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米飯物理性與官能品嚐之關係[1]

郭益全[2]　劉　清[2]　宋　勳[3]

摘要：本研究以分別插植於本省北、中及南等5個地點之9個水稻品種的材料供試。測定各該米飯樣品之硬度、凝集性、彈性、脆性、咀嚼性及黏性等諸種米飯物理性，再配合相同材料之官能品嚐成績（臺中場負責品嚐），以進行米飯物理性與官能品嚐關係之探討。試驗結果經變方分析後顯示：除凝集性僅品種間差異顯著外，餘官能品嚐、硬度、彈性、脆性、咀嚼性及黏性等性狀之地點間、品種間及地點與品種間之交感等三類變方成分均極顯著。官能品嚐與諸米飯物理性之相關關係，5地點間除彈性外餘均一致，皆呈極顯著負相關。官能品嚐依諸米飯物理性而變之淨迴歸係數，5個地點皆不顯著，但5地點綜合分析時則硬度、凝集性、脆性及咀嚼性等4性狀之淨迴歸係數具統計意義。路徑係數分析之數據指出，硬度之負的影響程度最大；而脆性的正的效應值則最大。

目前，國內各場所所新選獲有希望水稻材料之品質檢定，係由臺中區農業改良場米質研究室統籌辦理。其檢定項目係綜合美國農部及國際稻米研究所之方法，一般分成碾米品質（milling quality）、米粒外貌（grain appearance）及烹調與食用品質（cooking and eating quality）等三部份。其關係食用品質之檢定，往昔均以生米粒之直鏈澱粉含量（amylose content）及膠體硬度（gel consistency）爲主要判定之依據。此判定依據就秈稻言向稱適用[9,10,12]，但就粳稻言則不適用[14]，故目前均輔以食味官能品嚐。而食味官能之品嚐雖較恰當，但受供試品種數、基準品種及食味品嚐團組成員額與代表性等種種限制。栽培條件、碾米條件、煮飯方法及品嚐進行方式等人爲因素常左右正確之官能評價結果[6]。

概言之，米飯食用品質之決定，歸納而言可分成三方面：一爲米飯之外觀是否亮麗耀眼引人注目（即光澤）；二爲米飯之香味是否令人聞之涎垂，食指欲動（即香味）；三爲吾人咀嚼器官對米飯行咀嚼作用之感受是否適口（即所稱黏彈性或所謂Q）。據日人平野氏[5]之綜論：學者們對光澤、香味及黏彈性之與米飯食用品質關係之研究雖多且明確，唯，至目前止，吾人對米飯之香味及光澤尚未能予以客觀數值化，故此兩性狀可應用於育種選拔之客觀評估方法尚待開發。而所謂黏彈性（或稱Q），實爲米飯諸種物理特性之概括，其客觀之評估一般可用組織構造測定儀（Texturometer 或 Rheometer）來測定之。此類儀器係模擬人類口腔運動方式而設計發展成之機器，可直接測定米飯之硬度（hardness）、凝集性（chesiveness）、彈性（elasticity）、脆性（brittleness），間接誘導出咀嚼性（chewiness）及黏性（gumminess）等物理特性，是一種可將米飯物理特性客觀數值化之儀器，日本方面已有數篇報告描述其結果[6,7,8,11,13]。

國內經濟轉型，省民對於食米之消費已由量的吃得飽轉爲質的吃得好，稻米品質改進與分級之研

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究逐爲當前農業重點研究之一。而本研究之目的即在探討米飯諸種物理特性與官能品嚐成績之地點與品種變異，並進一步研究米飯諸動物理性與官能品嚐成績之關係，以爲稻米品質改進與分級之參考。

材料與方法

參試水稻品種爲臺南 5 號、臺南 7 號、臺農67號、臺中189號、高雄141號、吉野 1 號、臺中秈 3 號、臺南秈15號及臺中在來 1 號等 9 個品種。試驗地點爲桃園區農業改良場轄區後龍、臺中區農業改良場大村本場、臺南區農業改良場轄區西螺、高雄區農業改良場屏東本場及臺東區農業改良場轄區池上等 5 個地點。栽培管理統一。施肥量 $N : P_2O_5 : K_2O = 120 : 60 : 60$kg/ha 。氮肥（用硫酸銨）全量之25%當基肥，後於插秧後15天及30天分別追施全量之20%及30%之追肥，餘25%爲穗肥。磷肥則全量用爲基肥一次施用。鉀肥則全量之20%當穗肥，兩次追肥之量各爲全量之40%，施用時期如氮肥。田間設計採逢機完全區集設計，三重覆。小區面積 10m² 行株距 30×15cm。育苗箱育苗，每叢 4—5 支苗。適期收穫後，稻穀水分含量調整至14%後貯存六個月再調製成白米。

稻穀樣品以日本 Satake 脫殼機碾成糙米，再以美國 Seedburo McGill No. 2 Miller 碾白機，碾白30秒，磨光30秒。各該白米樣品以經去碎米 (broken rice) 後之完整米粒 (head rice or perfect rice) 備用。

米飯之製備仿 Okabe [11] 之方法烹煮。20g 之各該樣品的完整白米盛於 150ml 之燒杯 (Pyrex, No. 1000) 中，加二次蒸餾水25ml，以鋁箔紙封口並浸泡10分鐘。置於移去內鍋之大同電鍋 (TAC-6H) 烹煮之，每次可同時煮 6 個樣品，外鍋加水 42ml。電鍋自動開關跳開後，燜飯15分鐘後起鍋。去鋁箔紙，覆以白紗布，在室溫下熱平衡 (therml equilibration) 1 個小時。挑取燒杯中間部份米飯粒子完整，粒形一致之飯粒，以 Okabe 之單粒法 (one-kernel technique) 測試之。每次測試儀器測試部份進行 3 個循環 (cycle)，每個米飯樣品（每燒杯）測五個飯粒。每白米樣品均重覆兩次，所得數據之均值爲每白米樣品米飯物理特性值，亦即每一米飯樣品之物理特性值爲各樣品米飯十個飯粒測定值之平均。

所使用米飯物理特性測試儀爲日本不動工業株式會社之組織構造測試儀 (Rheometer, NRM-2020J-CW)。測試條件爲：測試範圍 (sensorg range) 0—500g；測試速度 30cm/M；黏彈測試棒直徑 10.0mm ；測試方式以上下 (UP & Down) 連續運動方式進行。

米飯官能品嚐由臺中區農業改良場米質研究室負責，方法詳謝等 [3] 稻米品質分級之研究。

結 果

供試 9 品種被栽培於 5 個試驗地點所得米飯樣品之官能品嚐成績及物理特性值的變方分析結果列於表 1。由表 1 可知，被評估諸性狀中，除凝集性僅品種間之差異達極顯著水準外；餘，如官能品嚐、硬度、彈性、脆性、咀嚼性及黏性等 6 個性狀之栽培地點間及品種間之差異均顯著。因知，參試 9 個水稻品種之米飯諸物理特性及官能品嚐成績確有不同；且五個試驗地點間所栽培出之稻米的米飯諸物理性及官能品嚐成績亦有差異。同表亦發現除凝集性外，餘，如官能品嚐成績等 6 被評估性狀之品種間與地點間之交感效應亦均極顯著。故知，各水稻品種米飯之諸米飯物理特性與官能品嚐成績在各地區之表現（優劣或順位）並不盡相同。

參試水稻材料米飯之官能品嚐成績及諸物理特性之地點變異如表 2。綜覽表 2 發現供試材料之米飯諸物理特性與官能品嚐成績之地點變異並無一致之趨勢存在。詳言之，就官能品嚐成績言，產自大村、西螺及屏東之稻米有較優之食用品質，產自後龍及池上者之官能品嚐成績則較差。米飯硬度方面，產自池上之稻米的米飯最硬，次硬者爲產自西螺及屏東之米飯，再次者爲產自後龍者，而以產自大村之稻米的米飯最軟。米飯之凝集性則 5 個栽培地點間無顯著差異存在。就米飯彈性言，產自屏東之

表1. 米飯官能品嚐成績與物理特性之變方分析

Table 1. Analysis of variance human panel testing score and physical properties of cooked rice.

Source of variance	DF.	Human panel testing score	Hardness	Cohesiveness	Elasticity	Brittleness	Chewiness	Gumminess
Location (L)	4	**	**	ns	**	**	**	**
Error (A)	10	—	—	—	—	—	—	—
Variety (V)	8	**	**	**	**	**	**	**
LXV	32	**	**	ns	**	**	**	**
Error (B)	80	—	—	—	—	—	—	—
Total	134	—	—	—	—	—	—	—

** : Significantly different at 1% level.

ns : Non-significant.

表2. 米飯官能品嚐成績與物理特性之栽培地點變異

Table 2. Variabilities of human panel testing score and physical properties of cooked rice of cultivated locations

Location	Human panel testing score	Hardness (g)	Cohesiveness	Elasticity	Brittleness (g)	Chewiness (g)	Gumminess (g)
Hol-Long	−1.45b[1]	453.7c	1.60a	1.85b	531.2c	1,315.8c	715.5c
Ta-Tsuen	−0.02a	386.8d	1.61a	1.86b	367.5d	1,148.0d	625.0d
Si-Lo	−0.02a	560.3b	1.60a	1.87b	444.1b	1,674.6b	900.5b
Ping-Tung	−0.07a	560.0b	1.60a	1.92a	541.2b	1,710.0a	892.0b
Chih-Sun	−1.49b	577.1a	1.60a	1.86b	562.3a	1,725.9a	926.6a

1 : Means in each column with the same letters are not significantly different at 5% level.

稻米的米飯有較高之彈性值，且顯著地高於產自後龍、大村、西螺及地上等 4 個地點者。米飯之脆性以產自池上之米飯有最高之脆性值，產自大村之米飯的脆性值則最低。產自西螺及屏東兩地稻米之米飯脆性值無顯著差異存在，然皆顯著地高於產自後龍者。米飯之咀嚼性方面則以產自屏東及池上兩地稻米之值較高，其次爲產自西螺者，再次者爲產自後龍者，而以產自大村之稻米的米飯咀嚼性值最低。米飯之黏性面方是以產自池上之稻米的值較高，其餘依次爲產自西螺及屏東者、後龍者及大村者。

表 3 爲參試 9 個水稻品種在 5 個試驗地點米飯諸物理特性及官能品嚐成績之綜合表現。表 3 數據顯示，臺農67號爲參試 9 個品種中官能品嚐成績值最高者，但其餘諸物理特性之表現順位則有相當之差異存在，此種諸被評估性狀表現順位不一致之現象不僅見諸臺農67號，亦見於諸參試品種。故知，供試材料米飯諸物理特性與官能品嚐成績之表現順位在品種內亦有不一致之現象存在。就品種別言，臺南 5 號、臺南 7 號、臺農67號、臺中189號、高雄141號及吉野 1 號等 6 個粳稻品種米飯之官能品嚐成績遠優於臺中籼 3 號、臺南籼15號及臺中在來 1 等等 3 個籼稻品種者。而粳稻羣中官能品嚐成績值由高而低依次爲：臺農67號＞臺中189號＞高雄141號＞臺南 5 號＞吉野 1 號＞臺南 7 號。其中臺農67號、臺中189號及高雄141號等 3 品種之官能品嚐值無顯著差異存在；高雄 141號與臺南 5 號等 2 品種

表3. 米飯官能品嚐成績與物理特性之品種變異

Table 3. Variabilities of human panel testing score and physical properties of cooked rice of different varieties

Variety	Human panel testing score	Hardness (g)	Cohesiveness	Elasticity	Brittleness (g)	Chewiness (g)	Gumminess (g)
Tainan No. 5	0.39bc[1]	502.1c	1.57c	1.85cd	487.9c	1,458.8d	799.7c
Tainan No. 7	0.24c	440.7f	1.55cd	1.83d	414.6f	1,247.8h	707.6e
Tainung No. 67	0.61a	480.9d	1.57c	1.82d	462.7d	1,349.1f	753.1d
Taichung No. 189	0.56a	441.8f	1.60c	1.87bc	424.9f	1,297.8g	697.3e
Kaohsiung No. 141	0.44ab	460.9e	1.52d	1.85cd	443.8e	1,307.6g	695.5e
Yoshino No. 1	0.25c	559.9b	1.57c	1.89b	547.5b	1,655.0c	869.0b
Taichung Sen No. 3	−1.01d	477.9d	1.58c	1.89b	462.0d	1,412.9e	741.3d
Taichung Native No. 1	−1.66f	607.0a	1.71b	1.90b	585.5a	1,922.8b	1,010.9a
Tainan Sen No. 15	−1.45e	596.9a	1.76a	1.94a	574.5a	1,981.9a	1,030.9a

1 : Means in each column with the same letters are not significantly different at 5% level.

間亦無顯著差異存臺在；臺南 5 號、吉野 1 號及臺南 7 號等 3 品種間之差異不顯著。秈稻羣官能品嚐值之順位則爲臺中秈 3 號＞臺南秈15號＞臺中在來 1 號。至於硬度、凝集性、彈性、脆性、咀嚼及黏性等米飯物理特性值大致亦可分爲秈粳兩羣，且秈稻諸物理特性值概較粳稻者爲高。同時，秈稻羣之臺中秈 3 號的諸米飯物理特性值均較趨近於粳稻，而顯著異於秈稻。9 個參試水稻品種諸米飯物理特性表現之變異請詳見表 3 。

表4. 米飯官能品嚐成績與物理特性之簡單相關係數

Table 4. Simple correlation coefficients among human panel testing score and physical properties of cooked rice

Location	Hardness	Cohesiveness	Elasticity	Brittleness	Chewiness	Gumminess
Hol-Long	− 0.74**	− 0.76**	− 0.79**	− 0.73**	− 0.84**	− 0.83**
Ta-Tsuen	− 0.80**	− 0.72**	− 0.32ns	− 0.75**	− 0.86**	− 0.70**
Si-Lo	− 0.69**	− 0.72**	− 0.44**	− 0.64**	− 0.85**	− 0.80**
Ping-Tung	− 0.54**	− 0.64**	− 0.17ns	− 0.50**	− 0.56**	− 0.58**
Chih-Sun	− 0.72**	− 0.63**	− 0.58**	− 0.70**	− 0.82**	− 0.81**
Combined data	− 0.48**	− 0.70**	− 0.37**	− 0.45**	− 0.59**	− 0.56**

*, **: Significantly different at 5 and 1% levels, respectively.
ns : Non-significant.

估得官能品嚐成績與米飯物理特性相關關係之結果列於表 4 。由表 4 知，官能品嚐成績與米飯諸物理特性皆呈負相關，但其顯著情形，則存有地點間或性狀別之差異。就地點別言，產自後龍、西螺

及池上三地點之相關係數顯著情形較相近；產自大村與屏東兩地點相關係數之顯著情形較近似。就性狀別言，官能品嚐與硬度、凝集性、脆性、咀嚼性及黏性之負相關值，五個地點皆達極顯著之水準。而官能品嚐與硬度之負相關之顯著情形則依產地之不同而異，產自後龍、西螺及池上等三地點者顯著；產自大村及屏東等兩地者不顯著。五個地點數據之綜合分析則官能品嚐成績與米飯硬度、凝集性、彈性、脆性、咀嚼性及黏性等 6 個物理性皆成極顯著之負相關。

表5. 米飯官能品嚐成績依物理特性而變的複迴歸分析

Table 5. The multiple regressions of human panel testing source on physical properties of cooked rice

Location	Hardness	Cohesiveness	Elasticity	Brittleness	Chewiness	Gumminess	R^2	F	b_0
Hol-Long	0.002ns	−1.506ns	−0.235ns	−0.003ns	0.001ns	− 0.004ns	0.79	12.68**	16.46
Ta-Tsuen	−0.007ns	−0.588ns	1.942ns	0.016ns	−0.006*	0.001ns	0.81	13.95**	0.04
Si-Lo	−0.002ns	−0.114ns	0.160ns	0.008ns	−0.004ns	0.001ns	0.80	129.6**	3.29
Ping-Tung	−0.008ns	−6.911ns	2.777ns	0.004ns	−0.002ns	0.006ns	0.47	2.99*	6.62
Chih-Sun	−0.190ns	2.883ns	−2.484ns	0.025ns	−0.001ns	− 0.005ns	0.76	10.32**	3.70
Combined data	−0.008*	−4.480**	1.494ns	0.010*	−0.002**	0.001ns	0.58	29.35**	5.61

*, **: Significantly different at 5 and 1% levels, respectively.
ns: Non-significant.

