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全球定位系統與即時定位傳輸技術在精準農業上之應用[1]

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摘要：在農業試驗所農化系頂樓及精準農業十公頃試驗田附近，各建立二個適當之基地站位置，並委託民間測量公司精密測定座標，可以供作每次採樣及施肥時動態插分定位所需。藉由無線電即時動態定位之傳輸，將定位座標傳至電腦程式，可以提供施肥決策專家系統用以估算施肥量。建立全球定位系統之基地站後，即可發展即時定位技術，藉以推廣及應用精準農業之相關研究事項。將空間資料與屬性資料相互結合，並普遍應用於農耕機械上，則可進一步達到農耕自動化控制的目標。

關鍵詞：精準農業、全球定位系統、即時動態定位。

前　言

精準農業 (Precision Agriculture) 是一個現代化農業發展的新方向，爲了節省時間、經費、人力及物力的耗費，及早實施精準農業對於農場管理實有極大之經濟效益，發展及應用精確而有效的方法，藉以評估農田區塊之空間變異、作物產量高低及作物品質好壞因素之相關性，實爲執行精準農業成功與否之重要因素[14]。而透過全球定位系統 (Global Positioning System, GPS) 標出農田方位及座標，並以即時 (Real-time) 方式獲得公分級的定位精度，將其顯示於地理資訊系統 (Geography Information System, GIS) 上，再藉由農耕及土壤資料庫組成之鑑定與決策，判斷農田與作物間之變異性[11]，並進一步配合具有變異率功能的自動化農機操作系統，實施小田區之變異性處理，即可達成精準機械農耕的需求[16]。

台灣在多變的氣候環境、地形地貌特性與集約且複雜之耕作制度下，土壤在田間的各項性質也會隨時間而產生種種變化及變異，尤其與肥力有關的性質，每每隨耕作期之改變而有大幅度的變動，因此，建立土壤性質及土壤肥力受耕作制度、氣候與地理特性的影響而隨時間變化的關係，將有助於預測整體土壤品質變化的趨勢，更可用於防範土壤品質惡化及估計農業生產成本效益。配合全省網格式調查、土壤品質監測網及長期監測點等計畫工作所得之相關資料，再利用多變量統計分析、地理統計分析、時間序列統計分析及主成份分析等多項技術，即可得到各項性質的空間變異特性、時間變動特性、土壤性質間的相關特性與土壤肥力之主要控制因子等[15]。如進一步以全球定位系統配合現地調查，確定調查地點座標，建立影像光譜與調查點現況之關係式，以達到由點而面，藉以節省調查人力並掌握時效。

全球定位系統 (Global Positioning System, GPS)，係利用位於地球同步軌道上的同步衛星，以其相

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對位置的關係，來測量出精確的位置。GPS在進行大範圍、大面積的地面測量時，往往受限於地球本身的弧度與測量儀器的精密程度，甚至於當時的天候狀況或測量人員本身的許多因素，而導致測量的結果產生或多或少的誤差，故透過GPS與地理資訊系統 (Geography Information System, GIS) 的結合應用，更可以使我們快速、精確而完整地定出遙測影像或其他發生問題農地的位置，並迅速配合農業機械的使用，處理農田肥力之差異性，以發揮其即時、有效及正確的優越性。而地理資訊系統是一個電腦化、大量化之空間資料管理系統，用來進行資料儲存、擷取、操作、分析及展示使用者所定義之各種需求資料。地理資訊系統發展已有一段歷史，距第一個地理資訊系統的雛形系統有四十年之久，目前已廣範的應用在各行各業[3]。

GPS動態定位技術已日趨成熟，應用亦極爲廣泛，有學者[2]即採用Leica及Ashtech等兩種GPS即時動態定位系統，依照傳統導線測量、地物及地形測量、高差測量及路線測量等方式，分別進行數據獲得、圖形繪製及現地放樣等工作，以檢驗GPS動態測量之適用性及不同GPS動態定位系統間之差異性，進而建立對GPS動態定位技術之信心及開發GPS動態測量之作業規範。而基於成本考量，且有些GPS之應用必須是在運動狀態下，如自然資源調查、土地利用管理、地面載具導航、航空攝影測量及海洋探測等，皆需要高精度之GPS動態測量[13]。而對於結合GPS與GLONASS的研究，Cook[10]及Gouzhva[12]以長時間對GLONASS衛星進行觀測顯示，GLONASS衛星壽命較短暫，而某些衛星也偶有訊號不良或資料脫落的情形發生。如針對與GPS相近之GLONASS(Global Navigation Satellite System)系統資料進行處理，使得GPS與GLONASS資料能納入同一相位二次差模式中聯合平差，而在GPS 6顆、GLONASS 4顆衛星以上之情況下，使得求解成功率最佳可達90%以上[5]。

由於導航單點定位係依據虛擬距離觀測量來求解，而虛擬距離爲訊號由衛星送出之時刻、接收儀接收之時刻以及衛星軌道資料等之函數所構成，其主要誤差之來源主要有衛星時鐘誤差、廣播星曆誤差、電離層及對流層遲滯誤差、雜訊與多路徑效應誤差等[7]。由於受到這些誤差來源之影響，當僅使用C/A電碼作導航單點即時定位時，其精度一般爲15～25公尺左右。因此，若欲獲得較佳之精度，則可改採相對定位觀念，針對單點定位之誤差來源作一有效之改正，而目前廣泛發展的差分GPS (Differential GPS, DGPS) 定位法不失爲一種有效的方式[1, 6]。DGPS的基本原理係利用架設在已知位置之固定GPS接收站 (Base station) 所接收到的觀測資料，來計算由於上述誤差造成之影響，再利用無線電通訊設備將此一誤差改正值，即時傳送至使用者隨身之電腦上，並將改正值與使用者所接收的觀測值合併計算，即可求得較高精度之導航定位解，其精度甚至可達到公分級之程度。DGPS依進行數據處理時間的不同可分爲即時性 (Real time) 與後處理 (Postprocessing) 等兩種方法[9]，其優缺點如表一所示。

表1. 插分全球定位系統 (DGPS) 即時性與後處理之比較

Table 1. The comparison between real time and post processing of Differential Global Positioning System (DGPS)

	Real time	Post processing
Merits	Obtain product immediately	Higher precision
	Without data save question	Differential detect easily
		Can analysis product detailed
		No real time data transmit question
Demerits	Worse precision	Can't apply product immediately
	Differential detect difficultly	With data save question
	Data unsaved	
	Require real time data transmit	

(趙志弘, 1992)

即時動態測量 (Real time kinematic, RTK) 係採用差分定位觀念，利用高精度的GPS雙頻載波相位觀測量，進行即時定位測量。而即時動態測量 (RTK) 係能夠透過通訊設備，將主站的觀測量訊號，利用無線電數據機，即時傳回移動站的電腦上，而在其移動的狀態下求解向未知周波未定值，即時獲得點位座標。此與其他GPS測定方法相較，除了具有其他GPS測量方法的優點外，還具有施測迅速、移動快速，產量大，且不需後續處理的內業計算工作，更重要的是可以達到公分級的精度，甚具發展潛力[4,8]。

故本研究之主要目的係希望利用全球定位系統、地理資訊系統與即時定位傳輸技術應用於精準農業，並進一步整合傳統農耕及農作物之栽培管理，以初步建立國內稻作生產之精準農耕作業模式，且推廣應用至相關產業，以保育生態、保護環境、提升農業資源使用效率，達成眞正永續農業之發展，以提高農民之經濟效益及台灣農業生產之競爭力。

材料與方法

一、基地站設置與座標精密測量

(一) 基地站設置：

分別於農業試驗所農化系頂樓以及精準農耕十公頃水稻試驗田區附近之適當位置，利用水泥以及不銹鋼樁，各建立二個適當之基地站地標。其架構包含不銹鋼架以及不含不銹鋼架之GPS控制點各一個，並進一步委託民間測量公司，進行精確之控制點（參考點）座標測定，以供每次採樣及施肥時之動態插分定位。

(二) GPS控制點（參考點）衛星定位精密測量：

1. 作業時間：89年6月14日。
2. 作業地區：霧峰、草屯地區。
3. 作業內容：新設GPS控制點共4點，編號由A1至A4。
4. 座標依據：內政部土地測量局88年12月公佈之TWD97 TM二度分帶座標成果。
5. 測量規劃：包括已知控制點、使用儀器及測量作業規劃等。
6. 測量作業：至測區點位上設置衛星定位儀接收衛星訊號。
7. 成果計算分析：依規定程序獲得四個控制點之正確座標及正高高程。

二、無線電傳輸系統架設與動態即時定位軟體之撰寫

(一)無線電傳輸系統架設：

1. 架設可在二公里內有效傳輸之無線數據傳輸系統之天線組構。
2. 傳輸系統除現有之ASHTECH GG24之外，尚可與其他廠牌之定位接收器相容。

(二)動態即時定位軟體：

1. GPS即時差分定位，在現有之ASHTECH GG24機型下，精度需達到公分級之定位。
2. 輸出定位資料，並可存爲檔案或傳輸至模擬施肥軟體，以茲後續應用所需。

結　果

一、GPS控制點座標精密測定

(一) GPS控制點（參考點）衛星精密測量工作：

1. 測量規劃：

(1)已知控制點：

引用內政部土地測量局921地震後檢測之控制點作爲已知參考點，點號爲L188、L196、L199等三點，並選取附近一等水準點8406及8407二點（約1公里範圍內），以相對應之大地起伏差值，計算未知點之正高值，其參考點之座標值，詳如表二所示。

表2. 參考點之座標值及正高值

Table 2. Coordinate and altitude of reference points on horizontal and vertical

Reference points of plane

Point No.	Abscissa	Ordinate	Ellipsoid altitude	Remark
L188	216999.608	2660573.132	84.104	Order 3 satellite oriented control points
L196	216774.274	2657513.126	98.025	Order 3 satellite oriented control points
L199	221101.092	2656638.234	138.579	Order 3 satellite oriented control points

Reference points of altitude

Point No.	Altitude	Remark
8406	69.46410	Order 1 bench mark
8407	88.68405	Order 1 bench mark

(2)使用儀器：

Leica System 300型雙頻GPS衛星定位儀七台。

(3)測量作業規劃：

a. 檢測已知點相互間之座標變異，選用變化量較少之點位，爲本研究座標測算之依據。

b. 採用衛星靜態 (Static) 測量，每15秒接收一筆衛星資料 (epoch)，共計觀測二個時段，每時段間 (Section) 至少均含4點以上連結重覆接收，爲求控制測量達二等精度成果，故每點間同步觀測時間至少需在60分鐘以上。

c. 觀測資料以PCMCIA磁片錄存，下載後以Leica-Ski計算軟體，計算及分析座標成果。

2. 測量作業：

依據以上之規劃至測區點位上設置衛星定位儀接收衛星訊號，紀錄至PCMCIA磁片中。

3. 成果計算分析：

先以自由網平差偵錯後，分析誤差橢圓較大點位之衛星訊號週波脫落情形及衛星接收狀況，刪除誤差較大觀測量後，重新計算邊長及平差。最終平面位置以L188及L196二點固定，以計算本研究中四個控制點 (A1, A2, A3, A4) 之座標成果，如表三所示。高程則以8406及8407二點之橢球高與其正高值相減，求得二點之大地起伏差平均值，A1至A4點之橢球高減去此二點大地起伏差值平均值，即得A1至A4點之正高值。而控制點座標測定圖示，則如圖一所示。

表3. 農試所農化系頂樓 (A1, A2) 及十公頃試驗田 (A3, A4) 控制點座標測定值

Table 3. The coordinates of four control points

Point No.	Abscissa (X)	Ordinate (Y)	Altitude
A1	218854.2419	2658263.724	89.1922
A2	218853.4169	2658266.442	89.1604
A3	218360.1306	2658648.193	72.8825
A4	218356.1845	2658648.275	72.8719

二、無線電傳輸系統架設與動態即時定位軟體

(一) 無線電傳輸系統架設：

利用現有帕斯卡ASHTECH GG24之機型，進行即時定位無線電傳輸系統之架設。

(二) 撰寫程式接收及動態即時定位軟體：

結合Windows作業系統下之人機介面發展軟體Visual Basic，以及配合MapObject GIS系統，撰寫

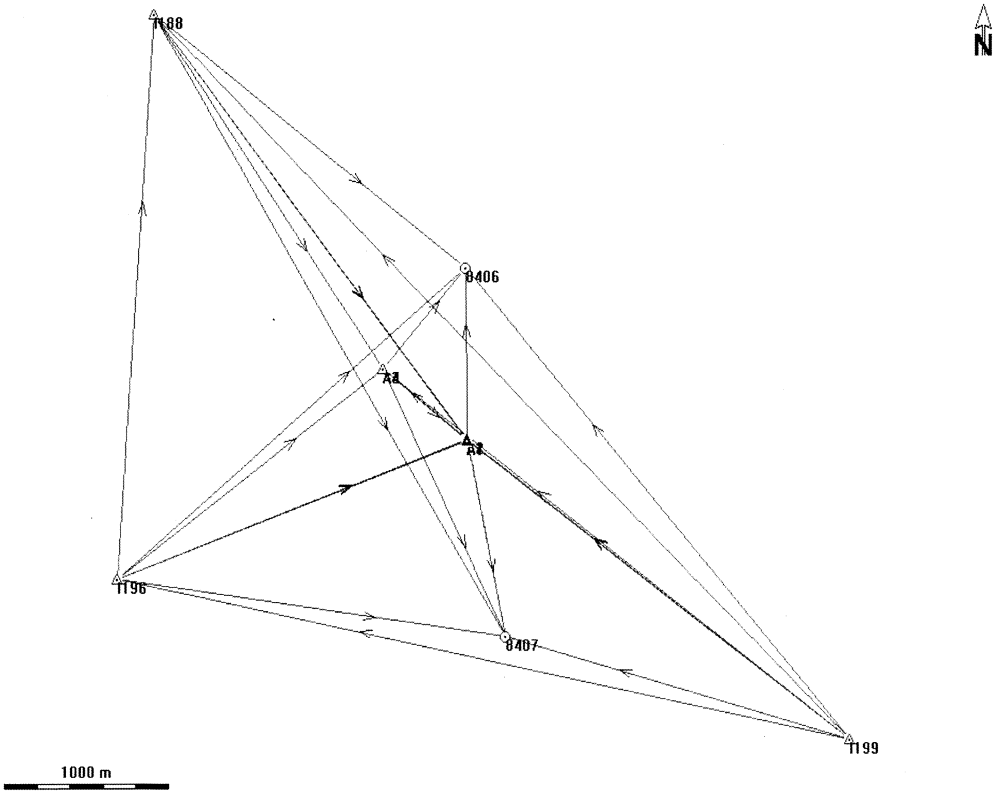


圖 1. 控制點座標測定圖示

Fig. 1. Determination of control points coordinate

一套適用於農機控制使用的動態即時定位套裝軟體，最終目的是要能夠將該套裝軟體普遍應用於農耕機械上，達到農耕自動化的目標。此外，對於GPS定位精度等級、GPS應具備之輸出入規格以及對於無線數據傳輸器材之規格，後續研究均應有所討論、建議及規範，以利各式GPS在精準農業試驗研究上之應用。

(三) 系統架構：

1. 參考站

參考站（如圖二所示）必須先有一已知控制點位的WGS-84經緯度座標，然後在該控制點位上架設GPS天線，主要的目的在接收該位置的GPS訊息，傳遞至GPS接收器上，而GPS接收器已知該控制點位置，經解算求得觀測量之相位與誤差值，透過無線電數據機調制成訊號，透過天線廣播給其他使用者接收解調，讓使用者能獲得更高的定位精度。

2. 移動站

移動站（如圖三所示）接收到由參考站傳來之修正資料後，經無線電數據接收機解調後，將資料傳遞給GPS接收器，與本身所接收衛星資料進行RTK，解算出精確座標後，將定位資訊NMEA 0183 Version 2.0 / 2.1格式傳遞給電腦，電腦除了在螢幕之電子地圖上顯示即時位置外，並可搜尋該位置所對應的屬性資料，以進一步達成農機自動化系統控制。

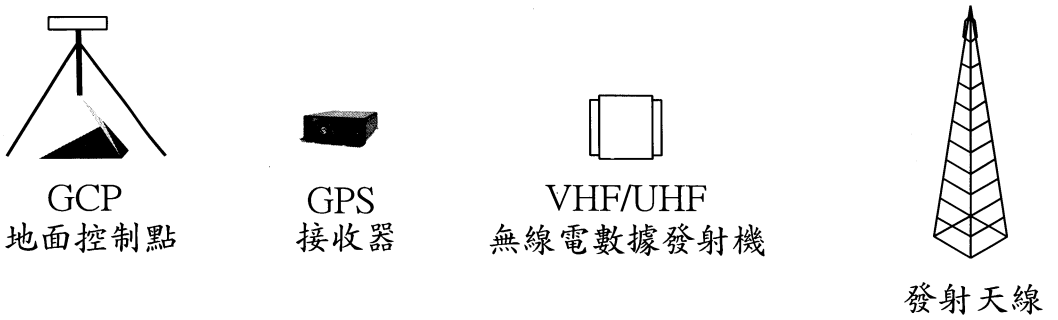


圖2. 全球定位系統參考站設置圖示

Fig. 2. Set up of base station for GPS.

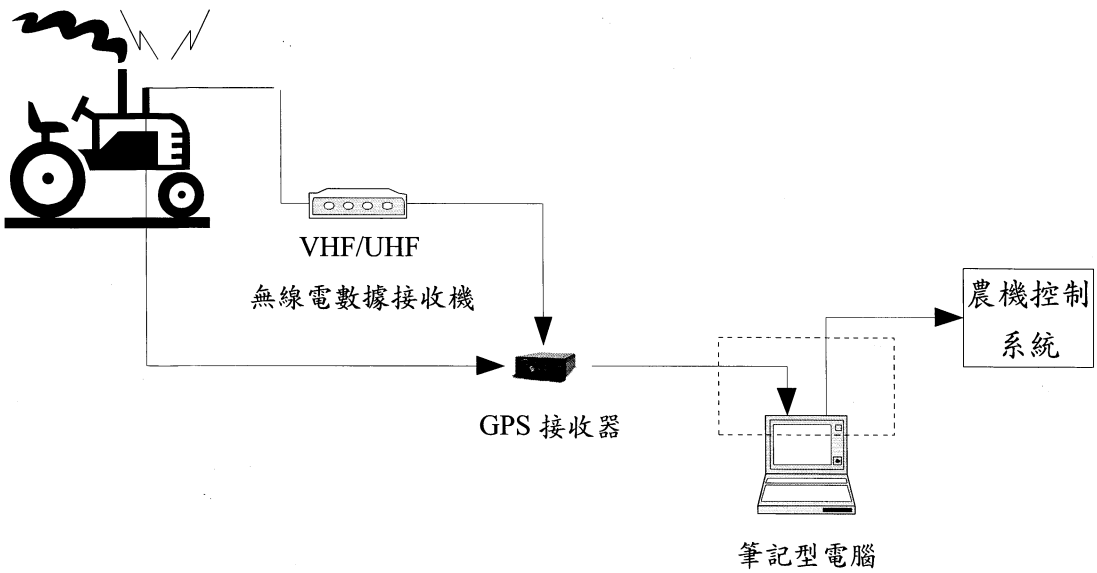


圖3. 全球定位系統移動站圖示

Fig. 3. The diagram of moving station for GPS

(四) 系統需求：

1. 參考站

(1) 具解算相位及即時廣播RTK資料的GPS衛星接收器，精度在1米以內。

(2) GPS RTK專用固定式天線。

(3) 輸入參考站座標控制模組（需經量測獲得）。

(4) UHF無線電數據發射機，傳輸速率在9600bps以上，發射功率則依服務區域而定。

(5) 發射無線電專用天線。

2. 移動站
   (1) 具接收相位及RTK資料解算的GPS衛星接收器，精度在1米以內。
   (2) GPS RTK專用移動式天線
   (3) 具備有兩個串列輸出、入埠，作業系統爲Windows 95/98/NT。
   (4) UHF無線電數據接收機，傳輸速率在9600 bps以上。
   (5) 接收無線電專用天線。
   (6) 手提電腦或工業級電腦：CPU Penitum II以上、RAM 32M Bytes以上、Hard Disk 10M Bytes Free、作業系統 Win95\98\NT以上版本、RS232C至少保留Com 1、Com 2皆未被其他裝置使用。

討　論

一、基地站架設之重要性

在試驗田附近設置完成適當之基地站位置，透過全球定位系統 (GPS) 標出農田方位及座標，以供每次採樣及施肥時動態插分定位，並以即時 (Real-time) 方式獲得公分級的定位精度，進一步藉由無線電傳輸將定位座標傳輸至電腦程式，以供施肥決策專家系統估算施肥量。

內政部所推動建立之衛星測量中心，目前尚未經由廣播系統提供即時動態衛星定位資訊，故建立本所衛星定位之基地站後，即可提供發展即時定位技術，藉以推廣及應用精準農業相關之研究事項。

二、即時定位傳輸技術之建立

爲了將現有的軟體能夠實際使用在農機機具上，我們必須制定一套能與農機控制閥溝通的介面，而又要考量到現有使用軟體的設備取得與方便性，於是考慮設備之間最常使用的溝通工具：串列埠(RS-232C)，以作爲與農機控制閥最好的控制模式。因爲，一般而言，農機的控制閥通常是由微處理器(Micro Processor) 所控制，而微處理器在對外的溝通上，最常使用的方法爲並列埠與串列埠兩種，其中，並列埠通常被當作開關控制使用，所以即時定位傳輸與精準模擬施肥軟體之使用，即以串列埠傳遞較爲恰當，而且不需特別的控制，僅需結合Windows作業系統下之人機介面發展軟體Visual Basic，並配合MapObject GIS系統，就可在不破壞原有軟體的完整性下，便能達到農機控制的實用性，以進一步調節農機控制閥，達到農機自動化控制的目標。

此外，移動站手提電腦原本只接收定位資訊NMEA 0183 Version 2.0 / 2.1格式，但爲了提供施肥資訊給農機控制系統使用，就必須將原本RS-232C 串列埠改裝，將一端接收端RX接至GPS、另一端傳送端TX接至農機控制系統，這樣電腦除了可以在顯示幕電子地圖上顯示位置外，並可搜尋該位置所對應的屬性資料，也可以進一步達成農機自動化系統控制的目標。

三、精準農業田區資訊庫之建立

利用GPS及GIS整合各項環境資料，並將空間資料與屬性資料相互結合，不僅只有看到幾何圖形，還可以獲得圖層間的屬性資料，即經計算分析獲得田地的施肥種類與數量，並且可由GPS獲知位於GIS圖形的所在位置，獲取對應幾何位置的施肥屬性資料，經過電腦分析判斷，以圖形方式來表示對應位置的施肥數量及大小。進一步經地理統計推估空間變異，即可建立空間資料庫，再經由傳輸資料並計算分析結果後，將可以繪製精確之田間施肥分佈圖。

四、精準施肥決策專家系統之建立

透過GPS標出農田方位並傳回座標位置，將其顯示於GIS上，再藉由農耕及土壤資料庫組成之鑑定與決策，判斷農田與作物間之變異性，依據精準農業專家系統輸出之施肥施藥決策圖、農機行進速度、所需控制之項目及變率多寡，並配合具有變異率功能的自動化農機操作系統，實施小田區之變異性處理，即可達成精準機械農耕的需求。

藉由GPS可精確地同步偵測農耕作業的狀態，並加以判斷某一田區的各項狀態，包括土壤、肥力及植生變化等，明確顯示農機機具在田區之位置、各項目之變率、施用率及達成率等，以作爲農場最佳化經營管理決策的依據，進而減少農業生產對生態環境的衝擊與節省農業資材。

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Application of Global Positioning System and Technique of Real Time Kinematics Positioning on Precision Agriculture [1]

Jen-Chyi Liu [2], Tsang-Sen Liu [2], Chien-Liang Chu [2], Horng-Yuh Guo [2] and Ming-Chih Yeh [2]

Summary

Two suitable base stations were set up on the rooftop of the building of Department of Agricultural Chemistry, TARI and adjacent the experimental plot of precision agriculture, respectively, to determine the accurate coordinate for sampling and fertilization. According to the best real time kinematics positioning, we could transmit the positioning coordinate to a field computer for estimating the fertilization rates by a decision support system. Setting up the base stations of the global positioning systems to develop the techniques of real time positioning could spread and applicant any relational research on precision agriculture. Combination and application spatial data and property data on agricultural machine could catch the objectives for auto control of agricultural cultivation.

Key words : Precision agriculture, Global positioning system, Real time kinematics positioning.

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水稻雜種弱勢基因 *Hwc-1*, *Hwc-2* 之表現及其利用之研究

I. *Hwc-1*, *Hwc-2* 基因對農藝性狀之影響及台灣水稻品種之基因型探討[1]

曾東海[2]　王強生[3]

摘要：水稻爲自交作物，但栽培種仍有0～6%的天然雜交率，爲預防花粉污染，黑糯稻等有色品種，被列爲不鼓勵、不輔導的品種。雜種優勢廣泛地被研究與利用，而雜種弱勢之研究雖偶可見，但鮮有其利用之報告。水稻雜種弱勢係受一組互補基因 *Hwc-1* 與 *Hwc-2* 所控制，當此互補基因皆爲顯性時，植株表現生育弱勢。本試驗探討 *Hwc-1*, *Hwc-2* 基因對農藝性狀之影響及台灣水稻品種之基因型，當 F_1 具 *Hwc-1*, *Hwc-2* 基因型時，其苗高及株高呈現生長弱勢，穗數多只1穗，平均穗長約10.6cm；產量構成要素中穗重、穗數、一穗穎花數及單株穀重亦顯現雜種弱勢，但千粒重及稔實率之差異較小。調查雜種弱勢之 F_2 苗高，分析其生育弱勢與正常株之分離比，符合受一組互補基因控制之9：7分離比。一般水稻品種之基因型爲 *Hwc-1*, *hwc-2* 或 *hwc-1*, *hwc-2*，而Jamaica則爲 *hwc-1*, *Hwc-2*，以107個台灣育成及4個引進之粳型與29個秈型水稻，共140個品種（系）與Jamaica進行檢交，結果有103組粳稻（佔92.79%）及4組秈稻（佔13.79%）之 F_1 呈現雜種弱勢，顯示，就 *Hwc-1* 基因而言，台灣極大部分粳稻爲顯性而秈稻多爲隱性。台灣水稻栽培約90%爲粳稻，若能育成具 *Hwc-2* 基因之黑糯品種，應可降低花粉污染問題，黑糯之栽培將可在粳稻栽培區列入輔導，提高農民栽培水稻之收益與意願。

關鍵詞：水稻、雜種弱勢、互補基因、秈稻、粳稻、花粉污染、天然雜交、有色米。

前　言

稻米爲國人的主食，亦爲臺灣主要農作物，栽培水稻已有數百年的歷史，但有系統的品種改良工作，則始於日本人據臺後；日人據臺初期除引進日本品種試種外，調查整理臺灣原有水稻品種，發現臺灣之水稻品種混雜情形甚爲普遍，尤其混雜紅米之程度極爲嚴重，影響稻米品質至鉅。自1906年起進行全島性的紅米去除工作(3)，至1921年計畫結束時，紅米幾乎從臺灣絕跡，對臺灣稻米品質之提昇有甚大的意義。

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水稻雖爲自交作物，其天然雜交率因種的不同而有差異，多年生野生稻如 *O. perennis* complex 或 *O. rufipogon* 等爲 7～56%，一年生的野生稻如 *O. breviligulata* 爲 3～20%，栽培稻亦有 0～6%[13]。因此，數年前當農民自行引進栽培「黑糯米」品種，因其具有「有色基因」，可能經由花粉飛散而污染一般栽培品種，致有降低稻米品質的疑慮。主管機關因此擔心，歷時 15 年動員全台相關人員『去紅米』的歷史在臺灣重演，而採取不推廣、不輔導的消極措施，但每期作仍有農民栽培「黑糯米」，雖然其栽培面積所佔比率極低，但問題仍懸而未決，實應謀防止或降低栽培「黑糯米」可能引起的花粉污染之良策，進而針對「黑糯米」栽培進行研究改良，以提昇農民的收益。隔離栽培，雖爲防止花粉污染的有效方法，但在自由意識下，實難有效地規範農民遵守；另一降低花粉污染率的方法，似可考量「如何使受花粉污染的天然雜交種子無法繁殖，以防止其擴散，或使其繁殖率降低而減少擴散」，而此功能須存在栽培品種（黑糯米）內，不待外力即可達成，才能達到目地。

雜種優勢已被廣泛地研究利用，種植雜種 F_1 提高作物產量，已然成爲趨勢。而雜種弱勢雖亦被研究，在 1943 年就有研究者指出作物具有雜種 F_1 弱勢，爾後許多學者亦提出相關的研究報告，在小麥雜種 F_1 有壞死 (necrosis) 或白化 (chlorosis) 現象 (Caldwell and Compton 1943[5]; Hermsen 1963[6] : Tsunewaki and Kihara 1962[19])，大麥則有雜種 F_1 致死 (lethal)、壞死或白化等三種弱勢現象[20]；1957 年 Oka 提出在某些秈稻雜交時，其雜種 F_1 呈現弱勢[10]，Amamiya 和 Akemin 於 1963 年報告[4]指出秘魯的栽培種 Jamaica 與日本品種如 Norin 8 等雜交，其雜種 F_1 之 15 日苗齡苗高爲其親本之 40～60%，而其根長亦只有 5～8%，呈現雜種弱勢，此種表現係受根抑制互補致死遺傳基因 (Root inhibiting complementary lethal genes) (*L-r*$_1$ 、 *L-r*$_2$) 所控制；1983 年 Sato 與 Hayashi[14]指出，日本品種有 94% 與 Jamaica 之雜種 F_1 具有弱勢，而亞洲栽培之粳稻屬於溫帶者與 Jamaica 雜交亦具雜種 F_1 弱勢，但屬於熱帶之粳稻品種或秈稻則多數沒有或全無此現象，氏等認爲係受一對顯性互補基因控制，將此基因命名爲 *L-2-a* 、 *L-2-b* 。

Jamaica 與某些品種呈現雜種 F_1 弱勢，係受一對顯性互補基因所控制，其互補基因型，Amamiya 和 Akemin 於 1963[4]認爲 Norin 8 之基因型爲 *L-r*$_1$ *L-r*$_1$ ++ ，Jamaica 之基因型爲 ++ *L-r*$_2$ *L-r*$_2$ ；Sato 與 Morishima[17,]在 1987 之報告爲首度將雜種弱勢基因以 *Hwc* 命名，且將 Jamaica 之基因型定爲 *Hwc-1*, *hwc-2* ，並認爲 *Hwc-1* = *L-r*$_1$ = *L-2-b*, *Hwc-2* = *L-r*$_2$ = *L-2-a* ，與一般基因命名之常理不符，推測應爲誤植，是爲錯誤造成之始；所幸 Sato 、 Sano 及 Nakagahra 在 1987 於 RGN (Rice Genteties Newsletter) 水稻基因符號統一命名報告中，將雜種弱勢基因三種符號予以統一，正確標示；亦即 Jamaica 之基因型爲 *hwc-1*, *Hwc-2* 【(*lr*$_1$ 、 *Lr*$_2$) (*l-2-a* 、 *L-2-b*)】，一般亞洲水稻爲 *hwc-1*, *hwc-2* 【(*lr*$_1$ 、 *lr*$_2$)、(*l-2-a* 、 *l-2-b*)】或 *Hwc-1*, *hwc-2* 【(*Lr*$_1$ 、 *lr*$_2$)、(*L-2-a* 、 *l-2-b*)】，爲本報告引用之依據。

目前臺灣栽培之粳稻品種，多自日本引進再經改良而成，而日本品種及多數溫帶之粳稻品種具有 *Hwc-1* 、 *hwc-2* 基因，而 Jamaica 則具有 *hwc-1* 、 *Hwc-2* 基因[4, 9, 14, 16]。因此，當 Jamaica 與前述粳稻品種雜交，其雜種 F_1 將因顯性互補基因之作用而呈現雜種弱勢。如能針對臺灣栽培之粳稻品種之基因型進行調查，探討其與 Jamaica 雜交 F_1 之表現是否呈現雜種弱勢。換言之，臺灣水稻品種中有多少比率是具有 *Hwc-1* 、 *hwc-2* 基因型，若主要栽培品種皆或有極高比率是具有 *Hwc-1* 、 *hwc-2* 基因，則將 Jamaica 的 *Hwc-2* 基因導入「黑糯米」品種，育成具有 *Hwc-2* 基因型的「黑糯米」品種，供農民種植，當其花粉飛到臨區具 *Hwc-1* 基因的粳稻田，產生天然雜交時，當此粳稻做爲一般白米使用時，因其雜種 F_1 種子外觀品質與母本相似，且比率極低，故對品質之影響極小。若做爲再繁殖用時，其雜種 F_1 種子混在一般種子中，由於爲雜種弱勢，其生長將被抑制，甚至在生育中即死亡，因此將可降低或甚而無花粉污染問題。本試驗自民國 80 年第二期作開始，以 Jamaica 與臺灣主要栽培品種雜交，探討 Jamaica 特有的 *Hwc-2* 基因，對臺灣主要的水稻栽培品種之影響，並評估主要栽培品種中有多少是具 *Hwc-1* 、 *hwc-2* 基因型，作爲決定是否將 *Hwc-2* 基因轉移到「黑糯米」品種，育成具 *Hwc-2* 基因的黑糯米等有色種皮品種之依據。

材料與方法

自民國80年二期作開始，以具有 *hwc-1*, *Hwc-2* 基因型之Jamaica品種與臺農67號 (TNG67) 等4個粳稻品種（系）雜交，而後陸續再與臺灣之其他栽培品種或引進品種（系）雜交，至84年二期作止，累計與111個粳稻（含4個引進品種）及29個秈稻雜交，總共檢交140組合，這些雜交組合分別調查雜種F_1之苗高、生育情形與產能或F_2之苗高分離比，經檢交之品種（系）名稱詳如表6。

雜交方法係以傳統的溫湯去雄（母本以43℃之溫水浸7分鐘），剪去已開花及未成熟之穎花，並將雜交當日開花之穎花剪穎（剪去上部三分之一），待父本花粉成熟時，以集團授粉法授粉，再套上半透明紙袋，而後培育於溫室，待雜交種子成熟後（一般爲雜交後28～30天），剪下雜交穗於45℃乾燥之。

栽培方法係將親本及F_1同時種植，行株距爲28×16公分，三行區，每行種10株，單本植，順序排列，無重複，栽培時期及管理方法皆按慣行法進行。

分別於適當時期調查親本及雜種F_1之苗高、株高、分蘗數、穗長、穗重、一穗穎花數、稔實率、千粒重等農藝性狀，並調查F_2之苗高分離比，試驗結果以小區單株之平均值表示。

結　果

一、雜種F_1的苗高

爲探討雜種弱勢基因對水稻秧苗生長發育之影響，於民國84年二期作，調查65個Jamaica與粳秈雜交F_1組合及其部分親本（6個粳稻，5個秈稻）之20日苗齡的苗高（表1），試驗結果依粳秈稻分述如下：Jamaica與粳稻雜交F_1有46組合 (87%) 的平均苗高介於6.4～13.7cm，其總平均苗高爲9.3cm，明顯地矮於其親本之平均值15.4cm；另7組合Jamaica與粳稻【臺中181號 (TC 181)、新竹54號 (HC 54)、嘉南2號 (CN 2)、嘉南4號 (CN 4)、高雄137號 (KH 137)、高雄139號 (KH 139) 及嘉農242號 (CNG 242)】雜交F_1的平均苗高介於26.2～29.2cm，其總平均苗高爲27.2cm，高於其親本之平均值，顯示雜種優勢。在秈稻與Jamaica雜交F_1中，有8組合平均苗高介於27.0～34.4cm，其總平均苗高爲29.5cm，高於秈型親本之平均值16.0cm；另4(33%) 組合【臺中秈3號 (TCS 3)、嘉農秈6號 (CNS 6)、臺農秈18號 (TNGS 18) 及臺農秈19號 (TNGS 19) 與Jamaica】雜交F_1的平均苗高介於10.8～12.5cm，其總平均苗高爲11.5cm，明顯地矮於其親本之平均值。

表1. 粳秈稻與Jamaica及其F_1之20日平均苗高與變域。(1995-II)

Table 1. The average and range of seedling height (cm) of Jamaica F_1 hybrids and their parents at 20 days after sowing. (1995-II)

Type	Parent or hybrid	No.	Seedling height (cm)			
			Mean	STD	Min.	Max.
Japonica	Parents	6	15.4	1.19	13.6	17.1
	F_1 (normal)	7[z]	27.2	1.06	26.2	29.2
	F_1 (weakness)	46	9.3	1.04	6.4	13.7
Indica	F_1 (weakness)	4[y]	11.5	0.75	10.8	12.5
	F_1 (normal)	8	29.5	3.45	27.0	34.4
	Parents	5	16.0	2.21	13.9	19.6
Jamaica		1	25.6	1.83		

STD : standard deviation

[z] Crosses of TC181, HC54, CN2, CN4, KH137, KH139, CNG242 and Jamaica.

