rest of flagellum brown. Palpi, tegula and legs yellow. Wings subhyaline, pterostigma and veins yellow.

Description of the female paratype. — Similar to the holotype. Body 3 mm long. Pterostigma three times as long as wide. Paratype damaged: right flagellum missing, fore left leg from femur together with middle left leg missing, hind leg and metasoma glued separately on the card.

Male and **host** unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus subglaber*, is nearest to *S. glaber* Chen et van Achterberg, 1997 (China), their specific distinction is presented in the subsequent key:

1 (2) Propodeum polished, at least and exceptionally medially rugulose-subrugulose (Fig. 71). Eye in dorsal view less long, 1.2–1.3 times as long as temple (Fig. 7). Pterostigma 3.4–3.6 times as long as wide (Fig. 36). Antenna with 22 antennomeres. Female: 3.2–3.5 mm ---------- *S. glaber* Chen et van Achterberg

2 (1) Propodeum posteriorly with a pair of carinae and its hind declivous part rugulose-subrugulose, its fore horizontal part polished (Fig. 75). Eye in dorsal view long, 1.7 times as long as temple (Fig. 11). Pterostigma 2.8 times as long as wide (Fig. 40). Antenna with (23–)26–28 antennomeres. Female: 3.1 mm ---------- *S. subglaber* sp. n.

With the help of Belokobylskij's key (2000b) *S. subglaber* runs to *S. lycteae* Cole, the two species are clearly distinct by the features keyed:

1 (2) Eye in dorsal view 1.3 times as long as temple (Fig. 8). Petiole more broadening posteriorly (Fig. 53). Antenna with (23–)28–30 antennomeres. Propodeum as in Fig. 72, along carina at most rugulose. Female: (2.8–)3.2–3.5 mm ---------- *S. lycteae* Cole

2 (1) Eye in dorsal view 1.9 times as long as temple (Fig. 11). Petiole less broadening posteriorly (Fig. 56). Antenna with 22 antennomeres. Propodeum as in Fig. 75, along carina transversely rugo-rugulose. Female: 3–3.1 mm---------- *S. subglaber* sp. n.

Syntretus temporalis sp. n. ♀ (Figs. 12, 22, 26, 41, 50, 57, 76, 81, 82)

Material examined (4 ♀). — Female holotype and one female paratype (in BP): Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 22–25 November 1982 leg. K. C. Chou et S. P. Huang (holotype) and 19–23 July 1982 leg. L. Y. Chou et T. Lin (paratype, in BP). — One female paratype: Central Taiwan, Nantou Hsien, Tsuifeng, 2300 m, 1–3 August 1981, leg. T. Lin et W. S. Tang. — One female paratype: Central Taiwan, Hwalian Hsien, Tayuling, 2560 m, 6–9 September 1983, leg. L. Y. Chou et K. C. Chou.

Holotype and two paratypes are deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan and one paratype in the Hungarian Natural History Museum, Department of Zoology), Budapest, Hym. Typ. No. 7880.

Etymology. — The species name "temporalis" refers to the temple equal in length with eye in dorsal view.

Description of the female holotype. — Body 3.2 mm long. Antenna about one-fifth shorter than body and with 23 antennomeres (left antenna damaged: with 22 antennomeres, i.e. ultimate flagellomere missing). First flagellomere slightly longer than second flagellomere and 2.3 times as long as broad apically (Fig. 81); further flagellomeres progressively attenuating, i.e. flagellomeres 2–10 1.6–1.7 times as long as broad, penultimate flagellomere twice as long as broad. — Head in dorsal view (Fig. 12) subcubic, 1.57 times as broad as long, eye as long as temple, temple rounded, occiput weakly excavated. Ocelli middle sized and elliptic, POL somewhat longer than OOL. Eye in lateral view twice as high as wide, temple beyond eye somewhat wider than eye and ventrally indistinctly narrowing (Fig. 22, see arrows). Face almost as high as wide. Intertentorial line three times as long as tentorio-ocular line. Clypeus 2.3 times as wide as high, its lower margin truncate (Fig. 26). Malar space somewhat longer than basal width of mandible. Head polished, face indistinctly hairpunctured.

Mesosoma in lateral view 1.5 times as long as high. Notaulix missing. Pronotum, mesonotum, scutellum, mesopleuron and metapleuron polished; fore and hind margin of mesopleuron finely crenulated. Propodeum polished, its hind margin with rugulae; above lunule with a pair of circular impressions (Fig. 76). — Hind femur 3.8 times as long as broad medially. Inner spur of hind tibia almost half as long as basitarsus.

Fore wing: Petrostigma (Fig. 41) 2.8 times as long as wide, issuing r-rs only somewhat distally from its middle, r-rs half as long as width of pterostigma, Rs3–4 approaching tip of wing and indistinctly sinuate. Basal and subbasal cells evenly setose. — Hind wing: Nervellus (or Cu & cu-a) antefurcal (Fig. 50, see arrows).

Metasoma about as long as mesosoma. Petiole 2.8 times as long as broad behind, clearly broadening posteriorly, i.e. one-third broader behind than basally, pair of lateropes behind middle of petiole (Fig. 57). Fore half of petiole transversely uneven, its hind broadening half together with further tergites polished. Ovipositor sheath in lateral view as long as hind tarsomeres 1–2.

Body dark coloured. Scape and pedicel brown, flagellum distally darkening brown. Head reddish yellow; frons, vertex and occiput dark brownish; palpi pale. Mesosoma and tergites black, sternites with rusty tint. Tegula blackish. Legs reddish yellow, middle coxa anteriorly and hind coxa entirely blackish. Tarsi brownish darkening. Wings hyaline, pterostigma and veins opaque yellowish brownish.

Description of the three paratypes. — Similar to the holotype. Body 3.2 mm long. Antenna with 26 (2 ♀) and 27 (1 ♀) antennomeres, flagellomeres 2–10 1.7–1.8 times (Fig. 82) and penultimate flagellomere 2.4–2.6 times as long as broad. Eye in lateral view 1.8 times as high as wide (Fig. 22). Pterostigma 3–3.3 times as long as wide. Pair of circular impressions above lunule of propodeum less distinct. Fore half of petiole also polished. Mesosoma reddish yellow, only dorsally (i.e. mesonotum, scutellum, propodeum, metapleuron) black.

Male and **host** unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus temporalis*, is nearest to *S. parvicornis* (Ruthe), their specific distinctions are disclosed in key-couplets 5 (6) — 7 (8) p.40.

Syntretus testaceus (Capron) (Figs. 13, 42, 58, 77)

Microctonus testaceus Capron, 1887: 82 (in key) and 85 (description) ♀♂, type locality: "at Shiere, near Guildford" (England), deposition of the type series (1 ♀ + 4 ♂) unknown, perhaps in The Natural History Museum, London or in Hope Department, University Museum, Oxford (England). — Shenefelt 1969: 132 (literature up to 1938). Tobias 1986: 246 (in key).

Material examined (2 ♀). — Two females (one female in BP): Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 20–22 June 1980, leg. C. C. Chen.

Female: Body 4.5–5 mm long. Antenna short, about half as long as body and with 19 antennomeres, first flagellomere just longer than second flagellomere. Head in dorsal view (Fig. 13) 1.6 times as broad as long, eye somewhat longer than temple, latter rounded. Propodeum with areola petiolaris on its hind two-thirds, above areola striolate and rugulose, otherwise propodeum polished (Fig. 77). Hind femur 4.6–5 times as long as broad. Pterostigma (Fig. 42) 2.8–3 times as long as wide, marginal cell short, along metacarpal vein (or R1) as long as pterostigma. Petiole 3.3 times as long as broad behind (Fig. 58). Body reddish yellow with rather effaced three brownish maculae on mesonotum, petiole faintly brownish.

Male: known, hitherto not captured in Taiwan.

Distribution: western Palaearctic Region, Taiwan (new to her fauna).

Syntretus transitus sp. n. (Figs. 14, 18, 27, 33, 43, 59, 78)

Material examined (2 ♀). — Female holotype and one female paratype: Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 16–20 April 1984, leg. K. C. Chou et C. H. Yung. — Holotype and paratype are deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan.

Etymology. — The species name "transitus" indicates its deceptive similarity to *S. parvicornis*.

Description of the female holotype. — Body 1.8 mm long. Antenna as long as body and with 17 antennomeres. First flagellomere as long as second flagellomere and both flagellomeres three times as long as broad apically, further flagellomeres progressively shortening so that penultimate flagellomere twice as long as broad. — Head in dorsal view (Fig. 14) subcubic, 1.5 times as broad as long, eye as long as temple, temple rounded, occiput weakly excavated. Ocelli small and elliptic, POL somewhat shorter than OOL. Eye in lateral view somewhat less high, 1.6 times as high as wide, temple beyond eye just a bit wider than eye and evenly wide (Fig. 18, see arrows). Face 1.7 times as wide as high, inner margin of eyes parallel. Clypeus 2.5 times as wide as high, its lower margin truncate (Fig. 27). Malar space a bit shorter than basal width of mandible. Head polished, face very finely hairpunctate.

Mesosoma in lateral view 1.4 times as long as high. Notaulix missing. Mesosoma polished. Propodeum areolated, areola petiolaris extending to two-thirds of propodeum, along medio-longitudo-anterior carina rugulose, along other carinae almost indistinctly uneven, otherwise propodeum polished (Fig. 78). — Hind femur 3.6 times as long as broad medially (Fig. 66). Inner spur of hind tibia half as long as basitarsus. Hind claw long downcurved (Fig. 33).

Fore wing: Pterostigma (Fig. 43) 3.1 times as long as wide and issuing r-rs distally from its middle, r-rs somewhat shorter than half width of pterostigma, marginal cell less long: along first section of metacarpal vein (or R1) 1.25 times as long as pterostigma, metacarpal vein (or R1–2) approaching tip of wing (Fig. 43). Basal and subbasal cells evenly setose. — Hind wing: Nervellus (or Cu & cu-a) antefurcal (cf. Fig. 46).

Metasoma as long as mesosoma. Petiole (Fig. 59) 3.2 times as long as broad behind, pair of lateropes large and clearly beyond middle of petiole, petiole itself relatively less broadening beyond lateropes, on its anterior two-thirds uneven, otherwise together with further tergites polished. Ovipositor sheath as long as hind tarsomeres 1–2 combined.

Body yellow. Scape and pedicel yellow, flagellomeres darkening yellow, rest of flagellum greyish brown. Vertex brownish; mesonotum, scutellum, metanotum, propodeum and metapleuron brownish to brown. Petiole brown, tergites 2–3 brownish, rest of tergites blackish brown. Palpi straw yellow. Tegula straw yellow. Legs yellow. Wings hyaline, pterostigma and veins yellow.

Description of the female paratype. — Similar to the female holotype. Body 1.9 mm long. Head in dorsal view 1.55 times as broad as long. Pterostigma 3.2 times as long as wide. Hind femur 3.8 times as long as broad. Petiole three times as long as broad behind. Tegula yellow, hind tergites dark brown.

Male and **host** unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus transitus*, is nearest to *S. choui* sp. n. and *S. parvicornis* (Ruthe, 1862) (Europe, Taiwan), the distinction from *S. choui* is disclosed in key-couplets 25(26)–26(25) p.42, the specific difference between *S. transitus* and *S. parvicornis* is presented in the subsequent key:

1 (2) Propodeum not areolated (Figs. 73–74), polished. Pterostigma 3.4–3.6 times as long as wide, marginal cell or vein R1–2 reaching tip of wing (Fig. 38). Hind femur 4.5–5 times as long as broad. Female and male: 1.5–3 mm ---------- *S. parvicornis* (Ruthe)

2 (1) Propodeum areolated, along carinae closely uneven to rugulose (Fig. 78). Pterostigma 3–3.1 times as long as wide, marginal cell or vein R1–2 approaching tip of wing (Fig. 43). Hind femur 3.6–3.8 times as long as broad (Fig. 66). Female and male: 1.8–1.9 mm ---------- *S. transitus* sp. n.