表6. 米飯物理特性對官能品嚐成績之路徑係數

Table 6. The path coefficients of physical properties of cooked rice influening human panel testing souce

Location	Hardness	Cohesiveness	Elasticity	Brittleness	Chewiness	Gumminess
Hol-Long	0.134	−0.185	−0.345	−0.241	0.178	−0.549
Ta-Tsuen	−0.508	−0.067	0.126	1.077	−1.534	0.170
Si-Lo	−0.126	−0.010	0.009	0.670	−1.384	0.053
Ping-Tung	−0.696	−0.726	0.381	0.366	−0.941	1.103
Chih-Sun	−1.661	0.312	0.188	2.224	−0.507	−0.983
Combined data	−0.972	−0.490	0.127	1.201	−0.843	0.243

官能品嚐成績依諸物理特性而變之迴歸分析如表 5，表 5 顯示諸物理特性之複迴歸 5 地點顯著（F 值），但決定程度（R^2）則略有地點之變異。而諸物理特性之淨迴歸係數之變異，則除大村地區咀嚼性之淨迴歸係數具統計意義外，餘 4 地點估得諸物理特性之淨迴歸係數均未具統計意義。但五個地點數據綜合分析時，6 個米飯物理特性中則硬度、凝集性、脆性及咀嚼性等 4 個性狀之淨迴歸係數具統計意義。其中，硬度、凝集性及咀嚼性等 3 個性狀對官能品嚐成績具負的效應；而脆性對官能品嚐成績則具正的影響。

表 6 爲路徑分析之結果，就產地別觀之，對官能品嚐成績有最大負影響之性狀，後龍地區爲黏性；大村、西螺及屏東等 3 地區爲咀嚼性；池上地區爲硬度。而對官能品嚐成績有最大正影響的性狀，後龍地區爲咀嚼性；大村、西螺及池上等 3 地區爲脆性；屏東地區爲黏性。就性狀別言，硬度對官能品嚐成績具正效應影響之地區僅後龍一區；餘大村、西螺、屏東及池上等 4 區均呈負效應，其中以產自池上者之負效應值最大。凝集性對官能品嚐成績具正效應影響之地區爲池上，其餘 4 區均呈負效應，就中以產自屏東地區者之負效應值最鉅。彈性與脆性對官能品嚐成績具正效應之地區均爲大村、西螺、屏東及池上等 4 地區。而對品嚐成績具最大正效應值之地區，在彈性方面爲產自屏東者；在脆性方面則爲產自池上者。彈性與脆性對官能品嚐成績均呈負影響之地區爲後龍。咀嚼性方面，對官能品嚐成績具正方向影響之地區僅後龍一地，餘 4 區均呈負方向之影響，中以產自大村者之負效應值最大，黏性方面，對官能品嚐具正效應之地區有大村、西螺及屏東等 3 區，中以產自屏東者之正值最大，具負效應之地區有後龍及池上等兩區，中以產自池上者之負效應爲大。綜合五地點數據分析之結果則：硬度對官能品嚐成績有最大之負影響；而脆性對官能品嚐成績則有最大之正的貢獻。

討　論

堀末 [6] 謂，米飯之食味評定中，米飯構造（texture）之重要性不亞於與食味有關之化學性質及香氣等，而所謂米飯構造實係米飯之硬度、凝集性、彈性、脆性、咀嚼性及黏性等米飯物理性之謂。Okabe [11] 指出：品種、米齡（age）、貯藏溫度、漬泡及烹煮之水基準（water level）及烹煮器（cooker）等爲影響米飯構造之重要因素。米飯存放期間，米飯之硬度值有逐漸變高之傾向；若以糙米及白米狀態封存容器內50天（30°C）後，其米飯物理性無顯著差異；自動瓦斯鍋（automatic gas cooker）與自動電鍋（automatic electric cooker）所煮之米飯的米飯構造無顯著差異 [13]；白米在自然條件下經一年之貯藏，則硬度與黏性會增高 [13]。穀粒之成熟度與米飯之硬度與咀嚼性有關，過熟，硬度與咀嚼性增高，黏性等降低 [8]。煮飯時所加水量較少時，米飯硬度增高；浸泡時間較長，則硬度降低而黏性增高 [11]。產自泥炭土（peaty soil)之米粒有較差之品質 [13]。青米及銹米（rusty kernel）之硬度及咀嚼性值稍低，黏性則略高 [8]。秈稻較粳稻有較高之硬度及咀嚼性值 [7]。凡此可知米飯之物理性（或米飯構造）除受內在品種因素 [6,11] 之支配外，尚受外在諸種環境條件所左右。郭等 [1] 則指出米粒之直鏈澱粉含量及膠體硬度等品質性狀有品種及栽培地點間之差異，而本試驗進行中，舉凡生育管理、適期收穫、加工調製及米飯製備等田間及實驗室操作均力求妥善一致之條件下，數據仍顯示供試米飯之官能品嚐成績及諸物理特性有極顯著之品種間與栽培地點間之差異存在，故此類差異之存在可被確認實係肇因於品種與栽培地點不同所致。同時本研究亦發現供試水稻、材料中，被評估諸種性狀卓然有秈粳兩羣之別（臺中秈 3 號除外），呈粳稻羣之官能品嚐成績優於秈稻羣者；秈稻羣之諸物理特性值均遠高於粳稻羣者。此等結果大致與堀末 [6]、Ebata and Hirasawa [7]、Okabe [11]、Suzuki [13] 及 Suzuki *et al* [14] 之結果相符合。並可進一步確證國內習稱西螺米、葫蘆墩米、池上米及吉安米較好吃，其來應有自。雖然，本研究中，產自池上之稻米的官能品嚐成績欠佳，原因有待明瞭，但本研究中被評估性狀有栽培地點間之變異則不容否認。

堀末 [5] 及 Okabe [11] 指出米飯之食味與硬度及黏性之關係很密切。Okabe [11] 進一步指出黏性對硬度之比值是一項很重要的均衡介量（balancing parameter）。米飯之硬度若高，但其黏性亦高，則食用品質仍佳；而若黏性值低，則食味不被接受。凝集性對米飯之食用品質亦有影響，其值若高，食用品質則趨低。本研究數據則發現米飯之官能品嚐成績與諸物理特性間皆成相關，但其顯著情形則有栽培地點間或性狀別之變異；而官能品嚐成績依諸物理特性而變之複迴歸 5 地點皆顯著，但決定程度則略有栽培地點間之變異。顯示本研究之數據指出，官能品嚐成績雖大致可由諸物理特性決定之，但決定之程度却存有栽培地點間之差異。亦即，食用品質（官能品嚐成績）之決定甚爲複雜，諸物理特性與食用品質（官能品嚐成績）之相關模式因栽培地點而不盡相同。

綜合本研究之論斷可知，米飯之官能品嚐成績及諸物理特性顯具栽培地點及品種之變異，而官能品嚐成績（食用品質）與諸物理特性亦存有密切關係存在，但決定程度却存在栽培地點間之變異，故知，國內欲推行稻米分級銷售，在目前相關資料闕如的情況下，遭遇之問題應很多。諸如：本研究中，官能品嚐成績係由臺中區農業改良場同仁品評之結果，雖說「一粒砂一世界」，但也因「一粒砂一世界」，臺中場同仁之品評結果之代表性，品評能力也是一值得考慮之問題；再者，本研究中亦發現評估性狀有品種與栽培地點之交感作用存在（本研究中未再進一步分析）但目前推行之良質米產銷計畫[2]下，逕選臺農68號、臺農70號、臺中189號、臺南9號及越光（試作）等品種爲各輔導區通用品種，似也值得考慮；同時，據堀末[6]及謝等[4]指出，良質米之生產有其異於與以高產導向之栽培管理方式，而目前國內良質米生產之栽培管理模式也尚待建立，現行仿以高產導向之栽培管理方式也亟待檢討。

總言之，民以食爲天，吃飯問題爲先求溫飽而後思良質，良質米之數據化判定與有關性狀之遺傳研究爲臺灣省農業試驗所遺傳研究室努力之目標之一，由現有之數據看來，良質之數據化判定尚須相當之投入與各方專家之不吝指導。

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The Relationship between Human Panel Testing Score and Physical Properties of Cooked Rice[1]

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Summary

Human panel testing score tested by Taichung District Agricultural Improvement Station and physical properties of cooked rice, such as hardness, cohesiveness, elasicity, brittleness, chewiness and gumminess, measured by Taiwan Agricultural Research Institute were for the purpose of elucidating the relationship between human panel testing score and physical properties. Nine commercial rice varieties grown at 5 locations distributed in the northern, central and southern parts of Taiwan were used as tested material.

The effects of variety, location and variety×location were significant for all evaluated traits except cohesiveness which was only significant in variety. It is found that human panel testing score was negatively correlated with all evaluated physical properties of cooked rice. The partial regression coefficients of human panel tesitng score on physical properties were not significant for all five locations, however, the partial regression coefficients of hardness, cohesivenessbrittleness and chewiness were significant for combined five locations data. The results of path analysis indicated that the trait with the largest negative effect was hardness and the trait with the largest poritive effect was brittleness among physical properties of cooked rice.

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不同接種季節對水稻花藥培養的影響[1]

葉常青　蔡新聲[2]

摘要：以週年栽培方式種植水稻臺農67號，探討水稻母株生育環境對花藥培養形成癒合組織及分化成植株的影響，結果發現4至12月份所接種的花藥癒合組織形成率約在27～42%之間；由於低溫造成水稻植株的生長遲滯，因而錯失了1月及3月份的資料，而在2月份低溫下產生的花藥，則完全無癒合組織的形成。以水稻母株生育環境的溫度而言，低溫較高溫對花藥癒合組織的形成能力有較爲不利的影響。就水稻母株的生育過程而言，生殖生長期對溫度變化的反應比營養生長期敏感，尤其以花粉母細胞減數分裂期爲最。經切片觀察，低溫會造成絨氈層細胞的異常增殖，及產生多核性巨大小孢子，而阻止小孢子發育。癒合組織的分化率並不隨着癒合組織形成率的高低而變化，亦無法找出其與外界溫度的關係。

爲了獲得足够的材料及調節人力的運用，週年種植母體植物，以充裕花藥培養育成單倍體的材料，實屬必要。花藥來源母株之營養及生理狀況均會影響花藥培養之成敗[7-8,10-11,14-20]，如以開花初期之水稻花藥進行培養，效果較佳[7,17]；Tsai and Lin[17] 報導水稻花藥培養之效果，一期作比二期作好；Hu *et al.*[12] 認爲水稻孕穗期之溫度及日照爲影響花藥培養的重要因子；Chaleff and Stolarz[5] 更指出水稻不同穎花，甚至同一穎花內不同花藥之癒合組織形成能力亦不一致。由此得知花藥來源母株的生理況狀，將影響花藥培養之成敗，須更進一步的探討。

本研究以週年種植水稻母體植株，進行花藥培養，以探討不同生育環境對水稻母株，其花藥癒合組織形成及分化成植株之影響，並配合石蠟切片，觀察不同生長季節水稻母株產生之花藥及花藥培養小孢子發育情形，探討其與癒合組織形成之關係，尋求適合母株之生長環境，以提高花藥培養效率，利於實際育種工作。

材料與方法

一、母體植株的種植及幼穗取樣方法

花藥培養所用的材料爲粳稻臺農 67 號品種，以單本植每盆三株方式種植於含有 18kg 壤土之 1/2,000 are塑膠盆中，施肥方式爲 $N : P_2O_5 : K_2O = 1g : 1g : 1g$，置於霧峰農業試驗所網室內生長。

幼穗取樣之標準，對水稻能正常抽穗之材料，俟植株發育至劍葉與下位葉之葉耳間長約 3～5cm 時連同劍葉取下，若因低溫造成抽穗不正常之植株，則輔以醋酸洋紅壓潰法檢定小孢子，取單核期者爲材料。

二、水稻母體植株之低温處理

將生殖生長期各個發育階段之水稻植株置於生長箱，以日溫20°C（16hr），夜溫15°C（8hr）的

1.臺灣省農業試驗所　研究報告第 1353 號。本文承蒙臺灣省農業試驗所農藝系研究員劉大江博士斧正，特此申謝。
2.臺灣省農業試驗所助理及研究員。臺灣省　臺中縣　霧峰鄉。

低溫處理之。各個處理代號之水稻植株發育時期如下：

L_1, L_{1b}：13.5葉齡，主桿尚無肉眼可見之幼穗，即幼穗形成初期之枝梗分化期[2]。

L_2, L_{2b}：14.5葉齡，主桿幼穗長約0.3cm，即幼穗形成後期之穎花分化期[2]。

L_3, L_{3b}：15.5葉齡，主桿幼穗長約7.5cm，即花粉母細胞減數分裂期[2]。

L_1, L_2, L_3 各處理一週，L_{1b}, L_{2b}, L_{3b} 處理至採樣。

三、花藥接種及培養基種類

取回之幼穗先以70%酒精擦拭葉鞘外部，取出稻穗浸漬於70%酒精中30秒，再以 0.5%次氯酸鈉消毒3分鐘，經無菌水沖洗3～4次後，將屬於單核期之花藥以 Chen *et al.*[6] 所述之方法接種於培養基上。

培養過程中採用兩種培養基，一爲誘導花藥形成癒合組織之培養基，簡稱NK 培養基，係以 MS 培養基的主要有機鹽類[13]，及 N_6 培養基之無機鹽類[9]，加上 4mg/l NAA, 2mg/l kinetin, 6% sucrose 及 0.8% Phytagar 所配製而成。二爲誘導癒合組織分化的培養基，簡稱 R-2，係以 MS 基本培養基加上 4mg/l kinetin, 1mg/l NAA, 40mg/l adenine sulfate, 3% sucrose 及 0.8% Phytagar 所配製而成。

培養於 NK培養基之花藥係於 26±1°C 之恆溫及黑暗下進行癒合組織之誘導。誘導癒合組織分化植物體之方式則取10日齡之癒合組織接種於 R-2培養基上，在26±1°C 恆溫，光照 1,500 lux 及光期 16hr 的環境下培養。

四、石蠟切片

將每批接種之花藥，分別取出經 0，7，14，21，28與 35 天培養之花藥各約 30 個，以 FAA 固定後，經 TBA 脫水、滲蠟、包埋等步驟，以轉動式切片機切取 10μ 厚度之連續蠟帶，以 Haupt's adhesive粘着於載玻片上，經 hematoxylin 染色，製成永久片觀察[19,20]。

結　　果

一、水稻週年栽培花藥培養

（一）對癒合組織形成之影響：

表1顯示於4～12月份所接種之花藥，癒合組織形成百分率在27～42%之間，其中又以5月下旬（如正常之一期作），9月（如正常之二期作），10月及11月份所接種之花藥其癒合組織形成率最高。由於低溫造成水稻植株生長遲滯，因而錯失了1月及3月份的資料，而2月份低溫下所產生的花藥，則完全無癒合組織的形成。

爲了探討溫度與水稻花藥癒合組織形成及分化之關係，在圖1中將每批水稻生長發育過程劃分成兩部份：①由插秧的4.0葉齡至12.5葉齡（包括營養生長盛期及部分營養生長遲滯期），②由 12.5葉齡至花藥採樣（包括部分營養生長遲滯期及生殖生長期）。而後將每批水稻生長過程與每十日均溫線配合比較，探討溫度對花藥癒合組織形成之影響。

A. 水稻母株營養生長期之溫度對花藥癒合組織形成之影響：

圖1之第5、6兩批水稻植株的營養生長期完全處於低溫下，其癒合組織形成率分別僅爲31%及27%，而第11、12兩批水稻植株之營養生長期處於高溫環境，癒合組織形成率分別高達40%及42%。

B. 水稻母株生殖生長期之溫度對花藥癒合組織形成之影響：

圖1之第2批水稻，其生殖生長後期（即孕穗期）適逢溫度急劇下降，使得癒合組織形成率也降至僅27%，而第3批水稻植株生殖生長期完全處於低溫下，更使花藥無法形成癒合組織。第9、10兩批水稻植株的生殖生長期處於高溫環境下，依然有29%及32%的癒合組織形成率。