[y] Crosses of TCS3, CNS6, TNGS18, TNGS19 and Jamaica.

二、雜種F_1的生長勢

爲探討不同基因型對水稻植株各生育期之影響，於民國81年一期作，調查臺農67號 (TNG 67)、臺粳2號 (TK 2)、臺中189號 (TC 189)、臺粳育1578號 (TKY 1578) 與Jamaica 等6個雜交組合F_1及親本於移植後28天、61天及收穫期之株高，調查結果詳如圖1，由調查資料顯示，臺農67號、臺粳2號與Jamaica正、反交之表現相同；在移植後28天，6個粳型F_1之株高均不及其較矮親本的二分之一；移植後61天F_1之株高差異更大，約爲其較矮親本的三分之一；收穫時F_1之株高皆矮於43.1cm，而其親本株高介於104.1～142.2cm，F_1之株高約爲其較矮親本的三分之一；在分蘖方面，6個F_1組合在全生育期皆無分蘖產生，其親本在插秧後61天就有9.5～14.9支分蘖，到收穫期的穗數則有9.2～13.0穗（表3）。

爲了解雜種弱勢基因在不同期作對水稻生長發育之影響，於81年二期作水稻生育期間，調查臺農67號、臺粳2號、臺中189號、臺粳育1578、臺中在來1號 (TN 1)、臺中籼10號 (TCS 10) 與Jamaica等8個雜交組合F_1之株高變化，結果詳如圖2，由調查資料顯示，粳稻親本之14日苗齡的苗高約20cm，Jamaica與籼稻親本苗高約27cm，Jamaica與粳稻之雜交F_1的苗高爲8.2～10.8cm，而與籼稻雜交F_1的苗高約30cm，6個粳型F_1皆呈現雜種弱勢，且正反交的表現相似，而2個籼型F_1則顯現雜種優勢。在移植後31天，Jamaica與臺農67號等雜交之6個粳型F_1的株高介於14.1～16.5cm，其親本之株高爲52.5～58.5cm，而籼型臺中籼10號及臺中在來1號與Jamaica之F_1株高則爲83.4～92cm。移植後59天粳型F_1之株高介於22.3～25.2cm，粳型親本之株高則爲84.2～89.3cm，而籼型F_1之株高則爲124.5～130.7cm。移植後92天時粳型F_1之株高介於29.6～35.6cm，粳型親本之株高則爲100.5～105.1cm，而籼型F_1之株高則爲138.6～145.4cm；兩類型之雜種F_1之株高，在全生育期皆呈現極大之差異，粳型F_1之株高在各時期皆約爲籼型F_1的五分之一，亦約只有其粳型親本之三分之一，在各時期皆呈現雜種弱勢，且與第一期作（圖1）之表現相似。而籼型F_1呈現雜種優勢。

三、雜種F_1的株高與分蘖

調查17個粳稻及6個籼稻與Jamaica之雜種F_1及相關親本收穫期之株高與分蘖數（民國84年一期作），表2結果顯示，收穫期之株高與有效穗數，可明顯地分爲雜種優勢與雜種弱勢兩群；Jamaica之株高爲137.0cm，粳型親本之株高介於92.8～111.4cm，平均爲104.2 ± 5.06cm，籼型親本之株高介於89.3～110.7cm，平均爲100.4 ± 8.78；Jamaica之有效穗數爲8.4 ± 2.32穗，粳型親本之有效穗數介於10.2～14.4穗，平均爲12.2 ± 1.48穗，籼型親本有效穗數介於11.6～14.4穗，平均爲13.1 ± 1.31穗；其中16個 (94%) 粳稻雜種F_1之株高介於33.9～55.2cm，總平均爲41.5 ± 5.86cm，有效穗數爲1 ± 0（即皆無分蘖產生），由資料顯示，此類雜種F_1之株高僅爲其親本之33～50%，分蘖數之差異更大，呈現明顯的雜種弱勢；其中有一粳型臺農育92433號 (TNGY 92433) X Jamaica雜種F_1爲例外，其平均株高爲137.7 ± 4.76cm，有效穗數爲11.4 ± 3.27，株高與較高親本近似，分蘖則較親本多。籼稻雜交組合中臺中籼3號 (TCS 3) 及臺農籼18號 (TNGS 18) 與Jamaica之雜種F_1平均株高爲49.4～65.9cm，約爲較矮親本之二分之一，其雜種F_1有效穗數爲1.0～1.4穗，與親本（11.6～14.4穗）有極大的差異，也呈現雜種弱勢；另4個籼型雜種F_1之平均株高爲137.4～161.5cm，總平均爲151.1 ± 12.10cm，有效穗數爲10.5～13.8穗，總平均11.7 ± 1.54，此類之雜種F_1的株高或分蘖皆高於其親本，呈現雜種優勢。

四、雜種F_1產量構成要素之表現

民國80年第二期作以臺農67號等4個品種（系）與Jamaica雜交，次年第一期作種植其F_1及親本，收穫時調查穗數、穗長、穗重、千粒重、一穗穎花數與稔實率，調查結果詳如表3，由資料顯示；五個親本之穗長介於21.0～27.9cm，以Jamaica最長、臺中189號最短，5組合F_1穗長則介於6.8～10.8cm。親本之平均穗重介於3.68～5.82g，以臺粳2號最輕，最重者爲Jamaica，而5組合F_1穗重

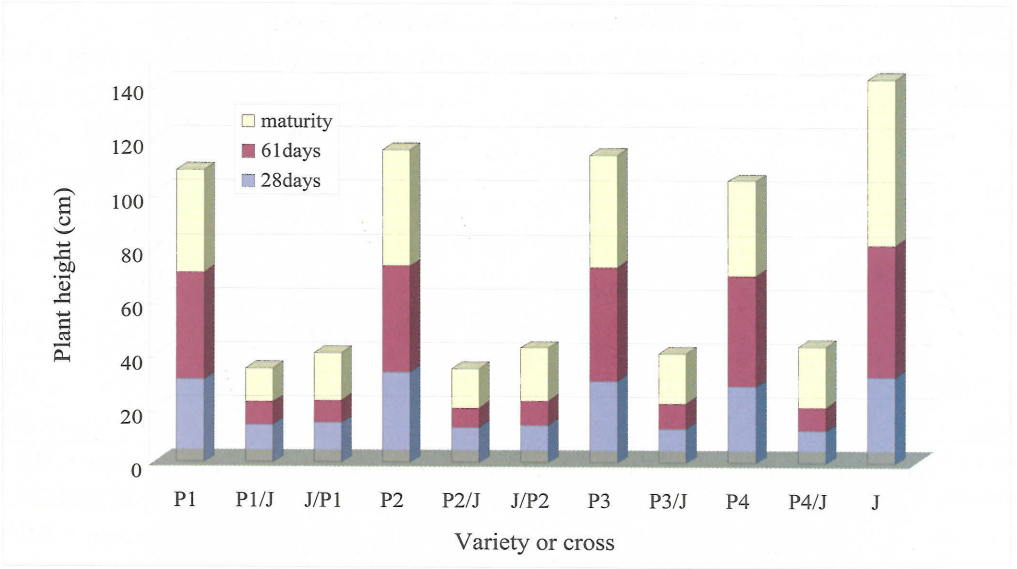


圖1. 秔稻與Jamaica及其F_1在移植後各時期之株高(cm)。(1992-I)

Fig. 1. The plant height (cm) of Jamaica, F_1 hybrids and parents at different days after transplanting. (1992-I) (P1=TNG67; P2= TK2; P3=TC189; P4=TKY1578; J=Jamaica)

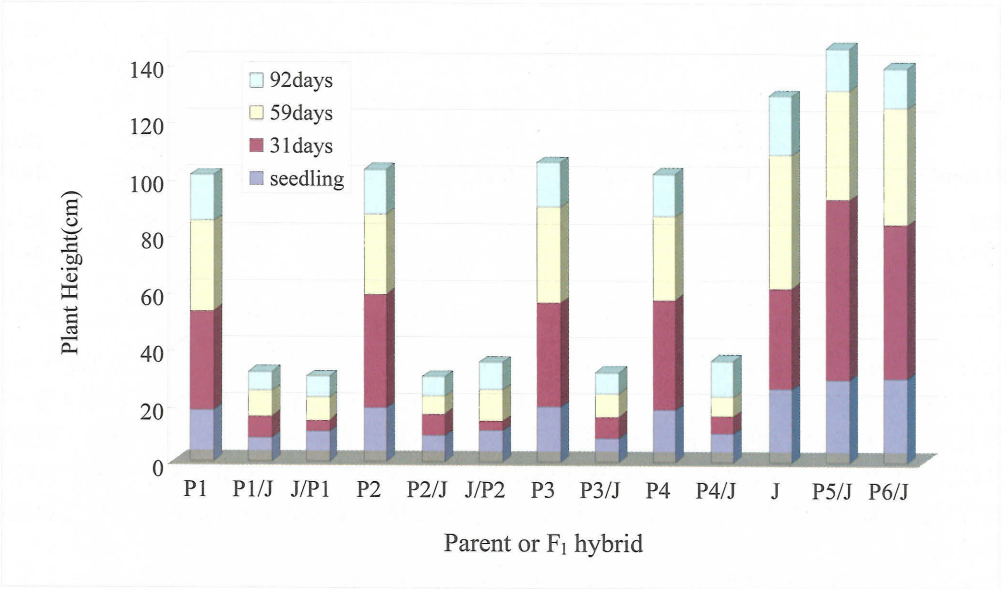


圖2. 秔秈稻與Jamaica及其F_1之苗高與在移植後各時期之株高(cm)。(1992-II)

Fig. 2. The plant height of Jamaica, F_1 hybrids and parents at seedling and different days after transplanting. (1992-II) (P1=TNG67; P2= TK2; P3=TC189; P4=TKY1578; P5=TCS10; P6=TN1; J=Jamaica)

表2. 粳秈稻與Jamaica及其F_1之株高與穗數。(1995-I)

Table 2. The average and range of plant height (cm) and panicle number of Jamaica, parents and their F1 hybrid at maturity. (1995-I)

Type	Parent or Hybrid	No.	Plant height (cm)				Panicle number			
			Mean	STD	Min	Max	Mean	STD	Min	Max
Japonica	Parents	15	104.2	5.06	92.8	111.4	12.2	1.48	10.2	14.4
	F_1 (normal)	1[z]	137.7	4.76			11.4	3.27		
	F_1 (weakness)	16	41.5	5.86	33.9	55.2	1.0	0.0	1.0	1.0
Indica	F_1 (weakness)	2[y]	57.6	11.70	49.4	65.9	1.2	0.28	1.0	1.4
	F_1 (normal)	4	151.1	12.10	137.4	161.5	11.7	1.54	10.5	13.8
	Parents	6	100.4	8.78	89.3	110.7	13.1	1.31	11.6	14.4
Jamaica		1	137.0	6.67			8.4	2.32		

[z] Crosses : TNGY92433 X Jamaica.

[y] Crosses of TCS3, TNGS18 and Jamaica.

表3. 粳秈稻與Jamaica及其F_1在收獲時之農藝特性。(1992-II)

Table 3. Agronomic characteristics of Jamaica, F_1 hybrids and their parents at maturity. (1992-I)

Parents or Hybrid	Plant height (cm)	Panicle			Spikelets / Panicle	Seed-set (%)	Kernel weight (g/1000)
		Number	Weight (g)	Length (cm)			
TNG67	108.3	12.7	4.42	21.1	208.0	86.4	22.94
Jamaica / TNG67	40.5	1.0	0.21	8.7	11.8	78.4	20.00
TK2	115.8	11.6	3.68	22.2	167.4	82.3	23.95
TK2 / Jamaica	34.8	1.0	0.13	6.8	9.3	70.3	15.38
Jamaica /TK2	42.5	1.0	0.19	8.3	11.3	77.1	21.37
TC189	114.1	11.3	4.32	21.0	204.2	88.9	22.03
TC189 / Jamaica	40.4	1.0	0.21	8.6	11.5	81.2	18.75
TKY1578	104.4	13.0	4.52	22.1	184.6	87.5	25.62
TKY1578 / Jamaica	43.1	1.0	0.37	10.8	17.0	85.3	24.14
Jamaica	142.0	9.2	5.82	27.9	201.2	94.8	29.14

介於0.13～0.37g，顯示雜種F_1穗重不及其親本之十分之一。親本千粒重介於22.03～29.14g，F_1之千粒重介於18.75～24.14g，各組合F_1之千粒重皆較其親本略輕。在一穗穎花數方面亦如同穗重，親本一穗穎花數介於167.4～208.0，F_1之一穗穎花數介於9.3～17.0，親本與F_1有極大之差異。稔實率亦以親本較高，介於82.3～94.8%，F_1之稔實率則介於70.3～85.3%。由這些資料顯示，雜種F_1除千粒重及稔實率與其親本之差異較小外，穗長、穗重、一穗穎花數等產量要素，皆與親本有極大的差異，呈現雜種弱勢。

爲進一步了解雜種弱勢對臺灣水稻品種之影響是否具有普遍性，於民國83年第二期作，將Jamaica與臺灣育成的17個粳稻及6個秈稻品種（系）雜交，次年第一期作收穫時調查各親本及F_1的穗部性狀發現，親本間的穗重、穗長、一穗穎花數、稔實率與千粒重都無明顯的差異（表4），但不論粳型或秈型的F_1，皆可明顯的分爲二群，此結果與株高及穗數之調查完全符合（表2）；秈稻與Jamaica雜交，其F_1僅有2組合（臺中秈3號、臺農秈18號大）顯現弱勢，另4組合則表現雜種優勢，其弱勢

表4. 粳秈稻與Jamaica及其F_1在收獲時之平均農藝特性與變域。(1995-I)

Table 4. The average and range of agronomic characteristics of Jamaica, F_1 hybrid and their parents at maturity. (1995 I)

Parents or Hybrid	No.	Panicle		Spikelets Per Panicle	Seed-set (%)	1000-kernel Weight (g)
		Weight (g)	Length (cm)			
Japonica						
Parent	15	3.64 ± 0.62	20.1 ± 1.52	155.8 ± 27.5	92.49 ± 4.11	23.92 ± 2.13
		2.54 ～ 4.54	17.5 ～ 22.4	111.8 ～ 212.2	82.27 ～ 96.35	19.53 ～ 27.34
F_1 (normal)	1 [z]	5.32	23.8 ± 2.59	201.1 ± 55.5	92.68	26.87
F_1 (weakness)	16	0.40 ± 0.17	10.6 ± 1.73	18.7 ± 6.77	84.90 ± 7.70	22.16 ± 2.90
		0.14 ～ 0.89	7.7 ～ 14.8	8.1 ～ 38.4	68.67 ～ 93.95	16.46 ～ 28.36
Indica						
F_1 (normal)	4	3.95 ± 0.55	26.7 ± 1.17	224.8 ± 31.09	45.18 ± 7.43	28.64 ± 0.28
		3.39 ～ 4.67	25.2 ～ 27.7	191.7 ～ 267.5	39.60 ～ 57.59	28.32 ～ 28.97
F_1 (weakness)	2 [y]	0.47 ± 0.25	13.9 ± 1.82	27.2 ± 10.335	49.23 ± 2.90	25.68 ± 5.89
		0.29 ～ 0.64	12.7 ～ 15.2	19.9 ～ 34.	47.18 ～ 51.28	21.52 ～ 29.85
Parent	6	3.73 ± 0.68	23.7 ± 2.66	150.6 ± 21.55	90.21 ± 1.34	25.32 ± 1.41
		2.78 ～ 4.58	20.3 ～ 27.4	124.0 ～ 179.4	88.48 ～ 91.52	23.02 ～ 26.87
Jamaica	1	3.33	23.8 ± 1.63	118.3 ± 34.4	93.71	28.31

[z] Crosses ： TNGY92433 X Jamaica.

[y] Crosses of TCS3 and TNGS18 with Jamaica.

F_1的平均穗重爲0.47 ± 0.25g，穗長爲13.9 ± 1.82cm，一穗穎花數爲27.2 ± 10.33個，稔實率爲49.22 ± 2.90%，千粒重爲25.68 ± 5.89g，而具雜種優勢之4個F_1的平均分別爲3.95 ± 0.55g、26.7 ± 1.17cm、227.2 ± 31.09個、49.12 ± 7.43%、28.64 ± 0.28g，其親本之平均值分別爲3.73 ± 4.58g、23.7 ± 2.66cm、150.6 ± 21.55個、90.21 ± 1.34%、25.32 ± 1.41g，由資料顯示此二群F_1除稔實率相似外，其他四項性狀皆有明顯的差異，秈型雜種弱勢F_1除千粒重與其親本相似外，穗重、穗長、一穗穎花數及稔實率皆呈現明顯差異。粳型親本之平均穗重、穗長、一穗穎花數、稔實率與千粒重分別爲3.64 ± 0.62g、20.1 ± 1.52cm、155.8 ± 27.5個、92.49 ± 4.11%、23.92 ± 2.13g，粳稻與Jamaica雜交，其F_1有16組合顯現弱勢，只有臺農育92433號 X Jamaica一組具雜種優勢，16個粳型雜種弱勢F_1的平均穗重爲0.40 ± 0.17g，穗長爲10.6 ± 1.73cm，一穗穎花數爲18.7 ± 6.77個，稔實率爲84.90 ± 7.70%，千粒重爲22.17 ± 2.90g，粳型雜種弱勢F_1除稔實率及千粒重與其親本略低外，而穗重、穗長、一穗穎花數則與親本呈現顯著差異，此與81年一期作的結果相符。而臺農育92433 X Jamaica之F_1每穗重5.32g，其穗長爲23.8cm，每穗平均具有201.1個穎花，而稔實率則爲92.68%，其千粒重爲26.87g，明顯地與其他16個組合不同，亦較其親本優，顯現雜種優勢。

五、雜種F_2苗高的分離

將Jamaica與臺農67號、臺粳2號及臺中189號、臺粳育1578號與Jamaica四組合之雜種F_2及其親本種子，經浸種催芽後播種於秧田，14天後經鏟秧沖洗後調查其苗高，結果示於圖3，親本苗高分別爲Jamaica 25.6 ± 1.83cm、臺農67號 17.9 ± 1.37cm、臺粳2號18.7 ± 1.61cm、臺中189號19.1 ± 1.03cm及臺粳育1578號18.2 ± 2.81cm；四組合F_2苗高皆在2.5cm～34cm之間呈雙峰分布，平均依序爲16.9 ± 8.19cm、17.0 ± 9.09cm、15.7 ± 7.10cm及18.8 ± 7.47cm。若以18cm（分布頻度爲0或較少）爲界，可將F_2苗高劃分爲兩部分，矮於18cm者爲弱勢，高於18cm者爲正常，則4組合的弱勢與正常

苗數分別爲 50 ： 49 、 60 ： 59 、 41 ： 31 及 83 ： 90 ，經 X^2 測驗檢測一組互補基因之擬說，其實測值分別爲 1.328 、 1.644 、 0.014 及 4.812 ，皆符合 9 ： 7 之分離比。

民國 85 年 7 月 10 日以臺農 67 號及臺農 70 號與 Jamaica 雜交之兩組合雜種 F_2 種子，經浸種催芽後點播於 49 × 39 × 15cm 之塑膠盆，點播行株距爲 3 × 2 cm ，每盆播 15 行計 285 粒之 F_2 種子，並分別播雙親各 9 或 10 粒，每組合各播 3 盆； 30 天後調查苗高，所測得親本與 F_2 苗高之分布如圖 4 ，臺農 67 號

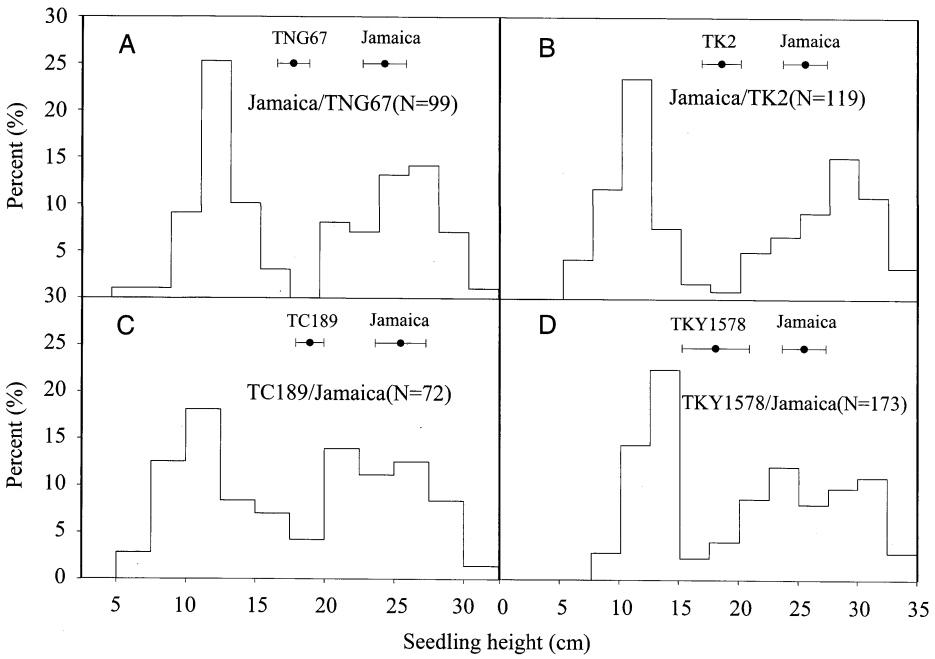


圖 3. 與 Jamaica 雜交的 F_2 族群之苗高分布。(1992-II)

Fig. 3. Segregation of seedling height (cm) of F_2 populations derived from crosses of Jamaica.(1992-II)

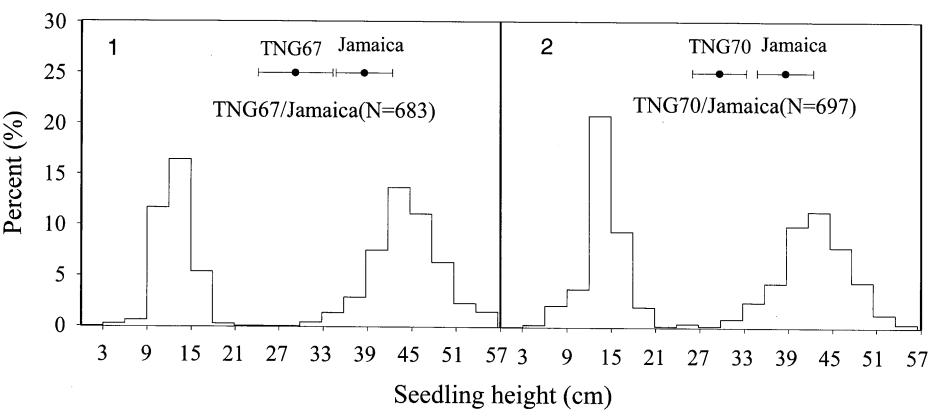


圖 4. 與 Jamaica 雜交的 F_2 族群之苗高分布。(1996-II)

Fig. 4. Segregation of seedling height (cm) F_2 populations derived from crosses of Jamaica.(1996-II)

之平均苗高爲29.4 ± 5.13cm，臺農70號之苗高爲29.9 ± 3.67cm，而Jamaica之苗高爲38.9 ± 3.87cm，F_2苗高雖略有差異，但皆呈雙峰分布在2.5cm～57cm之間，兩組合F_2苗高總平均分別爲28.4 ± 16.45及26.4 ± 15.01cm；若以21cm爲界，則二組合的弱勢與正常苗數分別爲462：393及485：370，經X^2測驗其實測值分別爲1.704及0.078，均符合9：7之分離比。

六、臺灣水稻品種之雜種弱勢基因型檢定

Jamaica爲已被確定爲具有*hwc-1*、*Hwc-2*基因型之水稻品種[4, 14, 16]，自民國八十年第二期作至八十四年第一期作，以Jamaica爲材料與臺灣主要栽培品種或新育成之品種（系）雜交，調查其雜種F_1苗高或在本田之生長情形，由各項農藝性狀表現是否呈現雜種弱勢，判斷其基因爲*Hwc-1*或*hwc-1*；歷次檢定之結果，彙整如表5所示，檢交親本共140個品種（系），其中粳、秈稻分別爲111與29個品種（系），多數爲臺灣之栽培種或新育成之品系（詳如表6），僅4個粳稻（3個爲日本品種，1個爲美國品種）、5個秈稻爲引進品種；經調查分析判定結果，共有103個品種（系）佔92.79 %或92.52 %（不含引進品種）的粳稻品種（系）具有*Hwc-1*基因，而秈稻品種（系）中則只有4個品種（系）具有*Hwc-1*

表5. 以檢交法分析臺灣140個水稻品種之*Hwc-1*基因型。

Table 5. Analysis of the *Hwc*-1 genotype for 140 rice cultivars in Taiwan by test cross.

Type	No. of cultivar contains		Total number of cultivars
	Hwc-1	*hwc-1*	
Japonica	103(92.79%)	8(7.21%)	111
Indica	4(13.79%)	25(86.21%)	29
Total	107	33	140

表6. 經檢測分析判定臺灣140個水稻品種（系）雜種弱勢基因 (*Hwc-1*, *hwc-1*) 之基因型。

Table 6. Determination of the genotype (*Hwc-1* or *hwc-1*) for the 140 rice varieties cultivated in Taiwan.

Cultivar contains the *Hwc-1* genotype

Japonica

Chianan(CN) 5, 6, 7, 8, 9, 10, 11, 13; J692153; Hsinchu(HC) 24, 50, 51, 55, 56, 62, 64; Kwangfu(KF)1, 401; Kaohsiung(KH)6, 18, 24, 27, 140, 141; Kaohsiung Sel. (KHS) 1; Taichung(TC) 65, 145, 170, 176, 178, 179, 180, 187, 189; Taichung Sel. 6; Taiken(TK) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11; Taiken Glutinous(TKW)1; Taiken Yu(TKY) 3540, 5207, 9198, 9386, 9480, 11942, 13034, 13120, 13212, 13345, 13556, 13632, 1578, 26415, 31961, 33787, 35025, 53231; Tainan(TN) 1, 2, 3, 4, 6, 9; Tainung(TNG) 16, 37, 38, 40, 45, 60, 61, 62, 67, 68, 69, 70, 72; Tainung Sel. (TNGS) 3; Tainung Yu(TNGY) 51103, 82649, 91636, 95031, 95049; Taipei(TP) 7, 8, 9, 10, 177, 301, 309; Taitung(TT)25, 26; Koshihikari; Kinuhikari; Hoshiyutaka; M401.

Indica

Chianung Sen(CNS)6; Taichung Sen(TCS) 3; Tainung Sen (TNGS)18, 19.

Cultivar contains the *hwc-1* genotype

Japonica

Chianan(CN)2, 4; Chianung(CNG)242; Hsinchu(HC)54; Kaohsiung(KH)137, 139; Taichung(TC) 181; Tainung Yu(TNGY) 92433.

Indica

Taichung Sen 10, 16, 17;Taichung Native(TN) 1; Taisen (TS)1; Tainan Sen(TNS) 15; Tainung Sen (TNGS)12; Tainung Sen Yu (TNGSY)115, 165; Tainung Sen Glutinous (TNGSW) 2; Kaohsiung Sen (KHS)7; Kaohsiung Sen Yu(KHSY) 392; Chianung Sen(CNS)11; Black waxy(BW)1, 2, 3; Ti-chueh-min-tang; Chu-tzu; Hsin-chu-ai-chueh-chien; I-kung-pao; IR8, 20, 56, 60; Java 14.