Syntretus varus sp. n. (Figs. 15, 28, 44, 60, 67, 79)

Material examined (1 ♀). — Female holotype: Central Taiwan, Nantou Hsien, Tungpu, 1200 m, 18–23 November 1981, leg. T. Lin et W. S. Tang. — Holotype is deposited in the Department of Applied Zoology, Taiwan Agricultural Research Institute, Taichung, Taiwan.

Etymology. — The species name "varus" refers to the subtle deviation from its nearest species, *S. choui* sp. n.

Description of the female holotype. — Body 2.3 mm long. Antenna nearly as long as body and with 17 antennomeres. First flagellomere a bit longer than second flagellomere, first flagellomere four times as long as broad, second flagellomere apically broadening and 2.8 times as long as broad apically, further flagellomeres progressively shortening and attenuating so that penultimate flagellomere twice as long as broad. — Head in dorsal view (Fig. 15) subcubic, 1.5 times as broad as long, temple long and rounded, 1.3 times as long as eye, POL = OOL. Eye in lateral view 1.7 times as high as wide, temple a bit wider than eye. Face just twice as wide as high. Intertentorial line three times as long as tentorio-ocular line. Clypeus twice as wide as high, its lower margin convex (Fig. 28). Malar space just shorter than basal width of mandible.

Mesosoma in lateral view 1.5 times as long as high. Notaulix missing. Pronotum medially rugose, otherwise polished. Propodeum also polished, basally with a pair of short carinae, its hind margin with short rugulae (Fig. 79). — Hind femur 3.8 times as long as broad medially (Fig. 67). Inner spur of hind tibia half as long as basitarsus.

Fore wing: Pterostigma 2.8 times as long as wide, r-rs a bit shorter than half width of pterostigma, R1–2 approaching tip of wing (Fig. 44). Basal and subbasal cells evenly setose. — Hind wing: Nervellus (or Cu & cu-a) antefurcal and hardly distinct.

Metasoma about as long as mesosoma. Petiole three times as long as broad behind, pair of lateropes beyond middle of petiole, petiole itself gradually broadening beyond lateropes (Fig. 60), together with further tergites polished. Ovipositor sheath as long as hind tarsomeres 1–2 combined.

Head dark rusty brown, meso- and metasoma blackish brown. Antenna faintly darkening brown distally. Palpi greyish yellow, mandible yellow. Tegula and legs reddish yellow. Wings subhyaline, i.e. faintly brownish fumous. Pterostigma and veins opaque yellow.

Male and **host** unknown.

Distribution: Taiwan.

Taxonomy. — The new species, *Syntretus varus*, is nearest to *S. choui* sp. n. viewing their dark corporal colour as well as their corporal size, the specific distinction between them is summarized in the subsequent key:

1 (2) Temple in dorsal view 1.3 times as long as eye, head in dorsal view subcubic, i.e. 1.5 times as broad as long (Fig. 15). Propodeum smooth and polished (Fig. 79). Marginal cell of fore wing somewhat less long, metacarpal vein (R1–2) approaching tip of wing (Fig. 44). Female: 2.3 mm ---------- *S. varus* sp. n.

2 (1) Temple in dorsal view as long as eye, head in dorsal view rather transverse, i.e. 1.6 times as broad as long (Fig. 5). Propodeum areolated (Figs. 69–70). Marginal cell of fore wing long, metacarpal vein (R1–2) reaching tip of wing (Fig. 34). Female and male: 2–2.5 mm---------- *S. choui* sp. n.

***S. varus* sp. n. is related to *S. parvicornis* (Ruthe), the two species are separated by the key as follows:**

1 (2) Eye in dorsal view as long as temple (or slightly longer) (Fig. 9). Hind femur 4–4.2 times as long as broad. Petiole somewhat broad (54). Body reddish yellow or yellow, dorsally with variable dark colour. Female: 1.9–3 mm---------- *S. parvicornis* (Ruthe)

2 (1) Temple in dorsal view 1.3 times as long as eye (Fig. 15). Hind femur 3.8 times as long as broad (Fig. 67). Petiole somewhat less broad (Fig. 60). Body rusty to blackish brown. Female: 2.3 mm----- *S. varus* sp. n.

CHECKLIST OF THE *SYNTRETUS* SPECIES OF THE EAST PALAEARCTIC AND ORIENTAL REGIONS

abbreviator Belokobylskij, 2000 — Russia (Primorski Territory)
amba Belokobylskij, 1993— Russia (Primorski Territory)
amoenus Belokobylskij, 1993 — Vietnam (Gia Lai-Con Tum)
areolatus Belokobylskij, 2000 — Russia (Primorski Territory)
bulbus Chen et van Achterberg, 1997 — China (Guizhou)
combinator Belokobylskij, 2000 — Russia (Primorski Territory)

conterminus (Nees, 1834) (*Perilitus*) — Palaearctic Region
= cultus Marshall, 1887
= vernalis (Wesmael, 1835) (*Perilitus*)
?= politus (Ruthe, 1856) (*Microctonus*)
choui sp. n. — Taiwan
daghestanicus Tobias, 1976 — Russia (Daghestan, Primorski Territory), Moldavia
= microphthalmus Tobias, 1986
elegans (Ruthe, 1856) (*Microctonus*) — Palaearctic Region
= transsylvanicus (Zilahi–Kiss, 1927) (*Perilitus*)
excavatus Belokobylskij, 2000 — Russia (Primorski Territory)
extensus sp. n. — Taiwan
glaber Chen et van Achterberg, 1997 — China (Zhejiang)
grodekovi Belokobylskij, 2000 — Russia (Primorski Territory)
hirtus Belokobylskij, 1996 — Russia (Primorski Territory), China (Guizhou)
= setosus Chen et van Achterberg, 1997
komarovi Belokobylskij, 1996 — Russia (Primorski Territory)
kui Belokobylskij, 2000 — Korea
lyctaea Cole, 1959 — Palaearctic Region
makarovi Belokobylskij, 1996 — Russia (Primorski Territory)
miscellus Belokobylskij, 1996 — Russia (Primorski Territory)
parvicornis (Ruthe, 1862) (*Microctonus*) — Palaearctic Region
planifacies Belokobylskij, 1993 — Russia (Primorski Territory)
sculptor Belokobylskij et Ku, 1998 — Korea, Russia (Primorski Territory)
secutensus sp. n. — Taiwan
splendidus (Marshall, 1887)* (*Microctonus*) — Europe, Russia
subglaber sp. n. — Taiwan
temporalis sp. n. — Taiwan
testaceus (Capron, 1887) (*Microctonus*) — Palaearctic Region
= niger Tobias, 1976
transitus sp. n. — Taiwan
varus sp. n. — Taiwan
venus Chen et van Achterberg, 1997 — China (Hainan)

* The name *S. splendidus* was placed in synonymy by Belokobylskij (2000b) with *S. parvicornis* (Ruthe). An examination of the female paralectotype of *S. splendidus* (housed in the Hungarian Natural History Museum) clearly revealed that it differs in a few clear-cut specific features from *S. parvicornis*.