（二）對癒合組織分化能力之影響：

由表1中癒合組織分化之結果顯示，綠苗，白苗及不定芽體所佔百分率似乎與癒合組織形成能力

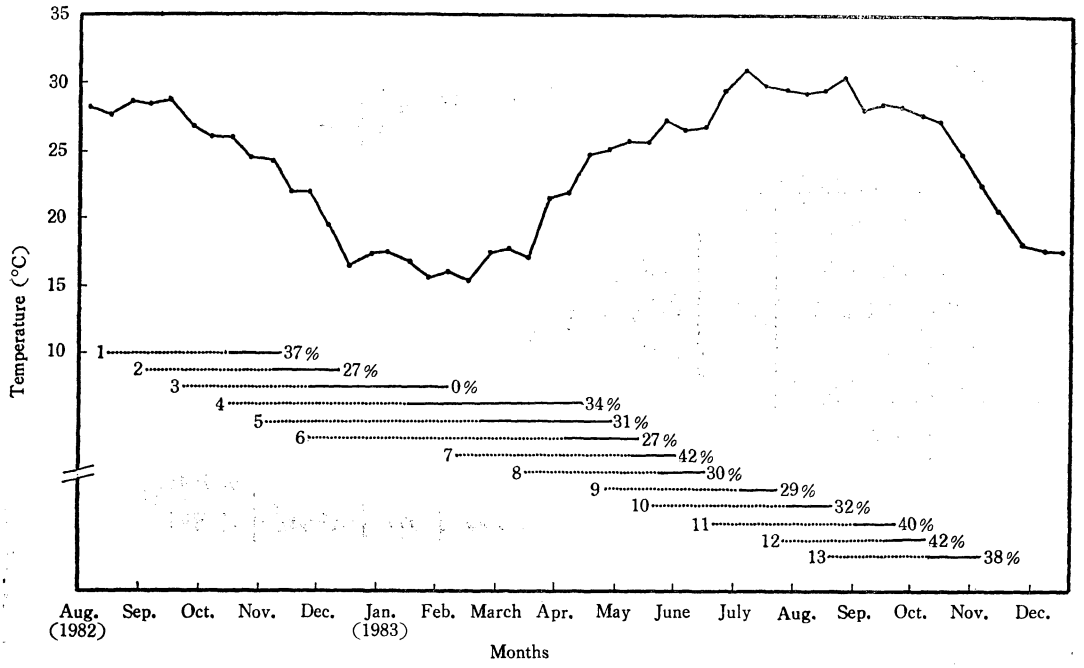


Fig. 1. Influence of mean air temperature during the growth period of donor plant on the callus formation ability in anther culture of Tainung 67 rice.
(-·—·-Average temperature, ·····Vegetative growth stage, ————Reporductive growth stage, %Callus inducing ability) 1-13: Batch No. of rice donor plant (same as table 1)

的高低並無關連。綠苗形成率較穩定，介於19%～34%之間，以第9、10、11與12批等溫度曲線往下降之月份較高。白苗分化率約4%～29%，在溫度曲線往上升之第5、6與7三批最低，其白苗分化率僅分別爲5%、4%及7%，然而這種低比率的白苗，對綠苗及不定芽體分化率的提高並無助益。

二、低温處理水稻母體植株對花藥培養之影響

表1及圖1顯示，水稻發育至生殖生長期若遭遇低溫，將嚴重影響花藥癒合組織之形成能力，因此將生殖生長期各個發育階段之水稻植株，在生長箱中以日溫 20°C (16hr)，夜溫 15°C (8hr) 的低溫處理，以探討水稻生殖生長期各個發育階段遭受低溫及低溫持續時間久暫對花藥培養之影響。

(一) 對癒合組織形成之影響：

表2顯示對照組之花藥癒合組織形成率爲40%。花粉母細胞減數分裂期爲低溫影響花藥能否形成癒合組織最重要的時期，此時施以低溫之 L_3 處理其癒合組織形成率僅達 28.4%。L_1 處理之幼穗形成初期次之，其癒合組織形成率爲34.1%。幼穗形成後期則無影響。隨着低溫處理時間的加長，低溫的影響具有累加性的效果，此可由 L_{3b} 處理尚有 29.4%的癒合組織形成率，而早一週處理的 L_{2b} 便降至14.2%得知。

(二) 對癒合組織分化能力之影響：

表2顯示，癒合組織形成能力與分化能力並無一致的關係。低溫處理發育階段愈早之水稻植株，則其花藥癒合組織之綠苗及白苗的形成率愈低。但當處理時間加長時，綠苗形成率依然下降，而白苗形成率反而異常增加。

三、水稻花藥培養切片觀察

(一) 正常花藥之小孢子發育情形：

Table 1. Influence of anther inoculation time (month of the year) on the abilities of callus formation and plantlet differentiation of Tainung 67 rice.

Batch No. of rice donor plants	Date of anthers cultured	Num. of anthers cultured	Num. and % of anthers browning	Num. and % of anthers forming callus	Num. of callus cultured	Num. and % of callus forming plants		
						Green	Albino	Roots & abnormal shoots
1	November 8-10 1982	2,244	1,186(53)b[1/]	822(37)bcd[1/]	110	23(21)bc[2/]	22(20)abc[2/]	18(16)cde[2/]
2	December 9-11 1982	2,244	704(31)gh	661(27)g	108	36(33)ab	27(25)abc	13(9)e
3	February 2-7 1983	1,024	890(87)a	0(0)h	—	—	—	—
4	April 13-14 1983	1,925	851(44)de	650(34)cde	126	27(21)bc	36(29)ab	32(25)ab
5	April 25-28 1983	1,932	565(29)h	604(31)efg	120	31(26)abc	6(5)e	22(18)bcde
6	May 11-12 1983	2,171	771(36)fg	582(27)fg	124	29(23)abc	5(4)e	16(13)de
7	May 29-30 1983	2,129	762(34)fg	890(42)a	122	31(25)abc	8(7)de	38(31)a
8	June 14-16 1983	2,087	692(33)fgh	619(30)efg	120	23(19)c	18(15)cd	23(19)bcd
9	July 20-22 1983	1,282	601(47)cd	372(29)efg	120	27(23)abc	21(18)bc	22(18)bcde
10	August 1-20 1983	2,034	800(39)ef	651(32)def	134	33(25)abc	27(20)abc	22(16)cde
11	September 20-22 1983	2,189	693(32)gh	876(40)ab	118	40(34)a	24(20)abc	19(16)cde
12	October 7-9 1983	1,998	711(36)fg	839(42)a	140	40(29)abc	29(21)abc	39(28)ab
13	November 4-7 1983	2,094	1,049(50)bc	792(38)abc	136	41(30)abc	40(29)a	13(10)e

[1/]: Means with the same letter are not significantly different at 5% level by Duncan's New Multiple Range Test.
[2/]: Means with the same letter are not significantly different at 5% level by Chi-square Test.

Table 2. Effect of low temperature treatment during reproductive growth on the anther culture of Tainung 67 rice.

Treatment*	Num. of anthers cultured	Num. and % of anthers browning	Num. and % of anthers forming callus	Num. of callus cultured	Num. and % of callus forming plants		
					Green	Albino	Roots & abnormal shoots
CK	2,189	693(31.7)b[1/]	876(40.0)ab[1/]	118	40(33.9)a[2/]	24(20.3)b[2/]	19(16.1)a[2/]
L_1	1,156	388(33.6)b	394(34.1)bc	134	16(11.9)c	13(9.7)c	31(23.1)a
L_2	1,263	390(30.9)b	532(42.1)a	160	25(15.6)bc	24(15.0)bc	23(14.4)a
L_3	1,218	347(28.5)d	346(28.4)d	140	36(25.7)a	31(22.4)b	24(17.1)a
L_{1b}**	—	—	—	—	—	—	—
L_{2b}	897	439(49.9)a	127(14.2)e	84	9(10.7)c	36(42.9)a	14(16.7)a
L_{3b}	1,213	344(28.4)b	357(29.4)cd	140	35(25.0)ab	32(22.9)b	23(16.4)a

*: CK: Rice plants were grown under normal environment of the 2nd crop season, 1983.
L_1, L_2 and L_3: Rice plants at 13.5, 14.5, and 15.5-leaf stage, respectively, were treated with temperature (20/15C, 16 hr photoperiod) for 1 week. L_{1b}, L_{2b} and L_{3b}: similar treatments were executed as L_1, L_2, and L_3, respectively, except that the plants were kept at low temperature until the anthers were collected for culture.
**: No anthers were available for culture due to prolonged low temperature treatment.
[1/], [2/]: Same as table 1.

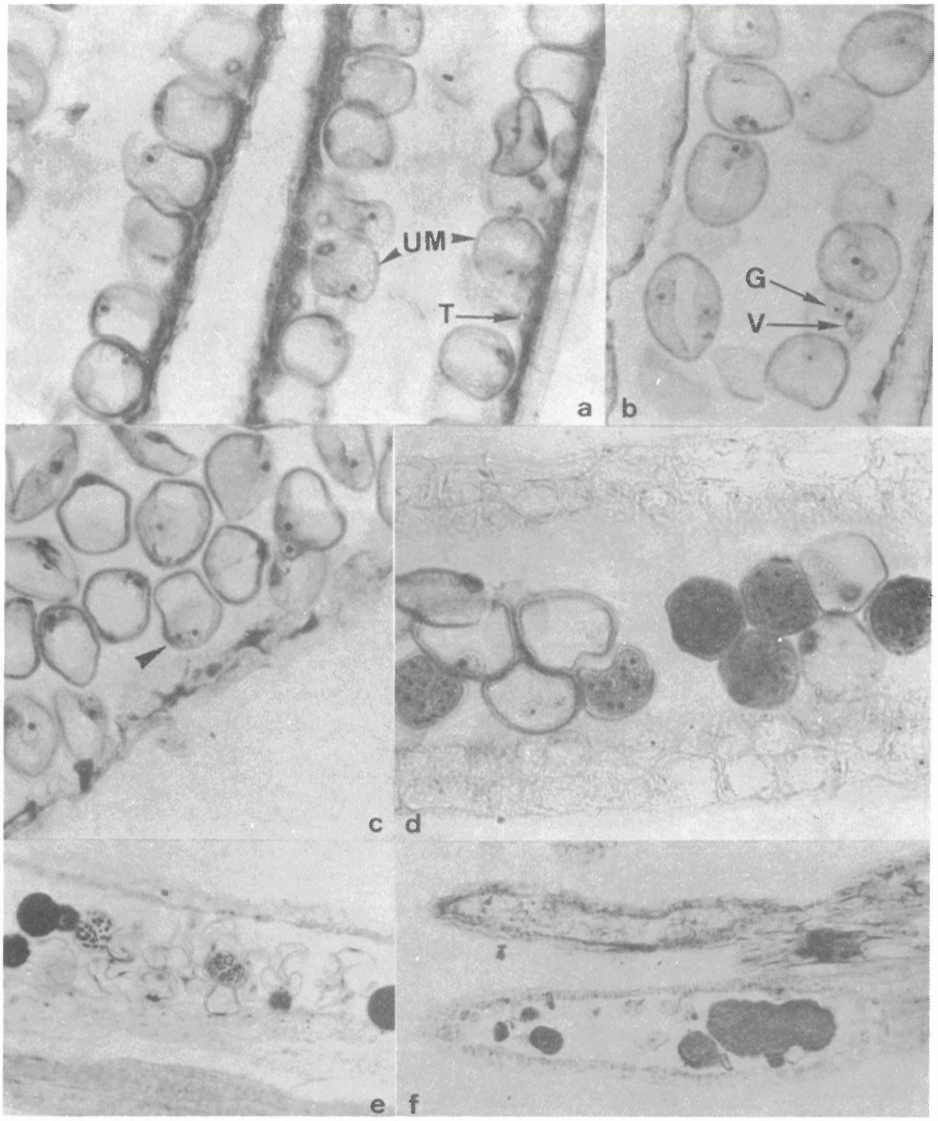


Fig. 2. The development of microspores in rice anther culture

a. Uninucleate microspores (UM) and degenerated tapetum layer (T). (400X).
b. Microspore with a large vegetative nucleus (V) and a small generative nucleus (G) after one week of anther culture. (400X).
c. Binuclei (arrow) of equal size in microspores after one week of anther culture. (400X).
d. Cell wall in multicellular microspores after two weeks of anther culture. (400X).
e. Two types of multicellular microspores after three weeks of anther culture. (200X).
f. Proembryoids formed in anther after three-week in culture. (100X).

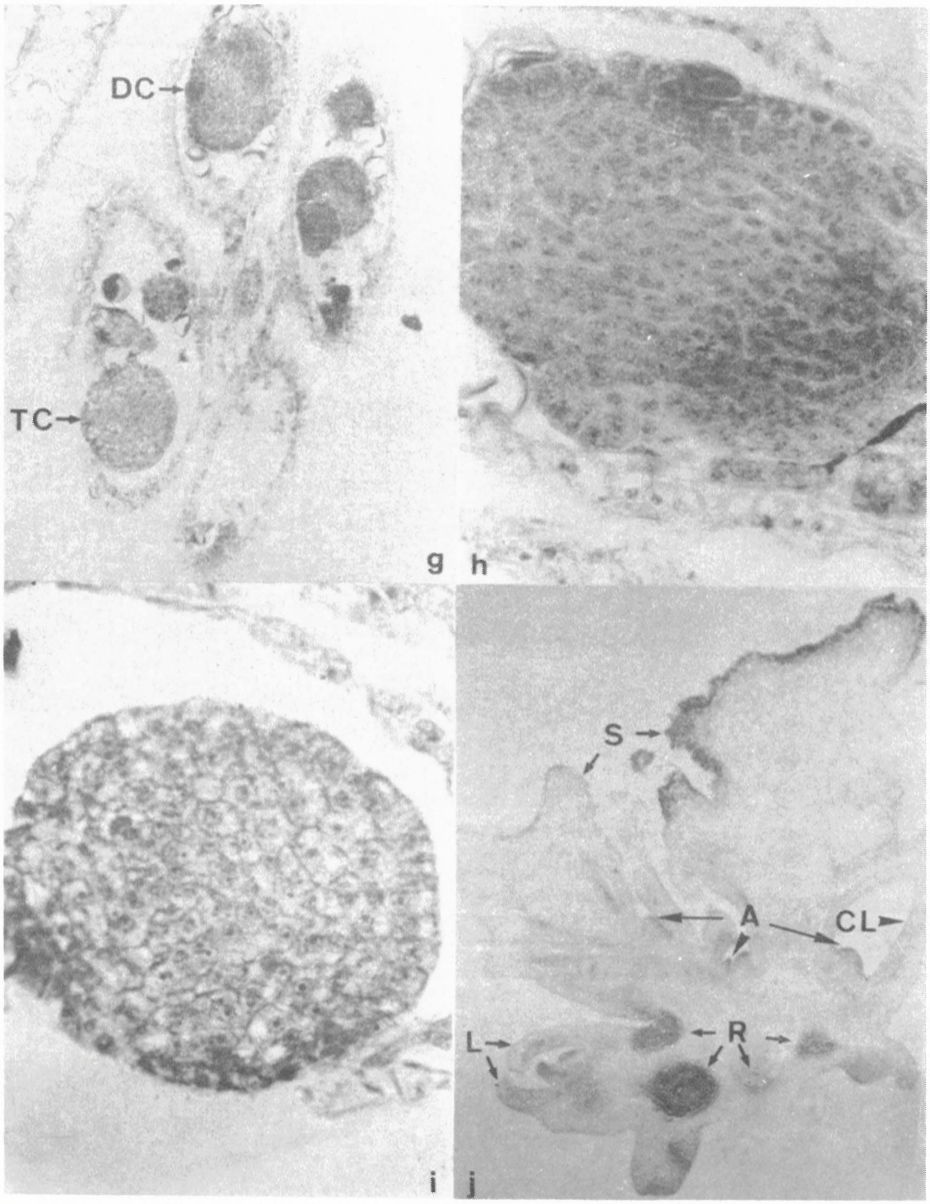


Fig. 2. The development of microspores in rice anther culture

g. Globular embryoids bursted out of anther wall with dense cytoplasm (DC), and with thin cytoplasm (TC). Observation made four weeks after anther culture. (100X).

h. High magnification of dense cytoplasm cell in Fig. 2g having uniform small nuclei. (400X).

i. High magnification of thin cytoplasm cell in Fig. 2g having uniform large nuclei. (400X).

j. Multiple apical meristem (A), radicle (R), leaf primodium (L), coleoptile (CL) and scutellum (S) of embryo five weeks after anther culture. (50X).