基因，佔 13.79 %，參試各品種（系）所具有之雜種弱勢基因（*Hwc-1* 或 *hwc-1*）之基因型詳如表 6。

討　論

水稻弱勢基因皆爲互補基因，已被提出的弱勢基因有顯性致死（*D-a, D-b* 或 *D-1, D-2*）、雜種白化 (*hca-1, hca-2*)、F_2 弱勢 (*hwb-1, hwb-2*) 及雜種弱勢[*Hwa-1*(*L-1-a*), *Hwa-2*(*L-1-b*) 及 *Hwc-1*(*L-2-a*), *Hwc-2*(*L-2-b*)]等[7, 8, 9]，這些弱勢基因多數僅在特定雜交組合出現。但 *Hwc-1*(*L-2-a*), *Hwc-2*(*L-2-b*) 這組弱勢基因之 *Hwc-2*(*L-2-b*) 僅存在特定品種中（如 Jamaica），而 *Hwc-1*(*L-2-a*) 則普遍存在粳稻品種中[4, 14]。Jamaica 爲秘魯的水稻品種，1963 年日人 Amamiya 及 Akemine 發現其與日本稻雜交呈現雜種弱勢，認爲係受一組互補基因所影響[4]，此雜種弱勢的表現異於常見的粳秈稻雜交之不和合性（一般爲雜種後裔的植株生育正常，而稔實率低）；其後雖有相關的雜種弱勢之研究，但未見有其利用之報告。本試驗主要目的在探討 Jamaica 特有的 *Hwc-2* 基因，對臺灣主要栽培品種的影響，及 *Hwc-1* 基因在臺灣水稻品種分布情形，進而了解其利用的可行性。

Jamaica 在臺灣的表現爲植株高大、分蘗略少、穗大、千粒重較重，本試驗以 Jamaica 分別與臺灣主要粳、秈稻雜交，其 F_1 稔實率分別爲 69～96% 及 40～58%，顯示明顯的差異性，Jamaica 與粳稻有較佳的親和性，與 Sato 等[16]將 Jamaica 歸類爲粳稻之結果吻合。

由表 1 及圖 2 調查 Jamaica 與栽培品種雜交 F_1 之苗高得悉，多數的粳型 F_1 呈現雜種弱勢，且正反交與一、二期作均有相似的表現，而秈型 F_1 顯現雜種優勢較多。當雜種弱勢 F_1 種子分別播種時，其苗高不及一般品種（親本）的二分之一，此結果與 Amamiya 及 Akemine 的報告相同[4]。因此，當 F_1 雜種弱勢之種子與一般種子混合播種時，推測將因生長競爭，兩者之差距應更大；當一般種子之苗高已至適當的插秧期時，雜種弱勢 F_1 的苗仍矮小，插秧後將無法與其他秧苗競爭，甚至將因浸於水中而死亡，故雜種弱勢 F_1 的種子，再繁殖的機會極微。

由調查 Jamaica 與栽培品種雜交 F_1 移植後的生長勢得悉，在移植後約一個月時，雜種弱勢 F_1 之株高不及其較矮親本的二分之一；移植後約二個月時 F_1 之株高約爲其較矮親本的三分之一；收穫時 F_1 之株高約爲其較矮親本的三分之一；且全生育期皆無分蘗產生；正反交後代的生長勢一樣；且在二個不同期作的表現也頗一致（圖 1、2），此結果亦與 Amamiya 及 Akemine 的報告相似[4]。民國 84 年第一期作，調查 17 個粳稻及 6 個秈稻與 Jamaica 之雜種 F_1 及相關親本收穫期之株高與分蘗數（表 2），結果亦如 82 年第二期作相同之調查結果；Jamaica 之株高爲 137.0cm、有效穗數爲 8.4 穗，粳型親本之平均株高爲 104.2 ± 5.06cm、有效穗數爲 12.2 ± 1.48 穗，粳稻雜種 F_1 中有 16 個 (94%) 組合之平均株高介於 33.9～55.2cm，總平均爲 41.5 ± 5.86cm，有效穗數爲 1，皆無分蘗產生；這係雜種 F_1 在單株栽培時的生長情形，若在混合播種移植的情形下，在生長競爭下，雜種弱勢 F_1 的生長勢將受抑制，生育更遲緩，或在生育期中因競爭弱勢而死亡。Amamiya 及 Akemine 的報告亦指出，雜種弱勢種子在溫室種植時，只有 17～80% 能抽穗結實，移至田間則僅有 7% 抽穗，故在早期 *Hwc-1, Hwc-2* 被稱爲互補致死基因[4]。

雜種弱勢 F_1 的種子，歷經苗期及本田期的生長競爭，如未因生長抑制、死亡，而達到成熟期，由歷次調查資料顯示，在分別種植下，雜種弱勢 F_1 幾乎沒有分蘗，且一穗穎花數皆少於 40 個，甚至少於 10 個，稔實率亦較低（比親本約低 10%），綜合評估其產能不及其親本的百分之一。推測，當具雜種弱勢種子在混合栽培條件下，其產能將更爲降低，故經由花粉污染而天然雜交的種子，再傳播擴散的機率應極低。

目前臺灣水稻皆以聯合收穫機收穫，因其刈取處理（脫粒）的適當範圍爲 55～130cm，因此收穫機割下的稻桿短於 55cm 時，將很難被脫粒，其稻穗將混雜於稻草中而被棄置於田中。具雜種弱勢 F_1 的植株，在個別栽培情形下，成熟期株高約只 40cm，聯合收穫機收穫時其刈取高度將不及 35cm，而在

混植競爭情形下，其株高將更矮，因此雜種弱勢的F_1植株，以收穫機收割時，其穗部將無法被送到脫穀部，其種子將混於稻桿中被切除而丟棄，因此所收穫的種子中，將不會混雜雜種F_2的種子，或其混雜機率微乎其微。

本試驗檢交的粳稻品種（系），以臺灣育成者爲主（詳如表6），檢測品種包括：臺粳1～11號等新育成與當前之主要栽培品種及常用的親本品種或新育成之品系，由本試驗結果得知，其中有92.79%具有*Hwc-1*基因，此一結果與1983年Sato與Hayashi[14]的報告相符。本試驗發現，具有*hwc-1*基因之粳稻品種爲：嘉農242號 (CNG 242)、嘉南2號 (CN 2)、嘉南4號 (CN 4)、臺中181號(TC 181)、高雄137號 (KH 137)、高雄139號 (KH 139)、新竹54號 (HC 54) 與臺農育92433號 (TNGY92433) 等8個品種（系），其中除了臺農育92433號外，皆爲老舊品種，這些老品種除了高雄139號，在東部地區仍有少量種植外，其他品種已鮮爲農民種植。因此，概括言之，臺灣一般農民栽培之粳稻均爲具有*Hwc-1*基因之品種，且臺灣水稻栽培品種以粳稻爲主，約佔總面積之90%，因此當栽培具有*Hwc-2*基因之黑糯品種時，其臨近之粳稻田，即使因天然雜交而受「有色基因」的花粉污染，產生具有色基因的雜種種子，亦將因前述之苗期競爭，本田期競爭，產能較低及收穫時漏失等諸因素，使花粉污染（天然雜交）產生雜種種子之問題得以解決，或將其污染可能性降至最低程度。因此，如能將*Hwc-2*基因轉移至黑糯等有色種皮之品種中，育成含「*Hwc-2*基因」的有色優良品種，應具有其意義，農民栽培黑糯稻將可受到輔導，提高農民之栽培選擇性及收益，亦可避免農民私自留種栽培所產生的問題。目前，我們已利用回交將*Hwc-2*基因轉移至數個黑糯稻之優良品系，正進行農藝性狀與產量之評估，期盼能育成具*Hwc-2*基因型的有色種皮之優良品種；若能達成目標，推介農民栽培，雖然無法全面防止花粉污染問題，但因臺灣以栽培粳稻爲主（約90%）[1]，在非秈稻栽培區，將可解決或減緩極大部分的農民私自種植「黑糯米」品種之潛在危險，農民亦可受良好的輔導，使稻作產業能多樣化，增加農民栽培的選擇性與收益。未來亦將利用分子遺傳技術篩選雜種弱勢基因之分子標誌與相關基因，並探討雜種弱勢基因之作用機制。

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Studies on the Influence and Application of the Hybrid Weakness Complementary Genes : *Hwc-1* and *Hwc-2* in Rice *Oryza sativa*

I. The Influence of *Hwc-1* and *Hwc-2* Genes on Agronomic Traits and Genotypic Investigation of Rice Cultivars in Taiwan [1]

Tung-Hai Tseng[2] and Chang-Sheng Wang[3]

Summary

As a self-pollinated crop, 0-6 % natural pollination rate were still observed in the cultivated rice, *Oryza sativa*. To prevent the pollen contamination, the colored rice such as red and black waxy rice varieties were neither encouraged nor introduced to the farmers. The hybrid vigor has been studied and utilized in rice for a long time nonetheless little was known on the hybrid weakness. It was reported that in rice the hybrid weakness is controlled by a set of complementary genes: *Hwc-1* and *Hwc-2*. In this report, we study the influence of the *Hwc-1*, *Hwc-2* genes on the agronomic traits of the F_1 and investigate the genotype of rice cultivated in Taiwan. When the F_1 plants contain both *Hwc-1* and *Hwc-2* genes they present significant hybrid growth weakness on the seedling and plant height. At maturity, most of F_1 hybrids of Jamaica have only one panicle with an average length at 10.6cm ; the yield components: panicle weight, panicle number, spikelet number per panicle, and grain weight per plant also showed hybrid weakness however 1,000 grain weight and seed-set rate were not influenced. The F_2 seedlings height segregated as 9 (growth weakness): 7 (normal) indicated that the hybrid weakness was controlled by a set of complementary genes. In general, rice varieties have either *Hwc-1*, *hwc-2* or *hwc-1*, *hwc-2* genotypes however the Jamaica variety contains *hwc-1*, *Hwc-2* genotype. To investigate the genotype of rice cultivated in Taiwan, 140 test crosses were made by crossing Jamaica with 111 Japonica (Ken-rice) and 29 Indica (Sen-rice) varieties (lines). The results showed that 103 (92.79%) crosses of Ken-rice and 4 (13.79%) crosses of Sen-rice showed F_1 hybrid weakness indicating that the dominant *Hwc-1* gene exits in most of the Ken-rice however, most Sen-rice contain the recessive *hwc-1* genotype. More than 90% rice cultivated in Taiwan are Ken-varieties therefore, if the *Hwc-2* gene of Jamaica were introduced to the colored rice varieties that will be able to eliminate the pollen contamination from colored-rice by the expression of hybrid weakness, and hence increase the farmers' willingness and earning of rice cultivation. .

Key words : Rice, Hybrid weakness, Complementary genes, Indica, Japonica, Pollen contamination, Natural pollination, Colored rice.

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肥培管理對珍珠菝番石榴果實品質之影響[1]

楊淑惠　謝鴻業[2]

摘要：在不同肥培管理下的珍珠菝番石榴，果實採樣後經官能品評，依優劣區分爲A至D四個等級，調查各等級果實比例並分析果肉成分差異。結果顯示，單施用化學肥料之栽培方式所生產之果實無A等級，而C等級約佔總採收量的65%；施用有機肥料爲主的栽培方式，A等級佔10%，B等級佔56%。成分分析顯示，施用有機肥料爲主的管理方式，其果實的維生素C、總醣、可滴定酸度的含量，平均比單施化學肥料爲高；而粗纖維及草酸含量則較低。施用有機質肥料栽培的果實，果肉有水浸狀情形發生。水浸狀果肉之蔗糖、檸檬酸及可滴定酸度含量明顯高於A等級果實，但果糖含量則遠低於A等級果實。

關鍵詞：番石榴、珍珠菝、化學肥料、有機肥料、組成份、水心病。

前　言

番石榴 (*Psidium guajava* L.) 爲一富含維生素 C、B 群及鈣、鐵等礦物質之熱帶、亞熱帶水果[4]，營養成份含量因品種、採收季節及成熟度等不同而有大的差異[1]。其可溶性醣包括果糖（含量最高）、葡萄糖及蔗糖[2, 7, 9]；成熟果實的維生素C含量變化由149.9至224.8 mg/100g[2, 10]，白拔品種含量則可達436 mg/100g[4]。栽培品種由具更年性的白拔、中山月拔、梨仔拔，演變至非更年性、果實碩大的泰國拔及二十世紀拔；加上栽培技術改進，不僅果實品質得以改善，更進而帶動市場消費及栽培規模，使番石榴成爲台灣的重要果樹產業[3]。

新育成之鮮食品種珍珠菝，其品質不論是口感或糖酸比，令多數消費者滿意；以有機肥料爲主化學肥料爲輔的栽培方式[6]所生產的產品，其食用品質與僅施化學肥料的管理方式有明顯差異；初步結果顯示，品質極佳的果品，酸、甜適中，風味如市售醃漬番石榴般的口感，具甘甜味道，且部分果實果肉有水浸狀現象；本試驗配合田間栽培試驗，共同探討不同肥培管理對番石榴果實品質、組成份之影響，並分析水浸狀果肉與正常果肉組成份之差異。

材料與方法

一、試驗材料

供試之番石榴品種爲珍珠菝，本試驗於民國八十六年十二月、八十七年一月、八十八年十月及十二月間，在高雄縣燕巢鄉與大社鄉，選擇單施化學肥料和以施有機質肥料爲主之果園進行採樣，果實大小以中型果爲標準，合計共450果。果實經八至十位品評人員品評分級後，全數取樣進行成分分析。供試驗之樣品，除灰分、粗纖維、粗蛋白質取切細果實外，均爲以果菜榨汁機直接榨汁之果汁樣品。

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二、試驗項目與方法

(一) 灰分測定 (Ash)：果實切細，精稱10.00g樣品，於85℃下烘乾後進行灰化，依中國國家標準CNS總號12571方法

(二) 可溶性固形物 (Soluble solids)：樣品榨汁、離心後，取上層澄清液，以數位式糖度計 (Refractometer) 測之。

(三) pH值 (pH value)：樣品榨汁、離心後，取上層澄清液係pH計測之。

(四) 可滴定酸度測定 (Titratable acidity)：樣品榨汁，取5.0 g汁液加10倍去離子水，以0.1 N氫氧化鈉滴定至pH值8.1。以檸檬酸（系數0.0064）爲計算標準。

(五) 有機酸測定：樣品榨汁，取澄清汁液，經離心後以0.45 μm濾膜過濾，以Varain 2550高效液相層析儀 (HPLC) 進行分析，分離管：Aminex-HPX 87H, 40℃，UV偵測器：210nm，流動相：5mM H_2SO_4溶液。經HPLC分析後積分所得面積與已知濃度標準品分析所得面積相比較，即得該樣品之濃度。

(六) 糖類測定：樣品處理同上，分離管：Aminex-HPX 87C, 80℃，RI偵測器，流動相：去離子水。

(七) 粗蛋白質測定 (Crude protein)：取1.0g新鮮樣品，依Semimicro-Kjeldahl法，於消化管中，加入催化劑及濃硫酸於氮分解爐中加熱分解，放冷卻後蒸餾，蒸餾液以0.1 N H_2SO_4滴定。

(八) 維生素C測定 (Vitamin C)：依AOAC之2, 6-Dichloroindophenol法，測定維生素C含量。

(九) 粗纖維測定 (Crude fiber)：依中國國家標準CNS總號5037方法測定。

(十) 品評分級：共分四等級，A等級：果實之酸甜比例佳，食用後有甘甜味，質地細緻；B等級：果實之酸甜比例佳，質地佳，食用後無甘甜味；C等級：果實之酸甜比例尚可，略帶澀味，質地略粗；D等級：果實酸甜比例不足，帶澀味，質地粗。

結　果

一、不同肥培管理果實品質分佈

不同肥培管理對果實等級分佈影響，如圖1所示，單施化學肥料所生產之產品，經品評分級後，以C等級品爲主，佔總採收量的65%；D等級產品次之，佔總採收量的25%；B等級產品只佔總採收

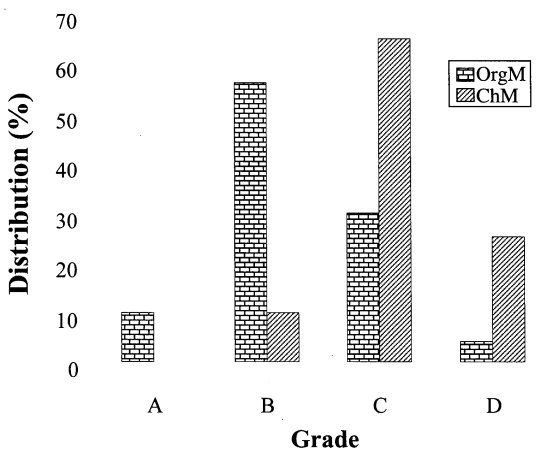


圖1. 不同施肥管理對珍珠菝等級之影響

Fig.1. Effect of fertilization on grade of 'Jen ju bar' guava fruit. OrgM= 施用有機肥料 ChM= 單施用化學肥料

量的10%，無A等級產品。而以有機肥料爲主所生產的產品，以B等級品最多，佔總採收量的56%；C等級品約佔總採收量的30%；有4%左右的產品被評爲D等級，A等級品則有10%。

二、不同肥培管理果實組成分比較

(一) 單施化學肥料

不同等級果實成分，如表1所示，維生素C含量在96 mg/100g上下，B等級者略高，可滴定酸度含量在B等級果實爲0.43 g/100g，可溶性固形物含量約爲11.7 °Brix；D等級果實可滴定酸度及可溶性固形物含量則降爲0.35 g/100g及9.6 °Brix左右，珍珠菝的糖類以蔗糖爲主，果糖及葡萄糖次之，B等級果實的蔗糖含量爲4.76 g/100ml，果糖及葡萄糖分別爲3.87 g/100ml，3.03 g/100ml；C等級果實的糖含量略微降低；D等級果實的總醣比B級平均下降1.37 g/100ml左右。有機酸類以檸檬酸(citric acid)爲主，B等級者含量約0.37 g/100ml，略高於C、D等級的果實。此種情形表現在酸鹼值時，B等級果實的pH值4.13，較C、D等級果實低，其他有機酸類包括蘋果酸 (malic acid) 及草酸 (Oxalic acid) 各等級平均含量分別爲0.06 g/100ml及0.05 g/100ml；粗纖維含量介於2.001-2.162 g/100g，D等級含量較高。其他成分如粗蛋白質、灰分等在不同等級間的差異並不明顯。

表1. 單施用化學肥料之各等級珍珠菝成分分析

Table 1. Effect of chemical fertilizer on the various components of different grade 'Jen ju bar' guava fruit.

Grade	Vit. C (mg/100g)	Titratable acidity (g/100g)	pH	Soluble solid (°Bx)	Crude fiber (g/100g)	Crude Protein (g/100g)	Ash (g/100g)	Sucrose (g/100ml)	Fructose (g/100ml)	Glucose (g/100ml)	Citric acid (g/100ml)	Oxalic acid (g/100ml)	Moisture content (g/100g)
A	–	–	–	–	–	–	–	–	–	–	–	–	–
B	98.3 ± 36.7	0.43 ± 0.01	4.13 ± 0.06	11.7 ± 0.2	2.002 ± 0.022	0.80 ± 0.07	0.346 ± 0.056	4.76 ± 0.02	3.87 ± 0.07	3.03 ± 0.06	0.37 ± 0.03	0.045 ± 0.005	84.7 ± 0.5
C	96.9 ± 28.1	0.38 ± 0.03	4.19 ± 0.01	11.2 ± 0.2	2.028 ± 0.005	0.81 ± 0.05	0.351 ± 0.038	4.40 ± 0.01	3.89 ± 0.04	2.89 ± 0.15	0.32 ± 0.03	0.055 ± 0.01	85 ± 0.3
D	92.2 ± 25.8	0.35 ± 0.03	4.24 ± 0.04	9.6 ± 0.3	2.162 ± 0.071	0.77 ± 0.02	0.351 ± 0.059	4.21 ± 0.03	3.42 ± 0.11	2.73 ± 0.09	0.32 ± 0.04	0.045 ± 0.015	85.7 ± 0.2

(二) 有機肥料爲主的栽培方式

各等級果實成分結果如表2所示，A-C等級果實的維生素C含量在104 mg/100g左右，D等級者含量較低90.7 mg/100g。A等級果實的可滴定酸度含量爲0.45 g /100g，酸鹼值則爲pH 4.09，可溶性固形物含量約爲12.7 °Brix；D等級果實可滴定酸度、酸鹼值及可溶性固形物含量，分別爲0.33 g/100g、pH4.15及9.7 °Brix左右。A等級珍珠菝的蔗糖含量最高可達4.87 g/100ml，葡萄糖含量4.34 g/100ml，果糖含量爲2.36 g/100ml；B、C等級果實含量略低；D等級者含量明顯較低。A、B等級的檸檬酸含量約0.35 g/100ml；C、D等級者含量0.31 g/100ml略低，各等級果實的草酸含量平均爲0.04 g/100ml。A、B等級者的粗纖維含量 (1.921 g/100g) 明顯較D等級者 (2.120 g/100g) 低，其他成分粗蛋白質、灰分等，在不同等級間的差異則不明顯。

三、水浸狀果肉之品質探討

調查發現施用有機肥料栽的珍珠菝，部分果實有果肉水浸狀情形，此種果實縱切面，介於果心與果皮之間會有半環或一環寬約0.3-0.8公分不等的透明水浸帶，如圖2所示。果實食用後的口感，類似市售醃漬番石榴，酸、甜口感很濃厚。將該區塊與該區外圍的果肉分離，進行成分分析。結果顯示，水浸區果肉的可滴定酸度爲0.49 g/100g，pH值爲4.02，可溶性固形物14.8 °Brix，檸檬酸含量爲0.47 g/100ml，高於水浸區外圍果肉含量；水浸區果肉的蔗糖含量高達6.81 g/100ml，比水浸區外圍的果肉的5.27 g/100ml高出1.5 g，也比A等級果實4.87 g/100ml高出近2 g左右；但水浸區果肉的果糖含量只

1.44 g/100ml，比水浸區外圍果肉的1.65 g/100ml含量低；水浸區果肉的維生素C含量爲58.6 mg/100g，明顯低於水浸區外圍的158 mg/100g，如表3所示。

表2. 施用有機肥料之各等級珍珠蕨成分分析

Table 2. Effect of organic fertilizer on the various components of different grade of 'Jen ju bar' guava fruit.

Grade	Vit. C (mg/100g)	Titratable acidity (g/100g)	pH	Soluble solid (°Bx)	Crude fiber (g/100g)	Crude Protein (g/100g)	Ash (g/100g)	Sucrose (g/100ml)	Fructose (g/100ml)	Glucose (g/100ml)	Citric acid (g/100ml)	Oxalic acid (g/100ml)	Moisture content (g/100g)
A	107.0 ± 33.9	0.45 ± 0.07	4.09 ± 0.01	12.7 ± 0.3	1.919 ± 0.135	0.91 ± 0.01	0.35 ± 0.109	4.87 ± 0.11	2.36 ± 0.01	4.34 ± 0.48	0.36 ± 0.05	0.04 ± 0.01	83.9 ± 0.15
B	106.6 ± 28.5	0.41 ± 0.03	4.15 ± 0.03	11.8 ± 0.3	1.921 ± 0.166	0.89 ± 0.03	0.36 ± 0.063	4.68 ± 0.11	2.12 ± 0.02	4.31 ± 0.46	0.35 ± 0.04	0.03 ± 0.005	84.5 ± 0.25
C	104.8 ± 26.2	0.39 ± 0.03	4.20 ± 0.01	11.3 ± 0.6	2.020 ± 0.02	0.89 ± 0.03	0.35 ± 0.028	4.48 ± 0.18	2.13 ± 0.08	4.14 ± 0.51	0.31 ± 0.06	0.03 ± 0.005	84.8 ± 0.15
D	90.7 ± 14.2	0.33 ± 0.01	4.20 ± 0.01	9.7 ± 0.15	2.120 ± 0.071	0.85 ± 0.05	0.37 ± 0.028	3.81 ± 0.04	2.07 ± 0.02	4.04 ± 0.39	0.32 ± 0.06	0.04 ± 0.01	85.3 ± 0.35

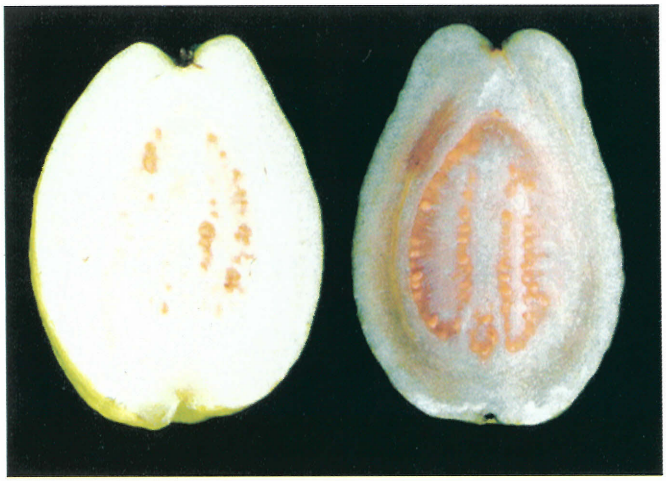


圖2. 珍珠蕨正常果肉(左)與水浸狀果肉(右)之比較

Fig.2. The appearance of normal (left) and watercore (right) symptom of 'Jen ju bar' guava fruit flesh.

表3. 珍珠蕨水浸狀果肉成分之分析

Table 3. The compounds of watercore fruit flesh of 'Jen ju bar' guava.

Area	Titratable acidity (g/100g)	pH	Soluble solid (°Bx)	Sucrose (g/100ml)	Fructose (g/100ml)	Glucose (g/100ml)	Citric acid (g/100ml)	Vitamin C (mg/100g)
Watercore flesh	0.49	4.02	14.8	6.81	1.44	3.29	0.47	58.6
Outside watercore flesh	0.43	4.11	11.0	5.27	1.65	3.41	0.35	158

討　論

果實的等級品評，與糖酸比、粗纖維含量等因子有關，試驗顯示品評爲A、B等級的珍珠菠，它們的可滴定酸、糖度等含量，平均比C、D等級者含量高；施用有機肥料爲主的果園，其果實的維生素C、總醣、可滴定酸等的含量，平均比單施化學肥料的果實含量高；且粗纖維及草酸含量較低，產品以A、B等級佔大宗，比單施化學肥料的管理方式有65%爲C等級產品相比較，明顯呈現出施肥管理對果實品質等級之影響。

成分分析顯示，珍珠菠糖類中蔗糖含量最高（佔總糖量的41.2%），葡萄糖含量次之，果糖含量較少，其比例與El Buluk及Golam[7, 9]之分析，果糖含量高、蔗糖含量低的結果明顯不同，可能與品系及栽培管理有關。Su等報告顯示[15]蔗糖在水解成果糖、葡萄糖後，甜度可增加20%，添加酸則有促進之功；A等級珍珠菠的蔗糖及檸檬酸含量高，兩者作用，延長甜味在口中停留的時間，可能是造成食用後有後味的重要因素。

珍珠菠果肉有水浸狀情形，推測可能與水心病 (watercore) 現象相類似，蘋果的水心病是一種生理疾病，一般發生在樹上成熟的果實和敏感性培育品種 (susceptible cultivars)[12]；Faust、Negm[8, 14]等人認爲這種現象可能與果實成熟時，山梨醇代謝減緩及山梨醇去氫酶 (sorbital dehydrogenase) 的活性有關；Marlow[13]實驗證明其活性與果實成熟時的乙烯、二氧化碳產生量相關；Kollas[11]認爲水心病組織的細胞膜成分改變，造成水心病組織的細胞有大量的山梨醇與葡萄糖漏失，是造成果肉水浸狀現相的重要原因；Wang[16]分析證實，水心病組織細胞膜的糖脂 (glycolipids)、磷脂 (phospholipids) 及固醇 (sterols) 含量很高，飽和脂肪酸轉成不飽和脂肪酸的比率較正常組織低。本試驗顯示，珍珠菠水浸狀部的蔗糖含量很高，但果糖、維生素C含量則遠低施用化學肥料的珍珠菠果實，此現象是否與水浸狀果肉的形成有關，其確切成因及對果實貯藏性的影響，有待更進一步探討。

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Effect of Different Fertilization on the Fruit Quality of Guava (*Psiduim guajava*)[1]

Shu-Hui Yang and Hung-Yeh Hung [2]

Summary

'Jen Ju Bar' guava fruit cultivated with different fertilizers were separated into four grades (A to D) according to sensory evaluation and the content of various compounds of fruit flesh. The proportion of C grade product of the guava fruit 'Jen Ju Bar' cultivated only with chemical fertilizers was 65% and that of A grade was absent. Whereas, the A grade was 10% and B grade was 56% for fruits produced by plants cultivated with organic fertilizers. Meanwhile, the content of ascorbic acid, total sugar, and titratable acidity of the fruit cultivated with organic fertilizers was much higher than those cultivated with chemical fertilizers. However, the crude fiber and oxalic acid content of the former were lower than those of the latter. Some of the guava fruit cultivated with organic fertilizers appeared watercore symptoms. The contents of sucrose, organic acids and titratable acidity of watercore flesh part were significantly higher than the A grade guava, but fructose content was lower than the normal fruit.

Key words : *Psidium guajava* L., Guava, 'Jen Ju Bar', Chemical fertilizer, Organic fertilizer, Composition, Watercore symptom .

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應用多型性分子標誌進行番椒屬 (*Capsicum*) 種原種內與種間遺傳歧異度之分析[1]

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摘要：國家作物種原中心所保存的番椒屬 (*Capsicum* spp.) 主要包括 *C. annuum* 、 *C. baccatum* 、 *C. chinense* 、 *C. frutescens* 、 *C. pubescens* 、 *C. chacoense* 、 *C. praetermissum* 及 *C. eximium* 等八個〝種〞（物種），由此八個〝種〞中選取94個品系，以RAPD分子標誌方法，進行〝種〞的鑑別及〝種〞間遺傳距離之探討。結果由580組逢機引子中篩選得17個引子具〝種〞間多型性，且再現性穩定；由RAPD產物中亦篩選得393個有效的特定片段，其中196條可用以區別上述八個〝種〞。有效條帶佔總條帶數之50%，將17個引子電泳結果合併進行以Nei和Li (1979) 所提之相似性係數爲計算基礎的UPGMA (Unweighted pair-group mean arithimetic) 集群分析 (Cluster analysis)，結果94個品系明顯依〝種〞別而呈現出集群趨勢；其中以 *C. eximium* 之〝種〞內最小距離 (Mini Distance; MD) 最小 (0.2783)，而以 *C. annuum* 之〝種〞內最小距離 (Mini Distance; MD) 之值最大則爲0.6009，以RAPD分群結果與以型態分類所得之〝種〞的分群關係及〝種間〞遺傳距離的遠近相符。再另以94個品系依據〝種〞爲基礎分成八個群，利用其多型性條帶於〝種〞間之出現頻度爲資料檔，進行折疊集群法 (Hierarchical clustering) 中之平均連結法 (Average linkage) 之集群分析，其結果亦呈現出上述以94個品系進行集群分析之結果相似；種間最小距離爲 *C. frutescens* 與 *C. chinense* 之0.747；而 *C. annuum* 與 *C. frutescens* 、 *C. chinense* 之距離爲0.812，均大於種內之距離。因此，綜合試驗結果顯示，利用分子層次之RAPD分子標誌所進行的集群分析結果，可與以植物性狀爲基礎所分類之〝種〞吻合；此結果亦可爲番椒品系間親緣遠近遺傳之評估及育種親本選擇之參考。

關鍵詞：番椒、逢機增殖多型性DNA、集群分析、遺傳歧異度。

前　言

番椒 (Pepper, *Capsicum* spp.) 爲茄科 (Solanaceae) 番椒屬 (*Capsicum*) 之一年或多年生作物，爲全球性之茄科三大作物，僅次於馬鈴薯和番茄。番椒原產於中美洲的墨西哥及南美洲的秘魯、玻利維亞等地區，而根據1999年FAO調查資料可知亞洲地區佔有全球番椒栽培面積的57%左右 (815,714ha)，而在產量方面亞洲亦佔一半以上（10,916,972百萬公噸）。番椒果實含有豐富的維他命C、維他命B及胡蘿蔔素等成份，可供作蔬菜食用、烹調的調味劑或藥材等多項用途，爲重要且具發展性的園藝作物(4)。

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番椒屬的花色、果型及果色歧異度較大，且易受環境影響，故傳統植物學上種的分類結果常不一致。McLeod等人 (1983) 依據植物形態及同功異構酵素電泳分析的結果，歸納出5個馴化栽培種及20~30個野生種，其中5個馴化栽培種分別爲：*C. annuum* 、*C. chinense* 、*C. frutescens* 、*C. baccatum* 及 *C. pubescens*[25]。國內現所蒐集及保存的番椒種原已達7,300份以上（表1），其中約有60%屬於 *C. annuum* ，而 *C. chinense* 、*C. frutescens* 和 *C. baccatum* 亦分別佔有5.5% 、5.1%和5.0% ，其他〝種〞所佔比率均甚低（包括 *C. chacoense* 、*C. eximium* 、*C. praetermissum* 、*C. pubescens* 等）。唯 *C. annuum* 、*C. chinense* 與 *C. frutescens* 等〝種〞間相似性高，不易由植株型態鑑別，爲提升育種效率，及瞭解保存種原的遺傳背景資料，故利用逢機增殖多型性DNA (randomly amplified polymorphic DNA ，RAPD) 標誌進行〝種〞之鑑別，及種原遺傳歧異度之評估。

表1. 台灣保存的番椒 (*Capsicum* spp.) 種原的〝種〞別和來源地區之數量分佈狀況[z]

Table 1. The statistics of *Capsicum* collection in Taiwan by species and areas of origin[z].

Origin area	Species									Total
	C. annuum	*C. chinense*	*C. frutescens*	*C. baccatum*	*C. chacoense*	*C. pubescens*	*C. eximium*	*C. praetermissum*	others	
Asia	1,640	10	89	7	0	1	0	0	902	2,649
Europe	1,518	11	11	21	9	3	3	4	130	1,710
North America	646	46	37	31	4	4	0	0	108	876
Central America	334	42	87	12	0	9	0	0	188	672
South America	121	277	82	286	12	9	0	0	92	879
Africa	164	10	47	5	0	0	1	0	24	251
Australia	8	2	2	1	0	0	0	0	3	16
Unknown	121	5	16	6	3	2			135	288
Total	4,552	403	371	369	28	28	4	4	1,582	7,341

[z] The data of species identified were collected by AVRDC.

近年利用同功異構酵素電泳分析及分子標誌（RAPD, RFLP, AFLP等）來作爲品種鑑定的技術已逐漸普及和成熟[3]；但是早期之同功異構酵素電泳分析能提供作爲遺傳標誌的基因座數目有限，且酵素表現易受環境影響[4]，故已較少被應用於鑑別品種。近十年來，應用RAPD進行種原鑑定及其相關的研究，如作物親緣關係的研究、栽培品種鑑定和分類、基因分析和鑑定等，已廣泛應用於：水稻[25]、小麥[8]、馬鈴薯[10]、落花生[7]、苜蓿屬[11]、蘆荀[18]、可可[21, 23]、油菜[26]、甜瓜[38]、芹菜[41]、木瓜[39]及薔薇科[9]等作物。

由於番椒〝種〞間的型態鑑別較爲困難，且往往需等到植株開花及結果期，才能利用園藝性狀做爲〝種〞間判別之用，極爲耗時費工；因此，近來利用生化特性探討及評估番椒之遺傳歧異度及其親緣相似度，有Tanksley (1984)[40]、Pickersgill (1988)[33]及Conicella (1990)等人[12]；利用細胞遺傳學及同功異構酵素兩種資訊之結合，做爲種原親緣及不同〝種〞間的判別。Panda等人 (1986) 則利用種子可溶性蛋白質電泳圖譜進行辣椒 (chili pepper) 〝種〞間遺傳相似度之鑑別[30]。但爲求獲得更多〝種〞間及〝種〞內的判別資訊與特殊分子標誌，有Ina等人 (1993) 利用RAPD marker 進行 *C. annuum* 與 *C.* chinense 〝種〞間雜交後裔中矮化因子之分析[16]； Prince 等人 (1995) 利用RFLP及RAPD之方法，分析21個源自不同地區之不同品系[35]，結果顯示 *C. annuum* 與 *C. frutescens* 遺傳相似性最高，但與 *C. baccatum* 之遺傳相似性較小；而Para等人 (1998) 以RAPD及AFLP兩個以PCR爲基礎之分子標誌方法，評估34個 *C. annuum* 栽培種之遺傳關係[31]； Lefebvre 等人 (1995; 1997) 更結合RAPD及RFLP所獲得之指紋標誌而建立 *C. annuum* 栽培種之完整染色體分子連鎖圖譜[19, 20]。綜合上述顯示，不論是生化特性或分子生物層次之結果，均與先前之分類學家以開花特性及以〝種〞間雜交難易程度做爲〝種〞的

分類結果相符[22]；且因其親緣遠近關係相似，故利用分子生物技術可獲得更多的〝種〞間及〝種〞內的資訊。

雖已有多位學者利用RAPD、RFLP等分子生物技術進行番椒親緣關係之鑑別，然而大多只針對5個栽培種（*C. annuum*、*C. chinense*、*C. frutescens*、*C. baccatum*及*C. pubescens*）[16, 19, 20, 25, 30]；本研究小組過去曾以RAPD分子標誌鑑定番椒種原（王等，1996, 1997）[1, 2]，結果顯示RAPD分子標誌可應用於番椒〝種〞間之鑑別。然以往所選取之樣品數較少，且以栽培種*C. annuum*爲主，故本研究之目的乃是針對目前國內已保存的8個主要番椒〝種〞，擴大*C. annuum*外的其餘各〝種〞之樣品數，結合RAPD分子標誌和集群分析來評估其遺傳距離的遠近，以做爲種原鑑別和資料庫建立之根據。

材料與方法

一、試驗材料

本研究採用的番椒供試材料，合計包含8個〝種〞共94個〝品系〞（表2）。採用的8個〝種〞中有5個栽培種，包括*C. annuum*（甜椒品種及多數辣椒商業品種）、*C. chinense*（辣椒）、*C. frutescens*（辣椒）、*C. baccatum*（辣椒）和*C. pubescens*（辣椒）；及3個野生種，包含*C. chacoense*、*C. eximium*和*C. praetermissum*。其中，*C. annuum*有18個品系，主要來源地爲墨西哥、瓜地馬拉、薩爾瓦多和英國等；*C. chinense*有14個品系，主要來源地爲墨西哥、玻利維亞和美國等；*C. frutescens*有11個品系，主要來源地爲哥斯大黎加、衣索比亞和薩爾瓦多等；*C. baccatum*有13個品系，主要來源地爲巴西、哥斯大黎加和牙買加等；*C. pubescens*有19個品系，主要來源地爲瓜地馬拉、祕魯、厄瓜多爾和德國等；*C. chacoense*有14個品系，主要來源地爲玻利維亞、英國、美國和荷蘭等；*C. eximium*和*C. praetermissum*分別有2個品系及3個品系，均來自英國。本研究所選用的供試材料是根據目前國內保存的番椒種原中〝種〞別的重要性與來源地區的不同而逢機挑選出具代表性的94個番椒品種（系），以使試驗結果能對各地番椒種原的特性作一廣泛的瞭解。

表2. 94個番椒種原品系之原單位代碼、〝物種〞別、供試代號、搜集來源地區及品系名稱

Table 2. RAPD code, accession number, species, original region and cultivar name of 94 varieties of *Capsicum* used in study.