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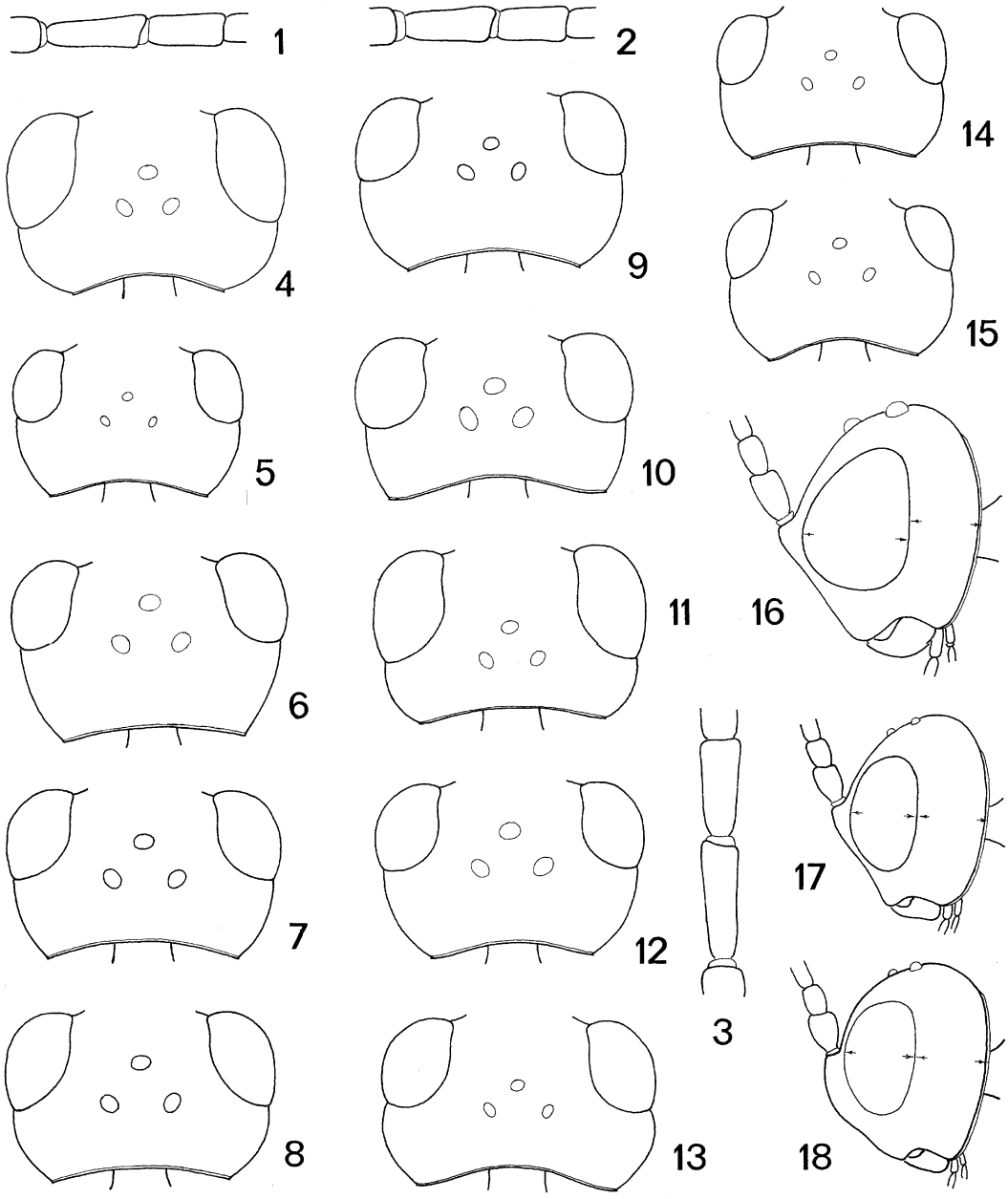
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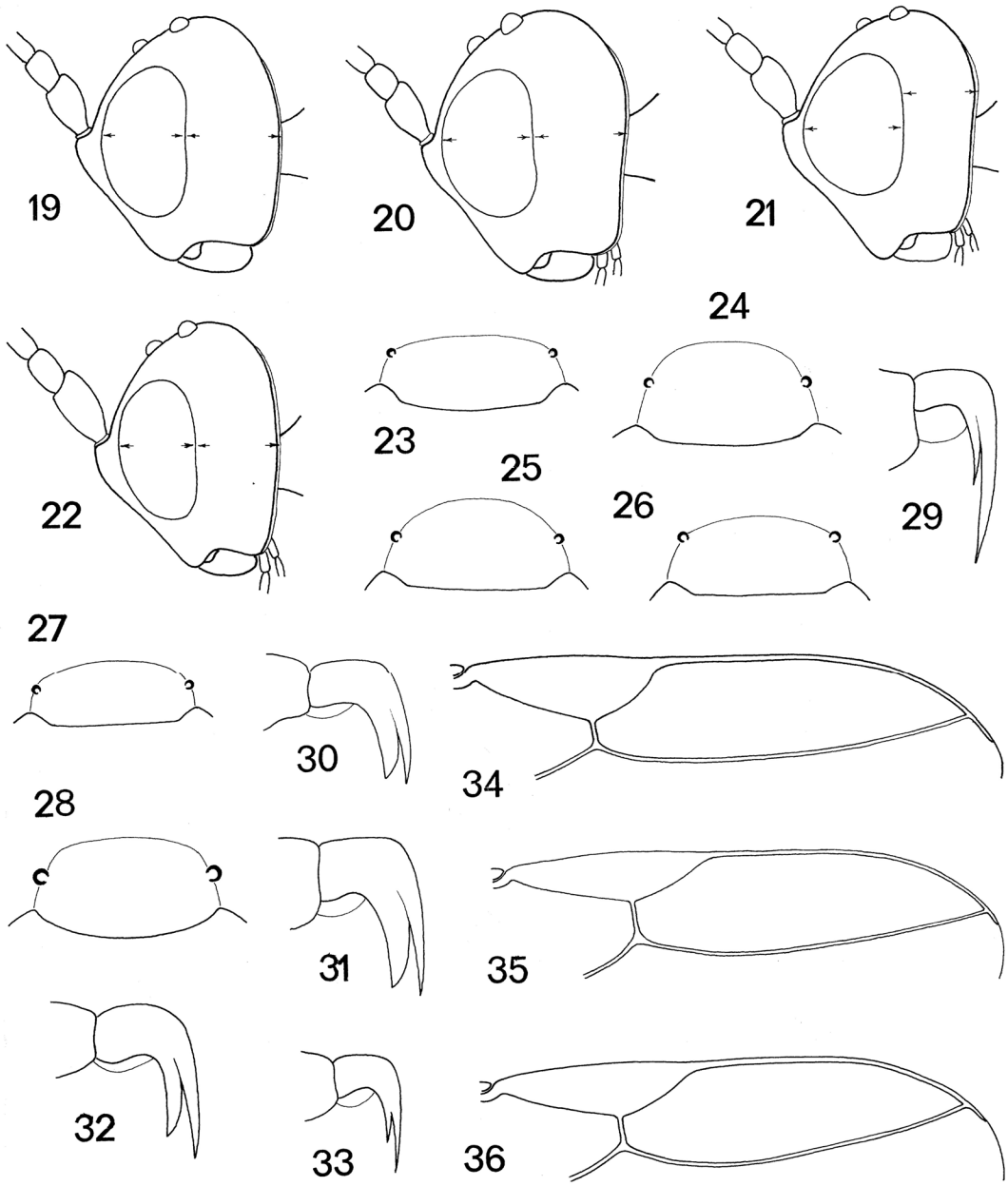
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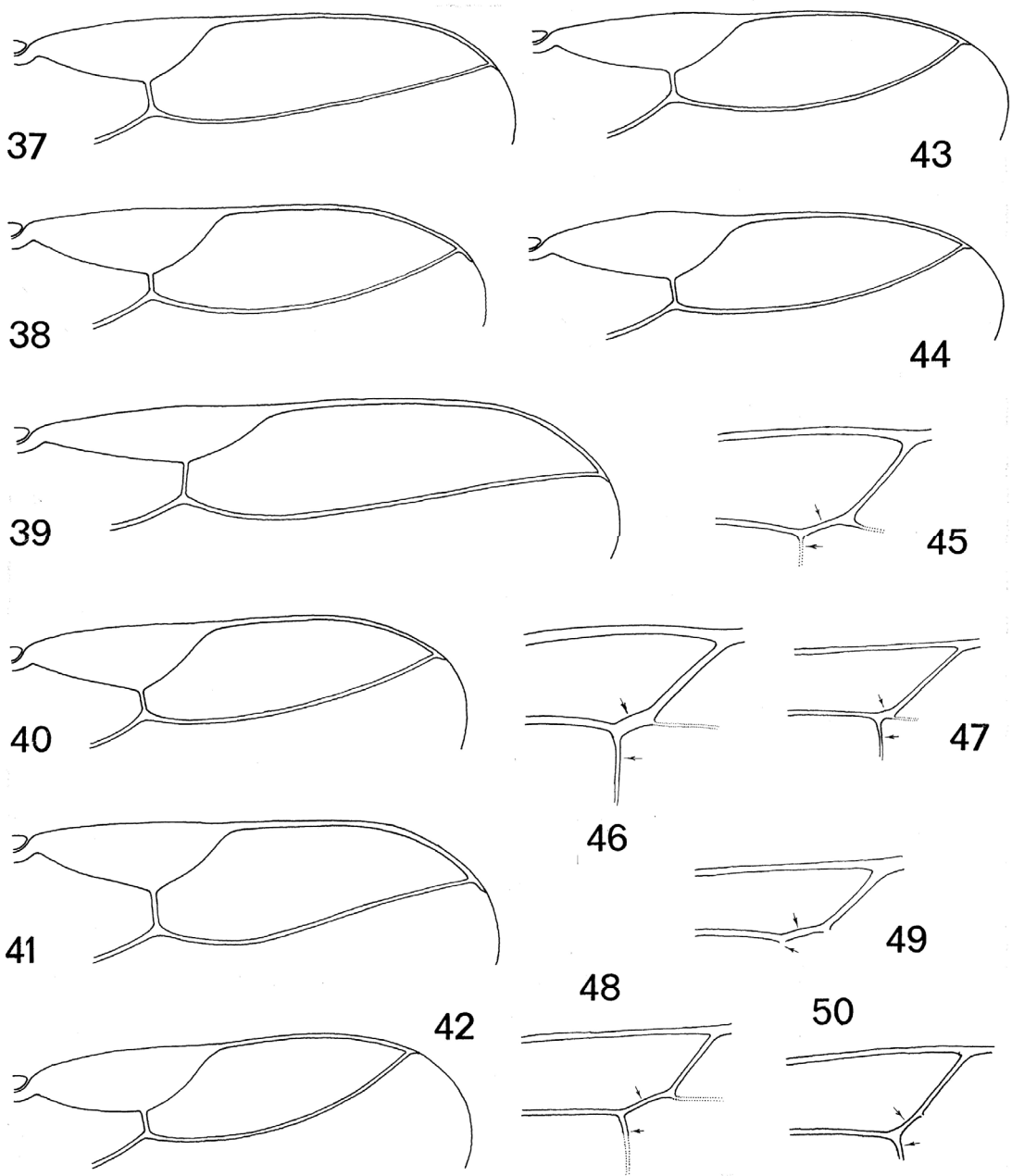
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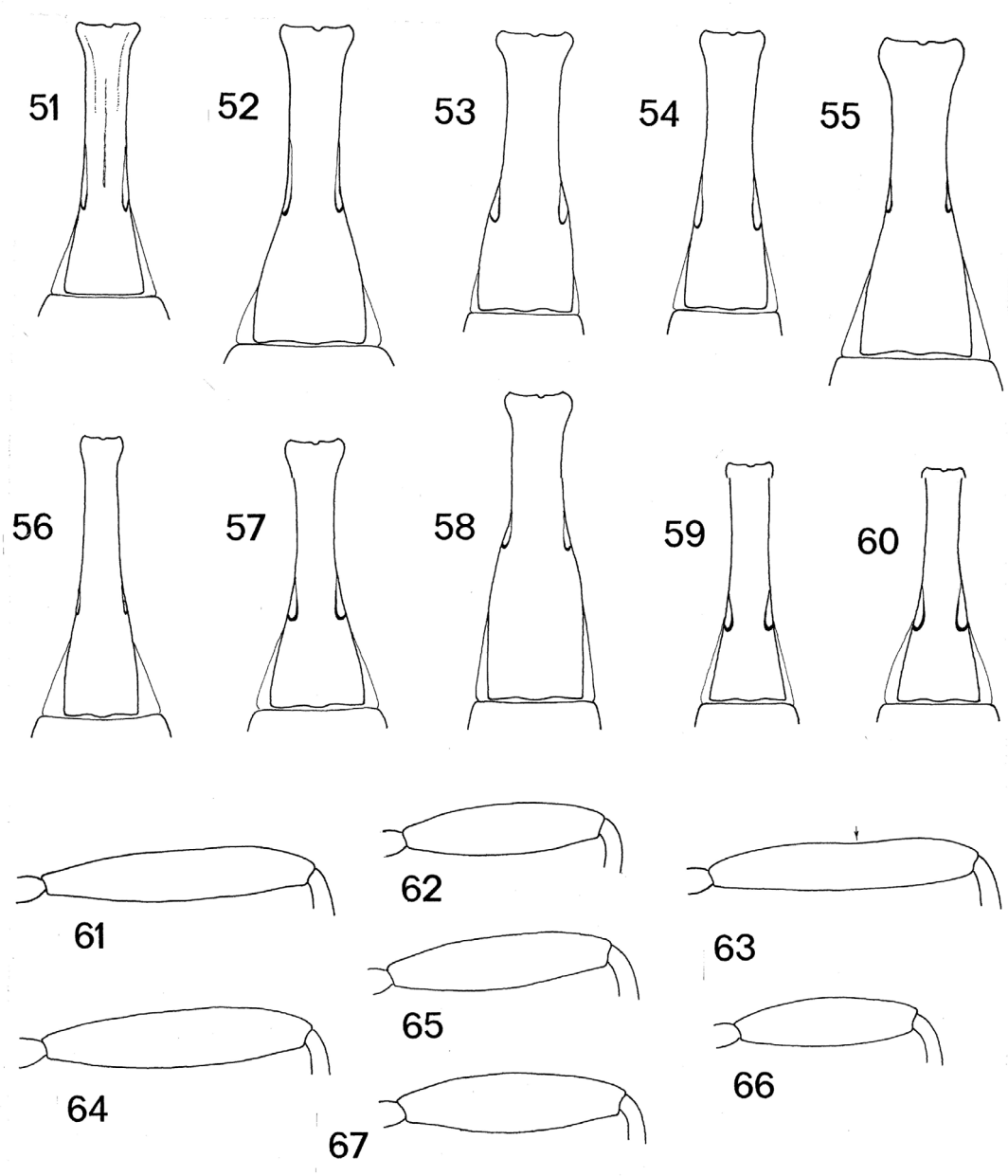
Figs 1–18. *Syntretus* species: 1–3 flagellomeres 1–2: 1 = *S. glaber* Chen et Van Achterberg, 2 = *S. parvicornis* (Ruthe), 3 = *S. subglaber* sp. n. — 4–15 head in dorsal view: 4 = *S. bulbus* Chen et van Achterberg, 5 = *S. choui* sp. n., 6 = *S. extensus* sp. n., 7 = *S. glaber* Chen et Achterberg, 8 = *S. lyctaea* Cole, 9 = *S. parvicornis* (Ruthe), 10 = *S. secutensus* sp. n., 11 = *S. subglaber* sp. n., 12 = *S. temporalis* sp. n., 13 = *S. testaceus* (Capron), 14 = *S. transitus* sp. n., 15 = *S. varus* sp. n. — 16–18 head in lateral view: 16 = *S. bulbus* Chen et van Achterberg, 17 = *S. choui* sp. n., 18 = *S. transitus* sp. n.