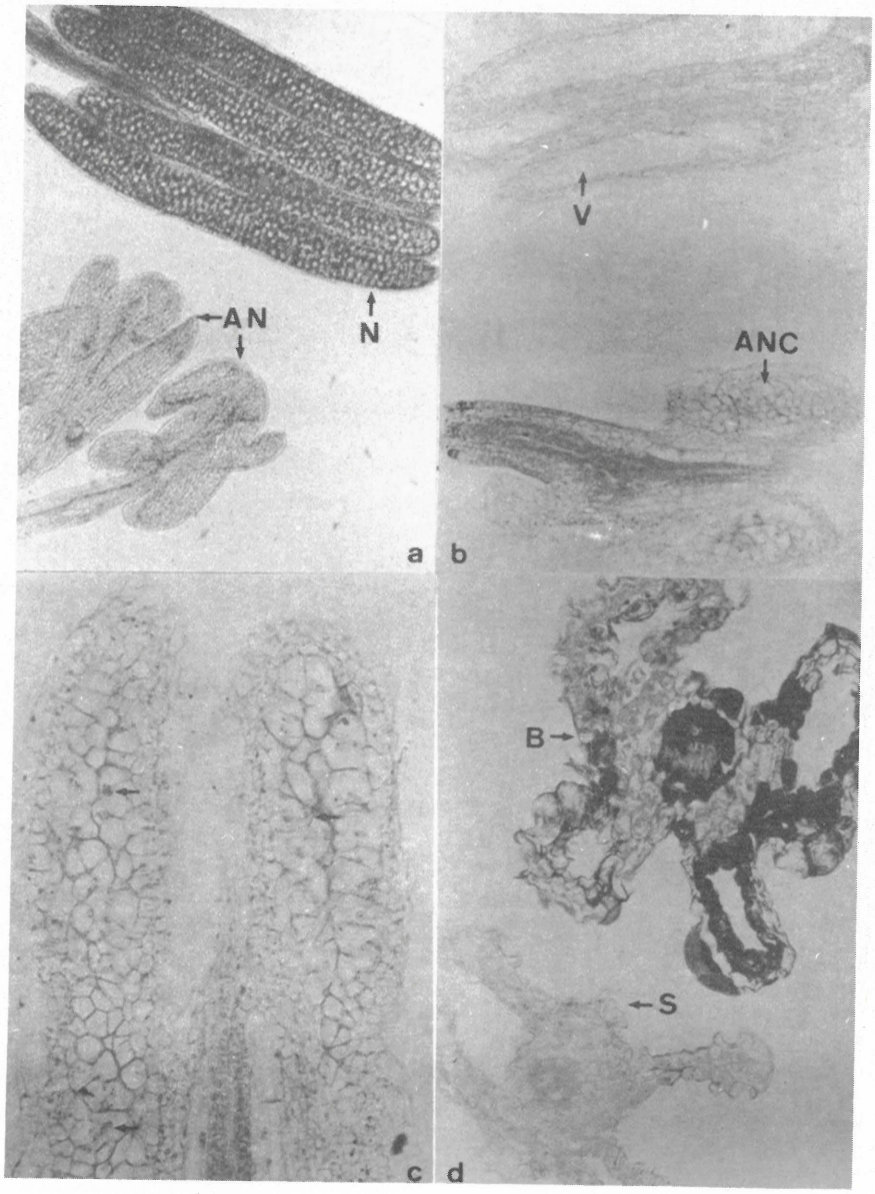


Fig. 3. The abnormality of anthers from donor plants grown under low temperature in February

a. Normal anther (N) filled with microspores and abnormal anthers (AN) without microspores by aceto-carmine squash. (35X).

b. Anthers with vacuoles (V) and anthers with abnormal cells (ANC) in pollen sacs during anthers excision and culturing. (100X).

c. Abnormal cells with multiple nuclei (arrow). (200X).

d. Browning (B) and shrinking (S) of anthers observed three weeks after anther culturing. (400X).

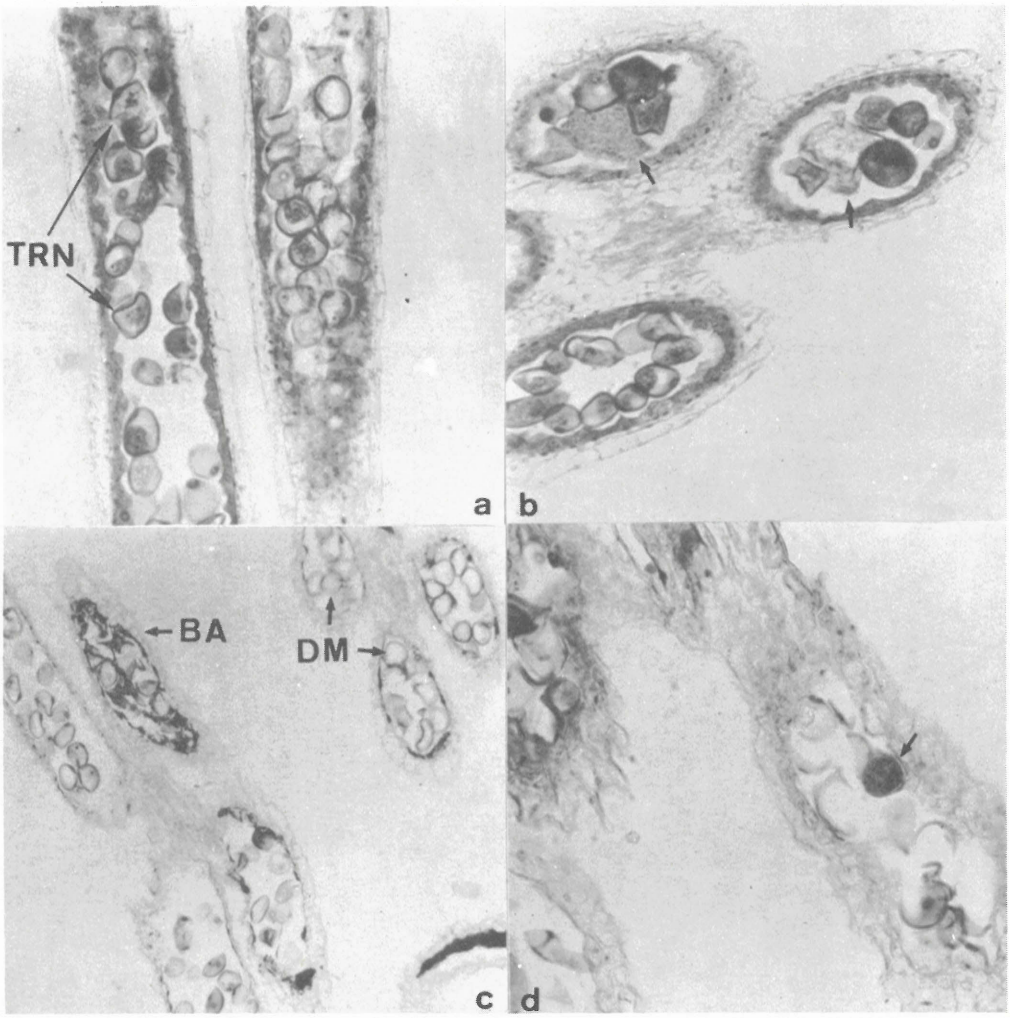


Fig. 4. Abnormality of anthers from rice plants grown under low temperature in growth chamber

a. Abnormal microspores with varied size from treatment L_{2b}. Some microspores had two or more nuclei. Note: two nuclei microspores (TN) and three nuclei microspores (TRN). (200X).

b. Abnormal cells (arrow) in pollen sacs from treatment L_{2b}. (200X).

c. Browning of anther (BA) and degenerating of microspores (DM) appeared one week after anther culture. (100X).

d. Multicellular microspores (arrow) and degenerated microspores after three weeks of anther culture. (200X).

單核期水稻花藥培養之初，圓球型之單核小孢子多緊靠花藥壁絨氈層上（圖2a）。培養7天後的花藥，小孢子經第一次有絲分裂形成一大（營養核）一小（生殖核）之雙核細胞（圖2b），或分裂成大小相同的兩個核（圖2c）。培養至14天的花藥，由小孢子發育成的多細胞小孢子仍包含在花粉的外粉層（exine）內（圖 2d）。培養至21天的花藥，多細胞小孢子的細胞數目急劇增加，多數仍包含在外粉層內，且在 hematoxylin 染色下可觀察出兩種不同型態的細胞，一種爲染色較淺，尙能分辨出核者，另一種呈染色極深的狀態（圖2e）；少數發育較快的小孢子已突破外粉層長成小型癒合組織（圖2f）。培養至28天，癒合組織不斷擴大，且突破藥壁（圖2g）；同樣的初期之癒合組織亦有明顯的不同染色型態，一爲染色深具小核之細胞（圖2h），另一種爲染色淺具大核之細胞（圖 2i）。培養至35天的花藥，小孢子形成的癒合組織已有根與芽的分化（圖2j）。

（二）2月份低溫下異常花藥之小孢子發育情形：

2月份低溫下所取得之花藥與正常花藥以醋酸洋紅染色壓片顯示，正常花藥的四個藥腔中皆充滿小孢子，而2月份產生的花藥則不含小孢子（圖3a）。經切片觀察顯示其藥囊爲空囊（圖3b），或充塞着許多不正常的多核細胞，故推測爲絨氈層細胞延伸所成（圖3c）。經取不同培養時期的花藥切片，只見花藥褐化及萎縮，而無任何生長（圖3d）。

（三）植物生長箱低溫處理之異常花藥的小孢子發育情形：

低溫處理除了 L_{2b} 處理外，其它處理之切片無法觀察出與對照組有何差異。L_{2b} 處理的水稻花藥於培養0天時，其小孢子與正常者比較，除排列凌亂大小不均外，且具有不正常之多核小孢子（圖4a），而部份花藥的藥囊中也發現絨氈層組織的異常增殖（圖 4b）。培養至7天的花藥，已有部份花藥褐化及萎縮，且許多小孢子之核已消失，而呈空胞化（圖 4c）。培養至14天，僅有極少數的小孢子能發育成多細胞小孢子，且不發育的小孢子迅速萎縮崩解（圖4d）。

討　　論

Tsai and Lin [17] 曾報導一期作水稻之花藥較二期作的花藥有較高之癒合組織形成率，但本研究未發現期作間的差異。許多報告指出植株營養狀況能左右花藥培養單倍體形成能力 [8,15,20]。本研究之結果顯示水稻母株營養生長期長期處於低溫環境生長，可能因生育不良而間接影響了癒合組織的形成，而營養生長期於高溫環境中生長，則無此不良影響。Hu *et al.* [12] 報導水稻植株孕穗期之溫度及日照爲影響花藥培養的重要因子，氏等認爲平均溫度 18.5～20°C，平均日照4小時，爲孕穗期最佳之生長環境；本研究發現臺農67號水稻母株的生殖生長期於 18～28°C 之間生長，均能有相當之癒合組織形成率，適溫範圍遠大於 Hu *et al.* [12] 所提出者，這種差異可能因水稻品種不同所致。本研究爲週年種植母體植株，每批水稻植株之生理狀況不同，因此對溫度的反應也各異，可能因而擴大了適溫範圍。如欲找出更精確之適溫範圍，應以植物生長箱測試，作更進一步的探討。

佐竹 [1] 於水稻生殖生長期以 12°C 的低溫處理，發現花粉母細胞減數分裂期受低溫影響時不稔率最高，幼穗形成初期次之。本研究以植物生長箱對水稻母株施以低溫處理，探討低溫影響花藥癒合組織形成率低下之結果，與佐竹所述之不稔率相符合。

植株在孕穗期如遇低溫，會造成花粉母細胞減數分裂時染色體不配對，減數分裂後細胞膜生成受阻，產生多核性巨大孢子，及絨氈層細胞的異常增殖，擴至藥腔，而阻止花粉發育等現象 [3,4]。本研究低溫處理所產生的花藥亦有相同的結果（圖 4a, 4b），且經切片觀察只有少數小孢子能分裂發育，而不發育的小孢子，在培養早期便已空胞化而迅速萎縮崩解（圖4c, 4d），並不形成具澱粉粒之營養細胞。Tsay [20] 及Tsay *et al.* [21]., 均曾指出這些具有澱粉粒之營養細胞的功能爲瓦解後供給發育中小孢子之養份，其數目與擬胚體之形成呈正相關。而週年栽培中2月份接種之第3批水稻母株的生殖生長期完全於低溫下生長，其花藥經切片結果爲空囊或充塞着不正常的多核細胞（圖 3b, 3c），以致完全無癒合組織的形成。

Tsai and Lin [17] 報告一期作水稻花藥癒合組織之分化率高於二期作。Hu *et al.* [12] 報導水稻孕穗期於高溫下生長之植株，其花藥癒合組織分化率低下，且有綠苗減少與白苗增加之現象。本研究週年栽培中7、8、9三個月份接種之水稻，其孕穗期均於高溫下生長，而花藥癒合組織之分化率並不低於其它月份，因而尚未能找出分化率與溫度變化的相關關係，可能是取樣數目較少，或外界其它因子（如日照、濕度等）與植株本身發育過程交感影響花藥癒合組織的分化，而非單從溫度一個因子便能探討的。在利用植物生長箱於水稻生殖生長期幾個階段施以低溫處理的試驗中，發現低溫不僅影響花藥癒合組織之誘導率，且影響癒合組織的分化能力。處理的階段愈早，癒合組織的分化能力便愈低。而處理時間加長則反而造成白苗比率的異常增加，可見在其它條件控制下，溫度確實能影響癒合組織的分化能力。若欲了解水稻母株對花藥癒合組織分化能力之影響，則需由更多的角度作更進一步的探討。

綜合本試驗結果得知，就臺農67號水稻之週年栽培，除了9月中旬至10中旬的一個月，其餘任何時間種植水稻均能產生正常健全的花藥，以供花藥培養之用，培養的花藥不但能有相當高的癒合組織形成率，且可正常的分化出植株，而不必僅限於每年兩次的期作，如此不但可提供充足的材料，更能調節人力、物力，充份運用空間，對實際的育種工作及提高育種效率上有莫大的助益。

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Effect of Inoculation Time during the Year on the Culture Efficiency of Rice Anthers[1]

C. C. Yeh and H. S. Tsay[2]

Summary

Rice plants (cv Tainung 67) were transplanted year round and anthers were collected and inoculated in different months of the year in order to investigate the effect of environmental factors, especially temperature, on the efficiency of rice anther culture. Results showed that the rate of callus formation for anthers inoculated between April and December ranged between 27 and 42%. Anthers collected in February failed to produce any callus. No anthers were available for culture in January and March due to the influence of temperature of the development of rice plants. Low temperature under which the rice donor plants were grown affected callus formation more severely than did the high temperature. The reproductive growth of rice plant, especially the meiosis of pollen mother cells, was more sensitive to extreme temperature than vegetative growth. Histological study indicated that low temperature inhibited microspore formation and caused abnormal multiplication of tapetum layer cells. No relationship was established between the rates of callus formation and plantlet regeneration in anther culture for rice plants grown in different seasons. The influence of average air temperature on the callus differentiation ability was also not clear.

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柴胡種子發芽勢之改良研究[1]

黃漢津　劉新裕[2]

摘要：發芽起始日數長及發芽不整齊爲柴胡發芽特性之一，本研究主要探討改良柴胡發芽勢之方法及適合之發芽溫度，其結果如下：柴胡可在 pH 值 4 至 9 之蒸餾水添加液中順利發芽，其發芽溫度以16°C 較爲恰當，20°C 以上則有明顯的抑制現象。日夜溫差有利於柴胡發芽，當夜溫 16—20°C而日溫 24—28°C 時有明顯的促進作用，但低夜溫（12°C或以下）較不適宜，顯示柴胡之播種期在秋末冬初或冬末春初較爲恰當。硝酸鉀（0.2，1.0%）溶液對柴胡發芽有不良影響，激勃素（GA_3）1000, 500ppm 與細胞分裂素（Kinetin）100, 50, 10 ppm 單獨或混合浸種24小時，對柴胡發芽並無促進作用，但經流水浸種後浸泡於激勃素 1000, 100ppm 24小時，却有增進發芽率的現象，此外單獨應用流水浸種處理亦可促進柴胡之發芽，而柴胡之種子浸出液有延遲發芽及降低發芽率等現象，顯示浸種處理有洗濾柴胡種子之發芽抑制物質的效果。低溫層育（0—4°C）對柴胡之胚發育及發芽有明顯的促進作用；經 8 星期層育後，其胚長度增爲對照之 3 倍，且到達最終發芽總數 9/10（T_{90}）的時間縮短至 4 天左右較對照之15天明顯快速，且高溫（24°C）亦可促進較長期的層育種子早日發芽而不再是限制因子。柴胡發芽率亦因低溫層育而增加，但 6 — 8 星期之層育有降低發芽率的趨勢。

柴胡爲繖形科多年生草本植物，神農本草經列爲上品，根爲主要藥用部位，其藥性苦、平、無毒，主治胸脇苦滿及肝膽疾病等，另有抗炎、鎮靜、抗過敏、解熱、鎮痙、鎮痛等作用[1,6]。本省每年需要量極多，大多仰賴進口，據國貿局海關73年統計資料，柴胡進口量爲67,941公斤，耗外匯15,200,000元（臺幣）。

柴胡於本省栽培年代甚短，栽培方法均仿效日本，然而日本之栽培亦未十分成功[6]。柴胡栽培常以大量種子先行育苗後，選擇生育期相近的植株移植，如此其種子用量及工作成本均相對的增加，且因移植時，主根易受傷害致其根形粗短、支根多、而影響市場價格。雖有研究指出栽培方法以移植法較直播法爲佳[2]，但究其原因可能在於無法克服柴胡種子發芽不整齊及其後期管理不便的緣故。因此改良柴胡發芽勢是爲柴胡直播栽培及改善品質的先決條件。

繖形科植物之種子於脫離母株時其胚通常尚未發育成熟，在發芽前必須經過一段時日之胚後熟作用[8]。但因胚發育程度之差異或內部生理活性之差別，造成發芽不整齊的現象[4]。本研究主要探討改善柴胡發芽不整齊之方法，以俾利柴胡之栽培。

材料與方法

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（1）試驗材料：以本所（臺中）12月中旬採收之三島柴胡（*Bupleurum falcatum L.*）成熟種子爲試驗材料。種子陰乾後以封口塑膠袋封存於0—4°C之冷藏庫貯存備用。