No.	RAPD code	Accession number	Species	Original region	Cultivar name
1.	1024ca	C01221	*C. annuum*	England	
2.	1924ca	C02090	*C. annuum*	Hungary	CSERESZNYEPAP PIKA
3.	2122ca	C02283	*C. annuum*	Mexico	CHILGAUCLE AMARILLR
4.	3767ca	C03862	*C. annuum*	Mexico	W-C 2065 DULCE
5.	3771ca	C03866	*C. annuum*	Mexico	W-C 2073 DULCE
6.	3782ca	C03877	*C. annuum*	Mexico	W-C 2154 CARIZO
7.	4826ca	C04908	*C. annuum*	Honduras	HARMONICA NA 39911
8.	4840ca	C04922	*C. annuum*	Guatemala	
9.	2313ca	C02466	*C. annuum*	Philippines	
10.	4480ca	C04563	*C. annuum*	British Virgin Islands	
11.	1595ca	C01770	*C. annuum*	Egypt	
12.	2321ca	C02474	*C. annuum*	EL Salvador	CHILE QUESILLO NUM-20 R.B.-J.E
13.	2322ca	C02475	*C. annuum*	EL Salvador	CHILE QUESILLO NUM-20 R.B.-J.E.
14.	4647ca	C04729	*C. annuum*	Ethiopia	ISCA 8730
15.	4661ca	C04743	*C. annuum*	India	
16.	4682ca	C04764	*C. annuum*	Afghanistan	ISCA K-1801
17.	4711ca	C04793	*C. annuum*	Guatemala	IGAA GUACA CHILI RED G2102

表2. 94個番椒種原品系之原單位代碼、〝物種〞別、供試代號、搜集來源地區及品系名稱(續)

Table 2. RAPD code, accession number, species, original region and cultivar name of 94 varieties of *Capsicum* used in study.(continued)

No.	RAPD code	Accession number	Species	Original region	Cultivar name
18.	4732ca	C04814	*C. annuum*	Guatemala	IGAA
19.	0044cb	C00044	*C. baccatum*	Costa Rica	CATIE 7201-1-3-4
20.	2275cb	C02432	*C. baccatum*		94
21.	3988cb	C04083	*C. baccatum*	Jamaica	1979
22.	4069cb	C04164	*C. baccatum*	Brazil	132 BGH 341 PIMENTA
23.	4070cb	C04165	*C. baccatum*	Brazil	134 BGH 366 PIMENTA
24.	4072cb	C04167	*C. baccatum*	Brazil	140 BGH 481 PIMENTA WILD
25.	4075cb	C04170	*C. baccatum*	Brazil	144 BGH 682 PIMENTA PITONGA
26.	4095cb	C04190	*C. baccatum*	Brazil	183 BGH 1638 WILD
27.	4096cb	C04191	*C. baccatum*	Brazil	185 BGH 1646 PIMENTA
28.	4193cb	C04279	*C. baccatum*	Brazil	IGAA MALAGUETA 209
29.	4194cb	C04280	*C. baccatum*	Brazil	IGAA MALAGUETA 210
30.	4195cb	C04281	*C. baccatum*	Brazil	IGAA MALAGUETA 212
31.	4240cb	C04326	*C. baccatum*	Brazil	IGAA HOT PEPPER NO. 1233
32.	0264cc	C00264	*C. chinense*	Peru	SCARLET LANTERN MISCUCHO
33.	0304cc	C00304	*C. chinense*	USA	ISCA 4673
34.	0755cc	C00970	*C. chinense*	England	
35.	1565cc	C01741	*C. chinense*	Netherlands	1565CC
36.	3770cc	C03865	*C. chinense*	Mexico	W-C 2068 HABANERO
37.	3775cc	C03870	*C. chinense*	Mexico	W-C-2137 HABANERO ROJO
38.	3778cc	C03873	*C. chinense*	Mexico	W-C 2147 HABANERO
39.	3784cc	C03879	*C. chinense*	Mexico	W-C 2167 HABANERO AMARILLO
40.	4353cc	C04436	*C. chinense*	Puerto rico	1774
41.	4420cc	C04503	*C. chinense*	Bolivia	IGAA PORTACHUELA 306
42.	4422cc	C04505	*C. chinense*	Bolivia	IGAA 313
43.	4465cc	C04548	*C. chinense*	Surinam	IGAA 1720
44.	4471cc	C04554	*C. chinense*	Venezuela	IGAA CHIREL 1496
45.	4619cc	C04701	*C. chinense*	Brazil	BGH 1725
46.	0971cco	C00572	*C. chacoense*	Netherlands	
47.	4304cco	C00572-1	*C. chacoense*	Argentina	IGAA 189
48.	0631cco	C00849	*C. chacoense*	USA	
49.	0961cco	C01174	*C. chacoense*	France	
50.	1107cco	C01304	*C. chacoense*	England	
51.	1367cco	C01554	*C. chacoense*	England	
52.	1368cco	C01555	*C. chacoense*	England	
53.	1550cco	C01728	*C. chacoense*	USA	
54.	1560cco	C01737	*C. chacoense*	Netherlands	
55.	4305cco	C04390	*C. chacoense*	Bolivia	COL No. 275 ARIVIVI
56.	4306cco	C04391	*C chacoense*	Bolivia	IGAA ARIVIVI 277
57.	4307cco	C04392	*C. chacoense*	Bolivia	IGAA 340
58.	4310cco	C04395	*C. chacoense*	Bolivia	ARIVIVI 348
59.	4315cco	C04400	*C. chacoense*	Bolivia	
60.	0736ce	C00951	*C. eximium*	England	

表 2. 94 個番椒種原品系之原單位代碼、〝物種〞別、供試代號、搜集來源地區及品系名稱(續)

Table 2. RAPD code, accession number, species, original region and cultivar name of 94 varieties of *Capsicum* used in study.(continued)

No.	RAPD code	Accession number	Species	Original region	Cultivar name
61.	1137ce	C01331	*C. eximium*	England	
62.	1110cf	C01307	*C. frutescens*	England	
63.	1173cf	C01367	*C. frutescens*	Costa Rica	CHILE PICANTE 343
64.	1528cf	C01706	*C. frutescens*	EL Salvador	
65.	2352cf	C02505	*C. frutescens*	Costa Rica	R.O.
66.	4655cf	C04737	*C. frutescens*	Ethiopia	ISCA 10192
67.	4678cf	C04760	*C. frutescens*	Ecuador	1285
68.	4715cf	C04797	*C. frutescens*	Ethiopia	IGAA 1881
69.	4722cf	C04804	*C. frutescens*	Mexico	1249
70.	4761cf	C04843	*C. frutescens*	Costa Rica	
71.	4765cf	C04847	*C. frutescens*	Costa Rica	
72.	4457cf	C04540	*C. frutescens*	Mariana	965
73.	1135cpr	C01329	*C. praetermissum*	England	
74.	1136cpr	C01330	*C. praetermissum*	England	
75.	1198cpr	C01391	*C. praetermissum*	England	
76.	0555cp	C00778	*C. pubescens*	Guatemala	
77.	0632cp	C00850	*C. pubescens*	USA	
78.	0737cp	C00952	*C. pubescens*	Germany	
79.	1111cp	C01308	*C. pubescens*	Peru	
80.	1127cp	C01322	*C. pubescens*	Guatemala	
81.	1128cp	C01323	*C. pubescens*	Guatemala	
82.	1129cp	C01324	*C. pubescens*	Guatemala	
83.	1181cp	C01374	*C. pubescens*	Peru	
84.	1246cp	C01439	*C. pubescens*	Peru	
85.	1268cp	C01461	*C. pubescens*		
86.	1385cp	C01572	*C. pubescens*	Guatemala	
87.	1414cp	C01599	*C. pubescens*	Peru	
88.	1425cp	C01608	*C. pubescens*	Germany	
89.	1561cp	C01738	*C. pubescens*	Netherlands	
90.	4813cp	C04895	*C. pubescens*	Ecuador	AJI 80025 SAM 2657
91.	4814cp	C04896	*C. pubescens*	Ecuador	AJIROCOTO SAM 2567
92.	4815cp	C04897	*C. pubescens*	Ecuador	AJIROCOTO SAM 2568
93.	4825cp	C04907	*C. pubescens*	Bolivia	W-931 LOCATO
94.	5430cp	C05500	*C. pubescens*	Indonesia	

二、試驗方法

(一) 供試樣品的RAPD分析

1. DNA 萃取

DNA之萃取方法主要參考Rogers 等人 (1994)[36]所提之方法。取植株嫩葉0.5公克放入1.5 ml已滅菌的小離心管，加入液態氮磨碎，加入500μl的DNA萃取緩衝液 (100 mM Tris-HCl, pH 8.0; 20 mM EDTA; 1.4 mM NaCl; 0.2% β-mercaptoethenol, v/v)，於60℃水浴30分鐘，輕輕混勻後加入500μl

chloroform/ isoamyl alcohol (24:1 ,v/v) 再輕輕混勻。以10℃，16,000g離心10分鐘 (SIGMA 2K15, rotor 12145)。取上清液加入300μl之冰冷isopropanol沈澱DNA。再於4℃，16,000 g離心10分鐘。沉澱之DNA，再加入1 ml的washing buffer (75% ethanol, v/v) 輕輕混勻後，於4℃下以16,000g離心10分鐘。留取DNA沉澱物乾燥後溶於50μl的無菌水再加入RNase (10μg/ml) 在37℃下反應30分鐘。將DNA溶液，稀釋並測定OD_{260} nm之讀值，及計算DNA含量濃度。

2. 聚合酵素連鎖反應 (Polymerase Chain Reaction, PCR) 與電泳

將萃取得之genomic DNA先稀釋作爲模板DNA (template DNA)，利用10個核苷酸序列的隨意引子580種（UBC RAPD primer set: 100/4及100/5 200種；Operon Kit：OPAO、OPAP、OPAQ、OPAR、OPAS、OPB、OPD、OPE、OPF、OPG、OPJ、OPK、OPO、OPP、OPQ、OPR、OPW、OPX及OPY 380種），並由八個〝種〞內挑選30個較具代表性之樣品，進行聚合酵素連鎖反應以篩選合適之primer，再將篩選出具有〝種〞間明顯多型性DNA片段之引子，進行整體94個樣品之聚合酵素連鎖反應，並自反應產物中篩檢出〝種〞鑑別專有之marker band。反應液參考Oard及Dronavalli (1992)[29]，反應總體積爲25μl，內含1 U Taq polymerase (Promega)、1×PCR buffer (50 mM KCl; 10 mM Tris-HCl, pH 9.0; 0.1% Triton 100)、2.5 mM $MgCl_2$、200 μM dNTP、0.2 μM primer及25 ng template DNA。聚合酵素連鎖反應儀器爲Perkin Elmer Cetus Thermal Cycler 9600，反應溫度條件設定如下：1次 (cycle)：94℃，3分鐘→42℃，1分鐘→72℃，1分鐘；44次 (cycles)：94℃，1分鐘→42℃，1分鐘→72℃，2分鐘。反應完成時自動保存在4℃。經聚合酵素連鎖反應而增殖之DNA，以2% 的 agarose 在TAE buffer (40 mM Tris acetate, pH 8.0；1 mM EDTA) 中進行DNA電泳。並以Boeheringer Mannheim公司之DNA molecular weight marker XVI (250 bp ladder) 做爲分子量標誌，電泳槽係採用Cosmo公司的Mupid-2，電壓設定100伏特，結束後置於0.5 mg/ml的ethidium bromide中染色，以UV光檢視agarose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) 進行分析。

(二) 供試樣品的集群分析

將儲存之RAPD分析影像經影像分析軟體 (GelCompar™, Applied Meth BVBA) 求得數位化之數據，再以1995年Mumm等[27]換算相異距離矩陣 (Dissimilarity Matrix) 的方法，採用Nei和Li (1979)[28]的公式 $[2N_{AB}/(N_A+N_B)]$；N_{AB}：A樣品及B樣品相同之條帶數，N_A：A樣品條帶總數，N_B：B樣品條帶總數]爲基礎所設計的PC-SAS電腦程式計算出歐氏距離矩陣 (Matrix for Euclidean Distance)。最後，採用摺疊集群法 (Hierarchical Clustering) 中的平均連結法 (Average Linkage) 來進行集群分析(Cluster Analysis)，並依據標準化的最小群間距離 (Mini Distance; MD) 來建立其樹狀圖[6]。

(三) 〝種〞間的集群分析

根據94個番椒品系的集群分析樹狀圖所顯示之趨勢結果，進而以8個〝種〞爲基礎進行集群分析，包含*C. annuum*、*C. chinense*、*C. frutescens*、*C. chacoense*、*C. praetermissum*、*C. pubescens*、*C. baccatum*及*C. eximium*等八集群。首先，亦採用RAPD分析後數據化的原始資料（0或1）計算出8個集群內所包含的全部品系多型性條帶之出現之一致性頻度 $(U)= (1/N)\sum_{i=1}^{n} V_i$ [9]。其次，以換算後8個集群間的多型性條帶出現頻度爲資料檔進行採用摺疊集群法中的平均連結法之集群方析[6]，並建立其樹狀圖做爲評估番椒〝種〞間遺傳背景遠近的根據。

結果與討論

一、番椒種原之蒐集數量和來源的分佈狀況

目前國內所蒐集和保存的番椒種原已達7,341份，其中以四個馴化栽培種的數量較多，分別爲*C.*

annuum 的 4,552 份、*C. chinense* 的 403 份、*C. frutescens* 的 371 份以及 *C. baccatum* 的 369 份。*C. annuum* 佔多數，而 *C. chinense*、*C. frutescens* 和 *C. baccatum* 次之。統計種原來源資料可知以亞洲和歐洲佔有較大的比例（表一），這也正和 1999 年 FAO 所調查的資料內容相符合。綜合國內外資料顯示番椒是目前亞洲地區相當重要的蔬菜作物，故爲提升番椒育種選拔效率，必須在選拔時對親本材料的親緣關係背景有更深入的了解，故本研究採用結合分子生物技術與多變數分析來做爲評估番椒屬內各〝種〞間與〝種〞內的品種（系）間之親緣關係遠近。

二、94 個番椒品系之 RAPD 分析

本試驗以 UBC primer set 200 條 random primer (UBC primer set：100/4 及 100/5 共 200 條），及 Operon kit 380 條 random primers（Operon kit：OPAO、OPAP、OPAQ、OPAR、OPAS、OPB、OPD、OPE、OPF、OPG、OPJ、OPK、OPO、OPP、OPQ、OPR、OPW、OPX、OPY）共 580 組逢機引子；篩選得 17 個引子具〝種〞間明顯多型性，且再現性穩定（OPB5、OPB6、OPB12、OPE14、OPE15、OPG13、OPG16、OPK12、OPO10、OPQ16、OPQ18、OPQ20、OPR2、OPR8、OPR13、OPX2 及 UBC457）；比率數爲 2.9％。由此 17 個引子之 RAPD 產物共獲得 393 個有效條帶，平均每個引子產生 23.5 個條帶，其分子量大小介於 253bp 至 2,741bp 之間；其中 196 個條帶爲〝種〞間多型性條帶，佔多型性條帶之百分率爲 50％，較 1998 年 Paran 等人以 RAPD 方法鑑別栽培種 *C. annuum* 獲得 22％之多型性條帶數爲高[31]，因此，本試驗所選取之多型性引子，具有較高之多型性表現，且以此 RAPD 分子標誌方法確實可應用於〝種〞間之種原鑑定。

三、94 個番椒品系之集群分析

依據番椒 94 個品系，綜合 17 個引子所產生之 393 個多型性條帶，以 Nei 和 Li[28] 的相似性係數爲基礎，計算歐氏距離矩陣所獲得之 RAPD 集群分析樹狀圖（圖 1），根據樹狀圖所呈現的最小群間距離遠近，來判斷 94 個番椒品系在 8 個〝種〞間所表現的遺傳背景程度與原先各品系在 8 個〝種〞間的歸屬分類資料是否符合（表 2）。樹狀圖顯示 *C. annuum* 的 18 個品系均有聚集起來，且群內距離不大於 0.601；*C. chinense* 的 14 個品系亦聚集在一起，且群內距離不大於 0.5766；*C. frutescens* 的 11 個品系亦聚集在一起，且群內距離不大於 0.585；*C. baccatum* 的 13 個品系當群內距離小於 0.645 時；*C. chacoense* 的 14 個品系當群內距離小於 0.592 時；*C. praetermissum* 的 3 個品系當群內距離小於 0.393 時和 *C. eximium* 的 2 個品系當群內距離小於 0.278 時均有聚集起來的趨勢；*C. pubescens* 則 19 個品系當群內距離小於 0.552 時全部亦有依〝種〞別而聚集起來的趨勢。將 17 個引子合併進行 94 個品系的集群分析，全部 94 個品系均會明顯依不同〝種〞別而呈現出集群趨勢，依本研究小組之結果可以瞭解，番椒種原〝種〞間之鑑定亦可以 RAPD 之結果來判別且可信度相當高。除了 *C. praetermissum* 及 *C. eximium* 種內歧異度估值有偏低之趨勢（分別爲 0.393 及 0.278）外，其他六個種之種內歧異度介於 0.65 至 0.55 之間，此數值可爲台灣番椒種原內遺傳歧異度之估值；同時，亦可表現出所蒐集番椒種原之種內變異性相當一致，意即在台灣所有之番椒屬種原材料內，各〝種〞內材料之遺傳變異性大致相同，並未有特殊集中於某些〝種〞。各〝種〞之種原份數以 *C. annuum* 佔 62％以上，但本試驗所得結果 *C. annuum* 〝種〞內歧異度並未大幅增加，此點給予了種原保存是否應考慮到〝核心收集 (core collection)〞之方向有若干啓示，此點將爲日後種原中心研究方向之一。

此外，由於 *C. annuum*、*C. frutescens* 和 *C. chinense* 是目前世界各地主要栽培的番椒，根據 1966 年 Lippert[22] 等人所提出番椒屬主要〝種〞間的交配和合性網狀關係圖，認爲 *C. annuum*、*C. frutescens* 和 *C. chinense* 三個〝種〞間確有可能會因天然授粉的發生，而使後代彼此具有較近的親緣關係。即 *C. annuum*、*C. chinense* 和 *C. frutescens* 等三〝種〞或因演化過程中其起始祖先相近，或演化的歷程中因可行基因互換故親緣關係較爲密切，經由種間的天然雜交，其基因組應有部分的置換，使得育種人員不易由植株外觀來辨認此三個種。本研究中番椒 94 個品系的 RAPD 集群分析樹狀圖中顯示，*C.*

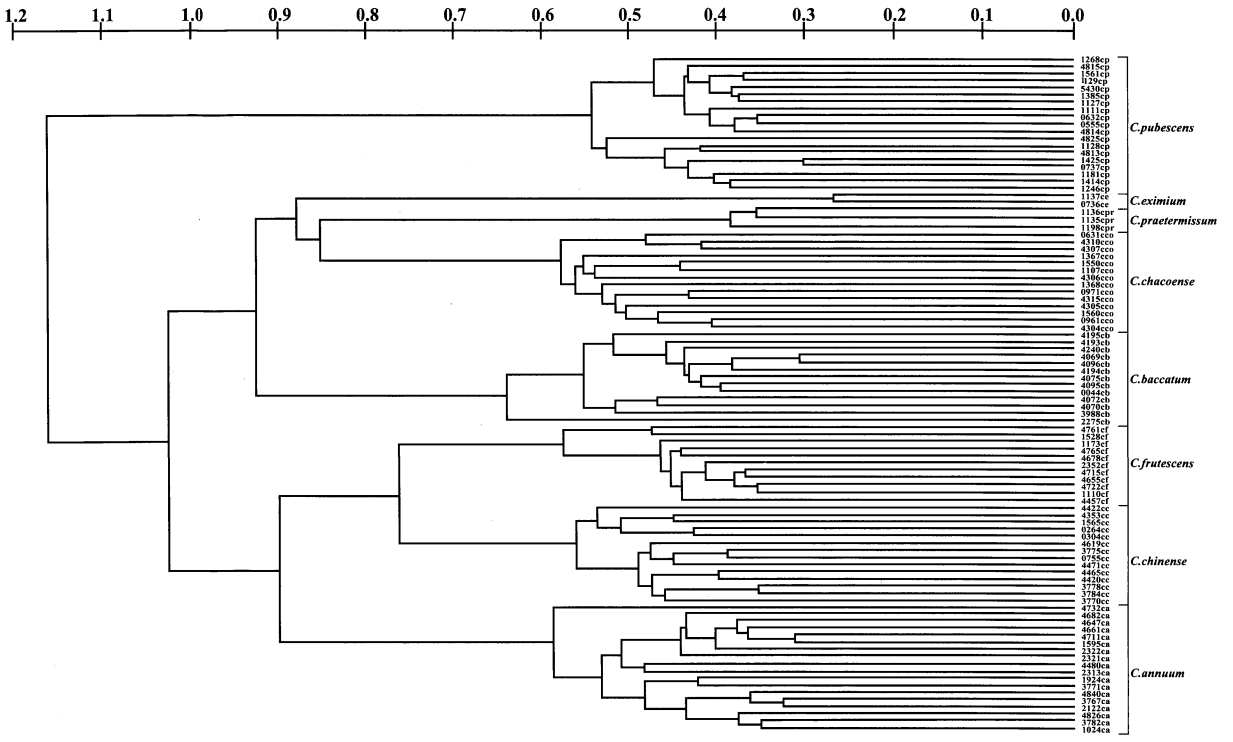


圖1. 番椒94個品系綜合十七個引子以Nei和Li相似性係數計算之歐氏距離爲基礎所構築之RAPD集群分析樹狀圖

Fig. 1. RAPD denrogram of 94 varieties of *Capsicum* by 17 primers and constructed by UPGMA cluster analysis based on Euclidean distances of Nei's and Li's Cofficient.

*annuum*的18個品系、*C. frutescens*的11個品系與*C. chinense*的14個品系均是相當緊密地聚集起來，並且依不同〝種〞別而表現出集中趨勢，且依RAPD之結果各〝種〞皆有其專一之標誌條帶，可作爲此三〝種〞間分類之參考。

四、8個番椒"種"的集群分析

按番椒屬內由於花色、果型、果色之分歧很大，故早期分類很紊亂。依DeWitt等人 (1993)[13]之文內指出1852年Dunal主張屬內應有50個〝種〞，Irish (1898) 則將番椒屬分爲多年生之*C. frutenscens*與一年生之*C. annuum*兩個種；而Bailey (1923) 認爲*C. annuum*也可多年生長，因此，將番椒屬歸爲只有1個〝種〞。目前較廣爲接受的分類方法，爲1957年Smith及Heiser等人[37]提出番椒屬有5個馴化栽培種，與1983年Esbaugh[14]根據阿根廷之植物學家Armando Hunziker之研究將番椒屬之野生種歸納爲22個種；其中番椒屬5個馴化栽培種即：*C. annuum* L.（包含全部甜椒品種及大部分辣椒商業栽培種），*C. frutenscens* L.、*C. chinense* Jacqin、*C. baccatum* L.和*C. pubescens* Ruiz & Pavon。其中除*C. pubescens*有黑色種子，最易於與其他黃色種子之番椒屬種原分辨。而上述相近之四個種中，僅*C. baccatum*的花冠基部有散漫黃斑較易辨認外，其餘*C. annuum*、*C. frutenscens*、*C. chinense*三者之間或同種內之種子、植株性狀均相似而不易分辨，僅能種植至開花結果期，再以花器特性區分之。在花序特性上*C. annuum*花朵多單生，而*C. chinense*及*C. frutescens*則每節花朵多2枚以上；另外*C.* frutescens花梗直立，而*C. chinense*及*C. annuum*則花梗下垂。除*C. frutescens*萼緣無齒突外，*C.*

表3. 番椒屬栽培種之類型特徵

Table 3. Characteristics of domesticated species of *Capsicum*

Species	Type	Corolla color			Corolla sport		Anther color			Calyx margin		Annular constriction at junction of calyx		Seed color			Number of pedicel/axil	
		Purple	White	Light-green	Yes	No	Purple	Blue	Yellow	Yes	No	Yes	No	Black	yellow	straw	Single	Multiple
C. annuum	1	*				*		*		*			*		*		*	
	2		*			*		*		*			*		*			*
	3	*				*	*			*			*		*		*	
C. baccatum			*		*				*	*			*		*		*	
C. chinense	1	*				*	*			*		*			*			*
	2		*			*		*		*		*			*			*
	3			*		*		*		*		*			*			*
	4			*		*		*		*		*			*		*	
	5			*		*		*		*			*		*			*
C. frustescens	1			*		*		*			*		*		*		*	
	2			*		*		*			*		*		*			*
C.pubescens		*				*	*			*			*	*				*

References: Greenleaf (1986) and IBPGR (1983) (張,1988)。

annuum 種內均有齒突。萼基上除部分 *C. chinense* 外，均無環縮（表3）[5]。但基本上以型態作爲〝種〞之鑑定，常有混淆及困難之處，利用分子生物技術，探討種間之遺傳及演進關係。

番椒94個品系，依〝種〞別區分成8個集群後，再根據綜合17個引子以393個條帶於各〝種〞內所有品系之出現頻率爲基礎，所構築之RAPD集群分析樹狀圖示如圖2，*C. frutescens* 與 *C. chinense* 其種間距離0.747爲最近，加入 *C. annuum* 之後，三個種間之距離爲0.812較其他種爲小，顯示 *C. annuum*、*C. chinense* 和 *C. frutescens* 等三〝種〞是較爲接近之族群。此點符合Pickersgill等人 (1979; 1988)[32, 33]所認爲該三〝物種〞是由同一祖先基因群所產生，且依其花色、花萼及每節花朵數等之園藝性狀亦顯示此三個〝種〞較爲接近；且McLeod等人(1979)[24]及Jensen等人 (1979)[17]以同功異構酵素鑑定之結果亦與上述之結果相同。根據1966年Lippert[22]等人認爲 *C. annuum* 和 *C. chinense* 間可雙向天然部分授粉，在 *C. annuum* 和 *C. frutescens* 間是則單向天然部分授粉，而在 *C. chinense* 和 *C. frutescens* 間亦是雙向天然部分授粉，除顯示 *C. annuum*、*C. frutescens* 和 *C. chinense* 三個〝種〞間確有可能會因天然授粉的發生，而使後代彼此具有較近的親緣關係；亦顯現 *C. frutescens* 和 *C. chinense* 二個〝種〞間之群間距離爲最小 (0.747)。*C. baccatum* 和 *C. chacoense* 二〝種〞群間之距離爲0.864，而 *C. baccatum* 和 *C. chacoense* 則距離上述三〝種〞較遠(群間相對距離爲0.921)，這正和Lippert等人 (1966年)[22]提出番椒屬主要〝種〞間的交配和合性網狀關係圖中 *C. baccatum* 和 *C. chacoense* 剛好位在 *C. annuum*、*C. frutescens* 和 *C. chinense* 三個〝種〞的周圍位置相呼應。1966年Lippert[22]等人以植物性狀進行分類時 *C. baccatum* 雖與 *C. annuum*、*C. frutescens* 及 *C. chinense* 三個〝種〞之距離較遠；而Pickersgill (1991)[34]及IBPGR (1983)[15]所提之番椒屬〝種〞間之交配和合性關係中顯示 *C. annuum*、*C. frutescens*、*C. chinense*、*C. baccatum* 和 *C. chacoense* 等五個種彼此間可雜交而產生正常且可存活之 F_1 植株，而Jensen等人 (1979)[17]認爲 *C. baccatum* 應與 *C. chacoense* 等距，除此之外，Panda等人 (1986)[30]以種子蛋白圖譜之分析結果進行集群分析亦顯示 *C. annuum* var 'glabriusculum' 介於野生與栽培種之 *C. baccatum* 之間，1979年Pickersgill[32]亦指出野生種之 *C. annuum* 花冠上亦有黃色之斑點，此爲 *C. baccatum* 之特有性狀，顯示野生種之 *C. annuum* 及野生種與栽培種之 *C. baccatum* 可能爲在不同地區產生共同演化，且某個野

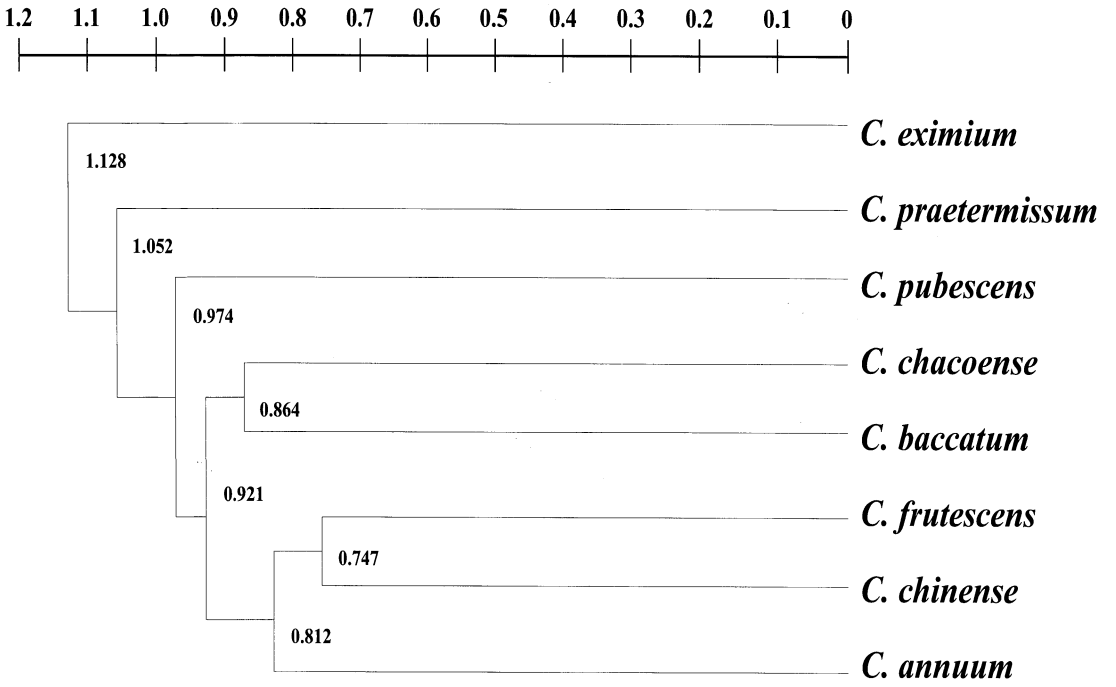


圖2. 番椒94個品系依種別分成八群後，綜合十七個引子以多型性條帶於〝種〞內出現之頻率爲基礎所構築之RAPD集群分析樹狀圖

Fig. 2. Dendrographic relationships among phenotypically unique RAPD patterns of 8 species of *Capsicum*. 94 isolates and 17 primers were used. The top scale is the percentage of similarity by Nei's similarity coefficient.

生種之 *C. annuum* 可能爲 *C. baccatum* 之祖先；而由其試驗結果亦顯示野生種之 *C. baccatum* 及 *C. annuum* 爲較接近之族群，可能因此，本試驗結果 *C. baccatum* 及 *C. chacoense* 二個〝種〞與 *C. annuum* 、 *C. frutescens* 及 *C. chinense* 等三個〝種〞共同聚集於一個較接近之集群中。

與上述五個種距離位置較遠的 *C. pubescens* 雖與 *C. baccatum* 、 *C. annuum* 、 *C. frutescens* 及 *C. chinense* 同屬於馴化栽培種，但可能因其具紫色花冠及黑色種子等較特殊之園藝性狀，加上與其他四個馴化栽培種間無法雜交產生可孕性種子，造成其遺傳物質型態與其他種分離，因此， *C. pubescens* 與 *C. chacoens* 、 *C. annuum* 、 *C. frutescens* 、 *C. chinense* 及 *C. baccatum* 所形成之集群距離較遠(0.974)。 *C. praetermissum* 和 *C. eximium* 在型態上本屬於野生型，經分子生物技術RAPD之結果亦發現此二族群之遺傳物質，明顯的與其他族群遠離（其遺傳距離分別爲1.052及1.028），而且亦與 *C. chacoens* 、 *C. annuum* 、 *C. frutescens* 、 *C. chinense* 及 *C. baccatum* 等五個族群間無法交配產生可孕性種子之結果相符。此外，在以各〝種〞內所有品系出現頻度爲基礎所構築的RAPD集群分析樹狀圖（圖2），亦可以顯示 *C. annuum* 、 *C. chinense* 、 *C. frutescens* 、 *C. baccatum* 與 *C. chacoense* 五種符合上述94個品系之RAPD集群分析樹狀圖（圖1）；但原來距離位置最遠的 *C. pubescens* 卻變成較爲接近 *C. baccatum* 與 *C. chacoense* 集群組合的〝種〞別(圖1)，這可能與 *C. eximium* 和 *C. praetermissum* 所選用的供試材料分別只有2個和3個，且RAPD集群分析樹狀圖是以不同的分析基礎爲背景資料（各〝種〞內所有品系的出現頻度與以Nei和Li (1979)(9)的相似性係數爲基礎所計算的歐氏距離矩陣）來進行集群分析產生的部分偏差有明顯的關係。但是，這結果與Lippert等人 (1966年)[22]提出番椒屬主要”種”間

的分類檢索表中 *C. eximium* 與 *C. annuum* 、 *C. frutescens* 和 *C. chinense* 三個〝種〞的親緣關係最遠亦剛好吻合。此外，根據8個〝種〞的集群分析樹狀圖中最小群間距離的遠近程度可看出 *C. chinense* 和 *C. frutescens* 之群間距離爲最小 (0.747)，遠較上述94個品系之最大種內歧異度爲 *C. annuum* (0.6009) 大，再次顯示本試驗所篩選之多型性引子，可以明確的應用作爲番椒之〝種〞間鑑別外，亦可呈現〝種〞內及〝種〞間之遺傳距離，可應用於種原相似性估測或品系間遺傳歧異度之探討。

進行品種間雜交，親本的選擇是決定育種工作成敗的關鍵，親本遺傳差異的大小，直接影響到雜種一代優勢的大小和以後分離世代中性狀變異幅度的大小。選擇遺傳差異較大的雙親進行雜交，是希望獲得強優勢雜種和分離世代中更多選拔的機會。然而，種間若遺傳差異太大則可能適得其反，不僅可孕性低且後代多爲不良性狀。故瞭解遺傳差異並適當的選擇是育種上重要的資訊。

由本研究之群集分析結果與植物性狀比對發現，種間的群集遠近與植物性狀之分類明顯具有吻合之處。故綜合言之，結合分生層次的RAPD分子標誌與多變數分析的集群分析，可與番椒以植物性狀爲基礎所分類之〝種〞吻合；此外，本研究在應用的層面上，除了可以幫助植物育種人員辨別番椒〝種〞間混淆的差異外，亦可由RAPD集群分析樹狀圖中供試番椒材料親緣關係的遠近來預先選拔出適合育種目的之親本材料，以避免育種親本因親緣關係較遠而無法達成雜交育種之目的，作爲選育種人員利用種原材料之參考。

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Analysis of the Genetic Diversity of *Capsicum* spp. (pepper) Using Random Amplified Polymorphic DNA Analysis [1]

Ming-Jen Fan [2], Jau-Yueh Wang [2], Shuen-Fang Lo [2], Ting Rong Shu [2] and L. M. Engle [3]

Summary

Pepper (*Capsicum* spp.) is one of the most important economical vegetables of Solanaceae family. The genus of *Capsicum* collected in Taiwan mainly includes 8 species. There are *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, *C. pubescens*, *C. chaccoense*, *C. praetermissum* and *C. eximium*. In order to identify and analyze the inter-specific and intra-specific genetic distance, a total of 94 lines representing these 8 species were selected for the study. From 580 random primers, 17 polymorphic primers were selected due to stable reproducibility of polymorphic bands. Three hundred and ninety-three effective polymorphic bands were obtained through RAPD analysis, and among them 196 bands could be used for species identification. The Cluster analysis based on Nei and Li's similarity coefficient from these 17 primers was calculated by the UPGMA method. The results showed that *C. eximium* has the smallest mini distance (MD) (0.2783) and *C. annuum* has the largest mini distance (0.6009) within *Capsicum* species. The genetic distance between species was also obtained from aforementioned results. The result of Cluster analysis from RAPD molecular marker is accordant with that based on horticultural characters. The cluster analysis of the 8 species (groups) was based on the frequencies of polymorphic bands appeared in the same "species", and calculated by the average linkage of hierarchical clustering. The result of cluster analysis of 8 groups is also similar to aforementioned results of 94 lines. In brief, the results indicate that RAPD markers are useful in estimating the genetic distances of the species between *Capsicum*, and can be used as the references for germplasm collection and breeding programs.