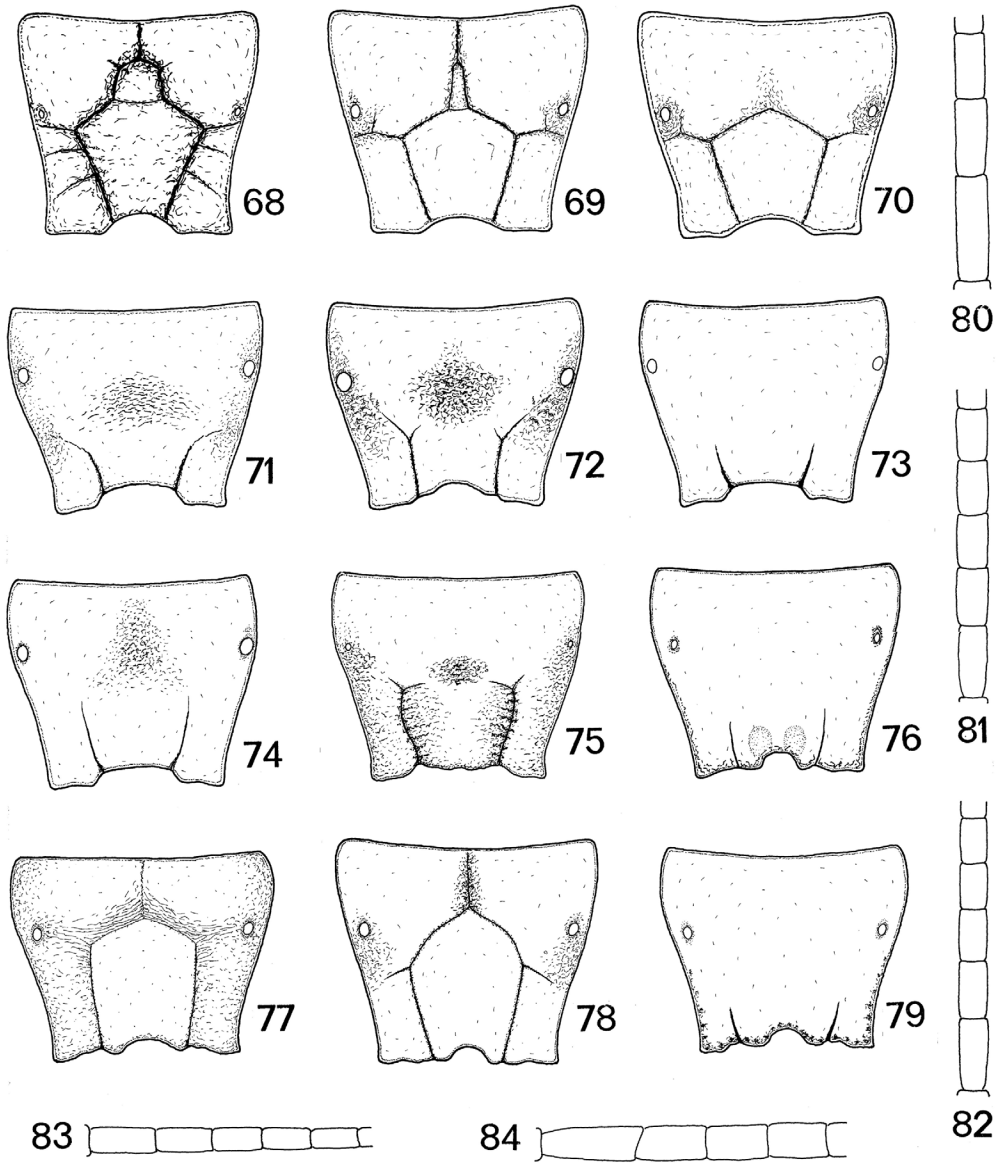
Figs 19–36. *Syntretus* species: head in lateral view 19–22: 19 = *S. extensus* sp. n., 20 = *S. secutensus* sp. n., 21 = *S. subglaber* sp. n., 22 = *S. temporalis* sp. n. — clypeus 23–28: 23 = *S. choui* sp. n., 24 = *S. extensus* sp. n., 25 = *S. subglaber* sp. n., 26 = *S. temporalis* (Capron), 27 = *S. transitus* sp. n., 28 = *S. varus* sp. n. — hind claw 29–33: 29 = *S. choui* sp. n., 30 = *S. extensus* sp. n., 31 = *S. secutensus* sp. n., 32 = *S. subglaber* sp. n., 33 = *S. transitus* sp. n. — distal part of right fore wing 34–36: 34 = *S. choui* sp. n., 35 = *S. extensus* sp. n., 36 = *S. glaber* Chen et van Achterberg



Figs 37–50. *Syntretus* species: distal part of right fore wing 37–44: 37 = *S. lyctaea* Cole, 38 = *S. parvicornis* (Ruthe), 39 = *S. secutensus* sp. n., 40 = *S. subglaber* sp. n., 41 = *S. temporalis* sp. n., 42 = *S. testaceus* (Capron), 43 = *S. transitus* sp. n., 44 = *S. varus* sp. n. — proximal part of right hind wing 45–50: 45 = *S. choui* sp. n., 46 = *S. extensus* sp. n., 47 = *S. glaber* Chen et van Achterberg, 48 = *S. secutensus* sp. n., 49 = *S. subglaber* sp. n., 50 = *S. temporalis* sp. n.



Figs 51–67. *Syntretus* species: petiole 51–60: 51 = *S. choui* sp. n., 52 = *S. extensus* sp. n., 53 = *S. lyctaea* Cole, 54 = *S. parvicornis* (Ruthe), 55 = *S. secutensus* sp. n., 56 = *S. subglaber* sp. n., 57 = *S. temporalis* sp. n., 58 = *S. testaceus* (Capron), 59 = *S. transitus* sp. n., 60 = *S. varus* sp. n. — hind femur 61–67: 61 = *S. bulbus* Chen et van Achterberg, 62 = *S. choui* sp. n., 63 = *S. extensus* sp. n., 64 = *S. secutensus* sp. n., 65 = *S. subglaber* sp. n., 66 = *S. transitus* sp. n., 67 = *S. varus* sp. n.



Figs 68–84. *Syntretus* species: propodeum 68–79: 68 = *S. bulbus* Chen et Van Achterberg, 69–70 = *S. choui* sp. n.: 69 female and 70 male, 71 = *S. glaber* Chen et Van Achterberg, 72 = *S. lyctaea* Cole, 73–74 = *S. parvicornis* (Ruthe), 75 = *S. subglaber* sp. n., 76 = *S. temporalis* sp. n., 77 = *S. testaceus* (Capron), 78 = *S. transitus* sp. n., 79 = *S. varus* sp. n. — Proximal flagellomeres 80–84: 80 = *S. extensus* sp. n. (flagellomeres 1–3), 81–82 = *S. temporalis* sp. n. (flagellomeres 1–5): 81 holotype and 82 paratype, 83 = *S. parvicornis* (Ruthe) (flagellomeres 1–5), 84 = *S. secutensus* sp. n. (flagellomeres 1–4).

臺灣的 *Syntretus* 屬瓢蟲繭蜂 (膜翅目：小繭蜂科：瓢蟲繭蜂亞科)[1]

Jenõ Papp[2]

摘 要

Papp, J.。2004。臺灣的 *Syntretus* 屬瓢蟲繭蜂 (膜翅目：小繭蜂科：瓢蟲繭蜂亞科) 。中華農業研究 53:39-62。

本文為對臺灣 *Syntretus* 屬種類的第一篇檢視報導，共檢視了 12 種，其中 *S. choui*、*S. extensus*、*S. secutensus*、*S. subglaber* 、 *S. temporalis*、*S. transitus* 及 *S. varus* 等 7 種為新種，其餘的 5 種為臺灣的新記錄種。對新種有詳細的描述，對已知種類也添加了分類註解。文中製作有此 12 種的分種索引，配以 84 幅鑑識用原圖。另外也將舊北區及東方區的 *Syntretus* 種類編纂成名錄。

關鍵詞：小繭蜂科、*Syntretus* 屬、新種、新記錄、分種索引、台灣、名錄。

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中華農業研究 (J. Agric. Res. China) 53:63~74 (2004)

利用植被反射光譜特徵估測稻株氮素含量[1]

張芳銘[2] 楊純明[3,5] 張愛華[4]

摘 要

張芳銘、楊純明、張愛華。2004。利用植被反射光譜特徵估測稻株氮素含量。中華農業研究 53:63-74。

本研究旨在量測近地面水稻植被高解析反射光譜，篩檢與稻株氮素濃度(重量百分比)有關的光譜特徵，並建立估測氮素濃度的光譜遙測模式。試驗結果發現水稻植被反射光譜類似於一般綠色植物的典型光譜，而施用不等量氮肥將改變水稻光譜曲線。無論一、二期稻作，大致上稻株葉片及全株的含氮百分比均隨著生育的進展而出現下降的趨勢。經反射光譜之各窄波段反射比與含氮百分比進行簡單直線相關分析，顯示各窄波段反射比對於葉片與全株的氮素濃度具有相似的敏感反應，惟各窄波段之反應強度不一，且一、二期稻作之間存在差異。整體來說，植被反射光譜各窄波段反射比與氮素濃度的相關性在一期稻作低於二期稻作，然兩期作皆以近紅外光波段(740-1300 nm)至短波紅外光波段(1300-1800 nm)的相關性高於其他波段；其中一期稻作以 1240 nm 窄波段位置之相關係數絕對值最高，然決定係數(R^2)低於 0.18，顯示存在相當變異，二期稻作以 1208 nm 窄波段位置之相關係數絕對值最高，R^2 可達 0.70 以上。續以多元線性複迴歸分析篩檢出與葉片及全株含氮百分比有關的多個特徵窄波段，發現兩期稻作均可利用 403 nm、558 nm 及 1607 nm 之反射比組成最佳的三元直線複迴歸模式，進行對葉片及全株氮素濃度的估測。模式估測值對一期稻作之稻株葉片及全株含氮百分比的估測準確度分別提升至 R^2=0.763 (P<0.0001)及 R^2=0.738 (P<0.0001)，對二期稻作則分別提升至 R^2=0.790 (P<0.0001)及 R^2=0.803 (P<0.0001)。經比較這些三元直線複迴歸模式的估測值與實測值後，發現機差均方根(RMSE)均小於 0.548，斜率則均大於 0.94，顯示無論葉片或全株的氮素濃度，模式估測可達近於 1：1 的比較結果。

關鍵詞：植被反射比光譜、光譜特徵、氮素含量、相關係數、多元線性複迴歸模式。

前 言

精準農業(precision agriculture or precision farming)的精髓係所謂特定地點的考量(site-specific aspects)，也就是針對某特定地區各定點的差異予以差異性處理以求達到既定標準，這是一種符合立

1. 行政院農業委員會農業試驗所研究報告第 2183 號。接受日期：93 年 4 月 21 日。
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足點平等為基準的農耕做法。以氮肥管理為例，即依照農田各定點農作物植體含氮狀態的變異，來施予差異量的氮肥，使達到既定的作物氮素需求標準。如此精準施用氮肥的方式，將可使得農田作物族群含氮狀態的空間變異趨向一致，進而獲得生產的充分表現、收穫物產量的穩定及品質的提升，並可因此減少或避免過量氮肥的使用，達到保育生態與保護生態的間接功效。