（2）試驗方法：發芽試驗：柴胡種子先以1%次氯酸鈉（NaHClO）浸種消毒10分鐘，復以蒸餾水清洗，浸泡10分鐘後置於上覆一層Whatman No. 1長型濾紙之壓克力發芽床上（已置入直徑15公分養皿中），每處理4重複，每重複40粒種子，培養皿中添加二次蒸餾水（pH 4.5—4.8）120ml，後加蓋以保持濕度，移入恆溫箱中行暗室紙上發芽，每日檢視發芽（胚根突出種皮）數目，三星期後統計其發芽率並計算T10、T50及T90；其各爲到達發芽總數 10/1、10/5及10/9的時間，用以表示其發芽速度 [10]。浸種處理：在室溫下以流水浸種 2—6日後進行發芽試驗。硝酸鉀（KNO_3）或種子浸出液處理：培養皿中添加硝酸鉀（0.2 %及1%）溶液或以種子浸種之濾液代替二次蒸餾水。日夜溫差處理：以日溫（16、20、24及28°C）8小時與夜溫（12、16、20及24°C）16小時組合處理。pH值處理：以HCl或NaOH調整蒸餾水之 pH 值爲2至9，以進行發芽試驗。GA_3及Kinetin 處理：種子消毒清洗後浸泡於不同濃度之GA_3或Kinetin或其混合溶液中（4.5<pH<5），24小時（16°C）再進行發芽試驗。低溫層育處理：種子消毒清洗後，置於前述培養皿中，於0—4°C下置放 1—8星期，發芽培養前取出復行消毒、清洗。胚長度測定：利用接目鏡附有標尺刻度表（micrometer）之光學顯微鏡測胚長度，每處理40粒種子。

結果與討論

（1）流水浸種與日夜溫差處理對柴胡發芽之影響：

在柴胡發芽過程中，往往發現濾紙上殘留黃棕色之潰跡，顯然柴胡在發芽過程中釋出某些物質。利用洗濾處理通常可去除種皮或種子內部之發芽抑制物質。本試驗應用流水浸種2天之結果如表1所示。表1中可明顯得知16°C爲柴胡發芽之較適溫度，溫度愈高發芽率愈低而到達 T50 的時間亦愈增長，此與黃等 [4] 之結果類似，但兩者之發芽起始日數却有明顯出入。郭 [3] 曾指出母株之生育環境對種子發芽有甚大之影響。本次試驗材料爲12月採收之種子，而黃等 [4] 之材料爲6月採收，是否因生育環境不同，有待進一步研究。在12°C之結果不如 16°C或20°C，唯仍優於 24°C或28°C，在24°C及

Table 1. Influence of pre-soaking and temperature treatments on the germination of *Bupleurum falcatum* L. seeds

Character	Duration of Pre-soaking* (day)	Germination Temperature (°C)				
		12	16	20	24	28
Germination Rate (%)	0	57.5d**	80.0a	65.6bcd	21.9f	5.6g
	2	56.9d	76.3ab	62.5cd	36.3e	13.1fg
Days to Beginning Germination	0	8	5	6	11	13
	3	8	5	5	6	6
Day to 50% of Final Germination Rate (T-50)	0	16.1a	10.9b	10.5b	16.4a	—
	2	14.8a	8.5c	8.6c	11.5b	—

*Seeds were soaked in running tap water.

**Means with the same letter across temperature and presoaking treatments for each character are not significantly different at 5% level by Duncan's New Multiple Rang Test.

28°C之發芽率僅及16°C之1/4或1/15且其 T50 明顯延長，顯示高溫抑制柴胡之發芽。邱[2]著稱柴胡發芽溫度約在18°C, Atwater[8] 亦謂20°C 左右爲繖形科及其它線狀胚種子(linear embryo)之適當發芽溫度均與本試驗之結果相近。流水浸種 2 天之發芽率與對照組之趨勢一致，無太大的差異，只在24°C及28°C 有較好的表現，但是到達 T50 的時間却有明顯的縮短現象，此可能與發芽培養前 2 天之浸潤 (imbibition) 有關。但是從發芽起始日數 (days to germinate) 探討。16°C 之條件下流水浸種及對照組同爲第 5 天開始發芽，而到達 T50 的平均日數在對照組爲 6 天左右 (10.9—5)，流水浸種則僅有3.5天 (8.5—5) 兩者差異顯著。此外，發芽溫度愈高時流水浸種與對照之發芽起始日數差距愈爲明顯。顯示流水浸種之效果將不僅僅是種子提早浸潤而已。欲明瞭是否有抑制物質被洗濾的作用，因此應用較長時間之流水浸種處理及其種子浸出液進行發芽，結果如表 2。比較表 1，表 2 之對照組，於24°C下之發芽率及T50有明顯的不同，此可能與後者曾經較長期之低溫貯藏而促進胚後熟作

Table 2. Influence of different presoaking treatments and seed leachate on the germination of *Bupleurum falcatum* L. seeds

Treatment*	Temperature (°C)	Germination Rate (%)	Days to% of Final Germ. Rate 10(T-10)	Days to% of Final Germ. Rate 50(T-50)	Days from T-10 to T-50
CK	16	75.0ab**	5.84b	8.66b	2.82ab
	24	59.4cdef	6.24b	9.44b	3.20a
S—2	16	71.9abc	3.36cde	4.86e	1.50d
	24	68.1bcde	2.51f	5.15e	2.64abcd
S—4	16	81.3a	3.58cde	5.21e	1.63d
	24	78.8ab	2.90ef	5.7ɕde	2.83abc
S—6	16	66.9bcd	3.81cd	5.49de	1.68cd
	24	68.8abcd	2.78ef	5.40de	2.62abcd
CK(L)	16	58.8def	7.56a	10.51a	2.95ab
	24	47.5f	8.18a	11.47a	3.30a
S—6(L)	16	66.9bcd	4.41c	6.27cd	1.86bcd
	24	55.6ef	4.21c	6.80c	2.65abcd

*CK, S-2, S-4 and S-6: pre-soaking in running tap water for 0, 2, 4 and 6 days, respectively, CK (L) and S-6(L): pre-soaking in running tap water fro 0 and 6 days, respectively, and watered with seed leachate afterwards.

**Means with the same letter within each collumn are not significantly different at 5%evel by Duncan's New Multiple Range Test.

用有關。種子浸出液對於柴胡發芽之影響（表 2），不論是否先經流水浸種處理，均有延長 T50的現象，而其原因主要在於延長發芽初期（培養至 T10）所需要的時間，對於 T10至T50之影響不大。就發芽溫度而言，高溫 (24°C) 之 T50仍然有較長的現象。此外，發芽率亦因種子浸出液處理而降低。顯示柴胡發芽初期及發芽率明顯受到種子浸出液的抑制。流水浸種（2，4，6天）之處理，其T50皆顯著的減少，究其原因乃由於發芽初期及T10至T50之發芽時間皆明顯縮短的緣故。但發芽初期(培養至 T10) 以24°C較16°C快速，與對照組因高溫而延長其 T50的時間迥然不同。T10至 T50之時間則以16°C較爲快速。比較不同天數流水浸種之結果，發現 4 天之處理效果較佳，其發芽率在 2 種溫度下

表現最爲良好，過長之浸種處理可能影響種子活之力。綜合以上結果，顯示流水浸種下，發芽抑制物質確有被洗濯的現象。柴胡之主要藥效成分爲皂苷素 [1,2,3] (saponin)。Nord *et al.* [14] 曾提出皂苷素爲種子發芽抑制物質之一。且另有報告指出由柴胡種子可分離出柴胡苷 (saikosides) [11]，這是多種苷的總稱，因此柴胡種子是否因累積此類物質而抑制胚發育及發芽，有待進一步研究。

自然的條件下日溫通常高於夜溫，且國際種子檢查規則中種子的發芽一般亦以日夜溫差處理。應用不同的日夜溫差之組合，進行柴胡發芽試驗，結果如表 3。比較表 1、表 3 之結果，發現以日夜溫

Table 3. Effect of different day/night temperatures on the germination of *Bupleurum falcatum* L. seeds

Temperature (°C)		Germination Rate (%)	Days to 50% of Final Germination Rate (T-50)
Day	Night		
16	12	54.4d*	13.6b
20	12	71.9bc	15.1a
20	16	72.5bc	8.8d
24	12	77.5ab	15.8a
24	16	75.0abc	8.4d
24	20	70.6bc	12.1c
28	12	63.8cd	15.1a
28	16	86.9a	11.7c
28	20	81.9ab	11.7c
28	24	24.4e	15.3a

*Means with the same letter within each column are not significantly different at 5% level by Duncan's New Multiple Range Test.

差處理之發芽率普遍高於以日溫或夜溫之恆溫處理，而到達 T50的時間亦有縮短的現象，顯示日夜溫差較利於柴胡之發芽。此與 Davis [11] 及Naylor *et al.* [13] 在其他種子發芽試驗之結果相類似。但是以日溫28°C、夜溫24°C之組合，其發芽及發芽勢 (T50) 仍然不理想。此外以夜溫12°C之各組合，其到達 T50之時間普遍延長，且與日溫16°C之組合，發芽率明顯的低於 16°C之恆溫處理 (表 1)，顯示低夜溫不利於柴胡之發芽。夜溫16°C之各處理組合表現良好，尤與日溫20°C及24°C之組合，到達T50之時間僅須8.4—8.8天，最爲快速，而與日溫 28°C之組合，雖然 T50略長於 16°C之恆溫處理，但其發芽率高達86.9%，顯著優於其他之組合，另外日溫 28°C與夜溫 20°C之組合，其發芽率亦高達81.9%，明顯的高於28或20°C之恆溫處理 (表 1)。綜合上述，可知日夜溫差在12—8°C，而夜溫爲16—20°C，對於柴胡發芽有明顯的促進作用。其促進機制可能在於溫差變化促使膜之通透性改良 [12]，而在較適之溫度中 (18°C或20°C) 發芽。

(2) 硝酸鉀、激勃素與細胞分裂素對柴胡發芽之影響：

硝酸鉀可促進某些繖形科植物之種子發芽 [8]，本研究以硝酸鉀0.2%及1.0%溶液代替蒸餾水添加於培養皿中，其結果如表 4 所示，硝酸鉀對於柴胡發芽有不良的影響，且其不良影響隨著濃度或溫度的升高而更加明顯，顯示柴胡種子應用硝酸鉀處理似無必要。

激勃素 (GAs) 在突破種子生理性及胚形態休眠上應用頗爲廣泛，而細胞分裂素 (Cytokinins)

Table 4. Infuence of temperature and concentrations of potassium nitrate on the germination of *Bupleurum falcatum* L. seeds

KNO3 (%)	Temperature (°C)				
	12	16	20	24	28
0.0	57.5bc*	80.0a	65.6b	21.9e	5.6fg
0.2	53.1bc	63.8bc	61.3bc	8.8fg	0.6g
1.0	38.1d	54.4bc	11.9ef	0.0g	0.0g

*Means with the same letter across temperature and concentration treatments are not significantly different at 5% level by Duncan's New Multiple Rnage Test.

在此方面並無太大的功效，但是當種子內部存在離層酸 (ABA) 之類似物質時，細胞分裂素之使用却是必要的 [5,7]。本研究使用激勃素 (GA_3) 1000, 500ppm 及細胞分裂素 (Kinetin) 100、50、10ppm單獨或混合浸種。由於細胞分裂素僅溶於强酸或强驗中，爲避免酸鹼值影響柴胡之發芽，因此，以NaOH 或 HCl調整蒸餾水之pH值，進行酸鹼度對柴胡種子發芽之試驗，結果如表 5 所示，柴胡

Table 5. Influence of different pH values on the germination of germination of *Bupleurum falcatum* L. seeds

Character	pH							
	2	3	4	5	6	7	8	9
Germination Rate (%)	0c*	70.0b	72.5ab	77.5ab	76.9ab	76.9ab	81.9a	75.0ab
Days to 50% of FinalGerm. Rate	—	10.0a	9.7a	9.9a	9.5a	10.2a	9.1a	9.7a

*Mean with the same letter within each row are not significantly different at 5% level by Duncan's New Multiple Range Test.

種子在 pH 值 4 — 9 間之酸鹼度中，其發芽並無明顯差異，顯示柴胡在弱酸至微鹼的環境中均可順利發芽。激勃素及細胞分裂素溶液之 pH 值依此結果調整爲4.5—5.0之間。將消毒，清洗後之柴胡種子於 16°C 下不同濃度組合之溶液中（表 6）浸泡24小時之後在 16°C 下培養，其結果如表 6，激勃素處理效果不彰，而細胞分裂素之使用，在高濃度下 (100, 50ppm) 有明顯不良影響。且細胞分裂素 (100ppm) 與激勃素 (1000、500ppm(之組合，其影響似乎更爲不利。由上顯示，柴胡之發芽不整齊現象，內生荷爾蒙可能不是一個主要限制因素。柴胡可能之發芽抑制物質可經由流水浸種被洗濯出來，已如前述，將流水浸種後之種子給予GA_3不同濃度浸泡處理，其結果如表 7，表 7 中 GA_3單獨處理之效果仍然不彰，而經流水浸種處理 6 天後應用GA_3 100ppm浸泡，其發芽初期較爲快速，而GA_3 1000ppm處理則有延長T_{10}的現象。但前文中述及因 6 天流水浸種後發芽率降低的現象，可由 GA_3 浸泡處理提昇，顯示GA_3在種子活力增强的效果。

（3）低溫層育對於柴胡胚發育及發芽之影響：

低溫層育 (0—4°C) 處理對柴胡發芽已有初步成效 [4]，本次試驗結果更證明低溫層育可明顯促進柴胡胚發育及快速的芽發（表 8）。Bryant [9] 指出低溫層育 (5°C以下)促使繖形科之*Heracleum sphondylium* 未成熟胚快速成熟，Simmands *et. al* [15] 亦指出低溫層育可增加糖槭 (*Acer sacch-*

Table 6. Influence of GA-3 and kinetin on the germination of *Bupleurum falcatum* L. seeds

Conc. (ppm) of GA-3	Conc. (ppm) of kinetin	Germination Rate (%)	Days to 10% of Final Germ. Rate	Days to 50% of Final Germ. Rate
0	0	77.5a*	6.73ab	9.15bcd
0	10	79.4a	6.12b	8.73cd
0	50	60.6cde	6.73ab	10.00ab
0	100	61.9bcde	6.80ab	10.01ab
500	0	76.3ab	6.06b	9.13bcd
500	10	66.3abcd	6.12b	8.37d
500	50	68.0abcd	6.62ab	9.38bc
500	100	51.3e	6.97a	10.57a
1,000	0	74.4abc	6.32ab	8.67cd
1,000	10	70.6abc	6.30ab	8.50cd
1,000	50	69.4abc	6.06b	8.73c
1,000	100	55.0de	7.04a	d9.98ab

*Means with the same letter within each column are not significantly different at 5% elvel by Duncan's New Multiple Range Test.

Table 7. Effect of pre-soaking and GA-3 treatments on the germinatio of *Bupleurum falcatum* L. seeds

Days of Presoaking	Days of GA-3 Treat. 100ppm	Days of GA-3 Treat. 1,000ppm	Germination Temp. (°C)	Days to 10% Final Germ. Rate	Days from T-10 to T-50	Germination Rate (%)
0	0	0	16	5.84a*	2.82ab	75.0abc
0	0	0	24	6.24a	3.20a	59.4def
0	1		16	5.75a	2.32ab	63.8cdef
0	1		24	6.34a	3.23a	53.0f
0		1	16	5.83a	2.71ab	67.5bcde
0		1	24	6.27a	3.14a	56.3ef
6	0	0	16	3.81b	1.68b	66.9bcde
6	0	0	24	2.78c	2.62ab	68.8abcd
6	1		16	2.32c	1.80b	76.9ab
6	1		24	2.05c	2.26ab	73.8abc
6		1	16	4.30b	1.86b	81.3a
6		1	24	4.24b	2.33ab	73.1abc

*Means with the sanme lettter within each c olumn are not sigfiicantly different at 5% level by Duncan's New Multiple Range Test,

arum) 胚軸的生長潛力 (growth potential)，糖槭之胚軸於 5°C 層育 10天後即逐漸的生長。柴胡種子經低溫層育處理其胚長度，在初期（1—2 星期）並無增加，但 3 星期後即有明顯的差異，尤其後期呈現快速的成長。經 8 星期層育後，其胚長度增爲對照之 3 倍左右。柴胡之發芽速度，一般而言，亦因層育的延長而加快；經過 1—2 星期層育之柴胡種子，雖然胚長度並無增加，但發芽速度仍然優於對照組，而 8 星期層育處理後，培養至最終發芽總數 9/10 (T90) 的時間僅需 4 天左右，較對照之15天 (16°C與24°C之平均) 明顯快速。此外，亦較對照組由培養至發芽初期 (T10) 所需要的時間 (6.5天) 短，顯示經長期低溫層育者已至發芽未了，而未經處理者却尚未發芽。前已述及柴胡之發芽溫度以16°C較爲恰當，20°C以上則有延遲發芽的現象，比較低溫層育種子在16°C與24°C之發芽速度發現，經 3 星期以上之層育種子在發芽初期 (T10) 不但不受高溫抑制，反有被促進的現象；8 星期層育處理者在 24°C下，其 T10 僅需0.4天而在16°C下却達0.9天，差異顯著。但隨著培育時間的延續，高溫不利發芽的現象又逐漸顯示出來，除了最終發芽率遍普低於16°C者外，其到達T90的時間亦有較長的趨勢。顯示高溫在基本上不利於柴胡發芽，但却可促進較長期層育種子早日發芽。此外，低溫層育亦可增進柴胡之發芽率（表 8），此與Thaphliyal*et. el.* [16] 謂低溫層育 (3—9°C) 可提高雪

Table 8. Effect of durations of cold stratification on the embryo development and germination of *Bupleurum falcatum* L. seeds

Stratification Period (week)	Embryo Length* (mm)	Germination Rate (%)**		Days to % of Final Germination Rate**					
				10(T-10)		50(T-50)		90(T-90)	
		16°C	24°C	16°C	24°C	16°C	24°C	16°C	24°C
0	0.46d	75.0abc	66.3c	6.4a	6.7a	9.0ab	9.3a	13.4b	16.7a
1	0.43d	80.6ab	68.8bc	5.1c	6.0b	7.3c	8.6b	11.3cd	12.6bc
2	0.47d	85.0a	75.6abc	4.3d	4.9c	6.7c	7.3c	10.9cd	10.5de
3	0.62c	77.5abc	73.8abc	3.8e	3.2f	5.8d	5.6d	8.0fg	9.1ef
4	0.79b	81.3ab	77.5ab	2.6g	2.1h	3.9e	4.1e	6.3h	7.5gh
6	0.82b	76.3c	67.5bc	2.4gh	2.4gh	4.3e	3.9e	6.4gh	7.0gh
8	1.48a	65.0c	64.4c	0.9i	0.4j	1.9f	1.7f	4.0i	4.0i

*Embryo length was measured after stratification, means with the same letter are not s ignificantly different at 5% level by Duncans New Multiple Range Test.