Key words : *Capsicum* spp., RAPD, Cluster analysis, Core collection

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Selection and Breeding of King Oyster Mushroom[1]

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Abstract: Three strains of the king oyster mushroom, *Pleurotus eryngii* (DC.:Fr.)Quél., were grown with bottle cultivation system for the evaluation of commercial production. They were the strains ATCC 36047, ATCC 90212 and Holland 150. One of them, i.e. the strain ATCC 36047, showed a high potentiality for commercial production. This strain produced medium to large fruiting bodies with an excellent feature, a firm and fine texture and a long shelf life, while the strain Holland 150 fructified fast and mostly produced large fruiting bodies with a good feature, a soft and fine texture as well as a short shelf life. The latter has a higher biological efficiency than the strain ATCC 36047 did, but it produced many warts on cap surfaces during high RH. In order to obtain a highly productive strain which can produce high quality fruit bodies, monokaryons from the strain ATCC 36047 and Holland 150 were isolated and their mating factors were also determined. Crossing between the monokaryons derived from the two strains and screening of the hybrids thus obtained were conducted. Four highly productive hybrids, i.e. B112, B012, B030 and B011, were obtained. The hybrid B112 produced large fruit bodies with a small cap, an equal to fusiform stipe, a medium soft and fine texture. The hybrid B012 produced stout fruit bodies with a prominent eccentric or lateral stipe and a fan-shaped cap of which surface produced warts during a high humid condition. Its stipe was equal and solid, and consisted of a white and very fine texture. The hybrid B030 produced slender fruit bodies with a medium size, and with a round cap, an equal and solid stipe. This hybrid was inclined to produce many small fruit bodies when the humidity of the cropping room was not properly controlled during cultivation. The hybrid B011 was inclined to produce stout fruit bodies with a large cap and a white and solid stipe. These productive hybrids of the fungus took 54.4 to 60 days from inoculation to the end of crop and they reacted differently to the composition of substrate in the aspect of productivity.

Keywords: Selection, Breeding, King oyster mushroom.

INTRODUCTION

The king oyster mushroom, *Pleurotus eryngii*(DC.:Fr.)Quél., is a typical fungus of the flora of the subtropics and steppes and is widespread in southern Europe and the areas of central Asia and North America

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[10]. It has been valued as the best one of all *Pleurotus* species due to its excellent consistancy of cap and stem, and pleasant aroma as well as culinary qualities[7, 10]. It has also the longest shelf life of any of the other oyster mushrooms due to its thick, firm flesh[8]. Recently the mushroom has been welcomed as a valuable addition to chefs's menus in the United States[6]. Besides, the mushroom has been demonstrated that both fruiting body extract and its powder were effective to repress the increase in blood cholesterol level[1]. The king oyster mushroom would have a great market potentiality in the coming years.

The king oyster mushroom has been commercially cultivated in Taiwan since 1993 using bottle cultivation technology[3, 4, 5]. It has also been successfully grown in Japan in the recent years[1]. Now this new *Pleurotus* species has become a popular mushroom on the traditional markets or supermarkets in the two countries. In order to increase the yield, mushroom quality and biological efficiency of this mushroom strain improvement would play an important role. The mating system of the king oyster mushroom has been shown as homogenic tetrapolar incompatibility system[2], therefore it would be possible to improve the mushroom strain by traditional breeding method. Three strains of the king oyster mushroom were grown with bottle cultivation technology for the evaluation of commercial production and monokayons were isolated from two of them for hybridization. Hybrid dikaryons thus obtained were subjected to a series of fruiting tests. The results are described in this paper.

MATERIALS AND METHODS

Strains of the king oyster mushroom

Strains ATCC 36047, ATCC 90212 and Holland 150 of the king oyster mushroom, ***Pleurotus eryngii*** were used for the evaluation of commercial production. They were maintained on PDA for regular subculturing and stored in liquid nitrogen for a long term storage.

Preparation of original and mother spawn

Sawdust from ***Trema orientalis*** was mixed with 40% of rice bran by weight and the water content of the mixture was adjusted at ca. 63% and its acidity was adjusted at pH 6.5 with calcium carbonate. The mixture was then filled into 200ml flask and sterilized at 121 ℃ for 30 minutes. After cooling down to room temperature the sterilized substrate was inoculated with the mycelium of each king oyster mushroom strain on agar slant and incubated at 25 ℃ for about 25 days. When the mycelium has grown throughout the sawdust substrate in flask, such sawdust culture is called original spawn which can be used to inoculate mother spawn. The mother spawn has the same ingredients of raw material as original spawn, however the substrate was filled in 1100 ml polypropane plastic bottle and sterilized in the same way as cultivation spawn described as following.

Preparation of cultivation spawn for fruiting

Sawdust from ***Trema orientalis*** was used as basic raw material in most experiments. The sawdust was mixed with rice bran or other nutritive supplements in various proportion by volume or by weight. Water content of each mixture was adjusted at 63% to ca. 71% varying with different experiments. These were illustrated in the note below each table of results. The mixtures were filled into 1100 ml polypropane plastic bottles with an automatic filling machine(including filling, pressing, holing and capping), then sterilized at 121 ℃ for one hour. The sterilized substrates were cooled down to room temperature and then inoculated with mother spawn using an automatic inoculator.

Incubation and cropping

The inoculated substrates were incubated at 18.5-21.5 ℃ for 32 to 42 days depending on the mycelial growth rate of each mushroom strain. After incubation the top layer(ca. 1.5cm in depth) of substrate in each bottle was removed by hand with a special spoon(bottle neck treatment, BNT) or was holed by an automatic holing machine in the center of bottle with a diameter of 1.8cm and a depth of ca. 2cm for fruiting. Then the bottles were moved to a cropping room of which temperature was controlled at 15.5-17.5 ℃, RH at 90 to 95% and light intensity at ca. 250 lux and carbon dioxide at 800-1200ppm during cropping.

Harvesting and determination of biological efficiency and production efficiency

Mushrooms in each bottle were harvested when the mushroom cap surfaces were flat to slightly up-rolled at the cap margins. In most experiments only one flush of mushroom, was harvested. The harvested mushrooms were then weighed for calculating biological efficiency and production efficiency. Biological efficiency and production efficiency were calculated as following:

Biological efficiency(%)= (the weight of fresh mushrooms harvested ÷ substrate dry matter content before inoculation) X 100

Production efficiency(%) = (the weight of fresh mushrooms harvested ÷ substrate dry matter content before cropping) X 100

Isolation and pairing of monokaryons and cultivation of hybrid dikaryons

One mature fruiting body was harvested from each of the two strains ATCC 36047 and Holland 150 which were cultivated singly in separate cropping houses. Basidiospores were collected separately in a sterilized large petri dish and stored at 2 ℃ in a refrigerator before use. Isolation of monokayons was conducted by dilution method and confirmed by the absence of clamp connections on mycelium with optical microscope. Monokaryons from each king oyster mushroom strain were paired in all possible combinations on PDA agar slant in test tube for mating type groupping. Seven monokaryons were selected from the strain Holland 150 to cross with those derived from the strain ATCC 36047 on PDA agar slant. The hybrid dikaryons with normal mycelial growth thus obtained were cultivated in bottle using the method described as above. The productive hybrid dikaryons with high qualities of fruiting bodies were subjected to a series of fruiting tests with various substrates of which ingredients were different.

RESULTS AND DISCUSSIONS

Comparisons of productivity and fruiting behavior seen in the strains ATCC 90212, ATCC 36047 and Holland 150 cultivated on a sawdust medium supplemented with 25% of rice bran by volume

The yield of the strain Holland 150 was significantly higher than those of the strains ATCC 36047 and ATCC 90212 including the yields of the first flush and of two flushes no matter what the time of uncapping after complete bottle neck treatment. The strain ATCC 36047 produced heavier first flush mushrooms than the strain ATCC 90212. The strain ATCC 90212 took the least time from bottle neck treatment to the first harvest (Table 1). After bottle neck treatment and recapping, removing the caps after appearance of pinheads or small mushrooms significantly reduced the time for harvesting 85% of total yield and for finishing the harvest of the first flush mushrooms This was resulted from the uniform fruiting caused by recapping treatment. The strain ATCC 36047 produced medium to large fruiting bodies with an excellent feature, a firm and fine texture and a long shelf life(Fig. 1a), while the strain Holland 150 produced mostly large fruiting bodies with a good feature

Table 1. Comparisons of productivity and fruiting behavior seen in the strains ATCC 90212, ATCC 36047 and Holland 150 of *Pleurotus eryngii* cultivated on a sawdust medium supplemented with 25% of rice bran by volume

Strain	Time of uncapping	Yield(g/24bottles)		Days from BNT to 1st picking	Days for harvesting 85% of total yield	Days for finishing the harvest of 1st flush
		1st flush	1st+2nd flushes			
ATCC 90212	BF	2117 e	4143 b	14.3 d	7.3 a	9.2 a
Holland 150	BF	3508 b	4892 a	17.3 b	6.7 a	7.8 ab
ATCC 36047	BF	2742 c	3925 b	16.7 bc	4.8 b	6.3 b
ATCC 90212	AF	2450 d	4141 b	15.8 c	3.0 c	4.2 c
Holland 150	AF	3791 a	4767 a	18.5 a	2.0 c	2.5 c
ATCC 36047	AF	3233 b	4208 b	17.3 b	2.5 c	3.2 c

[z] Sawdust from *Trema orientalis* and rice bran was mixed in a ratio of 3:1 by volume. The mixture was adjusted at pH 6.82 with calcium carbonate, while the water content at 71.91% by weight. The mixture was filled into 1100 ml polypropane bottles with an automatic machine(KPAC-50-2) for sterilization at 121 ℃ for one hour. Each bottles contained about 650g of substrate(wet).

[y] The sterilized substrate was inoculated with an automatic inoculator(RS-2000). All inoculated substrates were incubated at 20-22 ℃ for 32 days.

[x] After incubation the top layer (ca. 1.5cm in depth) of substrate in each bottle was completely removed by hand with a special spoon(bottle neck treatment, BNT). Then all the bottles were moved to a cropping room of which temperature was controlled at 15.5-17.5 ℃, RH at ca. 95% and light intensity at ca.250 lux during cropping.

[w] After bottle neck treatment all bottles were capped again, then four days after the treatment half of the bottles for each king oyster mushroom strain were uncapped for fruiting(BF), lids on the another half of bottles were removed until pinheads or small fruiting body appeared(AF). Each strain had 6 replicates for each uncapping treatment. Each replicates consisted of 24 bottles of substrate.

[v] Means with the same letter of a column are not significantly different at 5% level by Duncan's multiple range test.

and fine but soft texture and a short shelf life (Fig. 1b). The latter strain easily produced many warts on cap surfaces at high RH. The strain ATCC 90212 produced small fruiting bodies with a slender stipe (Fig. 1c), a firm and fine texture and a long shelf life (Table 2). According to these observations the strain ATCC 36047 showed a higher potentiality for commercial cultivation than the other two king oyster mushroom strains.

Determination of the mating types of the monokaryons derived from two *Pleurotus eryngii* strains

There were 23 and 38 monokaryons isolated from the strains ATCC 36047 and Holland 150 respectively. After intra-stock and inter-stock mating the mating types of these monokaryons were shown in Table 3. The result in Table 3 evidently indicates that ***Pleurotus eryngii*** is a tetrapolar heterothallic fungus of which incompatibility factors, A and B, have multiple alleles. This result is consistent with that of Hilbert (1982) and therefore it is possible to improve the agronomic characteristics of the fungus by traditional breeding.

Crossing of monokaryons and cultivation of hybrid dikaryons

Seven monokaryons were selected from the strain Holland 150 to cross all the monokaryons derived from the strain ATCC 36047. Totally 92 hybrid dikaryons with normal mycelial growth were selected for primary fruiting test. Yield, biological efficiency or both biological and production efficiency of each dikaryon, and shape, texture and taste of the fruit bodies produced were observed and recorded(Table 4 and 5). The productive hybrid dikaryons with good qualities of fruiting bodies were selected for the second fruiting tests using equal amount of sawdust from ***Trema orientalis*** and that from ***Liquidamber formosana*** as the basis of

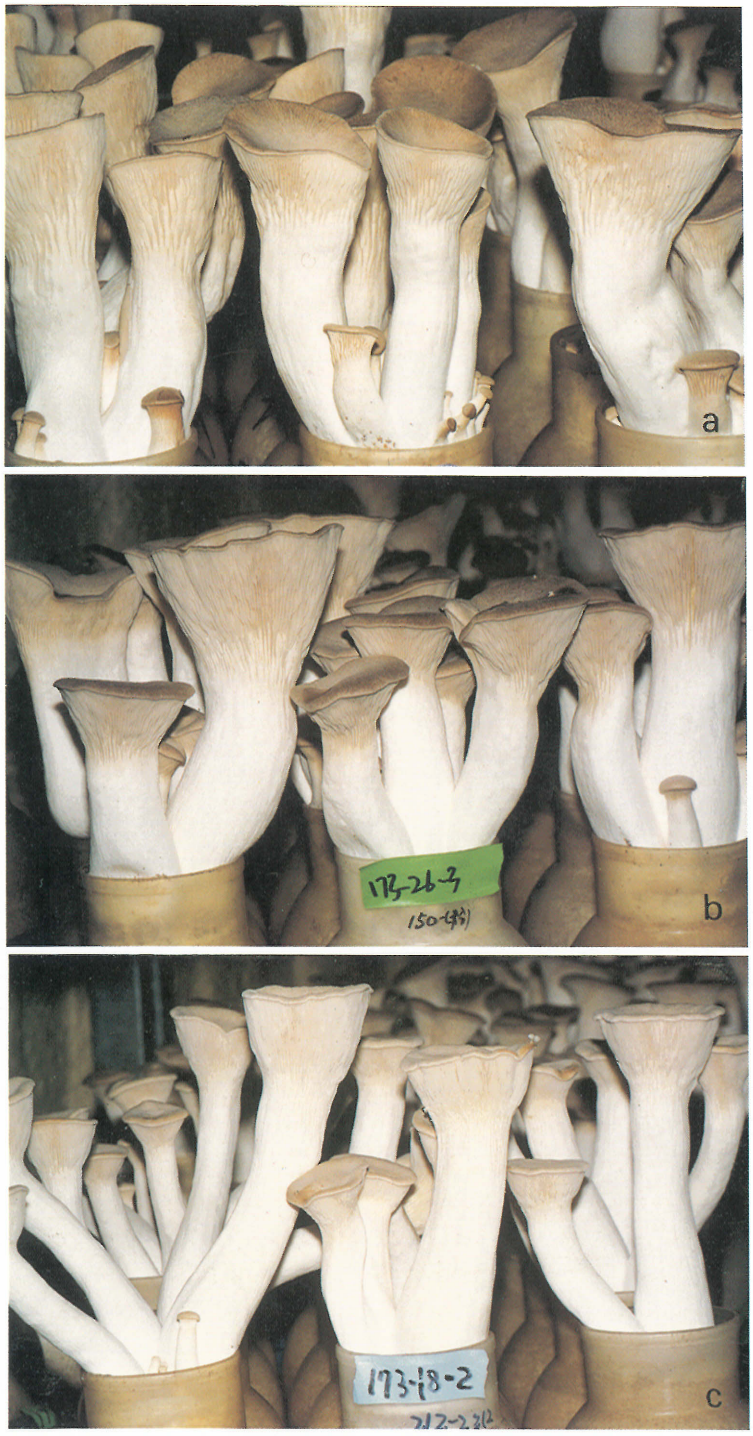


Fig. 1a-c. Fruiting bodies of the *P. eryngii* strains ATCC 36047(a), Holland 150(b) and ATCC 90212(c) grown on a sawdust medium consisted of 35% wheat bran and 15% rice bran by weight.

Table 2. Agronomic characteristics of three *Plearotus eryngii* strains

Items of characteristics	Designation of strains		
	ATCC 36047	Holland 150	ATCC 90212
Earliness of fructification	medium	medium	fast
Size of sporophores	medium>large	medium<large	small>medium
Appearance of sporophores	excellent	good	fair
Warts on cap at high RH	a few	many	few
Color of cap surfaces	light	dark	light
Firmness of tissues	solid	soft	solid
Texture of tissues	fine	fine	fine
Shelf life at 4 ℃	long(>2 weeks)	short(ca.1week)	long(>2 weeks)

Table 3. Mating types of the monokaryons derived from the *Plearotus eryngii* strains ATCC 36047 and Holland 150

Strain	Mating type	Monokaryon
ATCC 36047	A1B1	47003, 47004, 47019, 47023, 47026
	A2B2	47001, 47011, 47012, 47016, 47020
	A1B2	47005, 47009, 47010, 47017, 47021, 47022, 47024
	A2B1	47006, 47013, 47014, 47015, 47018, 47025
Holland 150	A3B3	15002, 15008, 15010, 15025, 15031, 15035
	A4B4	15007, 15013, 15015, 15016, 15023, 15022, 15033, 15036, 15037, 15038, 15040
	A3B4	15001, 15004, 15011, 15012, 15018, 15019, 15026, 15027, 15028, 15029, 15030
	A4B3	15003, 15005, 15006, 15009, 15014, 15017, 15024, 15032, 15034, 15039

Table 4. Primary fruiting test on some hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no.B-	Yield (g/bottle)	Biological efficiency (%)	Shape of fruiting body	Texture and taste of fruiting body
15008X47001	8	162.0f-m	67.79 f-m	G[u]	M.soft,sweett
15008X47003	5	172.2c-l	71.95c-l	G	M.soft
15008X47004	1	179.2b-j	74.87b-j	E	M.solid,sweet
15008X47006	7	120.6o-q	50.49o-q	F	M.solid
15008X47008	4	193.6a-c	80.89a-c	F	M.solid,sweet
15008X47010	3	159.0-m	66.43h-m	E	M.solid,sweet
15008X47013	11	180.6b-i	75.56b-i	G	M.solid
15008X47014	12	161.6f-m	67.52f-m	E	Solid, sweet
15008X47019	17	189.6a-f	79.35 a-f	E	M.solid
15008X47021	18	174.4c-l	72.95c-l	E	M.solid,sweet
15008X47022	19	148.0l-n	61.94l-n	G	M.solid
15008X47023	20	151.2j-n	63.28j-n	G	M.solid,sweet
15008X47024	21	193.4a-d	80.19a-d	G	M.solid,TL
15008X47026	22	171.4c-l	72.62c-l	G	M.solid,sweet
15025X47001	23	158.0h-m	67.22h-m	F	M.soft,TL
15025X47008	27	190.0a-f	79.49a-f	F	M.solid,sweet
15025X47009	28	96.8qr	41.55qr	G	M.soft, sweet
15025X47013	30	186.2a-h	78.89a-h	E	Solid, sweet
15025X47016	32	167.4c-m	69.95c-m	E	M.soft,sweet
15025X47019	34	164.4e-m	69.79e-m	G	Solid,sweet

Table 4. Primary fruiting test on some hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150(continued)

Pairing of monokaryon	Dikaryon no.B-	Yield (g/bottle)	Biological efficiency (%)	Shapeof fruit body	Texture and taste of fruit body
15014X47004	36	179.0b-k	74.89b-k	G	M.soft, sweet
15014X47011	40	110.2pq	46.15pq	F	M.solid,sweet
15014X47019	45	173.0c-l	73.38c-l	G	M.solid,sweet
15014X47026	47	149.4lmn	63.29lmn	G	Solid,M.sweet
15023X47001	48	75.8rs	32.77rs	B	Solid, sweet
15023X47003	49	61.2st	26.67st	B	Soft, sweet
15023X47004	51	210.6a	88.99a	G	Soft,M.sweet
15023X47012	58	142.8m-o	59.77m-o	B	M.solid,sweet
15023X47013	59	182.4b-i	77.32b-i	G	M.solid,sweet
15023X47016	62	155.6i-m	65.15i-m	F	M.solid,sweet
15023X47017	63	182.2b-i	77.23b-i	G	Solid.M.sweet
15023X47019	65	187.6a-g	79.48a-g	G	Soft,M.sweet
15023X47026	71	165.0d-m	69.47d-m	B	M.solid,sweet
15029X47001	72	181.4b-i	76.89b-i	G	Soft,M.sweet
15029X47003	73	124.2n-p	52.96n-p	F	M.soft,sweet
15029X47004	74	203.6ab	85.06ab	G	Soft,TL
15029X47005	75	185.4a-h	78.69a-h	F	Soft,TL
15029X47006	76	142.0m-o	61.34m-o	F	M.soft,TL
15029X47007	77	150.6k-n	63.21k-n	F	M.solid,sweet
15029X47008	78	184.4a-h	77.52a-h	F	Solid,sweet
15029X47009	79	141.6m-o	59.16m-o	G	Soft,M.sweet
15029X47010	80	178.0b-k	75.79b-k	G	Soft,sweet
15029X47011	81	171.0c-l	72.54c-l	G	M.soft,M.sweet
15029X47012	82	175.4b-l	73.92b-l	E	M.soft,TL
15029X47013	83	151.0j-n	64.86j-n	F	M.soft,M.sweet
15029X47014	84	155.4i-m	65.36i-m	F	Soft,sweet
15029X47016	86	160.4g-m	67.59g-m	F	Soft,M.sweet
15029X47017	87	189.4a-f	79.98a-f	G	Soft,M.sweet
15029X47019	89	192.2a-e	80.41a-e	G	Soft,M.sweet
15029X47022	91	51.8t	22.51t	B	M.soft,M.sweet
15029X47024	93	211.2a	88.49a	F	M.soft,M.sweet
15029X47025	94	142.6mn	61.79m-o	F	M.soft,M.sweet
15029X47026	95	121.4o-q	50.31o-q	G	Soft,M.sweet

[z] The substrate consisted of 35% of wheat bran, 15% of rice bran and 50% of the sawdust from *Trema orientalis*. Water content of the substrate was adjusted to 65% and pH to 6.5. The substrate was filled into polypropane bottles by an automatic filling machine and sterilized at 120 ℃ for 1.5hrs. Each bottle contained ca. 239.35g of dry matter.

[y] The substrate was inoculated by an automatic inoculator and incubated at 18.5-20.5 ℃ for 42 days.

[x] Before cropping the upmost central part of the substrate in each bottle was holed 1.5-2.0cm in depth with a aluminum rod of which diameter was 1.8cm. After holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 85-90% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 90.-96%. The temperature of cropping room was adjusted to 15.5-17.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 650-800ppm by heat exchanger and CO_2 controller.

[w] Each dikayon was cultivated in 24-36 bottles, experimental results from the five bottles with higher yield than others were used for statistic analysis.

[v] Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

[u] Shape of fruiting body: G= good; F= fair; E= excellent; B= bad.

[t] Texture and taste of fruiting body: M= medium; TL= Taste Less.

Table5. First fruiting test on the 39 hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no. B-	Yield (g/bottle)	Biological efficiency (%)	Production efficiency (%)	Shapeof fruit body	Texture and taste of fruit body
15026X47003	97	184.3e-h	74.60e-h	83.44d-i	G	M.soft,sweet
15026X47004	98	195.3c-e	79.05c-e	87.60c-e	G	M.soft,sweet
15026X47005	99	190.9d-g	77.27d-g	84.90d-g	G	M.soft,M.sweet
15026X47006	100	183.8f-i	74.39f-i	82.37f-j	F	Soft,M.sweet
15026X47007	101	178.8h-j	72.37h-j	80.51g-j	F	Soft,M.sweet
15026X47008	102	180.8g-i	73.18g-i	79.31h-k	G	Soid,sweet
15026X47009	103	169.0j-m	68.40j-m	75.02k-n	G	M.soft,sweet
15026X47010	104	200.5b-d	81.15b-d	89.82a-c	G	Soft,M.sweet
15026X47012	105	185.2e-h	74.96e-h	82.54e-j	F	M.solid,sweet
15026X47013	106	209.3ab	84.72ab	91.33a-c	E	M.solid,sweet
15026X47015	108	167.7k-m	67.88k-m	73.75mn	G	M.soft,sweet
15026X47017	110	193.8d-f	78.44d-f	83.28d-i	E	M.solid,M.sweet
15026X47018	111	200.7b-d	81.24b-d	87.37c-f	G	M.solid,sweet
15026X47019	112	166.0lm	67.19lm	71.62n	E	M.soft,M.sweet
15026X47021	113	190.6d-g	77.15d-g	84.30d-h	G	M.solid,sweet
15026X47022	114	200.2b-d	81.03b-d	87.59c-e	G	M.solid,sweet
15026X47024	116	214.86a	86.97a	92.50ab	G	M.solid,M.sweet
15026X47025	117	173.2i-l	70.10i-l	77.85j-m	E	Solid,M.sweet
15026X47026	118	178.4h-j	72.21h-j	80.64g-j	G	M.soft,sweet
15002X47001	119	205.6a-c	83.22a-c	83.01a	G	M.soft,M.sweet
15002X47003	120	160.7m	65.04m	73.46mn	G	M.solid,sweet
15002X47004	121	186.1e-h	75.33e-h	84.88d-g	G	M.solid,sweet
15002X47005	122	184.1e-i	74.52e-i	83.70d-i	G	M.soft,M.sweet
15002X47006	123	212.0a	85.81a	94.00a	G	Solid,sweet
15002X47007	124	91.3o	36.95o	42.41p	B	M.solid,sweet
15002X47008	127	163.2lm	66.06lm	73.95mn	G	M.solid,M.sweet
15002X47009	125	136.9n	55.41n	62.44o	G	M.solid,M.sweet
15002X47010	126	190.4d-g	77.07d-g	83.86d-i	G	M.solid,sweet
15002X47012	128	187.6e-h	75.93e-h	83.01d-i	E	Solid,M.sweet
15002X47013	129	177.5h-k	71.84h-k	78.94i-l	F	M.solid,sweet
15002X47014	130	162.3m	65.69m	73.96mn	F	M.solid,sweet
15002X47015	131	69.4p	28.09p	32.59q	B	Soft
15002X47016	132	135.4n	54.8n	60.72o	F	M.soft,M.sweet
15002X47017	133	159.0m	64.36m	71.79n	G	Soft,sweet
15002X47018	134	166.7lm	67.47lm	74.50l-n	G	M.soft,sweet
15002X47021	136	194.5d-f	78.73d-f	88.05b-d	E	M.soft,M.sweet
15002X47023	138	191.6d-g	77.55d-g	84.64d-g	G	M.solid,sweet
15002X47025	140	177.8h-k	71.97h-k	79.40h-k	E	M.solid,sweet
15002X47026	141	180.6g-i	73.10g-i	81.01g-j	G	M.soft,sweet

[z] The substrate consisted of 35% of wheat bran, 15% of rice bran and 50% of the sawdust from *Trema orientalis*. Water content of the substrate was adjusted to 65% and pH to 6.5. The substrate was filled into polypropane bottles by an automatic filling machine and sterilized at 120 ℃ for 1.5hrs. Each bottle contained ca. 247.06g of dry matter.

[y] The substrate was inoculated by an automatic inoculator and incubated at 18.5-20.5 ℃ for 41 days. Water content of the spawn run substrate was 65.35%.

[x] Before cropping the upmost parts of the substrate in bottles were holed 1.5-2.0cm in depth with a aluminum rod of which diameter was 1.8cm. Ater holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 90-92% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 93.-95%. The temperature of cropping room was adjusted to 15.0-17.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 600-800ppm by heat exchanger and CO_2 controller.

[w] Each dikayon was cultivated in 12-24 bottles, experimental results from the ten bottles with higher yield than others were used for statistic analysis.

[v] Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

[u] Shape of fruiting body: G= good; F= fair; E= excellent; B= bad.

[t] Texture and taste of fruiting body: M= medium.

substrate for cultivation. The results were shown in Table 6 and 7. The yields, biological efficiencies and production efficiencies of the most of the hybrid dikayons in the second fruiting test were obviously lower than those of the same dikaryons in the first test. This may be mainly related to the scale of experiment and the basis for statistic data analysis. In the first test only the data of the top 5 or 10 high productive plots were selected for statistic analysis and the repeating cultivation unit was one bottle, while 12 bottles in the second test. In addition, differences in the compositions of substrate might also be one of the reasons, however it is necessary to be confirmed further. Therefore three or five hybrid dikaryons were selected for further fruiting tests using various substrates with different compositions. These dikaryons were selected according to their good appearance and quality of fruit bodies, and higher biological efficiency and production efficiency or earliness of fruiting. The results of cultivation were shown in Table 8, 9, 11 and 12.

Table 6. Secondary fruiting test on 17 hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no.B-	Yield (g/12 bottle)	Biological efficiency (%)	Production efficiency (%)	Days from holing to 1st.harvest
15008X47024	21	1906.33a	70.40a	76.55a	23.67 bc
15008X47021	18	1779.33ab	68.12ab	72.19ab	22.17 de
15008X47019	17	1759.00abc	64.96abc	70.66abc	25.33 a
15008X47008	4	1753.83abc	64.77abc	69.15bc	19.33 gh
15025X47008	27	1706.33bcd	63.01bcd	67.76bcd	17.00 I
15029X47001	72	1702.00bcd	62.85bcd	68.25bcd	20.50 fg
15023X47004	51	1683.67bcd	62.18bcd	66.91bcde	20.50 fg
15029X47012	82	1682.00bcd	62.12bcd	66.89bcde	19.50 gh
15008X47014	12	1668.33bcd	61.61cd	67.57bcd	22.67 cd
15008X47004	1	1642.27bcde	60.67cd	67.77bcd	24.67 ab
15029X47011	81	1612.33bcde	59.54cde	64.49cde	19.00 h
15023X47017	63	1601.67cde	59.15cde	64.31cde	21.33 ef
15029X47004	74	1564.00def	57.76def	61.35def	17.00 i
15008X47013	11	1495.33efg	54.07efg	60.09ef	19.67 gh
15029X47010	80	1425.00fg	52.63fg	56.59f	22.67 bc
15029X47019	89	1397.00g	51.59g	57.42f	17.67 i
15029X47024	93	1381.00g	51.00g	54.83f	24.00 b

[z] The substrate consisted of 35% of wheat bran, 17% of rice bran, 24% of the sawdust from *Trema orientalis* and same proportion from *Liquidamber formosana*. Water content of the substrate was adjusted to 64% and pH to 6.0. The substrate was filled into polypropane bottles by automatic filling machine and sterilized at 120 ℃ for 1.5hrs. Each bottle contained about 225.6g of dry material.

[y] The substrate was inoculated by automatic inoculator and incubated at 18.5-20.5 ℃ for 41 days. Water content of the spawn run substrate was averagely 65.79%.

[x] Before cropping the upmost parts of the substrate in bottles were holed 1.5-2.0cm in depth with a aluminum rod of which diameter was 1.8cm. After holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 85-90% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 90-96%. The temperature of cropping room was adjusted to 15-16.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 650-800ppm by heat exchanger and CO_2 controller.

[w] Each dikayon had 6 replicates arranged by random, each replicate contained 12 bottles of substrate.

[v] Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

Table 7. Secondary fruiting test on 9 hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no.	Yield (g/12 bottles)	Biological efficiency (%)	Production efficiency (%)	Days from holing to 1st. harvest
15002X47013	129	2325.33a	85.88a	93.45a	19.50 cd
15026X47019	112	2243.00ab	82.84ab	89.40ab	19.83 c
15002X47001	119	2034.00bc	75.12bc	80.01bc	24.00 b
15026X47013	106	1923.67c	71.04c	83.59abc	27.33 a
15002X47004	121	1877.67c	69.34c	74.48c	24.00 b
15002X47012	128	1470.33d	54.30d	59.25d	28.33 a
15002X47026	141	1452.00d	53.62d	57.02d	17.50 d
15002X47022	114	1413.67d	52.21d	56.69d	27.17 a
15002X47025	140	1114.67e	41.17e	44.03e	23.17 b

[z]The substrate consisted of 35% of wheat bran, 17% of rice bran, 24% of the sawdust from *Trema orientalis* and same proportion from *Liquidamber formosana*. Water content of the substrate was adjusted to 64% and pH to 6.0. The substrate was filled into polypropane bottles by automatic filling machine and sterilized at 120 ℃ for 1.5hrs. Each bottle contained about 225.6g of dry material.

[y]The substrate was inoculated by automatic inoculator and incubated at 18.5-20.5 ℃ for 41 days. Water content of the spawn run substrate was averagely 65.79%.

[x]Before cropping the upmost parts of the substrate in bottles were holed 1.5-2.0cm in depth with after aluminum rod of which diameter was 1.8cm. A holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 85-90% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 90-96%. The temperature of cropping room was adjusted to 15-16.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 650-800ppm by heat exchanger and CO_2 controller.

[w]Each dikayon had 6 replicates arranged by random, each replicate contained 12 bottles of substrate.