氮素的缺乏或過量是作物生產過程常發生的現象，作物植被因缺氮或高氮而出現的徵狀亦相當明顯與固定，因此吾人可藉由植被反射光譜的特徵來評估作物的含氮狀態(李等 2002；陳等 2001；蔡&陳 1966；Baret & Guyot 1991；Blackmer 1988；Gausman *et al*.1969；Hergert & Ferguson 1997； Milton *et al*.1991；Xue *et al*. 2004)。在這些徵狀當中，最重要者乃氮素供給與葉面積大小的密切關聯，以及氮素含量與葉綠素濃度間的顯著關係(李&楊 2002；吳&林 1968；楊 2003；楊等 2003a；蔡&陳 1966；Baret & Guyot 1991)。藉由植被反射光譜中與上述徵狀相關的波段分析結果，可以篩檢出特定的光譜特徵，並進一步建立反饋估測作物含氮狀態的光譜遙測模式(楊 2003b；Xue *et al*. 2004)。以水稻而言，在營養生長期間氮素供應不足將妨礙生長潛力的發揮，有效分蘗期間將因減少分蘗數而使穗數下降，在幼穗形成至孕穗期間則降低穗軸分枝及每穗穎花數，嚴重影響生產容量(朱 1988；吳&林 1968；Yoshida 1981)。所以，植體氮素的調節及人為氮肥的供應，對於包括水稻在內的許多農作物的生產管理是非常重要的。

傳統上作物植體中的含氮狀態係以化學分析予以估算，然而此種傳統方法需要重複的取樣，耗費相當的人力及時間進行分析工作。倘若需要進行全生育期間或廣大地區的取樣時，金錢、勞力與人力的支出將相當可觀，亦有緩不濟急之虞。因此，前述物理性量測植被反射光譜的遙測方法提供另一種選擇，能夠在短暫的時間內獲得非破壞性的快速偵測結果，尤其能夠擷取大面積的農田資訊及評估作物族群外觀形態與內在生理生化性質的特點(朱 1988；湯&侯 1969；楊&蘇 1997；Blackmer 1988；Gausman *et al*. 1969)，更是遙測技術獨具的功能和優勢。經由遙測農田在時間與空間上的變異分佈，吾人即可迅速的展開相對應的農場作業，也能夠因此實施合理而有效的精準管理。就氮肥的管理而言，在栽培作物之前可先予遙測評估土壤中可供吸收利用的礦物性氮素含量，再追蹤作物生長過程的含氮狀態，便能估算出農田各定點應當追施的氮肥用量，而實施氮肥的精準管理。本研究著重於作物生長過程含氮狀態的估測，試以建立適用的光譜遙測模式。

由於水稻是臺灣地區栽培面積最廣而產值最大的農作物，米食亦為民眾的傳統主食，如何實施精準的肥料管理，以適地適時適量的施用氮肥，仍為現行栽培水稻的重點課題。氮肥的精準管理不但可以提高稻作生產及施肥的經濟效益，更可以減少過量肥料造成的非點源污染(non-point source pollution)，具有維護生態與環境的積極意義(李等 2002)。惟在水稻生育前期不易以目視方法判別稻株的氮營養狀況，即便以肉眼主觀的判斷決定施肥量，也難以依照稻株需求均勻合宜的施用於稻田中。本研究採用光譜遙測技術偵測近地面水稻族群植被高解析(high resolution)反射光譜，篩檢與稻株氮素含量相關的光譜特徵，以建立評估含氮狀態的光譜特徵模式，期以助益於未來實施氮肥精準管理用途。

材料與方法

水稻栽培及氮素化學分析

本研究之田間試驗係於 2000 年至 2002 年在位於臺中縣霧峰鄉之行政院農業委員會農業試驗所農場(24º45'N, 120º54'E, 海拔高度 85 m)進行，參試水稻品種為臺農 67 號(*Oryza sativa* L. cv. TNG 67)，

分別在一、二期稻作期間量測水稻族群植被之反射光譜(350-2400 nm)，同時於每次量測光譜後實施取樣調查，化學分析葉片及全株(不含穗)的氮素濃度(含氮百分比)。

將 3-4 葉齡之水稻秧苗以機插方式移植於本田，每叢 3-5 株，南北向，行距約 0.3 m、株距約 0.18 m，栽植密度 185,000 hills ha^{-1}。一期作分別於 02/26/2001 及 02/27/2002 插秧，再於 06/27/2001 及 06/18/2002 收穫，二期作則分別於 08/07/2000 及 08/06/2001 插秧，而於 11/23/2000 及 11/28/2001 收穫。以施用不等量氮肥來產生不同的稻株生長速率、含氮百分比及收穫產量，計有 0、30、60、90、120 及 150 kg N ha^{-1} 等六種氮肥處理，每處理小區面積約 400 m^2 (50 m 8 m)。試區為一壤質土壤，屬於 Fluvaquentic Dystrochrept，試驗前測定之酸鹼值約 5.60，有機質 1.2%，而土壤之 Kjeldahl-N 為 755 ppm、有效性磷為 9.5 ppm、可交換鉀為 44.3 ppm、可交換鈣為 623 ppm、可交換鎂為 107 ppm。將各變級之氮肥量分成三等份，基肥(臺肥 39 號，N:P_2O_5:K_2O= 12%:18%:12%，臺灣肥料公司，高雄市)於插秧後一週內施用，第一次追肥(硫銨，21% N，臺灣肥料公司，高雄市)於抽秧後 2 週(二期作)或 3 週(一期作)施用，第二次追肥(穗肥，硫銨)於抽穗前 2 週(二期作)或 3 週(一期作)施用。

試驗期間發生嚴重病、蟲、草害時，依植物保護手冊(2002)噴施農藥，另注意灌溉控制，避免發生土壤缺水狀況。其中，丁基拉草(butachlor，5%粒劑，1.5 kg a.i. ha^{-1})施用插秧後一週內，本達隆(bentazon，44.1%溶液，200X，1.3 L a.i. ha^{-1})施用於插秧後三週；施用有機氮劑培丹(cartap，6%粒劑，1.8 kg a.i. ha^{-1})防治螟蟲(stem borer, *Rupella* spp.)，噴施加保扶(carbofuran，40%水溶性粉劑，800X，0.48 L a.i. ha^{-1})防治褐飛蝨[planthopper, *Nilaparvata lugens* (Stal)]及瘤野螟[leaffolder, *Cnaphalocrocis medinalis* (Guenee)]，以維粒黴素(Validamycin A，50%溶液，1000X，0.5 L a.i.ha^{-1})防治紋枯病(sheath blight, *Rhizoctonia solani* Kühn)。

每次水稻植被反射光譜量測後，即自量測標的樣區割取 6 叢稻株，區分為葉片(身)及莖稈(葉鞘加上莖)等二部分，秤取鮮重後，經 70ºC 烘乾 72 h 後秤取乾重。將其磨粉過篩(0.1 mm)後的樣本，以修正的 Kjeldahl 方法(李等 2002)分析葉片及莖稈之含氮百分比(%)。即取前述各處理小區葉片或莖稈之磨粉樣本約 0.2 g，倒入 50 ml 分解瓶，加入 4 ml 之 36 N 濃硫酸及 0.2 g 催化劑(K_2SO_4:$CuSO_4{\cdot}5H_2O$:Se= 50:10:1, w/w/w)，置於 800℃電爐上加熱約 2 hr，至分解液呈淡青綠色時取出冷卻至室溫，加入去離子水(NANOpure, Ultrapure Water System, Barnstead/Thermolyne Inc., Iowa, USA)至 50 ml，均勻混合之。從中取 5 ml 稀釋液，加入 5 ml 之 10 N NaOH 溶液，以蒸餾方式收取冷凝之蒸氣於加溴酚藍之 4 ml 4%硼酸溶液。當顏色由紅轉成紫藍後，再以 0.01 N HCl 溶液滴定至中和，使顏色由紫藍轉成紅。如此，樣本之氮素含量(%)由下列公式計算: $N(\%)=0.01\times W_N\times V\times(50\text{ ml}/5\text{ ml})\times(1/W)\times100\%$，其中 V 為 HCl 之滴定量(ml)，W 為樣本重量(mg)，W_N 則為氮素原子量(14)。

水稻植被光譜量測

無論一、二期稻作，均自水稻插秧後約一個月起至收穫止，選擇晴朗時段(10:00 至 12:00 之間)，定期量測水稻族群植被之反射光譜。依照預備試驗結果，插秧後一月側視之植被覆蓋率近於 70%，此時之反射光譜曲線之大部分波段不受水田背景光譜之干擾，亦不影響植體含氮狀態之光譜特徵篩檢。光譜量測系統包括田間攜帶式高解析輻射光譜儀(spectroradiometer, model GER-2600, Geophysical & Environmental Research Corp., Millbrook, NY, USA)、10 度視角(FOV, field-of-view)掃描鏡頭、內建操作軟體之手提電腦、電池及電纜線等，掃描光譜區間 330-2600 nm。光譜儀本身具有二組分光儀組(spectrometers)，其一為 512 個矩陣排列之矽質感測器(silicon detectors)，量測波段介於 330-1050 nm，

光譜解析力達 1.5 nm；其二為 128 個硫化鉛質感測器(PbS detectors)，量測波段介於 1050-2600 nm，光譜解析力約 11.5 nm (GER-2600 User Manual 1996)。在實用上將窄波段之中心波長取至個位數，並選用 350-2400 nm 光譜範圍進行分析，以避開掃描光譜區間兩端之嚴重元件電磁干擾，使得窄波段數減至 537 個。

將光譜儀及各組件架設於可上下左右移動的高空作業車上，並將掃描鏡頭與水稻植被表層距離固定於約 5.8 m 位置，以垂直方式量測直徑約 1 m 的圓形面積(目標區)反射光譜。由內建於手提電腦之操作軟體控制量測流程，每次掃描均自動量測所有窄波段之輻射測值(radiance)，經內建校正值轉換成正確輻射值。每次掃描設定為 4 次連續測定之平均值，先後量測水稻植被及參考白板(reference panel, 'Spectralon', Labsphere, Inc., North Sutton, NH, USA)之反射輻射光譜後，由軟體計算反射比光譜曲線。每次每一處理小區皆逢機量測 15 個不同定點，取其等之平均值光譜曲線為當次量測代表，參與資料統計分析。

統計分析

試驗資料之統計分析由 Statistical Analysis System 8.1 版 (SAS 1998)及 Sigmaplot 2000 (SPSS ASC BV, The Netherlands)進行處理，先以虛擬變數檢(測)定(dummy-variable test)比較同期作之不同年份及不同期作之同一年份光譜資料間的差異，當檢定結果無顯著差異時，即將資料合併分析。在簡單直線相關分析上，求取稻株葉片(leaves)及全株(shoot)之氮素百分比與各窄波段反射比之相關係數(correlation coefficients)，並獲得相關強度曲線。續由此曲線篩檢最大相關係數絕對值所在窄波段，比較此特徵窄波段反射比與葉片及全株含氮百分比之互動關係。