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松 (*Cedusdeocara*) 之發芽率及發芽速度之結果相類似。但柴胡種子經 6—8 星期層育處理後，其發芽率有降低的現象，此與黃等 [4] 結果類似，顯示長期之層育處理會影響種子之活力，其原因可能與層育時之高濕環境及層育前之種子消毒不够徹底而導致發霉有關。

結 論

柴胡種子發芽不整齊造成栽培管理不便，因此柴胡發芽勢之改良實屬必要，經由各種處理發現，日夜溫差較利於柴胡發芽，於夜溫16—20°C而溫差12—8°C之條件下發芽情形雖爲良好而低溫 (12°C 或以下) 較不適宜，顯示柴胡適當播種期在秋末冬初或冬末春初。柴胡發芽勢可經由長期低溫乾燥貯

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3	0.62c	77.5abc	73.8abc	3.8e	3.2f	5.8d	5.6d	8.0fg	9.1ef
4	0.79b	81.3ab	77.5ab	2.6g	2.1h	3.9e	4.1e	6.3h	7.5gh
6	0.82b	76.3c	67.5bc	2.4gh	2.4gh	4.3e	3.9e	6.4gh	7.0gh
8	1.48a	65.0c	64.4c	0.9i	0.4j	1.9f	1.7f	4.0i	4.0i

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柴胡種子發芽不整齊造成栽培管理不便，因此柴胡發芽勢之改良實屬必要，經由各種處理發現，日夜溫差較利於柴胡發芽，於夜溫16—20°C而溫差12—8°C之條件下發芽情形雖爲良好而低溫 (12°C或以下) 較不適宜，顯示柴胡適當播種期在秋末冬初或冬末春初。柴胡發芽勢可經由長期低溫乾燥貯

藏，流水浸種及低溫層育而加以改良，其中以低溫層育效果最佳，而以流水浸種處理所需時間較短。硝酸鉀、激餾素及細胞分裂素之使用似無必要。柴胡可在 pH 值 4 至 9 之環境中發芽，然而栽培柴胡以何種酸鹼值之土壤爲佳，應另行探討。

致　謝

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Studies on the Improvement of Seed Germination of *Bupleurum falcatum* L.[1]

H. C. Huang and S. Y. Liu[2]

Summary

Bupleurum falcatum L. is an important medicina species under the family of Umbelliferae. Physiological immature embryo in the seeds of this family generally require an after-ripening period to germinate. The purposes of this experiment were to find ways to improve the germinating vigor of this species. Optimal temperatures for the germination of *B. falcatum* L. seeds were 16°C followed by 20°C. Germination rates at the two temperateurs were 80 and 65.5% and days needed to 50% gemination of final rates (T-50) were 10.9 and 10.5, respectively. Alternating day/night temperatures accelerated seed germination. Night temperature of 16 or 20°C and difference between day and night temperatures of 8 and 12°C were particularly effective. Presoaking seeds in running tap water for 4 days could increase germination rate singificantly at 24°C, presumably the result of leaching out some inibitory substances from the seeds. Soaking the seeds in GA-3 (500 or 1,000ppm) and/or kinetin (10, 50 or 100ppm) solutions or addition of potassium nitrate (0.2 or 1.0%) to the germination medium showed no positive effect on the germination performance of *B. falcatum* seeds. Cold stratification at 0—4°C, on the other hand, was of prominent importance in improving seed germination. After eight-week cold stratification, embryo length was three times longer than that of the untreated check treatment and time needed to reach T-90 was only 4 days, or 11 days shorter than the check. Cold stratification for eight weeks also enable seeds to germinate even at the unfavorable high temperature of 24°C. Germination rate also increased as a result. The most suitable duration of cold stratification was found to be four weeks.

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不同採收期與貯藏法對柴胡種子發芽之影響[1]

胡敏夫　黃漢津　劉新裕[2]

摘要：柴胡種子於開花後 55.62.69及76天分別採收，經充分陰乾後貯藏於冰箱（0-5℃）、乾燥器、定溫箱（36℃）及室溫下，並按貯藏後第 3、6、9及 12 個月等 4 期，測定其發芽率及發芽速度，發現(1)新鮮種子之發芽率及發芽速度，以開花後69天採收者比其他採收期表現優異，其發芽率高達71%，發芽率達50%所需日數爲21天。(2)乾燥種子之發芽率及發芽速度，則因貯藏法及貯藏時間不同而表現互異，一般以採用定溫箱貯藏法者較佳，其種子之平均發芽率達71%，平均發芽率達50%所需日數爲14天，依次爲採用乾燥器及冰箱貯藏法，而室溫貯藏者最差。(3)採用冰箱、乾燥器及室溫等貯藏法之乾燥種子，最高發芽率表現皆於貯藏後第 6 個月，而定溫箱貯藏者於第 9 個月。(4)不同貯藏法下之乾燥種子最佳採收期之研究結果顯示，則於開花後69天採收者，其種子發芽率較高，發芽速度亦較快。

三島柴胡（*Bupleurum falcatum* L.）爲一繖形科（Umbelliferae）多年生草本之藥用植物，其原產於日本富士山麓下之三島地方[7]。本所於1968年引進栽培，至目前已成爲本省重要藥材[3]。其根部成分早於1929年由日人惠澤氏等研究發現含有 2 %之脂肪油，構成脂肪酸係 Palmitic、Stearic、Oleic、Linolic 及 Linoleme acid等成分；1953年武田氏等分離出 Spinasterol, Stigmasterol 等 C_{29} 的 Sterol 類成分及 Adcnitol 等糖類；1965年東京大學柴田承二教授亦曾分離得皂素（Saponin）成分，並命名爲 Saikoside 1a 及 1b 等[4]。現在已知柴胡之有效成分爲 Saiko saponin a, c, d 其含量依產地及基源植物而異。其功能爲解熱、鎮痛、抗菌、抗病毒、升陽、解鬱及調經，主治肝膽等少陽病、寒熱往來、口苦耳聾、胸脇苦滿、頭眩嘔吐、瘧疾、月經不調、及中氣下陷等症[6]。又1956年小島氏等早就指出柴胡根確實有改善肝機能之功用[4]。

柴胡之栽培，通常以種子播種育苗再行移植本田被認爲是最適當栽培方法[2]，但播種後屢見種子發芽不整齊，且發芽日數過長而影響定植工作，因之，一般栽培者均用多量種子育苗法，即每公畝所需種子量高達 500g 以上。致使柴胡種子發芽有上述現象可能係因種子成熟度不一，貯藏方法不當及貯藏時間過長等因素所影響，而國內外針對此問題研究則闕如，實有必要詳加探討，經筆者一年研究頗有結果，特整理報告如下，以供有栽培興趣者參考，並乞方家有所指正。

材料與方法

供試材料爲三島柴胡花後（Days after flowering 簡稱 DAF）55、62、69及76 等不同日數採收之種子。種子採收方法，乃於八月間柴胡盛花後陸續選定當日開花之花朵，掛牌標示其開花日期，然後按上述日數採收不同成熟度種子，充分陰乾並測定其含水率，於民國75年 1 月 6 日同時貯藏。貯藏方法係將各不同採收期之柴胡種子裝入紙袋，每袋選裝飽滿種子25粒，每採收期種子計裝80袋，分別貯藏於冰箱（0-5°C），室溫、乾燥器及 36°C 定溫箱（Incubator）內；即各貯藏處理均貯存有

1.臺灣省農業試驗所　研究報告第 1360 號。
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不同採收期之柴胡種子20袋。種子貯藏前各貯藏處理均用雙層塑膠袋密封，袋內並盛氯化鈣以吸收濕氣。發芽率調查時間則分別於貯藏前及貯藏後第 3 、 6 、 9 及12個月行之；調查方法乃每貯藏處理之種子，依其不同採收期各取出 4 袋，即每袋25粒種子作爲一重複，先以 1 %次氯酸鈉 (NaHClO) 浸種消毒10分鐘，復以蒸餾水清洗10分鐘置於培養皿之濾紙上，放入 16°C 定溫箱內，待其種子胚根出現時記錄其種子發芽數目並累計而換算其發芽率。爲進一步瞭解其發芽勢並調查其 GT_{50} ，計算公式仿 Coolbear 氏等報告[11] ，即 $GT_{50}=ti+\frac{(N+1)/2-ni}{(nj-ni)}\times(tj-ti)$, N爲發芽總數， ni 及 nj 爲發芽數累計，當 $ni<(N+1)/2\leq nj$ 時，ti 及 tj 各爲 ni 及 nj 時之時間。

結　果

一、不同採收期之新鮮柴胡種子發芽率與 GT_{50}

柴胡花後55天種子呈黃色，花後62天呈淺褐色，花後69天呈褐色，花後76天呈深黑褐色，依此不同顏色即可辨別種子可能之成熟程度。本項試驗於74年10月17日採收新鮮種子後，隨即用 1 %次氯酸鈉消毒10分鐘，復以蒸餾水清洗10分鐘後置入培養皿之濾紙上，按前報試驗結果[5] 再置 16°C 定溫箱內以進行種子發芽觀察，發現 69DAF 之種子發芽率較高達71%，經顯著性差異分析達 5 %顯著水準（如表 1 ），次爲 55DAF 種子55%，再次爲62及 76DAF 之種子，其發芽率各爲 47%及 39%，後三者之差異均未達顯著性水準，可說試驗結果頗不規律。 GT_{50} 爲調查種子發芽速度之指標，即種子發芽率達50%所需日數愈短者，其發芽勢呈較強趨勢。從表 1 資料可得知62及 69DAF 之種子 GT_{50} 需21天，55DAF 爲22天，76DAF 爲23天，後二者之種子發芽速度較慢，但差異皆不顯著。

表1. 不同採收期之新鮮柴胡種子發芽率與發芽速度比較

Table 1. Comparison on the germination rates and Gt50 of fresh *Bupleurum falcatum* L. seeds harvested at different dates after flowering.

Harvest date (DAF)	Germination (%)	GT50 (days)[2]
55	55ab[1]	22a
62	47b	21a
69	71a	21a
76	39b	23a

1: Means in each column with the same letter are not significantly different at 5% level.
2: Days needed to 50% of total germination.

二、不同貯藏方法之柴胡種子發芽率探討

A. 冰箱貯藏

由圖 1 顯示，貯藏後第 3 個月 4 種不同採收期柴胡種子之發芽率均在60%—66%間，至第 6 個月時發芽率大幅提高，其中以 55DAF 最高達94%，次爲 69DAF 86%, 76DAF 76%, 62DAF 72%；但至第 9 個月時， 62DAF 之發芽率反而提高至90%與 55DAF 相同，而 69DAF 與 76DAF 之發芽率却微微降低爲80%與74%；貯藏一年後各採收期種子之發芽率均明顯降低，尤以 62DAF 與 76DAF 爲最，其發芽率僅55%左右。上述結果除貯藏後第 9 個月之 55DAF 與 62DAF 二採收期之種子發芽率達 5 %顯著提高外，其餘各貯藏期之不同期採收柴胡種子發芽率均未呈顯著差異。至於有效貯藏期限，即種子發芽率維持 80%左右之貯藏時間， 55DAF 與 62DAF 之種子可維持至第 9 個月，而 69DAF 與 76DAF 之種子僅能貯藏 6 個月期限，過此期間發芽率均降低。

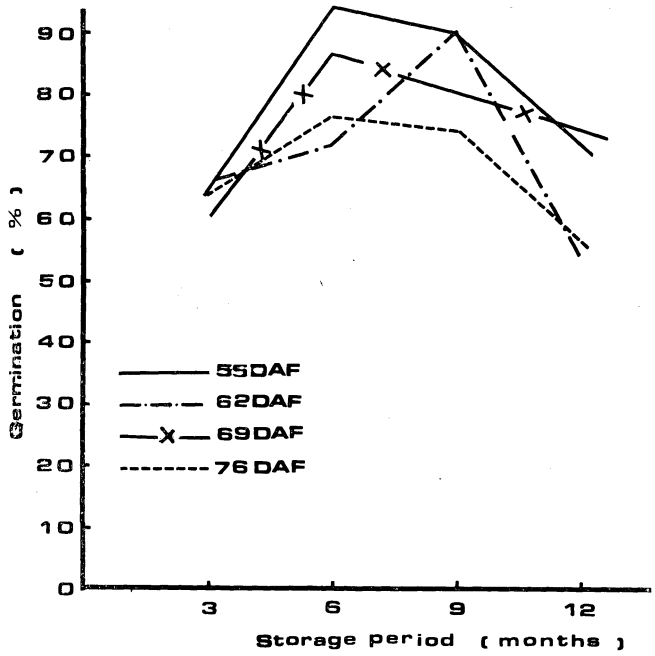


Fig. 1. Percentage germination of *B. falcatum* L. seeds against periods of storage in refrigerator at 0—5°C.

圖1. 採用冰箱貯藏之各採收期種子發芽率變化

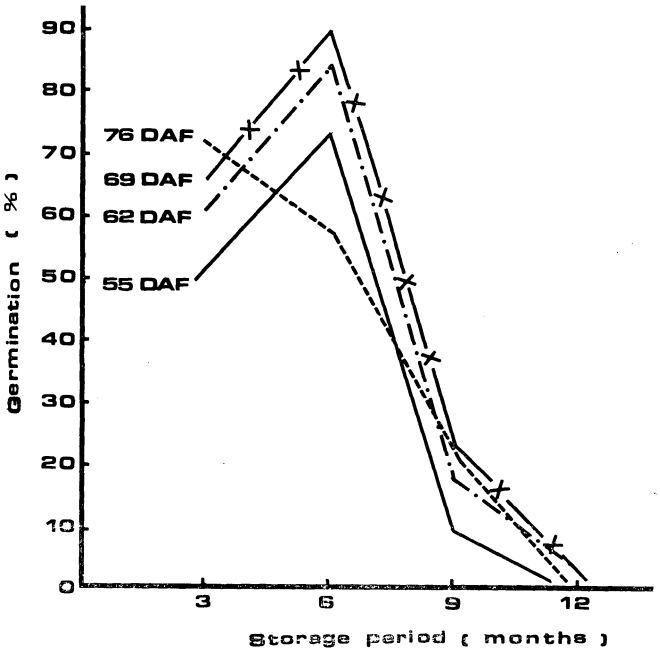


Fig. 2. Percentage germination of *B. falcatum* L. seeds against periods of storage under room temperature.