[v]Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

Table 8. Comparisons of productivity and fruiting behavior seen in eight *Plearotus eryngii* strains cultivated on a sterilized sawdust medium supplemented with 40% of rice bran

Designation of strain	Yield (g/12 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B112	2615.5 a (218.0)	105.08 a	92.83 a	18.0 b	3.75 d	20.75 c	5.00 c
B030	2430.5 ab (202.5)	103.4 a	86.27 ab	19.0 b	3.50 d	21.50 bc	4.75 c
B012	2425.0 ab (202.0)	98.13 ab	86.07 ab	19.5 ab	3.00 d	21.50 bc	5.25 bc
B011	2258.5 bc (188.2)	91.12 b	80.16 bc	18.0 b	4.00 cd	21.00 c	4.50 c
B045	1998.0 c (166.5)	81.24 c	70.91 c	18.0 b	3.25 d	20.25 c	4.25 c
ATCC 36047	1495.0 d (124.6)	62.47 d	53.07 d	20.5 a	5.75 bc	25.25 a	6.50 abc
Holland 150	1684.0 d (140.3)	69.34 d	60.00 d	16.0 c	8.25 a	23.25 ab	8.75 a
ATCC 90212	1169.5 e (97.5)	48.24 e	41.51 e	18.0 b	6.50 ab	23.50 ab	8.25 ab

[z]The substrate consisted of 40% of rice bran and 60% of sawdust from *Trema orientalis* by weight of dry matter. Water content of the substrate was adjusted to 64% and pH to 6.5. The substrate was filled into bottles by an automatic filling machine and sterilized at 121 ℃ for one hour in an autoclave. Each bottle contained ca. 234.77g of dry matter.

[y]The substrate was inoculated with an automatic inoculator and incubated at 18.5-20.5 ℃ for 34 days.

[x]Before cropping the upmost and central parts of the spawn run substrate in bottles were holed 1.5-2.0cm in depth, 1.8cm in diameter with a PLC automatic holing machine. After holing bottles were covered with white paper for 5days, the cropping room (negative pressure) was controlled at 16-17 ℃ in temperature, 90-95% in RH and under 1050 ppm in CO_2 concentration.

[w]Each strain had 4 replicates, each replicate consisted of 12 bottles of substrate.

[v]Duncan's multiple range test, columns with the same letter or letters were not significant at 5% level.

Table 9. Comparison of the productivity and fruiting behavior seen in seven *Plearotus eryngii* strains cultivated on a sterilized sawdust medium supplemented with 30% of barley flour and 20% of rice bran

Designation of strain	Yield (g/24 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B012	4348.6 a (181.2)	84.07 ab	82.50 a	15.6 cd	6.2 abc	20.8 ab	6.4 abc
B030	4482.8 a (186.8)	86.47 a	85.05 a	14.0 e	7.2 a	20.2 c	7.4 a
B045	4069.6 b (169.6)	81.95 bc	77.21 b	17.0 ab	4.2 d	20.2 c	4.4 d
B0112	3942.0 b (164.3)	77.82 cd	74.78 b	16.2 bc	5.0 cd	20.2 c	5.4 cd
ATCC 36047	3954.6 b (164.8)	73.90 d	75.02 b	17.8 a	4.2 d	21.0 a	4.2 d
Holland 150	4082.6 b (170.1)	81.71 bc	77.45 b	15.2 cd	5.8 bc	20.0 c	5.8 bc
ATCC 90212	3300.0 c (137.5)	63.03 e	62.61 c	15.0 de	6.4 ab	20.4 bc	6.8 ab

[z]The substrate consisted of 30% of barley flour, 20% of rice bran and 50% of sawdust from *Trema orientalis* by weight of dry matter. Water content of the substrate was adjusted to 64% and pH to 6.5. The substrate was filled into bottles by an automatic filling machine and sterilized at 120 ℃ for one hour in an autoclave. Each bottle contained ca. 219.63g of dry matter.

[y]The substrate was inoculated with automatic inoculator and incubated at 18.5-20.5 ℃ for 34 days.

[x]Before cropping the upmost central part of the spawn run substrate in each bottle was holed 1.5-2.0cm in depth, 1.8cm in diameter with an PLC automatic holing machine. After holing bottles were covered with white paper for 5days, the cropping room (negative pressure) was controlled at 16-17 ℃ in temperature, 90-95% in RH and under 1050 ppm in CO_2 concentration.

[w]Each strain had 5 replicates and each replicate consisted of 24 bottles of substrate.

[v]Duncan's multiple range test, columns with the same letter or letters were not significant at 5% level.

Table 10. Formulas of the substrates for studying the effect of addition of barley flour or wheat bran on the productivities and fruiting behaviors of six *Plearotus eryngii* strains

Substrate no.	Sawdust		Barley flour		Wheat bran		Rice bran		Water added (kg)	Substrate. DW (g/bottle)	Water content before holing(%)
	FW (kg)	DW %	FW (kg)	DW %	FW (kg)	DW %	FW (kg)	DW %			
1	660	50	160.7	35	0	0	69.41	15	239.73	245.33	See note
2	660	50	0	0	162.6	35	68.53	15	224.46	237.22	See note

[z]Sawdust from *Trema orientalis* was heaped for 3 months before use. Water contents of sawdust in the two different substrates were 68.33 and 68.73% respectively. Average water content in barley flour, wheat bran and rice bran were 8.95, 11.15 and 8.95% respectively. The two substrates were added with 3.97kg and 3.92 kg of calcium carbonate respectively. Water content of each substrate was adjusted to 63%. The substrates were filled with an automatic filling machine and sterilized in an autoclave for one hr at 121 ℃. Water contents of spawn run substrate of the strains B012, B045, B112, ATCC 36047, Holland 150 and ATCC 90212 were 63.2, 64.3, 63.53, 64.73,64.53 and 65.20% in the substrate no.1, while 66.27,64.9, 65.06, 66.17, 66.50 and 64.07% in the substrate no.2.

The dikaryons B112, B030 and B012 were not significantly different from each other in yield, production and biological efficiency, however they and the hybrid B011 were significantly more productive than their parents, ATCC 36047 and Holland 150 when the sawdust medium was supplemented with only 40% of rice bran (Table 8). The hybrid B112 produced large fruit bodies with a small cap, an equal to fusiform stipe, a medium soft and fine texture, being suitable for canning (Fig. 2d1-2). It took 56 days from inoculation of substrate to the end of crop. The hybrid B030 produced slender fruit bodies with a medium size, and with a round cap, an equal and solid stipe (Fig.2c1-2). This hybrid was inclined to produce many small fruit bodies in



Fig. 2a-d. Fruiting bodies of the four *P. eryngii* hybrids, B011(a1, a2), B012(b1, b2), B030(c1, c2) and B112(d1,d2) grown on a sawdust medium which consisted of 30% wheat bran and 20% rice bran by weight

one bottle when the humidity of the cropping room was not properly controlled during cultivation. It took 56.75 days from inoculation to the end of crop. The hybrid B012 produced stout fruit bodies with a prominent eccentric or lateral stipe and a fan-shaped cap of which surface produced warts during high humid condition. The stipe was equal and solid, and consisted of a white and very fine texture (Fig.2b1-2). This hybrid took 57.75 days from inoculation to the end of crop. The hybrid B011 was inclined to produce stout fruit bodies with a large cap and a white and solid stipe (Fig. 2a1-2). It took 55.5 days from inoculation to the end of crop.

When the selected hybrid dikaryons were cultivated in a sawdust medium supplemented with 30% of barley flour and 20% of rice bran the hybrids B012 and B030 were found to be significantly more productive than their parents and the hybrid B112 in yield and biological efficiency (Table 9). The hybrid B112 was not significantly different from its parent in their yield, production and biological efficiency. It took 55, 54.4 and 54.6 days for the hybrids B012, B030 and B112 respectively from inoculation to the end of crop (Table 9). The duration for each hybrid was obviously shorter than that for the same hybrid when cultivated on a sawdust medium supplemented with only 40% of rice bran. This might be due to the difference in the composition of substrate and needs to be confirmed further.

In order to examine the effect of different supplements on the productivity of the selected hybrid dikaryons and their parents, two formulas of substrate were used (Table 10). When grown with a sawdust medium supplemented with 35% of barley flour and 15% of rice bran the results of the comparisons of yield, production and biological efficiency were similar to those of the comparison when cultivated with a sawdust medium supplemented with 30% of barley flour and 20% of rice bran except that the hybrid B112 was significantly less productive than its parents ATCC 36047 and Holland 150 (Table 11). When barley flour was substituted by wheat bran in the medium, the yield and biological efficiency of the hybrid B112 was significantly higher than those of the hybrid B012, while not significantly different from those of its parents

Table 11. Comparisons of productivity and fruiting behavior seen in six *Plearotus eryngii* strains cultivated on a sawdust medium supplemented with 35% of barley flour and 15% of rice bran

Designation of strain	Yield (g/24 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B012	4584.67 a (191.03)	80.21 a	77.87 a	17.83 a	4.33 a	21.17 a	4.83 a
B045	4213.67 b (175.57)	78.95 ab	71.57 b	16.17 b	4.00 a	19.17 b	6.00 a
B112	3495.83 d (145.66)	63.59 d	59.37 d	17.00 ab	4.17 a	20.17 ab	5.17 a
ATCC36047	4066.17 bc (169.42)	74.92 bc	69.06 bc	17.00 ab	4.33 a	20.33 ab	5.17 a
Holland 150	3846.33 c (160.26)	71.80 c	65.33 c	17.00 ab	4.33 a	20.33 ab	5.50 a
ATCC90212	3469.50 d (144.56)	65.28 d	58.92 d	16.00 b	5.67 a	20.67 ab	6.17 a

[z] Formula of the substrates and related data see Table 10.

[y] Substrates were inoculated with an automatic inoculator and incubated at 18.5-20.5 ℃ for 38 days.

[x] Before cropping the upmost central part of the spawn run substrate in each bottle was holed 1.5-2.0cm in depth, 1.8cm in diameter with a PLC automatic holing machine. After holing bottles were covered with white paper for 5days, the cropping room(negative pressure) was controlled at 16-17 ℃ in temperature, 90-95% in RH and under 1050 ppm in CO_2 concentration until the end of cropping..

[w] Each strain had 6 replicates, 24 bottles of substrate in each replicate.

[v] Duncan's multiple range test, columns with the same letter or letters were not significant at 5% level.

Table 12. Comparisons of productivity and fruiting behavior seen in six *Plearotus eryngii* strains cultivated on a sawdust medium supplemented with 35% of wheat bran and 15% of rice bran

Designation of strain	Yield (g/24 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B012	3386.67 c (141.11)	68.21 c	59.48 c	17.33 a	5.17 ab	21.50 a	5.50 b
B045	4094.17 ab (170.59)	79.60 ab	71.97 ab	16.17 abc	4.50 b	19.67 b	5.50 b
B112	3808.67 ab (158.69)	73.47 bc	66.90 ab	15.00 c	5.33 ab	19.33 b	5.67 b
ATCC36047	3708.83 bc (154.53)	72.30 bc	65.15 bc	17.17 a	5.50 ab	21.67 a	6.50 ab
Holland 150	4159.83 a (173.33)	83.13 a	73.06 a	16.67 ab	5.00 ab	20.83 ab	6.17 ab
ATCC90212	2822.67 d (117.61)	52.46 d	49.58 d	15.67 bc	7.17 a	21.83 a	8.00 a

[z] Formulas of the substrates and related data see Table 10.
[y] Other illustrations see Table 11.

(Table 12). The difference in the result of such comparison is obviously ascribed to the difference in the composition of substrate. Since wheat bran contains higher percentage of nitrogen (3.59%) than barley flour (2.01%) (Shiuan-Yun Chien, personal communication) such difference might be related to the content of nitrogen or protein in the substrate for growing the fungus. This result also shows the difference in the preference for nutrient in the substrate as reported by Visscher(9) on other ***Pleurotus*** species. In this study the hybrid B012 seems to prefer a substrate containing less nitrogen or protein and more starch, while the hybrids B112 and one of its parents Holland 150 to prefer a substrate containing more nitrogen or protein and less starch.

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杏鮑菇新品系之選育[1]

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摘 要

三個杏鮑菇品系 (*Pleurotus eryngii*)，即ATCC 36047, ATCC 90212 及Holland 150，以食用菇自動化瓶栽系統栽培評估其商業化生產之價值，試驗結果顯示ATCC 36047品系最具商業栽培潛力，其菇體中至大型，菇形佳，組織堅實，質地細膩，貨架壽命長。Holland 150 品系出菇快，菇體大型，外形亦佳，組織較爲鬆軟且菌傘表面於高溼狀態下易形成細疣，貨架壽命短，但菇體質地細膩，生物效率一般較ATCC 36047爲高。ATCC 90212品系之菇體細長，但菇體組織堅實，質地細膩且貨架壽命長。爲獲得高品質及高產之杏鮑菇新品系，分別自ATCC 36047 及Holland 150品系各分離獲得23及38個單孢菌株，先經單孢菌株自交及兩品系四交配型代表性單孢菌株間雜交，獲知兩品系各單孢菌株之交配型後，經雜交獲得92個菌絲生長正常之雙核體，以一系列不同基質配方栽培比較其產量及出菇特性，獲得四個高產、質優之雜交雙核體，即B112，B012，B030及B011品系。B112品系之菇體大型，傘小，柄多呈紡錘形，菇體組織略軟，但質地細膩。B012品系菇體中大型，菇柄粗短，上下同寬，顯著偏生或側生，傘扇形，其表面相對溼度高時易生疣狀突起，菇體組織白而堅實，質地特別細膩，。B030品系菇體中小型，柄細長上下等寬，空氣溼度高時易生太多子實體，菇體組織白且結實。B011品系易生柄粗傘大之菇體，傘圓，傘緣上翻爲多，菇體組織白而結實。以上品系自接種至採收結束約需54至60天，各品系之產量或生物效率隨栽培基質不同而異。

關鍵詞：杏鮑菇、新品系、選育

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荔枝育種-親本對子代幼年期、果實成熟期、果實性狀之影響及優良品系選拔[1]

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摘要：荔枝人工雜交授粉以‘沙坑’及‘桂味’爲母本著果率較高。調查荔枝雜交育種所使用之親本對子代植株幼年期、果實成熟期、果實品質之影響，結果顯示以‘沙坑’、‘高雄早生’及‘港尾晚生’爲母本所得之子代幼年期較短。以‘沙坑’爲母本可獲得較高比例的早熟後代，且以沙坑種的實生後裔爲母本，則其後裔果實成熟期有更提前之趨勢。自然授粉實生苗果實性狀與原母本一致性較高者爲可溶性固形物，果重以小果品種之後裔表現與母本較爲相似，果肉率則以大果品種之後裔與母本較爲相近。實生品系評估結果已選獲1個早熟及4個中晚熟實生品系。

關鍵詞：荔枝、育種、子代、幼年期、果實成熟期、果實性狀。

前　言

荔枝原產中國大陸，由於果實色、香、味俱佳而聞名於世，成爲最名貴的亞熱帶果實之一。臺灣引進栽培已有二百餘年之歷史，光復前由於栽培管理之粗放，加上品種混雜，果實品質差，所以栽培不多且呈零星分佈。至民國40～50年代間嘉義農業試驗分所及中興大學承蒙農復會（農委會前身）的經費補助，篩選出高產之黑葉品種，使得荔枝的栽培面積大幅增加[2, 3]，年產量亦直線上升。據臺灣農業年報的統計，民國87年臺灣荔枝栽培面積已達12051公頃，年產量高達49241公噸[4]。雖然黑葉荔枝之品質中等，但由於適應性強、產量高而穩定，農民一窩蜂的種植，使得該品種之種植面積迅速擴張，目前已佔臺灣荔枝總栽培面積之90％以上，成爲臺灣最主要的經濟栽培品種。由於荔枝產期短，栽培品種過於單一化的結果，造成產期集中，加上荔枝果實保鮮貯運的不易，市場難於拓展，對銷售市場形成極大的壓力，導致盛產時果品價格低落不符生產成本。因此，有必要利用育種的方式選育出不同產期的高品質優良品種，提供果農栽培，以分散產期，來解決因品種單一，產期過分集中，所導致的盛產壓力。

荔枝育種多採自然授粉 (open-pollination) 實生苗選種[9]，因此，如能即時就現有的資料對不同母本來源之實生苗果實性狀予以評估，適度選擇母本並調整各品種種子收集及播種數量，將可大量節省投入育種所須的人力、時間、經費、土地，提高效率。另外，爲準確獲得有希望之雜交組合，本研究亦採用人工雜交授粉，希望能彌補自然授粉之不足。

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1. 行政院農業委員會農業試驗所研究報告第2093號。本文原在「提昇果樹產業競爭力研討會」（民國86年3月20、21日）發表，經增添資料修改整理而成。

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本文探討親本對荔枝人工授粉著果率的影響，並就育種之親本對子代植株幼年期、果實成熟期及果實品質之影響加以分析討論，亦報導育種方面的成果。

材料與方法

一、自然授粉實生選種

調查嘉義農業試驗分所荔枝品種基因庫中植物自然雜交後代的各種性狀。該基因庫內含有臺灣主要品種共15種，包括玉荷包、桂味、糯米糍、港尾晚生、黑葉、萬年紅、三月紅、高雄早生、竹葉黑、妃子笑、桂枝、獅頭、糖薄、淮枝、新興等，採取密植且同一品種不相鄰以提高雜交授粉率，同時亦自各地混植不同品種之荔枝園取種子播成實生苗，以供選種。

二、人工雜交授粉

開花前將花穗套袋，待雌花開後第二天或三天，選取當天開放之花，每花穗授粉15-20朵左右，其餘除去，再將花穗套袋，並於授粉前一天先採取雄花置於培養皿，以燈泡照射，使其於培養皿中裂開花葯以爲花粉源[6]，參試品種如表1。

三、實生苗處理

爲節省土地的使用採廣爲世界各國常用之密植方式[8, 10, 11]，民國83年以前採行株距2.5m × 0.25m，後因考慮到生長勢弱之實生苗常受到影響而淘汰，乃將行株距略爲擴大改採3.0m × 1.0m，並將生長勢弱者分區種植。實生苗生育期間定時施肥並依需要時防治病蟲害。

四、調查及選拔

調查植株幼年期。評估果實主要性狀，這些性狀包括果重、種子重、果肉率、可溶性固形物、肉質、成熟期等。

結果與討論

一、品種間人工雜交授粉著果率

以10個臺灣主要的栽培品種（系）當父母本，進行人工雜交授粉，結果顯示品種間雜交成功率【(結果穗數/授粉穗數) × 100%】差異極大（表1），以沙坑爲母本成功率最高，可達40%以上，桂味其次亦有36%，再其次依序爲三月紅、淮荔、黑葉、玉荷包、港尾晚生，較差的爲糯米糍及獅頭分別爲3.3%及0%；若比較父本的成功率，三月紅、高雄早生最高接近30%，玉荷包次之爲25%，黑葉、沙坑、糯米糍均不及20%，73-S-20、桂味、獅頭最差，均不及5%，再就特定組合之著果率而言，沙坑×三月紅，桂味×糯米糍、桂味×沙坑，均有60%以上的成功率，沙坑×糯米糍，三月紅×高雄早生，亦約有50%左右的成功率，三月紅×黑葉，港尾晚生×沙坑可獲得40%以上之成功率，其餘組合成功率大多爲20%左右（部份組合樣本數太少不列入比較）。最差的組合是玉荷包×沙坑及獅頭×沙坑雜交60-50穗無一結果，另外黑葉與獅頭雖同爲黑葉系統，彼此雜交卻幾乎不著果。從上述的結果似可看出下列兩項趨勢：1.著果率偏低的品種仍不宜當作人工雜交授粉的母本，此與顏等[6]的試驗結果趨勢相同，沈[1]認爲荔枝經授粉受精之後，其胚胎發育過程基本與母本品種相同，即正常型（大核）品種若授以敗育型品種的花粉，其種子發育正常，敗育型（焦核型）品種若授以正常型品種的花粉，其種子仍爲敗育，因此荔枝雜交育種不能選用敗育型品種作母本。本試驗糯米糍、73-S-20、獅頭、玉荷包，均屬胚胎敗育型品種，玉荷包本次試驗雖有約18%的成功率，然其種子多爲焦核播種後僅少數爲大核的種子出苗（資料未顯示）。2.以沙坑和桂味爲母本雜交成功率頗高，尤其沙坑×三月紅，沙坑×糯米糍，桂味×糯米糍等組合有助於獲得極端產期，產量穩定，品質較優良的後代，可多加利用。但若以沙坑及桂味當父本則成功率極低，須避免採用。

表 1. 荔枝品種間人工雜交授粉著果情形[z]

Table 1. Effect of various parental crosses on fruit-seting[z]

♀ \ ♂	Sun Yueh Hong (三月紅)	Yu Her Pao (玉荷包)	Kaohsiung Early (高雄早生)	Hak Ip (黑葉)	Sah Keng (沙坑)	No Mi Tsz (糯米糍)	73-S-20	Kwai Mi (桂味)	Shih Tou (獅頭)
Sun Yueh Hong (三月紅)	—	16/54[y](28)	9/19(17)	4/9(10)	2/7(4)	0/1(0)	—	—	—
Yu Her Pao (玉荷包)	25/99(49)	—	11/50(13)	7/80(14)	0/62(0)	10/45(11)	—	—	20/72(38)
Hak Ip (黑葉)	24/99(40)	13/47(16)	—	—	5/37(8)	13/28(36)	—	—	3/17(8)
Sah Keng (沙坑)	31/47(61)	5/21(15)	3/13(9)	8/29(12)	—	5/9(13)	—	—	—
No Mi Tsz (糯米糍)	1/6(3)	3/6(5)	—	6/53(7)	4/67(14)	—	—	0/288(0)	—
73-S-20	0/2(0)	0/1(0)	—	1/1(1)	0/3(0)	0/1(0)	—	—	—
Kangwei Late (港尾晚生)	—	—	—	6/15(16)	7/16(11)	3/155(3)	—	—	—
Kwai Mi (桂味)	0/25(0)	—	—	0/3(0)	8/13(11)	15/23(43)	—	—	—
Hawi Li (淮荔)	—	—	—	0/3(0)	1/2(1)	3/8(5)	2/53(2)	—	—
Shih Tou (獅頭)	—	0/21(0)	—	0/13(0)	0/49(0)	0/33(0)	—	0/161(0)	—

[z]The table was the total data which collected from 1991 to 1993.

[y]Denominator was the number of pollinated panicle, numerator was the number of fruit-seting panicle, the number inside parentheses denotes total fruit harvested.

二、不同母本來源之自然授粉實生苗植株幼年期、果實成熟期及果實性狀評估

嘉義分所自民國77年至85年所收集之自然授粉實生苗已結果者共計324株，各品種的後代分別爲三月紅21株、紅皮34株、高雄早生30株、黑葉42株、沙坑87株、妃子笑14株、獅頭4株、港尾晚生20株、糯米糍22株、桂味20株、其它品種9株、母本不詳及實生品系後代者21株。其植株幼年期、果實成熟期、果實性狀與母本來源間的關係，如表2、3、4所示。茲說明如下：

(一) 植株幼年期頻度分佈

已結果的實生苗中屬於播種6～8年後結果者佔大多數，其中又以播種後7年開花者爲最多，沙坑、高雄早生、桂味之實生後裔在播種5年後即結果者佔該品種總結果之實生苗數10％以上，港尾晚生的後裔播種後6年結果者亦佔該品種總結果者的50％（表2），顯示以這個品種爲親本幼年期可能較短，可縮短育種的時間。

(二) 果實成熟期

中熟期，亦即成熟期與黑葉相近者，仍佔多數，此可能與中熟或近中熟品種多，父本來源充裕有關；另一方面各品種的後代或多或少仍保留了母本成熟期的特性，雖然品種間仍有很大差異，三月紅的後代有14％極早熟，71％早熟，桂味的後代屬極晚熟及晚熟者各佔25％，糯米糍及港尾晚生約有60％的子代有晚熟性狀。值得注意的是沙坑種，母本雖爲中熟期，後代卻有8％極早熟，36.8％早熟（表3），且若持續以沙坑種的子代爲母本其後裔果實成熟期有更提早的趨勢，例如沙坑子代〞71-3-55" 果實成熟期約較沙坑早兩週，而其子代如 "71-3-55-18" 又較 "71-3-55" 早一週成熟（表5）。因此在育種效率的增進上，可充份運用上述各品種的特性，將三月紅、沙坑種在一起，糯米糍、桂味、港尾晚生種在一處，較易獲得極端成熟期的後代。

(三) 果實性狀

單果重介於11公克至26公克之間，果肉率介於56至67％之間，可溶性固形物介於16.3。Brix至18.3。Brix之間（表4）。至於供試的親本之果實性狀，單果重方面：大果品種如三月紅及沙坑在24至

表2. 荔枝不同母本來源之自然授粉實生苗幼年期頻度分佈表 (%)

Table 2. Distribution of seedlings in juvenile period of progenies from various mother plant varieties (%)

Mother plant varieties	Juvenile period (Years)						
	5	6	7	8	9	10	11
Sun Yueh Hong (三月紅)	—	28.6	57.1	14.3	—	—	—
Hung Pi (紅皮)	5.9	23.5	35.3	5.9	11.8	17.7	3.4
Kaohsiung Early (高雄早生)	13.3	13.3	13.3	40.0	13.3	6.8	—
Hak Ip (黑葉)	4.9	9.8	56.0	9.8	2.4	12.2	4.9
Sah Keng (沙坑)	11.7	23.3	26.7	28.3	5.0	1.7	—
Fei Tzu Hsiao (妃子笑)	—	—	64.3	14.3	14.3	7.1	—
Shih Tou (獅頭)	—	—	—	—	—	100	—
Kangwei Late (港尾晚生)	—	50.0	10.0	10.0	10.0	20.0	—
No Mi Tsz (糯米糍)	—	—	36.4	9.1	—	54.5	—
Kwai Mi (桂味)	10.0	15.0	35.0	40.0	—	—	—

表3. 荔枝現有品種實生後裔果實成熟期之變異分佈 (%)

Table 3. Distribution of seedlings in the mature seasons of progenies from various mother plant varieties (%)

Mother plant varieties	Maturity seasons[z](%)				
	Very-early	Early	Medium	Late	Very-late
Sun Yueh Hong (三月紅)(Very-early)[y]	14.3	71.4	14.3	—	—
Hung Pi (紅皮)(Early)	—	17.6	52.9	29.5	—
Kaohsiung Early (高雄早生)(Early)	—	6.7	73.3	20.0	—
Hak Ip (黑葉)(Medium)	—	4.8	61.9	23.8	9.5
Sah Keng (沙坑)(Medium)	8.0	36.8	46.0	17.2	—
Fei Tzu Hsiao (妃子笑)(Medium)	—	—	64.3	35.7	—
Shih Tou (獅頭)(Medium)	—	—	—	—	—
Kangwei Late (港尾晚生)(Late)	—	—	40.0	60.0	—
No Mi Tsz (糯米糍)(Late)	—	—	45.5	54.5	—
Kwai Mi (桂味)(Very-late)	—	—	50.0	25.0	25

[z] On the basis of "Hak Ip" maturity season each year, very early - 20 days earlier, early - 10 days earlier, late - 10 days later, very late - 20 days later.

[y] mother plant varieties maturity season.

表4. 不同母本來源之自然授粉實生苗果實性狀比較表[z]

Table 4. Main fruit characters of progenies from various mother plant varieties

Mother plant varieties	Fruit weight (g)	Aril ratio (%)	Total soluble solids (°Brix)
Sun Yueh Hong (三月紅)	19.7 ± 6.2	64.8 ± 5.7	16.3 ± 3.1
Hung Pi (紅皮)	15.4 ± 3.2	60.6 ± 8.3	16.3 ± 2.0
Kaohsiung Early (高雄早生)	18.9 ± 7.1	63.7 ± 7.1	17.2 ± 1.3
Hak Ip (黑葉)	19.0 ± 6.4	65.4 ± 8.7	16.5 ± 1.9
Sah Keng (沙坑)	21.1 ± 5.2	63.0 ± 10.2	16.2 ± 2.1
Fei Tzu Hsiao (妃子笑)	20.3 ± 6.4	62.9 ± 5.0	16.8 ± 1.6
Shih Tou (獅頭)	26.7 ± 2.5	61.3 ± 4.3	15.7 ± 1.8
Kangwei Late (港尾晚生)	18.7 ± 6.7	71.5 ± 4.4	16.6 ± 2.5
No Mi Tsz (糯米糍)	15.3 ± 4.8	60.9 ± 6.9	18.5 ± 1.9
Kwai Mi (桂味)	15.8 ± 1.7	63.3 ± 2.9	17.7 ± 2.1

[z] The total open pollinated seedlings were 324. Data were the means of total progenies with SD per variety.

28公克之間，中果品種如高雄早生、黑葉、妃子笑、獅頭、港尾晚生在23至24公克之間，小果品種如紅皮、糯米糍、桂味在17至20公克之間；在果肉率方面：獅頭、港尾晚生、糯米糍在75％左右，黑葉及沙坑在70％左右，其餘品種則在65％左右；可溶性固形物方面則除糯米糍及桂味在18。Brix以上，其餘則在17。Brix左右。若將子代與母本相較。小果品種如桂味、糯米糍、紅皮的後代有較小的單果重介於11至20公克之間，然大果品種如沙坑、三月紅之後代、卻未較中果品種如黑葉有較高的單果重。港尾晚生的後代有最高的果肉率可達75％，糯米糍的後代表現並未如預期，大果品種如沙坑、三月紅的後代則存有很大的變異，其中不乏果肉率70％以上者。至於可溶性固形物則以糯米糍後代爲最佳，達到17至20度Brix之間，次佳者爲桂味爲15至20度Brix，其餘各品種則多介於14至18度Brix之間（表4）。綜合以上所述可知，自然授粉實生苗果實性狀與原母本品種一致性較高者爲可溶性固形物，果重則以小果品種的後代表現較爲相似，反之，果肉率則以大果品種勉強與其母本相近。

由前述的調查結果可知，沒有任何一個品種的後代可輕易同時達到大果、高果肉率及高可溶性固形物等三項要求，亦即欲以一次雜交就選到理想品種十分困難，主要的瓶頸在於大果品種可溶性固形物常較低，而小果品種如糯米糍、桂味雖有較高的可溶性固形物，但果肉率卻不合乎理想，改善之道是擴大種植花期相似，較可能具有高果肉率子代及高可溶性固形物子代的親本於一處任其雜交，例如港尾晚生與糯米糍或沙坑與玉荷包（玉荷包產量低又多爲焦核，種子獲得不易，但可充當父本）；然另一方面，由於影響果肉率的主要因素小核率易受其他品種及環境的影響，高小核率之品種與其他品種混植時，小核率往往較單一品種種植時爲低，實生品系因密植的影響，小核率常更不易評估[7]；因此，高可溶性固形物但果肉率尚欠理想的實生品系，常有賴品種比較試驗做進一步的評估以決定是否淘汰；同理，由於密植時管理較爲粗放，除可溶性固形物欠佳，其餘性狀均極優良的實生品系，在正常管理時，可溶性固形物是否可進一步的提高亦有賴往後的評估。因此今後育種工作亦必須改進栽培環境，以進一步評估優良有希望單株之果實性狀及其潛能。另外，若再就現有實生品系資料分析可發現以沙坑、三月紅、港尾晚生爲母本之子代植株幼年期較短（表2），具有極端產期可能性較高（表3），果實性狀變異性大，大果且高果肉率比例較高（表4），尤其是沙坑種本身產量頗佳，因此，在決定收集及播種自然授粉園種子數量時，此三品種特別是沙坑應有較高的比例，較可提高育種效率。

三、實生品系評估

民國80年以前所選出的優良品系，顏及張[5]，顏等[7]已有有詳細討論，在此不予以詳述，80年以後所選出較爲優良者其果實性狀如表5，共計5品系，71-3-55-18屬早熟品系，產期比黑葉早18-21天，略晚於三月紅，但品質比三月紅佳。其餘4品系產期與黑葉相近或略晚，以76-C-2果大、子小，糖度高，最具有潛力。沈[1]認爲品質優良的荔枝品種最基本的要求應是果重20公克，果肉率70％，可溶性固形物17度Brix。顏等[7]認爲在臺灣甜度爲影響荔枝品質之最重要因素，17度Brix以上者多可令

表5. 荔枝優良實生果實主要性狀分析表

Table 5. Fruit characteristics of promising lines of litchi

Lines	Parents	Fruit weight (g)	Seed weight (g)	Aril ratio (%)	Total soluble solids (°Brix)	Maturity seasons
71-3-55-18[z]	71-3-55	26.9	2.5	66.8	15.3	Late of May
77-3-2-17	Sah Keng (沙坑)	18.5	0.8	76.0	17.1	Early of June
77-S-3	Unknown	21.9	2.0	70.0	19.0	Early of June
76-C-2	Hak Ip X Yu Her Pao (黑葉X玉荷包)	27.7	1.3	72.7	18.1	Mid of June
78-9-5-10	Fei Tzu Hsiao (妃子笑)	19.6	1.4	67.5	19.4	Late of June

[z]The first number of the line number was the year which got the seed, but the seed of 71-3-55-18 was gotten in 1990.

人滿意，若低於16度Brix者較不易受歡迎。若依上述觀點，表5中的品系僅76-C-2，77-S-3達到標準。但考量國人喜歡高甜度或種子小的習性，且市場極需異熟期的品種以調節產期，因此另外3品系以具有上述之一的特殊性狀而入選。

荔枝育種工作執行至今已育出幾個具有不同特性之實生品系，但面對荔枝一萬餘公頃的栽培面積且加入世界貿易組織 (WTO) 在即的今天，這些品系仍是不夠，主要是可供果農選擇的極端或異時產期、豐產、穩產、質優甚至退而求其次果實品質可爲市場所接受之品系仍然太少，雖然經過多年育種經驗，及對遺傳特性的了解，達到上述目標的育種年限或可縮短，但另一重要措施應爲再蒐集品種及擴大種源，例如自中國大陸引入一年兩收或兩年三收的四季荔或豐穩產大果的品種，將其特性導入現有的栽培品種，則育種效率應可大幅提昇。

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Litchi (*Litchi chinensis* Sonn.) breeding-The influences of mother plant on juvenile period, fruit maturity season and fruit characters, and the selection of promising lines [1]

Jer-Way Chang [2], Cheng-Nan Chao [2] and Chung-Ruey Yen [3]

Summary

In controlled pollination, 'Sah Keng' and 'Kwai Mi' used as mother plant could produce more fruits than other varieties. The influences of mother plant on juvenile period, fruit maturity season and fruit characters of progenies were studied. 'Sah Keng', 'Kaohsiung Early' and 'Kangwei Late' used as mother plant contributed to shorter juvenile period of progenies than other varieties. The progenies of 'Sah Keng' showed divergence toward early maturity season. When the progenies of 'Sah Keng' were used as mother plant, some seedlings of the 2nd progenies had maturity season one week earlier than mother plant and three weeks earlier than 'Sah Keng'. The contribution from mother plant to fruit size of progenies was not quite significantly correlated. However, the progenies of small mother plant produced smaller fruits more than the larger mother plant did. Aril ratio of progenies was not positively correlated with that of mother plant. Soluble solids in fruits of progenies were rather consistent with those in mother plant. Five promising lines were selected for advanced test in the breeding program.