在多元(變數)線性複迴歸分析(multiple linear regression analysis, MLR)上，以 SAS 中的共線性診斷(collinearity diagnostics)及不同變數組合決定係數(R-square selection)等程序篩選出若干合適的窄波段，再以逐步迴歸分析(step-wise selection)程序配合 Mallows' Cp criterion，將這些特定的光譜特徵組成最佳的多元線性複迴歸模式。本研究之最佳三元直線複迴歸模式如下列: $Y_3=a+bR_{\lambda 1}+cR_{\lambda 2}+dR_{\lambda 3}$，其中依變數(dependent variable, Y_3)為葉片或全株之含氮百分比，獨立變數(independent variables, $R_{\lambda 1}$, $R_{\lambda 2}$, $R_{\lambda 3}$)為水稻植被反射光譜中篩選出之特徵窄波段(λ1, λ2, λ3)反射比。機差均方根(root mean square error, RMSE)係由以下公式計算:

$$RMSE = \sqrt{\frac{1}{k}\sum_{i=1}^{k}(X_i - \hat{X}_i)^2}$$

用以比較實測值(X_i)與估測值($\hat{X}_i$)之間評估精度。

結　果

本研究量測的水稻植被反射光譜，類似於典型綠色植物光譜的變化趨勢，惟受到施用不等量氮肥的改變(資料未列出)。尤其在氮肥多寡直接影響光合作用高低的可見光區及間接影響植被覆蓋大小的近紅外光區，呈現較明顯的反射比差異。經分析稻株葉片及全株的含氮百分比，顯示兩期稻作生育期間的氮素濃度隨著生育進展而下降(圖 1)，換言之，單位重量的稻株含氮百分比隨株齡的增加而減少。

將營養生長期至生殖生長期分別量測的水稻植被光譜範圍(350-2400 nm)各窄波段的反射比與圖 1 所列稻株葉片及全株含氮百分比進行簡單直線相關分析，發現各窄波段反射比與稻株含氮百分比的相關性並不一致，惟對葉片及全株含氮百分比的相對敏感程度雷同(圖 2)，且皆以近紅外光波段(740-1300 nm)及短波紅外光波段(1300-1800 nm)的相關性較高。但是，一期稻作與二期稻作的相關強度不一，大體上一期稻作之反射光譜各窄波段反射比與含氮百分比的相關性低於二期稻作者；其中，相關係數在一期稻作介於-0.5 至 0.5 之間，在二期稻作則介於-0.75 至 0.5 之間。

在近紅外光波段至短波紅外光波段之中，與葉片及全株含氮百分比之相關係數絕對值最大的窄波段位置，在一期稻作落於 1240 nm，適用一次直線關係，決定係數(R^2)分別為 0.176 (P<0.005)及 0.146 (P<0.01)；在二期稻作則落於 1208 nm，適用於二次曲線關係，R^2 分別為 0.698 (P<0.0001)及 0.730 (P<0.0001)(圖 3)。

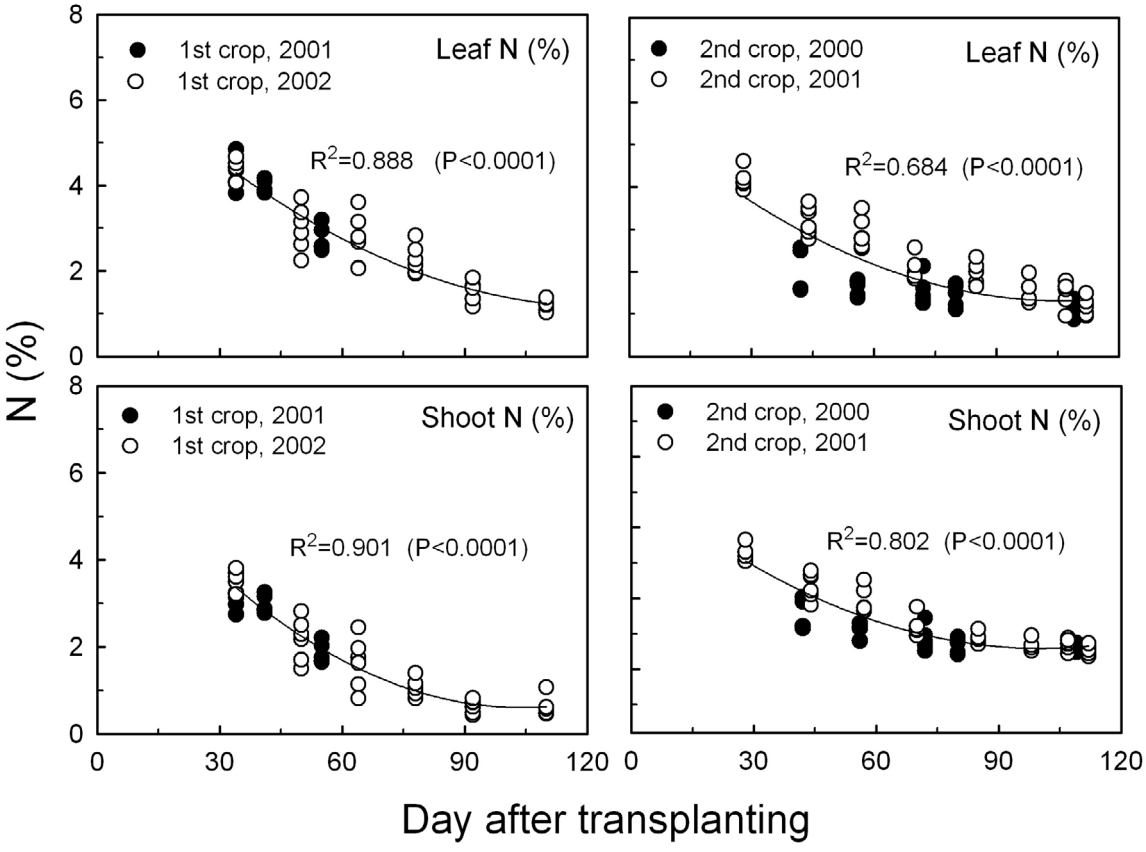


圖 1. 生長於 2000-2002 年兩期稻作期間施用不等量氮肥處理(0, 30, 60, 90, 120, and 150 kg ha^{-1})之稻株葉片及全株含氮百分比隨著生育進展的變化趨勢。

Fig. 1. Temporal changes of nitrogen concentrations in leaves and shoot for rice plants grown under different nitrogen fertilizer application rates (0, 30, 60, 90, 120, and 150 kg ha^{-1}) in the cropping seasons of 2000-2002.

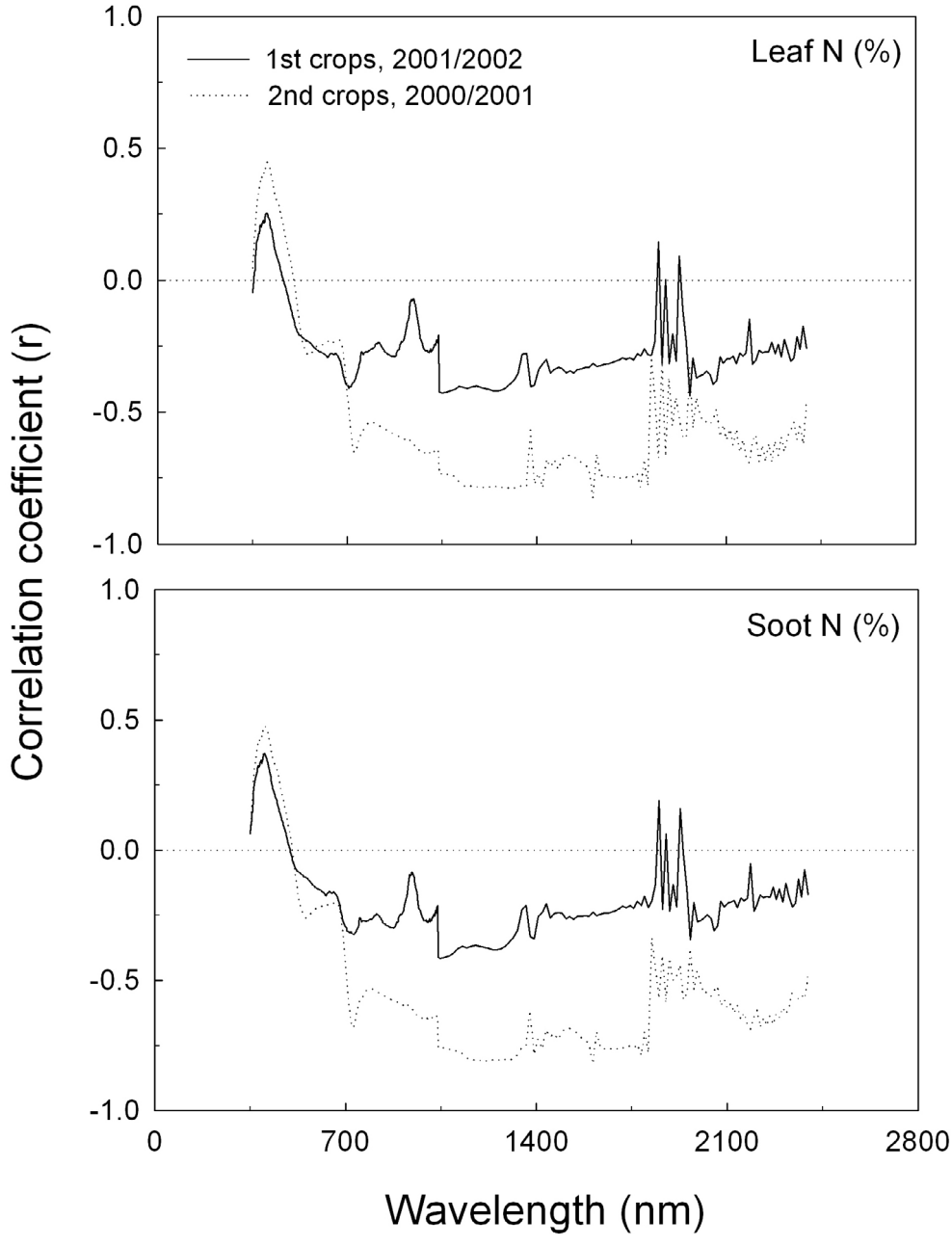


圖 2. 生長於 2000-2002 年兩期稻作期間施用不等量氮肥處理(0, 30, 60, 90, 120, and 150 kg ha^{-1})之稻株葉片及全株含氮百分比與植被反射光譜各窄波段反射比之簡單直線相關強度曲線。

Fig. 2. Correlation intensity curves between nitrogen concentrations in leaves and shoot and spectral reflectance of canopy for rice plants grown under different nitrogen fertilizer application rates (0, 30, 60, 90, 120, and 150 kg ha^{-1}) in the cropping seasons of 2000-2002.