圖2. 採用室溫貯藏之各採收期種子發芽率變化

B. 室溫貯藏

此法乃農民貯藏一般作物種子之慣用法，即使用塑膠袋密封並在袋內裝酌量乾燥劑置放於室內較乾燥地方，通常使用此法猶能維持種子壽命達一段時間，柴胡種子是否適用此法有待探討。由圖 2 曲線變化顯示，初期貯藏各採收期之柴胡種子發芽率隨著採收日數增長而增高，但經變方分析各採收期增高之發芽率並未呈顯著差異，貯藏至第 6 個月後各採收期之種子發芽率均提高，尤以 69DAF 與 62 DAF 較高分別達90%與84%與其他二者比較已達 5 %顯著水準，而 76DAF 之種子反而降低僅58%；貯藏達 9 個月後各採收期之柴胡種子發芽率皆大幅度下降，降幅達24—10%，貯藏達一年後各期之種子發芽率幾接近零，由此結果顯然可瞭解柴胡種子利用室溫貯藏法之劣變甚速，其有效貯藏期限僅能維持半年之久，其中 76DAF 較老熟種子似乎僅能維持 3 個月而已。

C. 乾燥器（Desiccator）貯藏

少量種子之貯藏，利用乾燥器是最簡便與最易行的，此乃因其內相對濕度低，此持續之乾燥狀態較易維持種子活力，一般試驗機構有採用此法貯藏一年生種子以供翌年播種。由圖 3 得悉各採收期之種子，貯藏一年後其發芽率猶能維持於70%—86%間，而全年期間之發芽率變化較小，各採收期之種子於不同時間所測定發芽率，經分別進行變方分析亦未達顯著差異，但最高發芽率之貯藏期限出現於貯藏後第 6 個月。

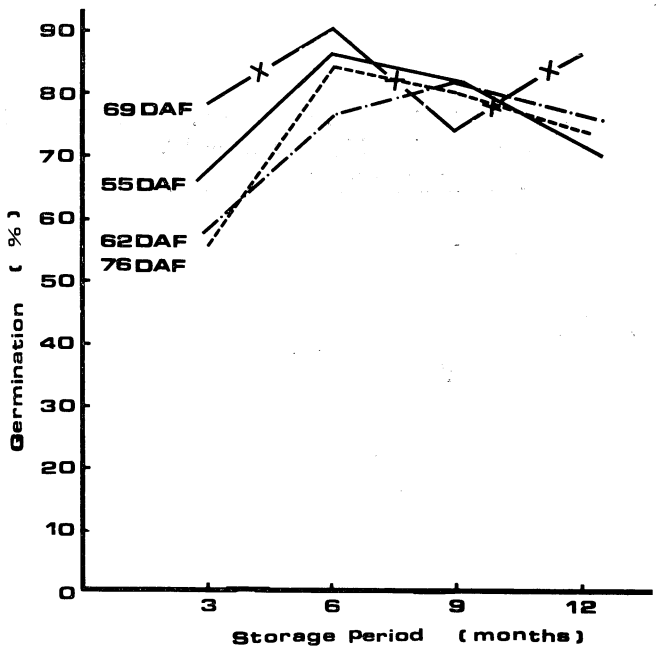


Fig. 3. Percentage germination of *B. falcatum* L. seeds against periods of storage in desiccator.

圖3.採用乾燥器貯藏之各採收期種子發芽率變化

D. 36°C 定溫箱（Incubator）貯藏

柴胡之開花期通常從 5 月份開始一直至12月還能繼續開花，早期採收之種子除供當年播種外，其餘之採種必需貯藏以供日後利用，其間有需經一年或以上之貯藏時間，故尋求特別貯藏方式以保持種子壽命是必要的，利用高溫 (36°C) 且乾燥之定溫箱貯藏結果由圖 4 可知，各採收期之柴胡種子發芽率經貯藏一年後亦維持於72—87%間與使用乾燥器貯藏相似，惟最高發芽率達90%，以上之貯藏期限係於第 9 個月，較後者晚 3 個月，由此可確定利用 36°C 定溫箱貯藏柴胡種子以維持發芽率是可行

方法之一。

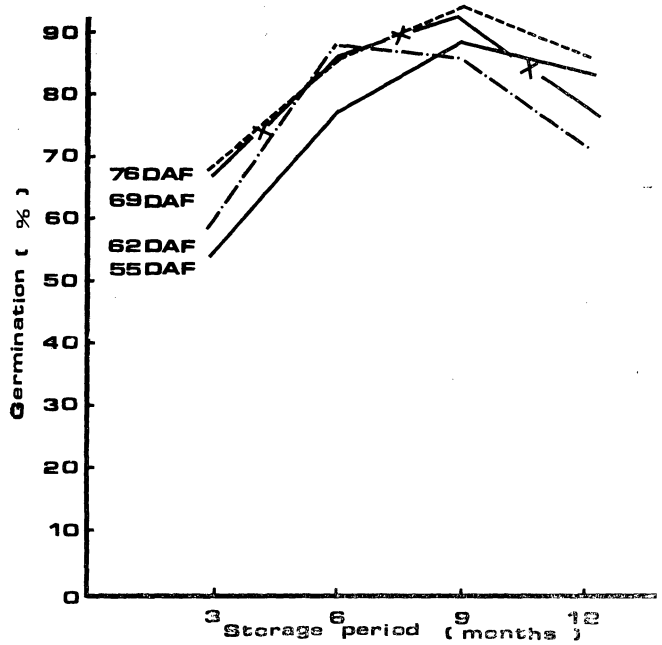


Fig. 4. Percentage germination of *B. falcatum* L. seeds against periods of storage in incubator at 36°C.

圖4. 採用定溫箱貯藏法之各採收期種子發芽率變化

三、各採收期、貯藏方式及貯藏時間對種子發芽率之影響

從表2資料顯示，55DAF 之種子發芽率於貯藏後第6個月，發現採用冰箱貯藏法最高達94%，依次爲用乾燥器貯藏法86%，36°C 定溫箱法77%，室溫貯藏法爲74%，其中更進一步發覺採用 36°C 定溫箱貯藏者，其種子之發芽率有隨著貯藏時間增長而提高之趨勢，餘三者貯藏法則無此現象，尤以採用室溫貯藏者，至第9個月降至10%，而至第12個月種子均不發芽。62、69 及 76DAF 之種子，採用室溫貯藏法亦有此現象，但 62DAF 種子之發芽率呈現最高係採用冰箱貯藏法於貯藏後第9個月達90%，乾燥器貯藏法爲82%，而 36°C 定溫箱貯藏法却於貯藏後第6個月88%最高。就整年間各期發芽率觀察，變異較小者爲採用乾燥器貯藏法。69DAF 種子之發芽率，除室溫貯藏法因貯藏時間增長變異較大外，餘三者均較穩定，尤以經貯藏後一年時間其發芽率仍維持於74～86%間頗屬不易。另 76DAF 種子之發芽率，採用 36°C 定溫箱貯藏法，於貯藏後第6、9及12個月均表現優異，分別爲86、94及86%，次爲用乾燥器貯藏法各爲84、86及74%，而用冰箱貯藏法其種子發芽率能維持70%以上之有效期間僅9個月，室溫貯藏法却僅3個月。上述資料以 Duncan 多變域顯著性測定結果，就貯藏方式而言，發現 36°C 定溫貯藏法平均發芽率爲77%，乾燥器貯藏法爲 76%，冰箱貯藏法爲 73%，而室溫貯藏法僅40%，前三者間並未顯著性差異間存在，可見柴胡種子貯藏均可採用上述三者，惟最優貯藏法似乎以 36°C 定溫貯藏法較佳。採收期之探討，則發現 69DAF 種子之平均發芽率爲71%，55DAF 種子爲66%，76DAF 種子爲65.7%，而 62DAF 種子爲63%，此顯示柴胡種子於花後69天採收發芽率較高。貯藏有效時間研究，經分析結果貯藏後第3個月種子平均發芽率爲63%，第6個月爲81%，第9個月爲67.8%，第12個月爲53.8%，即貯藏後第6個月種子之平均發芽率最高，分別對餘三貯藏期呈5%顯著性差異，但以個別貯藏方式而言，發現利用室溫貯藏有效貯藏時間最短僅6個月，冰箱及乾乾燥器貯藏約可維持9個月左右，而採用定溫箱貯藏者，其有效貯藏期間可達9

個月以上。

表2. 不同貯藏方式及貯藏時間下之各採收期種子發芽率比較

Table 2. Comparison on the germination rates of *B. falcatum* L. seeds harvested at various dates and stored in different ways and periods.

Harvest date (DAF)	Storage ways	Germination rate (%) storage period (months)			
		3	6	9	12
55	A[1]	64abcdefg[2]	94a	80abcdef	72abcdefg
	B	52fg	74abcdefg	10hi	0
	C	68abcdefg	86abcde	82abcdef	72abcdefg
	D	54efg	77abcdefg	88abcd	84abcdef
62	A	66abcdefg	72abcdefg	90abc	54efg
	B	62abcdefg	84abcdef	18hi	0
	C	58cdefg	76abcdefg	82abcdef	76abcdefg
	D	60bcdefg	88abcd	86abcde	46g
69	A	60bcdefg	86abcde	80abcdef	74abcdefg
	B	66abcdefg	90abc	24h	0
	C	78abcdefg	90abc	74abcdefg	86abcde
	D	68abcdefg	86abcde	92ab	79abcdef
76	A	64abcdefg	72abcdefg	74abcdefg	56defg
	B	72abcdefg	56cdefg	22hi	4h
	C	56defg	84abcdef	80abcdef	74abcdefg
	D	68abcdefg	86abcde	94a	86abcde

1. A: Refrigerator B: Room temperature C: Desiccator D: Incubator (36°C).
2. Means with the same letter across harvest dates, storage ways and germination rate are not significantly different at 5% level.

四、各採收期、貯藏方式及貯藏時間對種子 GT_{50} 之影響

表 3 資料顯示，69與 76DAF 較老熟種子之 GT_{50} ，採用乾燥器貯藏之初期分別達 18及 19天，顯然比其餘三種貯藏法長，但至後期却明顯縮短 5～8 天，而採用 36°C 定溫貯藏法則發現於貯藏後第 9 個月最短，各爲13及12天，分別比貯藏後第 3 個月短 2 天，冰箱貯藏法其貯藏後期種子之 GT_{50} 亦有縮短現象，而室溫貯藏法則無。55及 62DAF 種子之 GT_{50} ，變化較不規則，但其呈現之趨勢亦與69與 76DAF 種子略同，除了室溫貯藏法外，大致均以貯藏後第 9 個月種子發芽較快。此資料再經 Duncan 氏多變域顯著性測定，得知採用 36°C 定溫箱、乾燥器及冰箱等三種貯藏法，其 GT_{50} 平均皆爲14天，分別比用室溫貯藏法縮短一天而達 5 %顯著差異。採收期探討則以 76DAF 種子之平均 GT_{50} 最短13天，分別對其餘三採收期呈 5 %顯著差異，次爲 69DAF 種子14天，亦對餘二採收期呈 5 %顯著差異，而55及 62DAF 種子各爲16及15天，此試驗結果很明顯表示，較老熟種子經貯藏後其後熟作用之效果比較未完熟種子大。至於貯藏時間與種子之 GT_{50} 關係，發現貯藏後第 6 、 9 及12個

月等三貯藏期種子之平均 GT_{50} 均爲14天，而貯藏後第3個月長達16天，此因種子初期貯藏後熟作用尚未完全進行所使然。

3. 不同貯藏方式及貯藏時間下之各採收期種子 Gt50 比較

Table 3. Comparison on the seed Gt50 of *B. falcatum* L. seeds harvested at various dates and stored in different ways and periods.

Harvest date (DAF)	Storage ways	GT50 (days)			
		3	6	9	12 (months)
55	A[1]	17abc[2]	14cdefg	12efg	14cdefg
	B	15bcde	15bcde	17abc	—
	C	15bcde	14cdefg	14cdefg	16bcd
	D	15bcde	14cdefg	14cdefg	16bcd
62	A	17abc	15bcde	15bcde	13defg
	B	17abc	15bcde	16bcd	—
	C	16bcd	16bcd	13defg	15bcde
	D	18ab	15bcde	13defg	17abc
69	A	16bcd	14cdefg	12efg	12efg
	B	14cdefg	14cdefg	16bcd	—
	C	18ab	14cdefg	13defg	13defg
	D	15bcde	14cdefg	13defg	16bcd
76	A	16bcd	11fg	12efg	12efg
	B	14cdefg	13defg	14cdefg	—
	C	19a	12defg	11fg	11fg
	D	14cdefg	13defg	12efg	14cdefg

1. See the table 2 for description of storage ways.
2. Means with the same letter across harvest dates, storage ways and GT50 are not significantly different at 5% level.

—. Seeds were not or poor germinated at 30 days after treatment.

討　論

據 Buell 氏[10]研究 Dianthus Chinensis 之種子發育，發現授粉後第11天經解剖之種子已包含胚、子葉及胚乳三部分，柴胡種子發育雖未有人研究，但許氏[4]報告未完全成熟之柴胡種子，其發芽率比成熟種子高，本試驗採用不同成熟度柴胡種子進行發芽試驗亦發現花後55天之新鮮種子之發芽率爲 55 %，較花後76天者 39 %較高，但差異未達顯著，此結果與許氏之研究相類似，此或可能有如 Khan 氏[14]報告，未成熟種子富含有 GA9、GA17、GA20 及 GA29 等 4 種 GA 類似物較易發芽，而成熟種子僅含 GA29 一種作用較弱。另黃等[5]亦曾指出柴胡種子可能含有皂苷素 (Saponin) 之發芽抑制物質，是否不同成熟度之柴胡種子，其皂苷素含量或其他荷爾蒙含量不同而影響種子之發芽能力，此當有待近一步加以研究。另花後76天之種子亦可能如朱[1]氏所譯述指出係因延遲收穫而遭受惡劣環境，如溫、濕度之劇烈變化致使種子劣變而降低其發芽能力。另 Atwater[9]曾指出繖形

科之種子，於脫離母株時其胚通常未臻成熟，必須經一段時間之後熟作用。黃等 [5] 亦曾針對柴胡之胚進行觀察亦發現其胚大都未成熟，且不同大小與來源之種子間，胚大小亦有所差異。

柴胡原屬溫帶型作物，自然條件下開花結子落地後，翌年春季可發現株旁有甚多種苗出土。民間栽培農戶依據此特性，以柴胡種子收穫陰乾後混合細砂挖地埋藏以供翌年播種。筆者等亦於試驗前仿效此法，發現柴胡種子較易發霉不適當貯存。據 Hsu氏等 [13] 報告，Forage grasses 種子其發芽率及 GT_{50} 之提高，需冷凍處理。另 Abdelmagid 氏等 [8] 指出，棉花種子用袋裝貯藏，其種子活力僅可維持16個月。又 Denis 等 [12] 研究，發現澳洲 Channol millet 種子利用 50°C 與 36°C 貯藏時，36°C 貯藏其種子發芽率較佳。Nandi 氏等 [15] 亦指出胡麻種子貯藏於相對濕度90%及 30°C 條件下，種子發芽能力很容易損失。本試驗採用冰箱（0-5°C），室溫（廣泛溫濕度變化）、乾燥器、定溫箱（36°C）等四種不同貯藏方式，以探討尋求一簡便可行之貯藏方法，發現柴胡種子採用定溫箱、乾燥器及冰箱等三種貯藏方式，其種子之平均發芽率各爲77%、76%及73%，無顯著性差異存在，惟採用定溫箱貯藏方式似乎優於後二者，而室溫貯藏法僅40%，此乃因於室溫貯藏下易受廣幅之溫、濕度變動而造成種子劣變。GT_{50} 之調查，其結果與發芽率表現呈相同趨勢，即定溫箱、乾燥器及冰箱等三種貯藏法均爲14天，而室溫貯藏法達15天，這種現象當然亦爲影響發芽率隨伴因素所致成。貯藏有效時間之探討，發現所有柴胡種子貯存於上列 4 種方式下，其發芽率除了老熟種子（76DAF）之室溫貯藏外，於貯藏 6 個月後均有顯著提高之趨勢，於第 9 個月則發現除定溫箱貯藏法有增高外，餘三者均下降。由此結果可獲悉柴胡種子之最佳貯藏期間因貯藏方式不同，但若要貯藏較久，似乎可採用 36°C 定溫箱貯藏較佳，此乃較乾燥狀態下種子較不易吸濕受潮而劣變。採收期之探究，則發現 69DAF 種子平均發芽率較高達71%，比其餘三採收期高5.3～8%，此可能花後69天爲柴胡種子適熟期，過早採收之種子發育未充實，而過遲採收則可能因暴露於田間過久，易受外在環境影響其內生荷爾蒙量而降低其發芽能力。

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Influence of Harvest Date and Storage Method on Seed Germination of Chia-hu (*Bupleurum falcatum L.*)[1]

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Summary

The seeds of Chia-hu (*B. falcatum* L.) were harvested at weekly intervals from 55 to 76 days after flowering (DAF), They were dried and sealed in plastic bags with calcium chloride, and then stored (1) in a refrigertor at 0-5°C, (2) in a desiccator under room temperature, (3) in a incubator at 36°C, (4) on the bench of this laboratory under room temperature. Germination rate and Gt50 for each treatment were tested at 3, 6, 9 and 12 months after storage. The results were summarized as follows:

(1). The best germination rate and GT50 were obtained in the fresh seeds harvested at 69 days after flowering with a germination rate of 71% and GT50 of at 21 days.