Key words : *Litchi chinensis* Sonn., Breeding, Progeny, Juvenile period, Fruit.

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微生物接種對洋桔梗生長與植體磷變化之研究[1]

林素禎[2] 林淑媛[2] 吳繼光[3]

摘要：本試驗利用接種叢枝菌根菌、溶磷細菌及游離性固氮細菌，期能幫助洋桔梗生長。洋桔梗幼苗在4個葉片時進行接種試驗，栽培介質爲蛭石、珍珠石與泥炭苔的混合物（體積比爲6：3：1），生長溫度爲23℃/18℃（白天/晚上），每週以修正過的霍格蘭營養液澆灌。在接種116天後，單獨接種菌根菌或游離性固氮菌皆可明顯地增加洋桔梗地上部與根之乾重，地上部乾重分別增加17%與52%，根乾重分別增加56%與50%。單獨接種溶磷細菌之處理與對照組無明顯差異。複合接種菌根菌與溶磷細菌或游離性固氮菌，或三種菌一起接種，這三種處理皆可明顯增加洋桔梗之地上部與根之乾重，地上部乾重分別增加30%，42%與39%，根乾重分別增加53%，53%與56%。複合接種菌根菌與游離性固氮菌及複合接種菌根菌、溶磷細菌與游離性固氮菌這兩種處理，洋桔梗之花朵數最多，明顯比對照組多2.4朵與3.2朵，且比對照組提早14天開花。接種菌根菌後再接種游離性固氮菌或溶磷細菌，或三種菌複合接種，這三處理之菌根菌感染率皆比單獨接種菌根菌之處理者低，但地上部之乾重皆比單獨接種菌根菌之處理者重。不同的微生物接種處理對植體磷濃度皆無增加效果。

關鍵詞：洋桔梗、叢枝菌根菌、游離性固氮細菌、溶磷細菌、磷。

前　言

洋桔梗 (*Eustoma grandiflorum*) 是近年來在國內發展快速的新興切花之一，花形優美，花色多，且瓶插壽命長，除了以切花生產的品種外，亦有矮性品種供盆栽與花壇的佈置。洋桔梗屬於龍膽科，一、二年生植物，原產於美國中部內布拉斯加至墨西哥之間的石灰岩草原地帶，土壤pH值爲7.4~8，含有多量的鈣、鎂、鉀。育種工作主要在日本進行，育出的品種相當多而富變化。洋桔梗小苗生長緩慢，幼苗期長，小苗長至二對本葉以上才可定植，約需2~3個月[10]。洋桔梗的種子很小，發芽最適合的溫度爲25℃，屬於好光性種子，播種時不宜覆土，光量需在5000Lux以上，幼苗生長須在涼溫下才不會產生簇狀化，以日溫23℃，夜溫18℃最好[11]。

叢枝內生菌根菌能幫助宿主廣泛地吸收土壤中的養分特別是磷肥，進而對作物有促進生長的效用[23]。此外，叢枝內生菌根菌也可提高植物幼苗移植的存活率，加速健康種苗的生長，提早開花，增加花朵數及花朵大小，延長切花的插花壽命，幫助植物抵抗逆境等[1-3, 5-8, 23]。

土壤中除了菌根菌外，還有一些有益微生物可幫助作物生長，如溶磷細菌與游離性固氮細菌。磷在土壤中約佔0.05%(w/w)，但植物可利用的磷只佔全磷量的0.1%[32]，磷肥施入土壤後，僅5-15%爲當季作物吸收，其餘在酸性土壤中多與鋁及鐵結合形成難溶性鋁結合磷及鐵結合磷；在鹼性土壤中多與

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鈣結合，形成難溶性鈣結合磷，對植物吸收造成阻礙[31, 29]。接種溶磷菌後，可促進植物生長，增加磷的吸收，提高磷肥的利用率[13, 22, 25]。

游離性固氮細菌廣泛存在土壤中，是提供土壤氮肥重要來源之一[26, 27]。游離性固氮細菌亦可產生植物生長促進物質 (plant growth-promoting substances)，幫助作物生長，增加作物產量[16, 18, 19]。游離性固氮細菌 (*Azospirillum*) 產生的植物生長促進物質，如IAA， gibberellin和類似cytokinin的物質[33]，作物接種*Azospirillum*後，側根增加，側根上的根毛亦增多[28, 33]。

植物複合接種菌根菌與溶磷細菌後，其所吸收的氮量及磷量比單一接種者所吸收的多[14, 30]。亞洲型百合經複合接種菌根菌與溶磷細菌後，植株高度與葉片數明顯比對照組與單一接種者增加[7]。玉米單獨接種菌根菌 (*G. fistulosum*) 後，地上部乾重與總葉面積與對照組比較沒有明顯增加，但複合接種菌根菌與溶磷螢光細菌 (*Pseudomonas putida*) 後，可明顯增加地上部乾重與總葉面積，且菌根菌感染率亦顯著增加[21]。

植物複合接種菌根菌與游離性固氮細菌後，其根圈內的細菌與放線菌的數目比單一接種者多[15]。番茄接種菌根菌 (*G. fasciculatum*) 後，根圈內游離性固氮細菌 (*Azotobacter chroococcum*) 之數目增加，番茄根部接種游離性固氮細菌 (*A. chroococcum*) 後，菌根菌 (*G. fasciculatum*) 之感染率及產孢量增加，複合接種菌根菌與游離性固氮細菌的番茄，其乾物重明顯比未接種者大[15]。複合接種菌根菌與游離性固氮菌後，可明顯的促進文旦柚氮與磷的吸收量[8]。

對於將菌根菌及其他有益微生物複合接種於洋桔梗之研究尚未見報導，本報告除了探討菌根菌單獨接種之外，亦探討菌根菌與溶磷細菌、菌根菌與游離性固氮菌兩菌種複合接種及菌根菌、溶磷細菌與游離性固氮菌三種菌種複合接種，對洋桔梗生長與植體磷濃度之影響。

材料與方法

試驗作物：洋桔梗品種爲矮性品種（莉莎-藍），以種子播種育苗，俟洋桔梗幼苗長至4個葉片時，選取生長勢相當之幼苗進行接種試驗。

作物生長環境：種子灑播於育苗介質（蛭石、珍珠石與泥炭苔體積比＝1：1：1）上，以灑水壺澆水保濕，種子發芽溫度爲25℃，育苗60天後，選取生長勢相當之幼苗進行接種試驗。洋桔梗接菌試驗於農業試驗所大型生長箱中進行，生長箱光照強度8000~12000Lux，光照時間16小時，光照時溫度爲23℃，無光照時溫度爲18℃。

栽培介質：接菌後之栽培介質爲蛭石、珍珠石與泥炭苔之混合物（體積比6：3：1）。泥炭苔爲pH值近於6，無添加養份之商品。磷礦石粉、米糠與栽培介質一起混合，磷礦石粉的添加量爲每升介質加24g，米糠的添加量爲每升介質加12g。每盆栽培介質的量爲3升。磷礦石粉P_2O_5含量爲32~33%，由臺灣肥料公司購得。米糠氮含量爲1.95%，磷濃度爲1.77%，本試驗利用米糠作爲營養來源之一。

試驗處理：試驗處理有七種：(1) 對照組，不接種菌 (Control)，(2) 接種菌根菌 (VA)，(3) 接種溶磷細菌 (PS)，(4) 接種游離性固氮菌(NF)，(5)複合接種菌根菌與溶磷細菌 (VA+PS)，(6) 複合接種菌根菌與游離性固氮菌(VA+NF)，(7) 複合接種菌根菌、溶磷細菌與游離性固氮菌 (VA+PS+NF)。每一處理之洋桔梗各種20盆，每盆一株。

試驗菌種：(1) 菌根菌：試驗所使用之菌根菌菌種爲*Glomus intraradices*，菌根菌以高粱當宿主，高粱種在以砂加煉石爲介質的盆栽中，高粱收穫後，以塑膠袋密封，置於冷房4℃中保存，本試驗所使用之菌土在冷房中保存了6個月。(2) 溶磷細菌 (TARI-93111623-1)：由選擇性培養基篩選出可溶解磷酸鈣之菌株[35]，菌株來源爲農業試驗所之試驗田土與自製堆肥。(3) 游離性固氮菌 (TARI-94121508)：以選擇性培養基[17]篩選之菌株，再經氣相層析儀 (GC) 測定，確定菌株可將乙炔還原成乙烯，即爲可固

氮之菌株，菌株之來源亦由農業試驗所之試驗田及堆肥中篩選而得。溶磷細菌與游離性固氮菌培養一週後，菌液以離心法分離培養液與菌體，培養液倒棄，收集菌體，菌體加水稀釋至定量體積，菌液使用時之菌數爲 10^7cells ml^{-1}。

接種方法：(1) 菌根菌之接種：接種方法爲菌土置於洋桔梗根部下方 2~3 公分處，菌土的接種量爲每株幼苗接 100 個孢子。(2) 溶磷細菌、游離性固氮菌之接種：接種方法爲吸取處理好之菌液，延幼苗之莖部底端澆灌，使菌液流至根部。每株幼苗接種量爲 1×10^8cells。溶磷細菌與游離性固氮菌的接種時間爲菌根菌接種兩週後再行接種。

施肥方法：苗期每星期以Modified Hoagland營養液（含磷 16mg/kg）澆灑。接菌處理後，以 1/2 磷之營養液（含磷 8mg/kg）澆灌，生長初期，每週澆一次營養液，每盆 50ml，俟植株長至 10~12 片葉子時，每週澆兩次營養液，每盆 50ml。

生育調查與植體分析：洋桔梗開花後定期調查花朵數。於接菌後生長 116 天採收植株，每處理各取 5 株作菌根菌感染率調查，其餘植株，分地上部（不含花）與根兩部份，分別洗淨、烘乾、秤其乾物重。葉片與根分別磨碎，並以硫酸分解與過氧化氫氧化，分解液以鉬黃法測定磷濃度。

菌根菌感染率之測定：以酸性品紅 (acid fuchsin) 染色[24]，以格子線交叉法計算菌根菌感染率[20]。

結果與討論

一、微生物接種對洋桔梗花朵數之影響

洋桔梗經處理後定期調查其花朵數，結果如表 1，由表 1 可知，複合接種菌根菌、溶磷細菌與游離性固氮菌之處理 (VA+PS+NF)，在試驗處理 86 天後，每株花朵數平均爲 0.7 朵，爲所有處理中花朵數最多之處理，對照組 (Control) 尚未開花。在試驗處理 100 天後，複合接種菌根菌、溶磷細菌與游離性固氮菌之處理 (VA+PS+NF)，每株花朵數平均爲 2.7 朵，爲所有處理中花朵數最多之處理，另外，複合接種菌根菌與游離性固氮菌之處理 (VA+NF)，每株花朵數平均爲 1.8 朵，這兩處理每株花朵數皆明顯比對照組 (Control) 多，對照組尚未開花。以上這兩個處理皆比未接菌的對照組提早 14 天開花。其他四組處理，接種菌根菌 (VA)，接種溶磷細菌 (PS)，接種游離性固氮菌 (NF)，及複合接種菌根菌與溶磷細菌 (VA+PS)，這四組處理花朵數雖然比對照組多，但在統計上與對照組無顯著差異。對照組在本試驗第 100 天後才開始開花，因此所有接菌的處理，雖然在統計上並非全然有顯著的差異，但皆有提早 14 天開花的現象。有關菌根菌與溶磷菌接種可提早開花的報告很多，如非洲菊、一串紅、瓜葉菊與百日草等花卉接種繡球屬菌根菌可提早開花[1, 4, 9, 12]；而矮牽牛與一串紅接種溶磷菌亦可提早開花[3, 4]。但接種游

表 1. 洋桔梗接種不同微生物後花朵數變化的情形

Table 1. Effect of different microbial inoculation on the flower number of *Eustoma grandiflorum*.

Unit: flower no./plant

Treatment	Days after inoculation						
	86	91	96	100	105	112	116
Control	$0^{b\,Z}$	0^{b}	0^{c}	0.1^{c}	0.1^{c}	0.2^{d}	0.2^{d}
VA[Y]	0.2^{b}	0.3^{b}	0.4^{bc}	0.4^{bc}	0.7^{bc}	0.8^{bcd}	1.0^{cd}
PS	0.1^{b}	0.1^{b}	0.2^{bc}	0.4^{bc}	0.5^{bc}	0.7^{cd}	0.8^{cd}
NF	0.2^{b}	0.6^{ab}	0.8^{bc}	1.3^{bc}	1.8^{ab}	2.0^{abc}	2.1^{abc}
VA+PS	0.3^{b}	0.4^{b}	0.7^{bc}	1.0^{bc}	1.2^{bc}	1.2^{bcd}	1.3^{bcd}
VA+NF	0.4^{ab}	0.6^{ab}	1.3^{ab}	1.8^{ab}	2.0^{ab}	2.3^{ab}	2.6^{ab}
VA+PS+NF	0.7^{a}	1.2^{a}	2.1^{a}	2.7^{a}	3.1^{a}	3.3^{a}	3.4^{a}

[Z] Means each in column with the same letters are not significantly different by Duncan's multiple range test, 5% level.

[Y] VA: Vesicular arbuscular fungi; PS: Phosphorus solubilizing bacteria; NF: Free-living N_2-fixing bacteria.

離性固氮菌可提早開花的報告並不多見，因游離性固氮菌之接種試驗大多集中在禾本科作物[18]。

在試驗處理116天後，複合接種菌根菌、溶磷細菌與游離性固氮菌之處理，每株花朵數平均爲3.4朵，爲所有處理中花朵數最多之處理，另外，複合接種菌根菌與游離性固氮菌之處理，每株花朵數平均爲2.6朵，接種游離性固氮菌之處理，每株花朵數平均爲2.1朵，這三種處理之花朵數皆明顯比對照組多，對照組每株花朵數平均爲0.2朵。其他三組處理，接種菌根菌，接種溶磷細菌，及複合接種菌根菌與溶磷細菌，這三組處理與對照組無顯著差異。

在試驗處理116天後，複合接種菌根菌、溶磷細菌與游離性固氮菌之處理開花率爲65%，爲所有處理中開花率最高之處理，另外，複合接種菌根菌與游離性固氮菌之處理開花率爲60%，接種游離性固氮菌之處理，開花率爲55%，其他三組處理，接種菌根菌，接種溶磷細菌，及複合接種菌根菌與溶磷細菌，這三組處理開花率皆爲35%，而對照組之開花率爲10%。在本項試驗中發現單獨接種游離性固氮細菌或與菌根菌複合接種對於洋桔梗的開花及花朵產量皆有正面的影響。

二、微生物接種對洋桔梗乾重，磷吸收量與植物菌根菌感染率之影響

由表2可知，接菌116天後洋桔梗在地上部之乾重方面，除了接種溶磷細菌之處理與對照組無顯著差異外，其他五種處理之地上部乾重皆明顯比對照組重。對照組地上部乾重爲1.14g/plant，接種溶磷細菌之處理地上部乾重爲1.23g/plant。其他五種處理其地上部乾重爲1.33~1.73g/plant。在根之乾重方面，除了接種溶磷細菌之處理與對照組無顯著差異外，其他五種處理之根乾重皆明顯比對照組重。對照組根乾重爲0.36g/plant，接種溶磷細菌之處理根乾重爲0.38g/plant。其他五種處理其根乾重爲0.54~0.56g/plant。

表2. 洋桔梗接種不同微生物製劑後, 對於植體乾重、磷濃度及感染率之影響

Table 2. Effect of inoculation with different microbies on the dry weight, P contents and infection rate of *Eustoma grandiflorum*

Treatment	Shoot dry wt. (g/plant)	Root dry wt. (g/plant)	Leaf P (%)	Root P (%)	Infection rate (%)
Control	1.14 ^{dZ}	0.36^{b}	0.32^{a}	0.25^{a}	0^{c}
VA ^{Y}	1.33^{bc}	0.56^{a}	0.24^{bc}	0.20^{bc}	77^{a}
PS	1.23^{cd}	0.38^{b}	0.31^{a}	0.24^{a}	0^{c}
NF	1.73^{a}	0.54^{a}	0.31^{a}	0.20^{bc}	0^{c}
VA+ PS	1.48^{abc}	0.55^{a}	0.26^{b}	0.22^{ab}	50^{b}
VA+ NF	1.62^{a}	0.55^{a}	0.21^{bc}	0.16^{d}	36^{b}
VA+PS+NF	1.59^{ab}	0.56^{a}	0.23^{bc}	0.19^{c}	36^{b}

^{Z} Means in each column with the same letters are not significantly different by Duncan's multiple range test, 5% level.

^{Y} VA: Vesicular arbuscular fungus; PS: Phosphorus solubilizing bacterium; NF: Free-living N_2-fixing bacterium.

在葉的磷濃度方面，對照組，接種溶磷細菌，及接種游離性固氮菌之處理，這三組處理之磷濃度較高，爲0.31~0.32%，明顯比其他四組處理高，其他四組處理磷濃度爲0.21~0.26%。在根的磷濃度方面，對照組，接種溶磷細菌，及接種菌根菌與溶磷細菌，這三組處理之磷濃度較高，爲0.22~0.25%。接種菌根菌與接種游離性固氮菌這兩處理之磷濃度皆爲0.20%。複合接種菌根菌與游離性固氮菌及複合接種菌根菌、溶磷細菌與游離性固氮菌，這兩處理之磷濃度最低，分別爲0.16%與0.19%。在七種處理中，複合接種菌根菌與游離性固氮菌，及複合接種菌根菌、溶磷細菌與游離性固氮菌，這兩處理地上部與根之乾重皆明顯比對照組重，但葉與根之磷濃度卻比對照組低，這可能因部份磷已移至花朵及種子，而這兩處理之花朵數最高，故在葉與根之磷濃度較低。

在菌根的感染率方面，接種菌根菌之處理，其感染率爲77%，明顯比其他處理高，接種菌根菌與

溶磷細菌之處理，其感染率爲50%，複合接種菌根菌與游離性固氮菌之處理，其感染率爲36%，複合接種菌根菌、溶磷細菌與游離性固氮菌之處理，其感染率爲36%，而對照組，接種溶磷細菌，及接種游離性固氮菌，這三組處理之感染率爲0。接種菌根菌後再接種游離性固氮菌或溶磷細菌，或三種菌複合接種，皆可降低菌根菌之感染率。Gryndler and Vosatka[21]所做之玉米接菌試驗中，接種溶磷細菌 *Pseudomonas putida* 之活細胞後，可使 *Glomus fistulosum* 感染率明顯提高；Bagyaraj and Menge[15]所做的番茄接菌試驗中，接種 *Azotobacter chroococcum* 亦可增加 *Glomus fasciculatus* 之產孢量與感染率，此結果與本試驗結果不同。吳等人[5]於火鶴花組織培養苗接種菌根菌 *Glomus intraradices*，其菌根菌感染率爲41.7 %，複合接種菌根菌、溶磷細菌與游離性固氮菌後，菌根菌感染率下降爲22.5 %；王等人[3, 4]於一串紅與矮牽牛之微生物肥料接種試驗中，發現接種溶磷菌或枯草桿菌皆可降低菌根菌之感染率。此結果與本試驗結果相同。造成這兩極化的結果，有待進一步研究，推測可能與菌根菌及其所搭配的微生物種類有關。菌根菌感染率的提高並未必對植物有促進生長的作用，相反的，由於較高的感染率，菌根菌爲了生長會消耗植物較多的碳源，反而增加植物的負擔。在這種情形下，對作物的生長未必有利。

此外，本試驗也證實在栽培介質中添加12g的米糠 (212.4mg-P/L) 並不會影響菌根菌感染洋桔梗。在接菌116天後，菌根菌感染率可高達77%，且接種菌根菌與不接菌之對照組比較，可明顯增加洋桔梗地上部與根之乾重，花朵數亦增加，但未達顯著。

單獨接種溶磷細菌之處理與不接菌之對照組比較，洋桔梗地上部乾重、根之乾重與花朵數雖然比不接菌之對照組多，但在統計上無明顯差異。

單獨接種游離性固氮菌之處理與不接菌之對照組比較，可明顯增加洋桔梗之地上部與根之乾重，花朵數亦明顯增加。根據Tien等人[33]試驗指出，游離性固氮菌 (*Azospirillum brasilense*) 可產生植物生長促進物質，如IAA，gibberellin和類似cytokinin的物質。本試驗中所使用之游離性固氮菌亦可產生IAA（未發表資料），故有關游離性固氮菌之作用機制，除了固氮作用之外，植物生長促進物質亦是原因之一。

接種菌根菌後再接種溶磷細菌，在接菌116天後與不接菌之對照組比較，可明顯增加洋桔梗之地上部與根之乾重，花朵數亦增加，但未達顯著；複合接種菌根菌與溶磷細菌之處理，與單獨接種菌根菌或溶磷細菌之處理相比較，其地上部之乾重與花朵數略增加，但無顯著差異。由此可見雖然本試驗所使用之溶磷菌株在功能上較弱，但仍有因共同接種所表現出的加成效果 (synergistic effect)。筆者在文旦柚與百合之微生物接菌試驗，以及Raj等人在小米之微生物接菌試驗中皆有類似之結果[7, 8, 30]。而王等人[3, 4]於一串紅與矮牽牛之微生物肥料接種試驗中，複合接種菌根菌與溶磷菌則無加成效果，這可能與菌根菌及其所搭配的微生物種類有關。

接種菌根菌後再接種游離性固氮菌，在接菌116天後與不接菌之對照組比較，可明顯增加洋桔梗之地上部與根之乾重，花朵數亦明顯增加。複合接種菌根菌與游離性固氮菌之處理，與單獨接種菌根菌之處理比較，可明顯增加洋桔梗地上部之乾重與花朵數。複合接種菌根菌與游離性固氮菌之處理，與單獨接種游離性固氮菌之處理比較，可增加花朵數0.5朵，但在統計上不顯著，在地上部與根之乾重方面亦無明顯差異。由此可見本試驗所使用之游離性固氮菌株在功能上較強，與菌根菌共同接種後所表現出的加成效果 (synergistic effect) 較弱。在Bagyaraj and Menge[15]所做的番茄接菌試驗中，亦有類似之結果。

接種菌根菌後再接種溶磷細菌與游離性固氮菌，在接菌116天後與不接菌之對照組比較，可明顯增加洋桔梗之地上部與根之乾重，花朵數亦明顯增加。而三種菌複合接種之處理與單獨接種菌根菌之處理比較，洋桔梗地上部與根之乾重則無明顯差異，但花朵數明顯增加；三種菌複合接種之處理與單獨接種溶磷細菌之處理比較，洋桔梗地上部與根之乾重皆明顯增加，花朵數亦明顯增加；三種菌複合

接種之處理與單獨接種游離性固氮菌之處理比較，洋桔梗地上部與根之乾重則無明顯差異，花朵數增加1.3朵，但差異不顯著。顯然三種微生物的複合接種較單一接種者有助於花朵產量。

由於複合接種微生物的效果是一綜合性的考量，所有微生物間的相互作用應予重視。近來Toro等人[34]所做的試驗結果顯示兩種菌根菌 (*G. mosseae*) 和 (*G. fasiculatum*) 與溶鈣磷的溶磷菌複合接種效果，前者較後者表現佳。不同的溶磷細菌相比較，鐵磷的溶磷細菌單獨的接種效果較與菌根菌複合接種的效果佳；反之鋁磷的溶磷菌與菌根菌複合接種效果較單獨接種者佳。本試驗所用的溶磷細菌係以鈣磷選擇性培養基所篩選之菌株，而所使用之菌根菌爲*G. intraradices*。本試驗中，溶磷菌與菌根菌的共同接菌效果有增加但不顯著，這也可能是因菌株的組合非最佳所致。

洋桔梗複合接種菌根菌、溶磷細菌與游離性固氮菌之處理，尚未見文獻報導，有關其詳細的生長促進機制，尚待進一步研究。

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Microbial Inoculation Effect on the Growth and Phosphorus Concentration of *Eustoma grandiflorum* [1]

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Summary

The main purpose for this experiment was to evaluate the effect of VA mycorrhizal fungi (VA), phosphate-solubilizing (PS), and free-living nitrogen-fixing (NF) bacteria on the growth of *Eustoma grandiflorum*. Plants with 4 true-leaves were used for inoculation. Bulbs of calla lily were grown in the vermiculite-perlite-peat medium (6 : 3 : 1, v/v/v) with day/night temperatures as 23/18 ℃ and were fertilized with modified Hoagland's solution weekly. The growth effect of the microbes to calla lily was investigated and measured 116 days after inoculation. Treatments inoculated with either VA mycorrhizal fungus (VA) and free-living nitrogen-fixing bacteria (NF) have significantly higher dry weight of shoot (17% and 52%, respectively) and dry weight of root (56% and 50%, respectively) than those of uninoculated treatments (control). The plant growth of the treatment inoculated with phosphate-solubilizing bacteria (PS) was not significantly different from that of control. Any combination of VA+PS, VA+NF, or VA+PS+NF could promote 30%, 42%, and 39% dry weight of shoot, and 53%, 53% and 56% dry weight of root, respectively. Treatment inoculated with VA+NF and VA+NF+PS produced 2.4 and 3.2 flowers per plant, respectively, more than that of controls. Furthermore, these two treatments flowered 14 days earlier. Adding PS or NF to the VA inoculation, or mixing the three inoculants together caused slower in colonization rate. However, the shoot dry weight of each treatment is heavier than that of single VA inoculation. There is no promoting effect on phosphate uptake by plants among those different microbial inoculations.

Key words : *Eustoma grandiflorum*, Arbuscular mycorrhizal fungi, Free-living nitrogen-fixing bacteria, Phosphate-solubilizing bacteria, Phosphorus.

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藥用植物－銅皮石斛人工授粉時期及果莢發育之研究[1]

楊淑如[2] 蕭翌柱[2] 陳威臣[2] 蔡新聲[3]

摘要：銅皮石斛 (*Dendrobium moniliforme* (L.) Sweet) 爲蘭科 (Orchidaceae) 石斛屬 (*Dendrobium*) 具藥用之經濟植物。授粉後之果莢發育，其寬度、厚度及長度皆呈現S型生長曲線 (sigmoid curve)，生長速度皆以授粉後第1~2週呈加速度，並分別於授粉後第3、8及9週達到較大值，以後變化很小。銅皮石斛之自交授粉，以在開花後2~7日內進行較爲適當。而較佳時期則爲開花後2~5日，此時授粉之著果率約爲10%；人工異花授粉則以開花當日至開花後5日進行較爲適當，此時可交性較強，果莢著果率在30%~100%之間。

關鍵詞：銅皮石斛、人工自花授粉、人工異花授粉、果莢發育、S型生長曲線。

石斛類藥材爲蘭科 (Orchidaceae) 石斛屬 (*Dendrobium*) 有用之藥材植物，其假球莖是一種常見之中藥，以新鮮或乾燥莖入藥，有益胃生津、茲陰清熱之效果。石斛屬爲蘭科第二大屬，多年生草本植物，全球計有1500種；中國大陸地區約有80種石斛屬植物，主要分布於華南及西南地區，目前供作藥用的石斛屬植物有33種[6, 12]；台灣的山區亦可見石斛之蹤跡。

銅皮石斛 (*D. moniliforme* (L.) Sweet) 又名細莖石斛、白花石斛、金石斛、竹節草、圓莖乾石斛、黃草石斛、黃草、細黃草[12]，其莖粗在5mm以內、莖長4~15cm、不分枝、顏色爲清灰色或古銅色；總狀花絮具花1~6朵，花序短，花色白，花梗纖細長2~10mm，花小，直徑約2.5~3.5cm，唇瓣基部有突起胼胝體，花被片長1.1~2.5cm[2, 12]。

石斛生理活性的強弱與其多糖的含量有一定的關係[2]。近年由其藥理作用研究中發現，石斛具有以下多種功能：(1) 石斛多糖具有增強T細胞及巨噬細胞免疫活性的作用，是一種前景可期的中藥免疫增強劑[2, 4]；(2) 對眼科疾病石斛有明顯的治療作用，如治療半乳糖性白內障[2]；(3) 消除腫瘤、抑制癌症[1, 4]；(4) 恢復噪音、抗聲帶疲勞[4, 12]；(5) 治療胃炎及十二指腸炎[9]等功效。

自然生長的石斛屬植物，喜生長於熱帶及亞熱帶高溫多濕森林的樹上及林下岩石上，特別是半陰半陽的地方且附生在有蘚苔植物的石灰岩上及樹上的石斛品質較好；石斛主要分佈於海拔900~1500m之間，年平均氣溫12℃~18℃，相對溼度在60%~75%之間，林間透光度爲60%左右，生長季節溫度20℃~25℃，冬季氣溫9℃~12℃，在無霜多霧、年雨量爲1100~1500mm之長綠闊葉林中附生於樹幹上，以及少數上述的石灰岩上[3, 4, 5]。自然界中，石斛開花多結果少，但結果之果莢可含數萬粒種子，

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種子細如粉每粒重約0.3~0.4μg，成熟果莢內種胚發育不超過球形胚階段，如同一般蘭科植物呈發育不全需後熟現象。由於缺乏胚乳組織，須與眞菌共生 (symbiosis) 才能萌發，自然條件下發芽率不及5%。自然實生繁殖幼年期長，從種子萌發、生長至成熟供採收約需5~7年。石斛的特殊生活環境及自身繁殖困難是造成產量低、生長區域小之重要原因[4, 11]。本研究以銅皮石斛爲材料，探討授粉適期與果莢之發育，以利實生苗之大量繁殖。

本試驗之試驗期間爲1~3月，銅皮石斛材料取自棲蘭山之原生種，並選取大小均一，生育健壯但尚未開花之銅皮石斛植株爲材料20株，待開花後紀錄每朵花之開花日期，依不同開花日數進行人工自花、異花授粉，栽培在日溫/夜溫爲22℃/18℃之生長箱中，自授粉之日算起，約經7~8週，調查各個授粉期所得之果莢數，所得之著果率採用二項分布 (Binomial distribution) 95% 信賴區間 (confidence limits) 檢定處理間之差異。

以開花2~5日後之花朵進行人工異花授粉成功所得之果莢，於授粉後每隔一週以游標尺測量果莢之長度、寬度及厚度，以建立果莢生長曲線及相對生長速率 (Relative growth Rate, RGR)。

$RGR = (\ln L_2 - \ln L_1) / (T_2 - T_1)$

L_1：第1次取樣時長度　　T_1：第1次取樣時間

L_2：第2次取樣時長度　　T_2：第2次取樣時間

正常著果之果莢發育，不論果莢之長度、寬度或是厚度皆呈現S型生長曲線，於第12週所測得之果莢寬度、厚度及長度平均分別爲4.57mm、6.68mm及21.74mm（圖1），從圖1中果莢膨大之生長曲線及圖2之果莢相對生長速度得知，果莢寬度、厚度及長度生長速度皆以第1~2週較快，而後之生長則有個別之差異。寬度於授粉後第3週幾乎已達到最大，以後變化很小；厚度生長則於授粉後第8週漸趨於穩定，長度之發育則較慢，在授粉後第8週才趨於穩定。

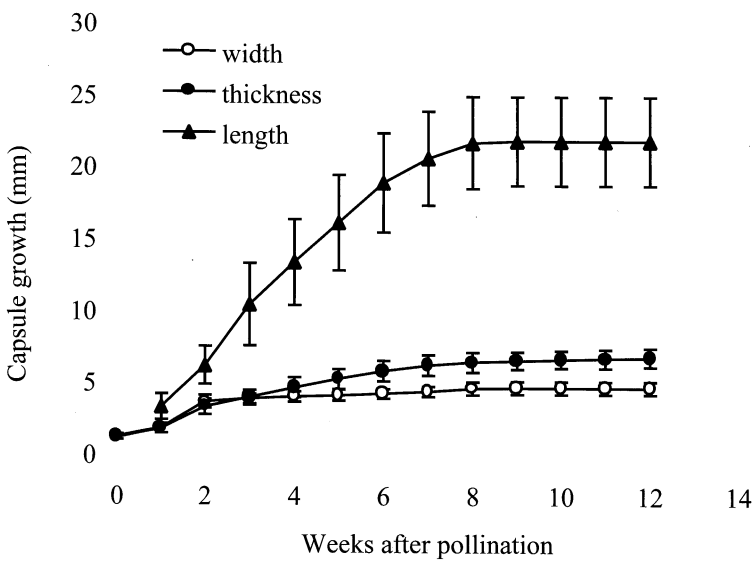


圖1. 銅皮石斛開花授粉後果莢膨大之生長曲線

Fig.1. The growth curve in pollinated capsule of *D. moniliforme* (L.) Sweet.

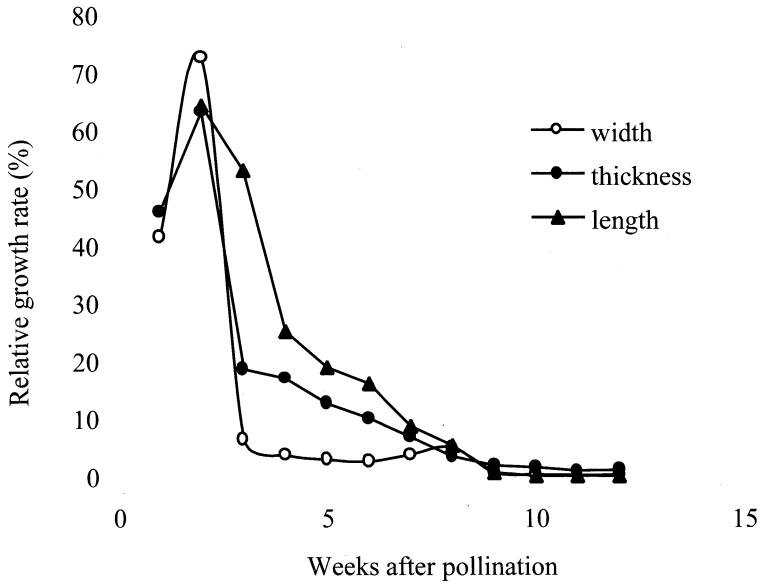


圖2. 銅皮石斛開花授粉後果莢發育之相對生長速率曲線

Fig.2. The relative growth rate in pollinated capsule of *D. moniliforme* (L.) Sweet.