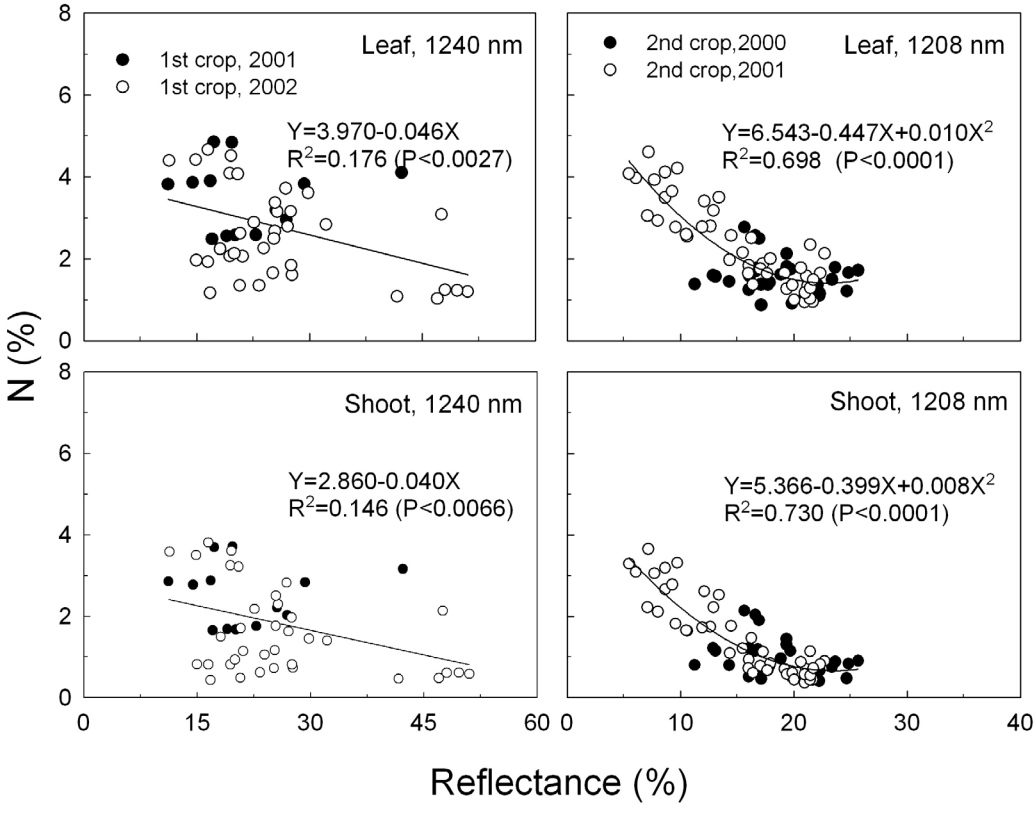


圖 3. 生長於 2001-2002 年一期稻作和 2000-2001 年二期稻作之稻株葉片及全株含氮百分分別與 1240 nm(一期作)及 1208 nm(二期作)窄波段反射比之關係。

Fig. 3. Changes of nitrogen concentration in leaves and shoot to spectral reflectance at 1240 nm and 1208 nm for rice plants grown in the first cropping seasons of 2001-2002 and the second cropping seasons of 2000-2001, respectively.

又由多元線性複迴歸分析結果，篩檢出最佳三元直線複迴歸模式的植被光譜特徵窄波段，如表 1 所示。這些三元直線複迴歸模式提高了對稻株氮素濃度估測的準確度，即增進了對稻株葉片及全株含

表 1. 生長於 2000-2002 年兩期稻作期間量測之水稻植被反射光譜特徵窄波段與稻株葉片及全株含氮百分比之多元線性複迴歸分析結果

Table1. Summary of multiple linear regression analysis for relationships between nitrogen concentration in leaves and shoot and spectral characteristics of canopy reflectance spectra for rice plants grown in the cropping seasons of 2000-2002

Item	$Y=a+bR_{\lambda1}+cR_{\lambda2}+dR_{\lambda3}$ [z]				R^2	P
	a	b	c	d		
First crops, 2001 and 2002						
Leaf N（%）	1.783	1.828	-0.804	0.091	0.763	<0.0001
Shoot N（%）	0.560	1.745	-0.703	0.077	0.738	<0.0001
Second crops, 2000 and 2001						
Leaf N（%）	2.632	0.874	-0.308	-0.097	0.790	<0.0001
Shoot N（%）	1.673	0.863	-0.293	-0.082	0.803	<0.0001

[z] Y represents leaf nitrogen or culm nitrogen; λ1=403 nm, λ2=558 nm, λ3=1607 nm.

氮百分比變異的估測。無論一、二期稻作，403 nm、558 nm 及 1607 nm 等三窄波段的反射比組合皆可適用，一期稻作的 R^2 分別為 0.763 及 0.738，二期稻作的 R^2 則分別為 0.790 及 0.807。而這些複迴歸模式的估測值與實測值的關係十分密切，斜率大於 0.94，機差均方根(RMSE)值小於 0.548(圖 4)。

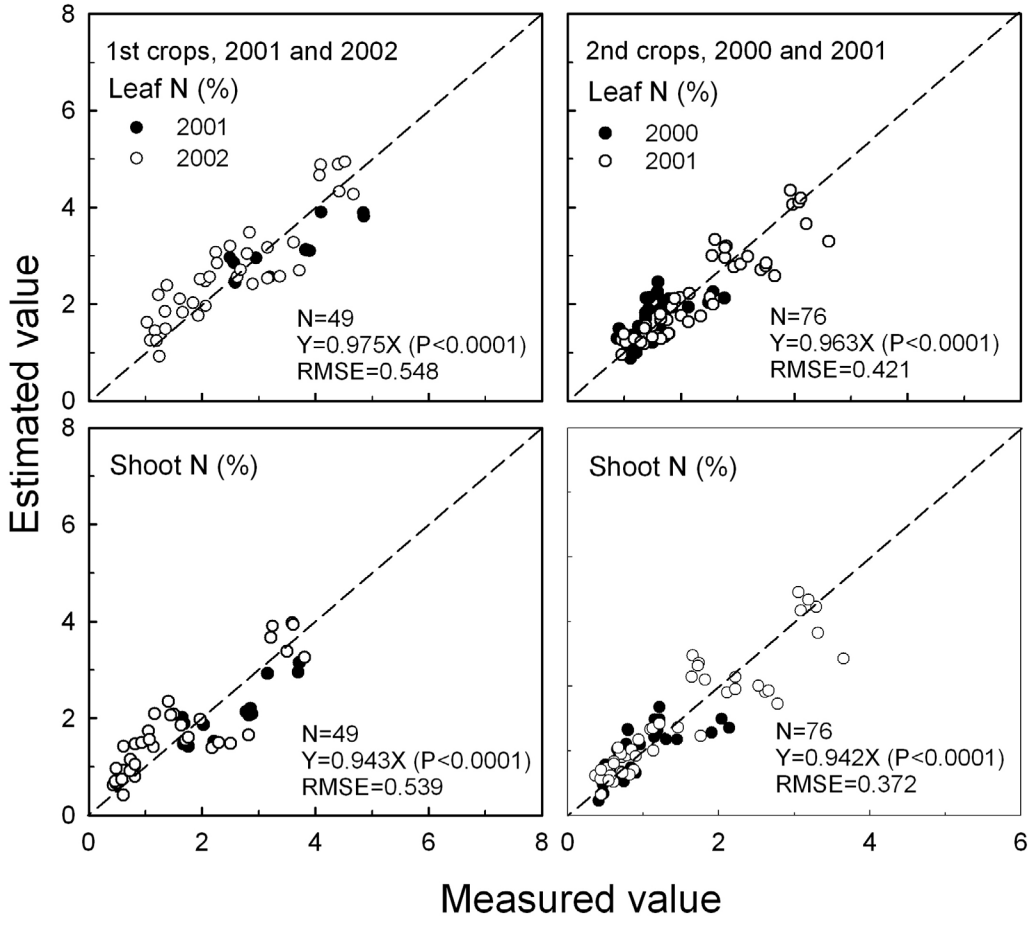


圖 4. 生長於 2000 年至 2002 年兩期稻作期間之稻株葉片及全株含氮百分比實測值與最佳三元線性複迴歸模式估測值之間的比較。

Fig. 4. Comparisons between the estimated values, from the best three variables linear regression models, and the measured values of nitrogen concentrations in leaves and shoot for rice plants grown in the cropping seasons of 2000-2002.

討　論

作(植)物植被的顏色、光澤、組織結構、組成分及生理代謝的實際表現，將影響其反射光譜特性，而族群幾何構造及周遭環境隨著時空的變異，亦將綜合反應在反射光譜質與量上的變化。一般綠色植物的健康植株，因為含有豐富的葉綠體(素)及其他色素(包括胡蘿蔔素、花青素及葉黃素等)，因此在可見光區(400-700 nm 或 400-740 nm)有超過 80%的吸收率，惟因色素與細胞壁在近紅外光區

(740-1300nm)幾乎不吸收，植被反射光譜在近紅外光區會出現一個反射高峰區(陳等 2001；Blackmer *et al.* 1996；Gausman 1982；Gausman *et al*. 1969；Sinclair 1971)。又由於葉綠素 a 及葉綠素 b 的主要吸收峰位於可見光區的藍光波段及紅光波段，分別以 450 nm 及 670 nm 為中心，所以植被約在 550 nm 附近會有較強的反射值(陳等 2001；楊&蘇 1997；Arnon 1949)。

本研究量測的水稻植被反射光譜，具有類似於上述綠色植物光譜的變化趨勢及陳等(2001)在水稻植被光譜量測之試驗結果，但是也發現施用不等量氮肥將改變光譜曲線(資料未列出)，如李等(2002)試驗之不同氮肥用量對稻株植被反射光譜的影響。許多文獻報告均顯示，施用高量氮肥將促進植被覆蓋，使其在可見光區因旺盛的光合作用而具有較低的反射比，在近紅外光區則因複雜植叢的多重反射與折射作用而具有較高的反射比；反之，缺氮的作物植被在可見光區的反射會因為葉綠素濃度的降低而增加，在近紅外光區的反射卻因為葉片細胞層數的減少而降低(Baret & Guyot 1991；Guyot 1990；Hergert & Ferguson 1997；Milton *et al*. 1991；Sinclair 1971)。因此，可見光及近紅外光波段的起伏變化，可作為作物植株氮素狀態的初步判斷。

從稻株葉片及全株的含氮百分比在生育期間的改變，發現無論一、二期稻作，氮素濃度均隨著生育的進展出現下降的趨勢(圖 1)。顯然地，稻株植體的氮素總量雖因體積的加大而增加，就單位重量而言，稻株的含氮百分比將隨株齡的升高而減少。推測在生育前期可視為發展營養器官(如葉、莖)的容積導致單位重量濃度隨著生長進展而逐漸下降，生育後期則更分別因為轉移至生殖器官(如穗)及老化組織枯萎掉落損失等，導致營養器官單位重量濃度的降低。

在量測的水稻植被光譜範圍內(350-2400 nm)，各窄波段之反射比與稻株葉片及全株的含氮百分比關係，可利用簡單直線相關強度分析，得到水稻全生育期間各窄波段與氮素含量的相關強度(圖 2)。大致上，葉片及全株含氮百分比與反射比光譜之相關強度曲線變化雷同，顯示各窄波段反射比對於葉片及全株的氮素濃度具有相似的敏感反應，皆以近光外光波段(740-1300 nm)及中紅外光波段(1300-1800 nm)的相關性較高，但是一期稻作與二期稻作的相關強度不一。整體來說，一期稻作的反射光譜各窄波段反射比與含氮百分比的相關性(相關係數介於-0.5 至 0.5 之間)低於二期稻作者(相關係數介於-0.75 至 0.5 之間)。在相關性較高的近紅外光波段至短波紅外光波段，一期稻作以 1240 nm 窄波段位置的反射比與葉片及全株的含氮百分比之相關係數絕對值最大，適用於一次直線關係(R^2 分別為 0.176 及 0.146)(圖 3)。惟因決定係數(R^2)不高，顯示具有相當大的變異，不適合僅以一次迴歸模式即可應用。二期稻作則以 1208 nm 窄波段位置的反射比與稻株葉片及全株的含氮百分比之相關係數絕對值最大，適用於二次曲線關係(R^2 分別達到 0.698 及 0.730)。因此，欲以單一窄波段反射比來估測與追蹤一期稻作的植株氮素濃度，將因變異範圍太大而不宜；二期稻作期間，若選取 1208 nm 窄波段，則可以獲得對葉片及全株的氮素濃度近 70%之估測準確度。由於兩期作之間的不同，反應各期作對氮素吸收與利用的效率並非一致，而在生長上的明顯差異亦造成含氮百分比的不同，總此使得一、二期稻作之最大相關窄波段位置並不相同。

本研究續利用多元線性複迴歸分析方法，期以篩檢出更多相關的光譜特徵窄波段，組合成複迴歸模式來提高對氮素濃度估測的準確度。由表 1 可知，無論一期稻作或二期稻作，均可建構出最佳的三元直線複迴歸模式，增進對稻株葉片及全株含氮百分比變異的估測。當選用 403 nm、558 nm 及 1607 nm 等三窄波段的反射比組合，對一期稻作植株葉片及全株的含氮百分比變異估測的準確度(R^2 表示)，將分別由 0.176 及 0.146 上升至 0.763 及 0.738，二期稻作植株的葉片及全株則分別由 0.698 及 0.730 升高至 0.790 及 0.807。經比較這些三元直線複迴歸模式的估測值與實測值後，發現二者關係十分密切，斜率大於 0.94，而機差均方根（RMSE）值均小於 0.548(圖 4)。根據此一結果，在本項研究之試

驗資料範圍內(低於 5%含氮百分比)，無論葉片或全株的含氮百分比，模式估測準確度近於 1：1 之比較。

綜合各項試驗結果，顯示可藉由簡單直線相關強度分析，篩檢出反射光譜中與稻株氮素濃度相關性最高的單一特徵窄波段。並可再藉由多元線性複迴歸分析，篩檢出與氮素濃度相關的多個特徵窄波段，組合成最佳的多元直線複迴歸模式，提高對稻株含氮狀態估測的準確度。本研究結果證明了利用光譜遙測於估測及追蹤稻株含氮狀態的可行性，也彰顯了光譜遙測於水稻氮肥精準管理的應用潛力。然而，為了增進光譜遙測模式對不同環境的適用性，未來應當增加更多栽植環境條件的量測資料，找出更適當的光譜特徵波段及數目，使建立的光譜遙測模式更能掌握氮素濃度的變異。其次，由於抽穗前後出現的明顯形態上差異，若能以抽穗期為界將水稻生育期切割成營養生長相(vegetative growth phase)及生殖生長相(reproductive growth phase)，將可能篩檢出不同生長相更直接相關的光譜特徵波段，進而提高光譜遙測模式的估測準確性與精確性。

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Estimation of Nitrogen Content in Rice Plants from Spectral Characteristics of Canopy Reflectance Spectrum[1]

Fan-Ming Chang[2], Chwen-Ming Yang[3,5] and Ay-Hwa Chang[4]

Summary

Chang, F. M., C. M. Yang, and A. H. Chang. 2004. Estimation of nitrogen content in rice plants from spectral characteristics of canopy reflectance Spectrum. J. Agric. Res. China 53:63-74.

This study was to measure near-ground remotely sensed canopy hyper-spectral reflectance data during the growing periods under varied treatments of nitrogen (N) fertilizer and to identify spectral characteristics in related to N concentration so as to establish spectral models for estimating N status in rice plants. Results indicated that reflectance spectra of rice canopy were similar to other green plants. Applications of different amounts of N fertilizer changed the spectral patterns, but did not change the decreasing trend of N concentrations in leaves and shoot following plant development in both cropping seasons. Correlation intensity curve between spectral reflectance in range of 340-2400 nm and N concentration was similar in leaves and shoot, suggesting that responses of reflectance along the spectral domain to N concentrations in leaves and shoot were quite alike. However, the correlation intensity curve of the first crops was not conformed to that of the second crops, and varied correlation coefficients existed in different narrow bands. In general, correlation coefficients from near-infrared (740-1300 nm) to short-wave infrared region (1300-1800 nm) had larger values than the other bands. Change of correlation coefficient was in between –0.5 and 0.5 in the first crops, with the maximum absolute value of correlation coefficient located at 1240 nm (R^2<0.18). The values of correlation coefficient in the second crops had greater variability, with the maximum absolute value found at 1208 nm (R^2>0.70). Results also indicated that estimation of N concentrations in leaves and shoot of rice plant may be improved by using multiple linear regression (MLR) models. The spectral wavebands suitable for the best three variables MLR models were 403 nm, 558 nm and 1607 nm for both cropping seasons. The slopes for the relationship between the estimated and the measured values of N concentrations were greater than 0.940, with the root mean square error (RMSE) less than 0.548.

Key words: Canopy reflectance spectrum, Spectral characteristics, Nitrogen content in rice, Correlation coefficient, Multiple linear regression model.

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中華農業研究稿約

一、本刊為行政院農業委員會農業試驗所發行之學術性季刊，刊登農業試驗研究相關之研究稿件：

論　　說：由本所邀請專家撰寫。

研究報告：關於農業科學未曾發表之研究報告，按題目、作者、摘要、前言、材料與方法、結果、討論、結論(可略去)、誌謝(可略去)、引用文獻等依序撰寫，圖與表附於其後，全文以不超過十印刷頁為原則。

研究簡報：關於農業科學未曾發表之簡短原始研究報告，不需分章節撰寫，但需附中英文摘要，全文以不超過四印刷頁為原則。

二、稿件應以中文或英文撰寫。來稿請送原稿一份及影印稿二份(內各附照片原圖，繪製圖版可附原圖與影印圖片二份)。文稿以 A4 大小之白色紙張(長 29.6 公分，寬 21 公分)空一行(double space)打字，上下應預留 3 公分，左右 2 公分空間，並自第二頁起在右下角以阿拉伯數字註明頁數。英文撰寫時，左邊對齊、字距一格，句距二格，以 Times New Roman 字體打字，以便審查。審查接受之稿件，隨文請附文稿內容磁片或電子檔案，以利電腦排版等作業。

三、稿件格式：

題　　目：宜簡短，能充分反應文旨，並附簡題(Running title)。英文題目除介系詞、連接詞等字外，每字第一字母應大寫。題目附小註，說明研究報告編號。

作　　者：作者姓名置中，中文名字間空一格；英文姓名則名在前姓氏在後，作者二人或二人以上時，以“，”相隔，最後一人前加“and”字樣，其表示方法如 Chen-Seng Huang, Chen-Chang Chen and Chyr-Guan Chen。每一作者需附小註，以說明所屬機關單位，通訊作者須附電子郵件信箱與傳真機號碼。題目與作者同置第一頁。

摘　　要：不分段落、宜簡潔明確。摘要之前應加作者、年代、題目、期刊、卷期及頁數，摘要之後應附關鍵詞 (Key words)，關鍵詞不超過五個，每個英文關鍵詞第一字母應大寫。摘要以不超過五百字為原則。以中(英)文發表者須附英(中)文摘要，置於本文之後，且內容須與中(英)文摘要相符，並附稿件編號、作者、通訊作者之小註。

本　　文：依序為前言 (INTRODUCTION)、材料與方法 (MATERIALS AND METHODS)、結果(RESULTS)、討論(DISCUSSION)、結論(CONCLUSION)、誌謝(ACKNOWLEDGEMENTS)、引用文獻(LITERATURE CITED)。除學名、專有名詞、化學式、數學公式、數字、度量衡單位及縮寫外，文句中不得中外文並用，須使用英 (外) 文以助明瞭時，須將英 (外) 文括號置於中文之後。材料與方法、結果兩部份如有必要須分章節，每一章節內並可再分小章節，以助明瞭文稿內容。章節題目(heading)單行置於該章節之前，小章節題目(sub heading)直接置於小章節之首。

表　　格：

1、每一表格應分別繕製，上方須有標題，並按文中出現順序加上表 1 (Table 1.)，表 2 (Table 2.) 等冠號。

2、中文稿之表格標題應中英文並列，冠號與表幅齊頭，表內項目及內容以英文書寫。英文標題及各欄英文細目，除第一字之第一字母大寫外，餘均小寫。

3、表格應加分析、簡化與歸納後始可列表。每一表格應在文中標示清楚，以便對照。

4、表中內容如需說明，均須附加小註，以 z, y, x,...等縮小小寫字母置於右上角，並於表下依次敘述。

5、表格僅列橫線。

6、性質類同之圖與表務請精簡與合併。

圖　片：

1、圖版以黑色墨水分別繪圖紙或描繪紙(白色透明紙)上。如以電腦繪圖，請以鐳射印表機或同規格之印表機列印，力求清晰。

2、圖中加註者，應於圖中說明，或以括號註明於標題之後。

3、照片以光面紙為原則，清晰度以縮小 50%可判讀為宜。多幅照片儘量製成併版；如有特別指明，應加圈或箭頭表明。顯微照相，應加比例尺大小；照片大小以不超過版面(25cm X 18cm)為宜。

4、圖版及照片下方應有標題 (或另頁繕寫)，並按文中出現的順序加上圖 1(Fig.1)、圖 2(Fig.2)等冠號，在文中須標示清楚，以便對照。中文稿應中英文併寫，力求明確。

5、除併圖外，每一圖片應單頁製作。

6、照片、圖片背面應註上冠號及作者姓名，以免排印錯誤。

誌　謝：不分段落，包括人物、經費來源等。誌謝可略去。

引用文獻：

1、引用文獻(LITERATURE CITED)－列舉確實曾引用之文獻。文獻以登載於學術性期刊、書籍、研討會之文章及學位論文為限。文中涉及引用文獻時，根據該文獻的作者與年代加以括號：如 (Sung 1991) (Lo 1991，1992) (陳 & 胡 1983) (Sung 1991；Hsu *et al.* 1992)，置於引用文處。

2、中文稿可列中、英文文獻，文獻原文為中(英)文者，須以中(英)文列文獻。英文稿之引用文獻須全採用英文。

3、中文文獻以作者姓氏筆劃為序，英文文獻以姓名字母為序。中英文併陳時，中文在前，英文銜接其後。作者相同時以年代先後為序。

4、文獻之書寫方法以期刊為例，依序為作者、年代、題目、期刊、卷期、頁數。

5、西文期刊名須用縮寫，其縮寫方式參照美國出版之〝Biosciences Information Service of Biological Abstracts〞規定，中文期刊用全名。

6、文獻的書寫範例如下：

(1)文獻出自學術性期刊

陳隆澤、胡念才。1983。水稻品種(系)對稻熱病抵抗性反應之研究。中華農業研究 32:1-13。

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四、單位、縮寫格式及統計分析

1、所有度量衡均採公制，數量如用中(英)文數字，單位以中(英)文全名表示；數量如用阿拉伯數字，單位以 SI units 為準，如一公尺(one meter, 1 m)、二公克(two grams, 2 g)、三公升(three liters, 3 L)、四毫升(four milliliters, 4 ml)。各常用之縮寫單位參照說明如下：

(1)長度：公里(km)、公尺(m)、公分(=厘米)(cm)、公釐(=毫米)(mm)、微米(μm)。

(2)重量(W)：公斤(kg)、公克(g)、μg。

(3)容積(V)：公升(L)、公撮(=毫升)(ml)、μl。

2、溫度：攝氏(C)、絕對溫度(K，但須加以說明)。

3、時間：年(yr)、月(mo)、週(wk)、日(天)(day,days)、時(hr)、分(min)、秒(sec)。

4、濃度：莫耳(M)、千分之一莫耳(mM)、當量(N)、千分之一當量(mN)。

5、重力加速度：*g*。

6、其他之縮寫：平均(*av*)、大約(*ca*)、直徑(diam.)。

7、統計分析：達顯著性水準以*、**或處理間顯著差異區分，以 a、b、c、…表示者均應加註顯著機率值 *P*(例如 $P<0.01$)；各統計量請採慣用符號，例如相關係數(*r*)、決定係數(R)、變異係數(cv)、標準機差(se)等。

五、生物學名以斜體字表示，屬名第一字母大寫。學名於摘要、本文、每一圖表中第一次出現時，須寫全名，如 *Citrus sinensis*；第二次出現時，屬名保留第一字母後加句點“.”，種名全寫，如 *C. sinensis*。生物學名於本文中首次出現時應加註命名者。

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