銅皮石斛不同成熟度之花朵，人工自花授粉後之著果率，結果如表1所示，以開花當日爲起算基準，每兩日爲一期，在經過7~8週後調查每一供試時期之著果率，結果以2~3日及4~5日兩個時期之著果率較高爲10%；初開及開花8日以後之花朵授粉後之著果率爲0%，故開花早期及開花1星期後的銅皮石斛花朵不利於自花授粉。由此推斷，若要進行銅皮石斛自交，可能在開花後2~7日內進行較爲適當，而較佳時期應爲開花後2~5日，此時授粉之著果率較高。

人工異株異花授粉之著果率結果如表2所示。當雌蕊處於A時期（開花當日至開花後1日），以不同成熟度之之異株花粉進行雜交，結果A時期花粉授粉著果率爲30%；B時期（開花後2日至開花後3日）花粉之授粉著果率爲40%；C時期（開花後4日至開花後5日）花粉之授粉著果率爲40%；D時期（開花後6日至開花後7日）花粉之授粉著果率爲30%；E時期（開花後8日至開花後9日）花粉之授粉著果率爲10%，平均著果率爲30%。依此類推，進行交互授粉試驗，當雌蕊成熟度處於B時期，

表1. 銅皮石斛不同成熟度花朵人工自花授粉之著果率

Table 1. Effect of self-pollinated timing on fruit set of *Dendrobuium moniliforme* (L.) Sweet

Pollination (DAF)[x]	No. of flowers pollinated	Fruit set		
		Formed	%	95 % CL[y]
0~1	20	0	0	0~17
2~3	20	2	10	1~31
4~5	20	2	10	1~31
6~7	20	1	5	0~25
8~9	20	0	0	0~17
10~12	20	0	0	0~17

[x]DAF: Days after flowering.

[y]95 % Confidence limits of Binomial distribution.

表2. 銅皮石斛不同成熟度之花粉與雌蕊人工異花授粉之著果率

Table 2. Effect of pollen and ovule maturity on fruit set in crossed pollination *D. moniliforme* (L.) Sweet

Pollinia age (DAF)	Ovule age (DAF)[x]																	
	A(0~1)			B(2~3)			C(4~5)			D(6~7)			E(8~9)			Total & Average (0~9)		
	Fruit set[y]			Fruit set			Fruit set			Fruit set			Fruit set			Fruit set		
	No.	%	95 % CL[z]	No.	%	95 % CL	No.	%	95 % CL	No.	%	95 % CL	No.	%	95 % CL	No.	%	95 % CL
A(0~1)	6	30	12~54	20	100	83~100	6	30	12~54	4	20	6~44	1	1005	0~25	37	37	28~47
B(2~3)	8	40	19~64	18	90	69~99	11	55	32~77	8	40	19~64	2	10	1~31	47	47	37~57
C(4~5)	8	40	19~64	11	55	32~77	9	45	23~68	5	25	9~49	1	5	0~25	34	34	25~44
D(6~7)	6	30	12~54	7	35	15~59	8	40	19~64	2	10	1~31	1	5	0~25	24	24	16~33
E(8~9)	2	10	1~31	3	15	3~38	3	15	3~38	1	5	0~25	0	0	0~17	9	9	4~16
Total & Average (0~9)	30	30	21~40	59	59	49~60	37	37	28~47	20	20	13~29	5	5	2~11			

[x] Days after flowering (DAF).

[y] Number of flowers pollinated in all treatment were twenty.

[z] 95 % Confidence limits of Binomial distribution.

用A時期花粉授粉著果率爲較高者達100%；用B時期授粉亦有90%之著果率；如改用C時期之花粉授粉，其著果率則劇降爲55%；如再以更老熟之花粉行之，D、E時期花粉之著果率分別爲35%及15%；平均著果率爲59%。當雌蕊成熟度處於C時期時的著果率，用A、B、C、D及E之花粉雜交，其著果率分別爲30%、55%、45%、40%及15%，平均著果率爲37%。當雌蕊成熟度處於D時期時，不論是何時期之花粉授粉，著果率已明顯下降，平均著果率更降爲20%。因此，就表2之結果顯示，處於A、B時期之花粉（開花後0~3日）授粉於處於B時期（開花後2~3日）之雌蕊可得到較佳之結莢率，此時進行雜交授粉較爲適當。

蘭科花朵壽命依不同種屬而有極大之差異，如台灣原生種蝴蝶蘭 (*P. amabilis*) 長達120日[14]。蘭花之大孢子在小孢子之後分化，花朵盛開時小孢子已分化完全，大孢子之分化須藉由授粉刺激，授粉後，花粉管所分泌之IAA刺激胎座邊緣之胞原細胞 (archesporial cell) 分裂[16]。授粉後的蘭科植物於果莢成熟時，其內種子的胚發育到圓球體 (globular stage) 階段，細胞數目極少，核大且細胞質濃，胚柄 (suspensor) 端的細胞較大，液泡亦較大，稱爲後端 (posterior)；另一端則爲前端 (anterior)，細胞較小質濃，以後分化成幼苗之頂端分生組織[8]。至於蘭科植物的果莢發育至何種程度始可採收播種，受遺傳及環境影響甚鉅，Harvais (1980) 指出 *Cypripedium reginae* 在果莢尚綠，種皮內之澱粉剛消失時，採收果莢較好[14]；美國加州數種地生蘭和萬代蘭 (*Vanda*) 於果莢未成熟採收，種子發芽及幼苗生長均較成熟裂開者爲佳[13, 15]。

在進行銅皮石斛人工栽培中，由於缺乏自然界中之媒介昆蟲，自然雜交率偏低，人工授粉則可增加授粉率。人工授粉方式即是以尖嘴鑷子於蕊柱之頂端將花藥帽掀開，將花藥取出經稍微擠壓後使花粉塊分散，再塘塞於母本柱頭所在之蕊柱基部凹陷處，即完成人工授粉。授粉後1天即花朵開始枯萎，1週後子房開始膨大，迅速充實而形成紡錘形果莢；而未授粉成功之果莢會在1~2週後逐漸委凋。果莢之生長曲線一般可分爲S型生長曲線及雙S型生長曲線兩大類，白花蝴蝶蘭、台灣金線蓮之果莢發育呈現S型生長曲線[7, 10]，本研究得到銅皮石斛果莢寬度、厚度及長度之發育亦皆呈現S型（圖1），與前述兩種蘭科植物得生長曲線極爲相似。

銅皮石斛之自花授粉著果率介於0%~10%間，較適期（開花後2~7日）授粉之著果率僅有5%~10%；而異花授粉著果率爲0%~100%之間，較適期授粉之著果率介於（開花當日至開花後5日）

30%~100%，平均著果率爲30%，因此，推斷銅皮石斛應屬於較適宜進行異交授粉之作物。

隨著科學技術的進步，國內外的新藥研究發展面臨愈來愈激烈之競爭與挑戰，研究重點逐漸轉向傳統中藥及天然產物的研發與利用，再加上野生藥用植物資源瀕臨枯竭，進行物種保存及大面積栽培是今後藥用植物資源發展與應用的重點之一。對石斛的研究，除發展組織培養之大量繁殖外，進行科學化管理以滿足市場需求，並對其有效藥用成分及含量作更深入之探討極爲必要。

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Studies on Pollination Time and Capsule Growth in A Medicinal Orchid Plant *Dendrobium moniliforme* (L.) Sweet [1]

Shu-Ru Yang[2], Yih-Juh Shiau[2], Uei-Chin Chen[2] and Hsin-Sheng Tsay[3]

Summary

The width, thickness, and length of capsule showed sigmoid development in a medicinal orchid *Dendrobium moniliforme* (L.) Sweet. The most rapid growth of capsule was 1~2 weeks after pollination. The width, thickness, and length reached maximum in 3rd, 8th, and 9th week, respectively. The optimum timing for self-pollination was 2~7 days after flowering, but it only achieved 10 % fruit set. The optimal cross-pollination was 1~5 days after flowering (DAF) and the fruit set varied between 30% to 100%.

Key words : Artificial self-pollination, Artificial cross-pollination, *Dendrobium moniliforme* (L.) weet, Fruit growth, Sigmoid development.

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Insecticide Resistance in *Plutella xylostella* L.

XI. Resistance to Newly Introduced Insecticides in Taiwan (1990~2001)[1]

Ching-Hua Kao and Edward Y Cheng[2]

Abstract: The diamondback moth (*Plutella xylostella*, DBM) has developed resistances to almost all conventional insecticides, for example, carbamates, organophosphorus compounds, synthetic pyrethroids, and insect growth regulators. The resistance and cross-resistance amongst these insecticides had been studied and clarified. A strategy by rotating selected insecticides to manage the resistant DBM has been proposed and tested in field, and the result is acceptable to farmers. From 1990 to 2000, many new insecticides with unique mode of actions were registered, and DBM control is no longer a problem for farmers. However, whether DBM also slowly develops resistance to those new insecticides is worth to study. DBM strains were collected from two largest vegetable districts, Hsi-hu and Lu-chu, for testing the resistance profile to newly registered insecticides. The test results confirmed that field DBM has developed resistance to new insecticides except diafenthiuron. The resistance ratios of abamectin and emamectin benzoate between two field DBM populations are 2500~5000 and 300~150 respectively; the resistance ratio of another GABA inhibitor, fipronil, between these two populations is 65~104. The resistance ratio of derivatives of cartap and carbamate, bensultap and benfuracarb between two different populations are 20~30 and 10~30 respectively. The resistance ratio of chlorfenapyr and spinosad between two different populations are 10 and 60 respectively. The DBM resistance to commercial product of neem extract, azadirachtin, is too high for field application practically since the high viscosity of the extracted product plugged the spray nuzzle of sprayer when the concentrated product was used.

Key words: *Plutella xylostella*, Insecticide, Resistance, Resistance monitoring.

INTRODUCTION

Crucifer crop is the most important group of vegetable in Asian countries. The major insect problem of crucifer crop is the diamondback moth (*Plutella xylostella* L., DBM). During 1950s to 1960s, synthetic insecticides such as DDT and parathion were introduced for DBM control. Bt products also were used since

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1960s. Following more restricted safety consideration, many early registered insecticides were banned in recent years. Nevertheless, there're still a great number of carbamates and organophosphorus insecticides registered for DBM control[21], meanwhile synthetic pyrethroids were introduced around 1980s. All of them had confronted with strong resistance problem[4, 12, 20], and DBM has become a perennial insect problem for crucifer production. Taiwan Agricultural Research Institute (TARI) has monitored and studied this resistance problem since 1980[2, 3]. After sorting out the interrelation of cross-resistance among various insecticides, we are able to propose a resistance management strategy[5] by rotating selected insecticides with minimum cross-resistance effect, and the results are acceptable by farmers[7].

On the other hand, pesticide industry has been continuous making efforts in developing new insecticides to counter the resistance problem. For example, the BPU-type IGRs were introduced in early 1980s, although very promising at the beginning, it met strong MFO resistance and the effectiveness was vanished in just one year[10]. Later on, abamectin, a new GABA inhibitor, was registered, and this biological-originated insecticide had been very effective. However, the low-quality generic products of abamectin were massively marketed without the consideration of resistance management[13]. Another GABA inhibitor, fipronil, was subsequently registered in late 1990s. From 1990 to 2001, there are several other insecticides with new modes of action been registered for DBM control, which are either chemicals or biological-originated products (Table 1). Currently, DBM control is not a significant problem in Taiwan due to the introduction of these products[22], but whether DBM has gradually developed new resistance is a question that needs to be studied. TARI has re-initiated the resistance study on DBM in two major vegetable production areas, Lu-chu and Hsi-hu, on the above mentioned

Table 1. DBM control agents registered in Taiwan

Insecticide categories	Common names	Number
Chemical insecticides		
Organophosphorus compounds	Diazinon, salithion, phenthoate, quinalphos, cyanophos, profenofos, naled, pirimiphos-methyl, prothiophos, mevinphos, acephate, pyridaphenthion, mephosfolan	13
Carbamates	Methomyl, carbofuran, benfuracarb[z]	3
Synthetic pyrethroids	Fenvalerate, permethrin, cypermethrin, deltamethrin, flucythrinate, fenpropathrin[z]	6
Organonitrogens	Cartap, thiocyclam hydrogenoxalate, bensultap[z]	3
Mixtures	Phenthoate+dimethoate, teflubenzuron+fluvalinate, prothiophos+mevinphos, chlorpyrifos+cypermethrin	4 x 2
Insect growth regulators	Teflubenzuron[z], chlorfluazuron[z]	2
Others	Fipronil[z], chlorfenapyr[z], diafenthiuron[z]	3
Bioinsecticides		
Bacillus thuringiensis (Bt)	Dipel, Thuricide, Javelin, Turex[z], MVP[z], Cutlass[z], Xentari[z], Quark[z], Batik[z]	9
Streptomyces avermitilis	Abamectin[z], emamectin benzoate[z]	2
Neem extaract	Azadirachtin (Neemix)[z]	1
Saccharopolyspora spinosa	Spinosad[z]	1
Total		47

[z]Compounds registered after 1990.

newly registered insecticides such as abamectin, emamectin benzoate, bensultap, benfuracarb, diafenthiuron, fipronil, chlorfenapyr, spinosad, azadirachtin and the new Bt products.

MATERIALS AND METHODS

Insecticides used:

Commercially formulated insecticides were used for comparison which include abamectin, 2% EC; emamectin benzoate, 2.15% EC; bensultap, 50% WP; benfuracarb, 20% EC; diafenthiuron, 25% FP; fipronil, 4.95% FP; chlorfenapyr, 10% SF; spinosad, 2.5% SF; azadirachtin, 4.5% EC; Javelin, 6.4% WP; Thuricide, 3% WP; Turex, 3.8%WP; Xentari, 10.3% WP; MVP, 10% FP; Cutlass, 10.3% SW; and Batik 10.4% SF.

Insects used:

Two field-resistant DBM strains collected from Hsi-hu and Lu-chu were tested against a native susceptible IL strain. Both areas are major vegetable growing districts in Taiwan, and are nearly 150 kilometer apart and geographically separated by non-vegetable growing areas.

I. Susceptible strain: I-lan (IL) DBM strain was collected in 1983[9].

II. Field-collected resistant strains: Collected in 1996, 1997, 1998 and 2001, respectively, and only the offspring of the first and the second generation were used.

i. Hsi-hu strain: collected from vegetable growing district in central Taiwan.

ii. Lu-chu strain: collected from vegetable district in southern Taiwan.

Bioassay methods:

I. Spray tower method:

The susceptibilities of DBM to bensultap, benfuracarb, diafenthiuron, fipronil and chlorfenapyr were tested as described by Cheng *et al.*[14]. One milliliter of insecticide solution was pipetted into the holding tube of Burkard Potter Spray Tower and sprayed on leaf disc. After air-dried, the leaf disc was inverted and loaded with fifteen third instar DBM larvae, and sprayed with another milliliter of the same insecticide solution. Treated larvae and leaf discs were transferred into the petri dish, and total of 60 larvae in 4 subsets was treated for each dosage. The post-treatment holding condition was controlled at 25 ℃ in a constant temperature growth chamber. The leaf discs used were 24 cm^2 and changed to fresh untreated leaf discs after 48 hours.

II. Leaf dipping method:

The susceptibilities of different DBM strains to abamectin, emamectin benzoate, Javelin, Thuricide, Xentari, MVP, Cutlass, Batik, azadirachtin and spinosad were determined as described by IRAC Method No. 7[6]. Cabbage leaves were washed and air-dried first, and leaves were punched into discs of 3.5 cm in diameter. Total of 20 leaf discs were prepared for each dosage and leaf discs were dipped for 5 seconds in testing solutions containing 333ppm Triton X-100 and left to air-dry. For the control test, cabbage leaf discs were dipped in a 333ppm Triton X-100 solution only and air-dried on wire rack. In each petri dish, three leaf discs were used in the beginning and 15-20 third instar DBM larvae were put on the treated leaves. Forty-eight hours after treatment, these leaf discs were discarded and changed to the other two treated leaf discs that been stored at 5 ℃ until pupation.

Assessments:

Mortalities of DBM to besultap, benfuracarb, diafenthiuron, fipronil and chlorfenapyr were recorded at 48 and 72 HR after the spray tower treatment. For abamectin, emamectin benzoate, Bt products, azadirachtin and spinosad, the mortalities were recorded at 24, 48, 72, 96 and 120 HR after the start of treatment. Analysis was

made by correcting with the control first, then analyzed by Probit analysis[15]. If the DBM mortality in the control test was over 8%, the test was abandoned and a new test was repeated for compensation.

RESULTS AND DISCUSSION

It has been demonstrated in the past that DBM is capable of developing resistance to all conventional synthetic insecticide groups such as organophosphorus compounds, carbamates, pyrethroids[4, 20], insect growth regulator[10, 12, 17], and even the biologically originated Bt products[16, 24, 25, 26]. A program of rotating selected insecticides for managing resistant DBM has been mandated in several counties and gained successful results [7], which confirmed that certain insecticides are still useful in rationally rotation to minimize the resistance problem.

When TARI re-started the DBM resistance study program on these new insecticides in 1996, we experienced a five-year work span because previous reports showed that field DBM did develop resistance to insecticides within one to three years[5]. The monitored result confirmed DBM has developed resistance to the most potent insecticide ever registered for DBM for example, abamectin. The resistances to fipronil and spinosad are also noticed, even they have different modes of action. The sensitivities of the susceptible and resistant DBM to eight new insecticides were presented in Tables 2 and 4, as well as the resistance ratios were calculated and listed in Tables 3 and 5.

Two derivatives of traditional insecticides, bensultap and benfuracarb, are discussed first. Bensultap, an analog of cartap, was registered in 1995 and rapidly developed 20 to 30 times resistance in 1997, which was much higher than previously recorded cartap-resistance[8, 9, 14]. This high resistance may build upon the already existed cartap-resistance; hence bensultap was not considered to be an effective insecticide for DBM control anymore. Benfuracarb belongs to the carbamate group, and its effective dosage for DBM has already exceeded 2000 - 5000 ppm in two monitored areas. The obvious cross-resistance from carbamates[11] made the bensultap application not realistic in practice.

The field DBM was not very sensitive to azadirachtin (neemix), a natural product extracted from neem, even without too extensive usage. Strong resistance in DBM had occurred as the LC_{50} is 0.24 ppm in the susceptible strain, while the resistance ratio in both field strains exceeded 1000. This material also has high viscosity that caused spray problem in operation at higher dosage.

Table 2. Susceptibilities of Lu-chu strain DBM to newly registered insecticides

Insecticide	LC_{50} in ppm/slope				
	Suscep. -IL	1996	1997	1998	2001
Abamectin	0.01/1.58	2.02/1.46	3.30/1.58	6.06/1.49	49.88/1.88
Emamectin benzoate	0.004/1.67	-	-	-	0.61/1.90
Bensultap	194.73/2.11	1214.70/1.32	4450.36/1.28	-	-
Benfuracarb	164.03/1.30	610.40/3.87	1990.82/2.59	1594.58/2.20	-
Diafenthiuron	346.77/2.71	406.58/2.12	362.98/2.05	337.28/1.84	-
Fipronil	0.82/3.60	2.18/4.62	12.53/4.49	22.91/1.95	85.57/3.59
Chlorfenapyr	17.32/3.93	-	29.72/5.52	24.45/3.57	167.05/1.70
Spinosad	0.41/3.80	-	-	-	24.06/2.41
Azadirachtin	0.24/0.047	-	-	-	>300ppm

Table 3. The resistance ratios of Lu-chu strain DBM to newly registered insecticides

Insecticide	Resistance ratio				
	Suscep. -IL	1996	1997	1998	2001
Abamectin	1.0	202.0	330.0	606.0	4988.0
Emamectin benzoate	1.0	-	-	-	152.5
Bensultap	1.0	6.2	22.9	-	-
Benfuracarb	1.0	3.7	12.1	9.7	-
Diafenthiuron	1.0	1.2	1.1	1.0	-
Fipronil	1.0	2.7	15.3	27.9	104.4
Chlorfenapyr	1.0	-	1.7	1.4	9.6
Spinosad	1.0	-	-	-	58.7
Azadirachtin	1.0	-	-	-	>1000

Table 4. Susceptibilities of Hsi-hu strain DBM to newly registered insecticides

Insecticide	LC_{50} in ppm/slope				
	Suscep. -IL	1996	1997	1998	2001
Abamectin	0.01/1.58	1.44/1.04	3.33/1.85	3.71/1.30	24.97/1.50
Emamectin benzoate	0.004/1.67	-	-	-	1.22/1.07
Bensultap	194.73/2.11	-	6123.52/1.39	-	-
Benfuracarb	164.03/1.30	4755.60/1.53	4976.68/1.73	2262.06/2.13	-
Diafenthiuron	346.77/2.71	562.88/1.83	345.11/1.96	319.71/1.45	-
Fipronil	0.82/3.60	7.61/3.09	9.38/4.80	6.87/2.68	53.62/4.34
Chlorfenapyr	17.32/3.93	-	20.56/4.76	32.91/4.50	159.86/1.71
Spinosad	0.41/3.80	-	-	-	26.77/3.24
Azadirachtin	0.24/0.047	-	-	-	>300ppm

Table 5. The resistance ratios of Hsi-hu strain DBM to newly registered insecticides

Insecticide	Resistance ratio				
	Suscep. -IL	1996	1997	1998	2001
Abamectin	1.0	144.0	333.0	371.0	2497.0
Emamectin benzoate	1.0	-	-	-	305.0
Bensultap	1.0	-	31.5	-	-
Benfuracarb	1.0	29.0	30.3	13.8	-
Diafenthiuron	1.0	1.6	1.0	0.9	-
Fipronil	1.0	9.3	11.4	8.4	65.4
Chlorfenapyr	1.0	-	1.2	1.9	9.2
Spinosad	1.0	-	-	-	65.3
Azadirachtin	1.0	-	-	-	>1000

There're compounds with only minor resistance problem e.g., diafenthiuron did not show any sign of resistance up to 1998. Since diafenthiuron was not used extensively, this may be the reason that resistance was not found and hence was not tested in 2001.

Chlorfenapyr belongs to a new category of insecticide developed by the American Cyanamid Company. The commercial product is a pro-insecticide (AC303.630), which has to be activated by MFO to AC303.268,

then acted on mitochondria and influenced the electron flow in the cell[1]. The unbalance in ion concentration finally blocked the production of ATP and caused death. Processes of actual poisoning of chlorfenapyr include at least three steps i.e., the absorption through stomach, the activation by MFO, and the biochemical lesion on mitochondria. Hence, more than one step may be involved in the resistance mechanism. The multi-steps poisoning makes things more complicated and possibly lengthens the resistance development, which may be the reason why only minimum change in susceptibility of field DBM to chlorfenapyr were recorded in 1998. However, almost 10 times resistance was recorded in both DBM in 2001. The high resistance causes the effective dosage of chlorfenapyr reached the upper limit of its registered concentration, and currently the field efficacy of chlorfenapyr for DBM control is remained in question.

Both abamectin and fipronil act on the GABA transmission in the neural system[19, 23]. Aabamectin resistance in both DBM strains of Hsi-hu and Lu-chu areas has increased progressively from 100/200-fold in 1996, 400/600-fold in 1998, and has already reached 2500/5000-fold in 2001. Since the generic abamectin products once occupied 90% of abamectin market, the resistance induced in DBM might be the consequence of those generic products rather than abamectin. These generic products contain a lot of toxin impurities[13], and may induce more complicated resistance mechanism in DBM. The purified B1a/B1b mixture, emamectin benzoate, was registered for DBM control in March 2000, and is the most potent DBM control agent as its LC_{50} is 0.5-1.0 ppm for both Lu-chu and Hsi-hu strains. Emamectin benzoate suffered 150~300 fold resistance of DBM in the test of 2001, and the cross-resistance from abamectin is obvious but in lesser degree. The difference may be contributed from purified B1a/B1b active ingredients, and suffered less cross-resistance from other toxins.

Fipronil, another GABA inhibitor, was registered in Taiwan in 1996 but has already confronted with 10- to 30-fold resistance since 1998, and its resistance ratios increased to 60/100 fold in 2001. Although current DBM resistance did not hamper the effectiveness of abamectin and fipronil seriously, the trend of resistance development is alarming. It is unclear whether the cross-resistance has developed between abamectin and fipronil, but the introduction of abamectin for field application is much earlier. Since both compounds share the similar action site, the GABA transmission, the resistance mechanism study is needed. The registered concentrations of both abamectin and fipronil for DBM are comparatively high, and that is why farmers are still able to use these compounds despite the strong resistance.

Spinosad was registered in 1996 and tested for resistance for the first time. This bio-originated insecticide acts as a stimulator on central nerve system. Surprisingly, we found the spinosad-resistance has occurred in both tested field strains and the resistance ratio between resistant and susceptible populations is around 60-fold. Whether this resistance is the cross effect from other insecticide is unknown, but this consequence already raised the LC_{50} dosage to 25 ppm, which is comparable to the current LC_{50} of abamectin .

The Bt resistance in DBM has been studied and reported[16, 17, 18, 23, 24, 25], and TARI has developed an empirical testing system. Four discriminating dosages, 2000-, 4000-, 32000- and 128000-fold dilution, were chosen for all tested Bt products, and the 50% mortality is used to distinguish the resistance development. Two higher dosages are used mainly for identifying the field resistant strains, and the two lower dosages are used for confirming the test standard for the susceptible strain. When the 50% mortality occurred between 4000- and 32000-fold dilutions of Bt, we consider there is no alarm for Bt resistance. While the 50% mortality occurred at the dosage higher than the 4000-fold dilution, a warning for Bt resistance should be issued.

Bt mortalities in DBM collected from Lu-chu and Hsi-hu from 1996 to 2001 were recorded in Tables 6

and 7, which confirm at that field DBM did develop resistance when compared to the susceptible strain. More than 50 % mortality in the susceptible strain was found in the first three dosages of all Bt products; while in both field strains, only two higher dosages caused more than 50% mortality. For the past five years, most Bt products still maintained reasonable efficacy in Hsi-hu DBM, while Xentari, Turex and Cutlass had met resistance in Lu-chu DBM since 1998. In general, the result of Xentari testing in 2001 is almost the same as in 1998, and the Bt resistance of field DBM is rather stable when compared to chemical insecticides.

Table 6. Susceptibilities of Lu-chu strain DBM to commonly used Bt products (1996-2001)

Bt products	Dilution	% Mortality					
		Suscep.-IL	1996	1997-1	1997-2	1998	2001
Javelin, 6.4%	2000X	100	95.7	100	96.3	87.9	-
	4000X	100	90.5	95.0	79.1	79.1	-
	32000X	93.5	51.2	63.1	15.6	41.7	-
	128000X	77.4	20.7	39.7	13.3	35.8	-
Thuricide, 3%	2000X	100	89.3	83.1	63.0	82.1	-
	4000X	100	72.7	73.9	48.7	80.0	-
	32000X	70.6	25.2	20.5	26.5	25.0	-
	128000X	30.9	13.4	19.5	18.3	18.2	-
Xentari, 10.3%	2000X	100	88.5	95.9	83.6	91.9	77.7
	4000X	95.4	65.3	63.7	68.5	42.3	52.0
	32000X	55.5	18.6	16.2	22.8	18.4	14.7
	128000X	24.7	7.2	2.6	17.2	6.3	5.0
MVP, 10%	2000X	100	81.0	83.1	66.0	-	-
	4000X	89.7	63.3	81.3	56.9	-	-
	32000X	85.6	13.5	26.4	23.6	-	-
	128000X	64.0	7.7	11.2	12.3	-	-
Turex, 3.8%	2000X	100	95.3	98.3	95.2	74.5	-
	4000X	80.9	84.4	88.1	78.3	50.4	-
	32000X	69.1	31.7	24.2	31.3	18.7	-
	128000X	40.9	14.3	20.4	6.9	6.4	-
Cutlass, 10.3%	2000X	100	96.3	99.2	96.9	50.0	-
	4000X	97.6	94.3	93.2	83.2	32.8	-
	32000X	68.4	52.2	54.2	31.3	13.4	-
	128000X	32.3	28.8	30.5	16.0	1.6	-
Batik, 10.4%	2000X	100	-	-	-	87.2	-
	4000X	95.0	-	-	-	67.4	-
	32000X	59.5	-	-	-	26.2	-
	128000X	22.0	-	-	-	11.9	-

Table 7. Susceptibilities of Hsi-hu strain DBM to commonly used Bt products (1996-2001)

Bt products	Dilution	% Mortality				
		Suscep.-IL	1996	1997	1998	2001
Javelin, 6.4%	2000X	100	91.8	97.4	95.7	-
	4000X	100	69.7	93.3	88.7	-
	32000X	93.5	15.7	43.3	49.7	-
	128000X	77.4	13.2	28.8	36.7	-
Thuricide, 3%	2000X	100	92.4	79.3	-	-
	4000X	100	71.6	50.8	-	-
	32000X	70.6	28.7	25.7	-	-
	128000X	30.9	19.3	16.9	-	-
Xentari, 10.3%	2000X	100	98.4	100	-	82.0
	4000X	95.4	65.0	85.0	-	71.1
	32000X	55.5	21.7	29.2	-	19.8
	128000X	24.7	16.7	30.5	-	10.9
MVP, 10%	2000X	100	85.3	82.5	-	-
	4000X	89.7	68.0	42.9	-	-
	32000X	85.6	31.3	26.8	-	-
	128000X	64.0	11.7	15.5	-	-
Turex, 3.8%	2000X	100	99.2	100	-	-
	4000X	80.9	77.0	83.1	-	-
	32000X	69.1	20.4	23.0	-	-
	128000X	40.9	13.0	22.2	-	-
Cutlass, 10.3%	2000X	100	98.7	91.7	-	-
	4000X	97.6	90.9	84.9	-	-
	32000X	68.4	32.1	39.2	-	-
	128000X	32.3	10.1	28.2	-	-
Batik, 10.4%	2000X	100	-	87.1	87.0	-
	4000X	95.0	-	62.9	63.3	-
	32000X	59.5	-	14.3	8.2	-
	128000X	22.0	-	12.1	8.1	-

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小菜蛾抗藥性之研究

十一、對數種新型殺蟲劑之抗性(1990~2001)[1]

高靜華　鄭　允[2]

摘要

小菜蛾對傳統殺蟲劑氨基甲酸鹽劑、有機磷劑、合成除蟲菊精劑及昆蟲生長調節劑等抗性之偵測研究已於1980~1990年間完成，並經田間測試減緩抗性發展之對策，以因應此一易發生抗藥性害蟲之防治。而農藥工業界也於1990~2000年間陸續推出各類作用不同的新殺蟲劑，其中登記於小菜蛾者包括免速達、免扶克、阿巴汀、汰芬隆、芬普尼、克凡派、賜諾殺、因滅汀、印楝素及多種新型蘇力菌。但此一期間，本省之小菜蛾田間族群對上列新型藥劑是否已產生抗性則尚有待瞭解。本研究選定溪湖及路竹兩大蔬菜專業區進行採集及藥效測試，結果顯示除了汰芬隆因使用不多，未發現明顯抗藥性外，兩地區之小菜蛾對所有新登記藥劑均產生不同程度之抗性，尤甚者爲效果極佳之阿巴汀及經純化之同型藥劑因滅汀，分別有2500~5000倍及150~300倍之抗藥性，而作用同爲抑制GABA 傳導之另一藥劑芬普尼，也有65~100倍抗性產生。分屬氨基甲酸鹽及培丹類似物之免扶克及免速達，於1997年時之抗性倍數已達20~30倍。作用於粒線體之克凡派抗性約10倍，小菜蛾對賜諾殺之抗性也已有60倍。印楝素亦有抗性產生，且因該藥劑本身極爲黏稠，提高濃度更使噴藥不易，而無法使用。1996~97年監測時發現，溪湖及路竹小菜蛾對蘇力菌均有中等程度之抗性，路竹地區「向前走」及「獨佳」兩種製劑之效果均有下降趨勢，但2001年之測試結果仍維持原有之抗性程度。由以上資料研判，近10年來小菜蛾之防治因新藥劑不斷推出，而獲良好之控制，惟因對各類新藥之抗性已逐漸形成，未來5~10年間小菜蛾仍可能因對新藥劑之嚴重抗藥性，再度成爲十字花科蔬菜之首要害蟲，故吾人應持續對新型藥劑進行抗性監測及抗藥性對策之研究。

關鍵詞：小菜蛾、新型殺蟲劑、抗藥性、監測。

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1. 行政院農業委員會農業試驗所研究報告第2096號。本研究之論文於2001年8月在吉隆坡舉辦之第四屆亞太昆蟲學會中宣讀。

2. 本所應用動物系副研究員及研究員。臺灣省　臺中縣　霧峰鄉。

中華農業研究稿約

一、本刊爲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報告及研究簡報。

二、來稿可用中文或英文撰寫，包括原稿一份及影本二份（內各附照片原圖）。稿件以未在其他刊物發表者爲限。稿紙以A4紙張（長29.6公分，寬21公分）打字列印，每頁以不超過二十五行爲原則，上下應預留3公分，左右2公分空間，以便審查。

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㈡第二頁爲中（英）文稿之中（英）文摘要與關鍵詞。中、英文摘要均不分段落。中文摘要以不超過五百字，英文摘要(Summary)以不超過三百字爲原則。關鍵詞(Key words)不能超過五個，每個英文關鍵詞第一字母應大寫，其表示方法如Key words:Heritability, Indirect selection.

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㈣第四頁爲行政院農業委員會農業試驗所研究報告刊登之編號（編號謄空）、作者之註腳，如通信作者、發表場合、服務機關、及其他相關事項等，以小號數字表示順序。

㈤第五頁起爲本文，依序爲前言(INTRODUCTION)、材料與方法(MATERIALS AND METHODS)、結果(RESULTS)、討論(DISCUSSION)、誌謝(ACKNOWLEDGEMENTS，含計畫編號）、引用文獻(REFERENCES)、表、圖之說明(LEGEND)及圖，其中結果及討論兩項可合併爲「結果與討論(RESULTS AND DISCUSSION)」；研究簡報則不分章節，內容簡短，以不超過四印刷頁爲度（約6,000字）。農推、農經研究報告參考本方法書寫。

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⑴文獻出自學術性刊物

1.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。

2.Hsu, S. H., T. Y. Chung, T. C. Kuo and S. F. Wang. 1992. Studies on the micro-climate of greenhouse. I. Light environment of greenhouse (in Chinese). J. Agric. Assoc. China 157:34-43.

3.Weng, J. H. 1992. CO_2 exchange characteristics of C_3 and C_4 vegetable crops in response to light and nitrogen (in Chinese). J. Agric. Assoc. China 157:21-33.

⑵文獻出自著作性書籍

1.楊清白。 1980。家畜飼料學。 74-108 頁。藝軒。台北。

2.Sung, J.M. 1991. Nitrogen Metabolism of Rice. p.100. Chinese Agronomy Press, Taipei.

⑶文獻出自編輯性書籍

1.Lo, W.S. 1991. The effect of calcium on nitrogen metabolism pp.100-110. in:Biology of Rice. (Sung, J. M. and D. J. Liu, eds.) Chinese Agronomy Press, Taipei.

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