(2). The germination rates and GT50 of dry seeds varied with time and mtehods of storage. The best storage method was found with incubator at 36°C, which resulted in a mean germination rate of 77% and GT50 of 14 days. The results stored in desiccator under room temperature and in refrigertor at 0-5°C were better than that stored on the bench in this laboratory, which lost their viability in 6 months, that meight due to the great fluctuation of ambient temperature and relative humidity.

(3). The maximum germination rate for the seeds stored in the refrigerator. desiccator and under room temperature were found in the periods of 6 months after storage. The seede stored in the incubator, however, had lost their vigour in 9 months after storage.

(4). Among the four harvesting date, the best germination rate and GT50 were observed in the seeds harvested 69 days after flowering.

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玉米單葉面積與全株葉面積之簡易估算法[1]

蕭柊瓊[2]　劉　清[3]

摘要：本研究的主要目的在於探討臺農 351號玉米品種單葉面積及全株葉面積的簡易估算方法，並比較在不同栽植密度及不同採收時期所求得之估算係數間的差異。本試驗於民國七十四年秋作在本所試驗田區進行，在開花期至子粒充實期間隔週取樣調查，計取樣三次；取樣方法採雙重取樣法(double sampling method)，一爲破壞性取樣（destructive sampling），一爲非破壞性取樣（nondestructive sampling），前者測量樣品之各葉長、寬、面積及乾重，後者則只於田間測量各葉之長及寬。以破壞性取樣所得之資料利用統計方法找出與玉米單葉面積相關最大的性狀，並求此性狀與玉米單葉面積之迴歸關係；再綜合非破壞性取樣及破壞性取樣所得之資料，計算與玉米全株葉面積相關最大的性狀及其迴歸關係。結果顯示玉米葉片之長寬乘積與其單葉面積之相關最大（r＝0.9822），且在不同密度及不同採收時期所求得之迴歸係數均無顯著差異，故取其平均值0.76做爲估算玉米單葉面積的有效介值；而玉米之第八葉面積與全株之葉面積有很大的相關（r＝0.9499），且在不同密度及不同採收時期所求得之迴歸係數亦無顯著差異，故也取其平均值9.27做爲估算玉米全株葉面積的有效介值。

綜合以上結果可知，若欲測量玉米全株之葉面積僅須測量其第八葉之葉長及葉寬，取其乘積再乘以估算單葉面積之介值 0.76 估算出第八葉之面積，再乘以估算全株葉面積之介值 9.27即可很準確地估出全株葉面積。除了以第八葉面積來估算全株葉面積外，本研究亦發現植株葉乾重、全株各葉長之和及葉寬之和與全株葉面積均有很好的直線關係，唯其估算的準確程度皆不如以第八葉面積來估算全株葉面積。

葉面積是表示作物生長潛能最有效的測量項目，育種學家可根據葉面積指數的大小來選擇生長潛力大的品種，而生理學家亦可以藉著葉面積的變化及大小，來模擬作物生長的模式及利用太陽能的效率；然而直接測量葉面積不僅會破壞植株，而且非常耗費時間和人力，而限制了試驗的規模和取樣的大小。

國外學者曾針對不同的作物，利用統計方法探討葉面積和一些田間非破壞性可測的性狀之關係，再配合迴歸模式[10]有效而準確地估算出葉面積[2,3,4,5,6,7,9,11,12]。有關玉米葉面積的估算，Mckee[5]根據 Montgomery[6] 所提出的迴歸模式求得玉米的單葉面積可由0.73×葉長×葉寬估算之；Pearce 等人[7]則發現玉米全株的葉面積與其第八葉之面積相關最大，並以迴歸方法求得將玉米第八葉面積乘以9.39即可估算全株的葉面積。然不論是估算玉米單葉面積或全株葉面積的係數，皆會因品種[5]或栽培的地點、年度、期作[4]而有所不同，本研究的主要目的即在探討臺灣本地玉米品種單葉面積及全株葉面積的簡易估算方法，並比較在不同栽培密度及不同採收時期所求得估算係數的差異。

基於研究需要，在農業試驗上經常利用破壞性取樣來獲取資料，或割取植株研製成粉末進行生理分

1.臺灣省農業試驗所　研究報告第1361號。本計畫承本所農藝系生理研究室之同仁協助試驗工作，謹致謝意。

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析，或考種進行遺傳育種研究，而植株的乾重都是必測的項目，Aase [1] 和 Rhoads et al. [8] 分別發現多小麥和棉花全株葉面積與其乾重及葉乾重均有很高的正相關存在，故可以以植株乾重或葉乾重來表示葉面積。本研究亦希望找出玉米植株乾重或葉乾重與全株葉面積的關係，使進行破壞性取樣時，可利用已知乾物重的資料直接估算全株的葉面積。

材料與方法

試驗材料爲臺農 351號，於民國74年秋作在臺中縣霧峰鄉臺灣省農業試驗所試驗田進行，田間管理及肥料用量按一般慣行法實施，生育期間曾加適時之病蟲害防治。

本研究爲探討在不同密度和不同採樣時期，玉米單葉面積及全株葉面積的簡易估算法，故播種時分別以行株距80×10cm（每公頃125,000株）、80×20cm（每公頃 62,500 株）與 80×40cm（每公頃31,250株）之栽培密度進行，田間設計採逢機完全區集法以減少土壤差異，於開花期與子粒充實期間隔週調查，共調查三次。調查分爲兩部分，第一部分爲破壞性的取樣，各處理自田間逢機割取24株玉米植株，依葉序（由上而下）量取全株各葉之長及最大寬，並利用葉面積測量儀（LI-COR Model LI-3000）測量各葉之葉面積，植株烘乾後測其株重及全株葉重；第二部分爲非破壞性取樣，各處理逢機取30株，在田間依葉序量取全株各葉之長及最大寬。

結果與討論

（一）玉米單葉面積的估算

由各性狀資料與玉米單葉面積相關分析的結果，可找出與玉米單葉面積相關最大的性狀，再配合迴歸模式求出此性狀與玉米單葉面積的迴歸關係，所獲得之迴歸係數即爲估算玉米單葉面積的有效介值。

表一 玉米臺農351號單葉面積與葉片各性狀間之相關係數

Table 1. Correlation coefficients between individual leaf area and other leaf characters of maize hybrid Tainung 351

Treatment[a]	Leaf Length	Leaf Width	Square Leaf Length	Square Leaf Width	Leaf Length × Leaf Width
D1—1	0.9135	0.9232	0.9145	0.9187	0.9889
D2—1	0.9147	0.9234	0.9162	0.9186	0.9762
D3—1	0.9377	0.9337	0.9458	0.9321	0.9797
D1—2	0.9484	0.9469	0.9502	0.9444	0.9915
D2—2	0.9325	0.8965	0.9327	0.8963	0.9755
D3—2	0.9241	0.9043	0.9307	0.9070	0.9729
D1—3	0.9337	0.9190	0.9329	0.9149	0.9865
D2—3	0.9356	0.9219	0.9331	0.9188	0.9880
D3—3	0.9170	0.9237	0.9227	0.9248	0.9810

[a]: DN-M: the Mth sampling of density treatment N. Plant spacings of D1, D2 and D3 were 80×10, 80×20 and 80×40cm, respectively.

表二 不同處理間玉米單葉面積對葉長乘葉寬之迴歸係數的比較（t-測驗）

Table 2. Comparisons (t-tests) of regression coefficients of maize individual leaf area on leaf length×leaf width between different treatments

Treatment[a]	D1—1	D2—1	D3—1	D1—2	D2—2	D3—2	D1—3	D2—3	D3—3	Reg. Coef.
D1—1	.....	1.150	0.043	0.733	−2.543*[b]	−1.606	1.799	1.315	1.071	0.7706
D2—1		.....	−1.003	−0.607	−3.213**	−2.367*	0.383	−0.041	−0.121	0.7567
D3—1			.....	0.572	−2.357*	−1.521	1.492	1.084	0.921	0.7701
D1—2				.....	−3.205**	−2.184*	1.244	0.697	0.496	0.7637
D2—2					.....	0.704	3.843**	3.526**	3.255**	0.8036
D3—2						.....	2.855**	2.543*	2.343*	0.7924
D1—3							.....	−0.513	−0.539	0.7519
D2—3								.....	−0.095	0.7572
D3—3									.....	0.7583

[a]: See Table 1 for description of treatments.

[b]: *and**, significant at 5% and 1% level, respectively; otherwise, nonsignificant at 5 % level.

根據第一部分的調查資料，將玉米單葉面積分別和其葉長、葉寬、葉長的平方、葉寬的平方及葉長乘以葉寬等性狀進行相關分析，結果（表1）發現玉米單葉面積（LA）和其葉長乘以葉寬之值（LW）相關最大，其各處理之平均相關係數高達0.9822，此結果顯示LA和LW間有很好的直線關係，遂進行LA和LW之迴歸分析，迴歸模式爲：$LA=LW\times b+\varepsilon$，所得之迴歸係數（b）即爲估算玉米單葉面積的介值。分析結果表(2)發現各處理所得之估算介值經差異顯著性測驗除第二次採樣之第二及第三密度外，其餘七個處理所得之估算介值間均無顯著差異。由於第二次採樣時，葉面積測量儀之轉軸發生故障，測量第二及第三密度處理樣品之葉面積只好改以手測，手測的結果易偏高，可能使得所求之估算介值因人爲誤差而偏高，故去除第二次採樣之第二及第三密度處理所求得之估算介值，仍可推論玉米（臺農351號）在開花期至子粒充實期間 LA 和 LW 的關係相當固定，故取其餘七個處理所求得之迴歸係數的平均值0.76，做爲估算玉米單葉面積的有效介值，即玉米各葉之面積皆可測其葉長及葉寬，取其乘積再乘以0.76而估算之。

（二）玉米全株葉面積的估算

由各葉面積與玉米全株葉面積相關分析的結果，可找出與玉米全株葉面積相關最大的葉片，再配合迴歸模式求出此單葉面積與玉米全株葉面積的迴歸關係，所獲得之迴歸係數即爲估算玉米全株葉面積的有效介值。

合併第一及第二部分調查的資料，各葉之面積均由估算單葉面積有效介值 0.76 乘以葉片之長寬乘積估算。將各葉之面積分別和玉米之全株葉面積進行相關分析，結果（表3）以第八葉面積（LA_8）和玉米全株葉面積（PA）之相關最大，其各處理平均相關係數高達 0.9499，此結果顯示 PA 和 LA_8 間有很好的直線關係，故進行 PA 和 LA_8 之迴歸分析，迴歸模式爲：$PA=LA_8\times b+\varepsilon$，所得之迴歸係數即爲估算玉米全株葉面積的介值，分析結果（表4）發現各處理所得之估算介值間均無顯著差異存在，即在不同密度或不同採次，於開花期至子粒充實期間，PA 和 LA_8 之關係很固定；取所求得之九個估算介值的平均值 9.27 做爲估算玉米全株葉面積的估值，日後若欲測量玉米全株的葉面積時，僅須測量其第八葉之葉長及葉寬，將該葉長及葉寬之乘積乘以0.76即可估算第八葉的面積

，再將所估得之第八葉面積乘以9.27即可估算出玉米全株的葉面積。

表三 全株葉面積與單葉面積之相關係數

Table 3. Correlation coefficients between total plant leaf area and individual leaf area

Leaf order	Treatment[a]								
	D1—1	D2—1	D3—1	D1—2	D2—2	D3—2	D1—3	D2—3	D3—3
1	0.449	0.565	0.599	0.449	0.642	0.580	0.688	0.484	0.568
2	0.598	0.661	0.582	0.575	0.713	0.548	0.734	0.585	0.625
3	0.698	0.724	0.623	0.672	0.795	0.613	0.775	0.715	0.701
4	0.734	0.811	0.721	0.767	0.810	0.701	0.828	0.783	0.724
5	0.836	0.905	0.836	0.841	0.889	0.769	0.872	0.820	0.717
6	0.904	0.905	0.882	0.883	0.911	0.862	0.915	0.877	0.869
7	0.961	0.915	0.956	0.926	0.955	0.905	0.931	0.904	0.928
8	0.970	0.961	0.956	0.953	0.968	0.947	0.959	0.929	0.906
9	0.950	0.948	0.891	0.966	0.959	0.909	0.936	0.896	0.917
10	0.903	0.930	0.890	0.933	0.930	0.881	0.875	0.873	0.879
11	0.841	0.869	0.851	0.857	0.877	0.837	0.590	0.841	0.807
12	0.847	0.847	0.821	0.843	0.834	0.803	0.755	0.813	0.760
13	0.834	0.752	0.771	0.824	0.712	0.751	0.625	0.681	0.626
14	0.821	0.693	0.712	0.618	0.633	0.567	0.484	0.447	0.359

[a] : See Table 1 for description of treatments.

表四 不同處理間玉米全株葉面積對第八葉面積之迴歸係數的比較（t- 測驗）

Table 4. Comparisons (t-tests) of regression coefficients of maize total leaf area on the 8th leaf area between different treatments

Treatment[a]	D1—1	D2—1	D3—1	D1—2	D2—2	D3—2	D1—3	D2—3	D3—3	Reg. Coef.
D1—1	.....	−0.086[b]	−0.501	0.316	0.058	−0.210	0.565	0.094	−0.059	9.2803
D2—1		.....	−0.426	0.395	0.142	−0.129	0.629	0.166	−0.002	9.3447
D3—1			.....	0.801	0.557	0.280	1.024	0.515	0.295	9.6530
D1—2				.....	0.256	−0.507	0.238	−0.159	−0.270	9.0400
D2—2					.....	−0.265	0.499	0.048	−0.099	9.2347
D3—2						.....	0.732	0.268	0.088	9.4436
D1—3							.....	−0.345	−0.418	8.8566
D2—3								.....	−0.126	9.1919
D3—3									.....	9.3468

[a] : See Table 1 for description of treatments.
[b] ; See Table 2 for notations of test result.

本研究所得之估算玉米單葉面積及全株葉面積的介值 0.76及9.27，與 Montgomery [6] 和Mckee [5] 所求得之估算玉米單葉面積的介值0.75和0.73，及Pearce [7] 所求得之估算玉米全株葉面積的介值9.39不盡相同，此乃試驗所採用之品種及臺灣本土與美國栽培環境之不同所造成。唯本試驗既以本地品種在臺灣本土栽培，所得之結果自將更適合於本地研究者所採用。

除了以第八葉之面積來估算玉米全株葉面積外，本研究亦將玉米全株之葉面積分別和其植株乾重、植株葉乾重、全株各葉長之和及各葉寬之和等性狀進行相關分析，結果（表 5）以植株葉乾重與全株葉面積之相關最高，全株各葉寬之和（ΣW）及全株各葉長之和（ΣL）次之；將此三個性狀分別和全株葉面積進行迴歸分析，得下列三個迴歸方程式：

全株葉面積＝205×植株葉乾重 …… （1）

全株葉面積＝63.6×全株各葉寬之和 （ΣW） …… （2）

全株葉面積＝6.11×全株各葉長之和 （ΣL） …… （3）

（1）式表示將植株葉乾重乘以205即可估算玉米全株葉面積，其估算之準確程度達 92%；（2）式則以全株各葉寬之和來估算全株之葉面積，其估算介值爲63.6，估算之準確度爲88%；（3）式以全株各葉長之和乘以6.11來估算全株之葉面積，估算準確度爲87%，唯在非破壞性的取樣試驗中，測量葉長遠較葉寬之人爲誤差少，也較易於實行，故此方程式仍有其特殊的意義。

綜合上述可知，無論是破壞性取樣或是非破壞性取樣的試驗皆有簡易的方法來估算玉米全株葉面積，然本試驗所獲得之各個估算介值是否可適用於不同生育期、不同期作、不同品種或不同的試驗地點，皆將於日後做進一步的探討。

表五 全株葉面積和各項性狀間之相關係數

Table 5. Correlation coefficients between total plant leaf area and other leaf characters

Treatment[a]	Sum of Leaf Length (L)	Sum of Leaf Width (W)	Leaf Dry WT per Plant	Total Dry WT per Plant
D1—1	0.8455	0.8791	0.8345	0.6211
D2—1	0.9067	0.9054	0.9460	0.8604
D3—1	0.9269	0.9553	0.9371	0.7053
D1—2	0.9031	0.9488	0.9223	0.6609
D2—2	0.7709	0.7596	0.9011	0.7897
D3—2	0.9356	0.8989	0.9311	0.7235
D1—3	0.8749	0.8187	0.9377	0.6464
D2—3	0.8612	0.8932	0.9633	0.8217
D3—3	0.8326	0.9031	0.9490	0.6136

[a]: See Table 1 for description of treatments.

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A Simple Method for Estimating Individual and Total Leaf Area of Maize[1]

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Summary

This study was aimed to find a rapid, nondestructive and accurate method of measurement for estimating the individual leaf area and total leaf area of maize. The experiment was conducted by using maize hybrid Tainung 351 in the fall crop of 1985 at Taiwan Agricultural Research Institute. Three samplings were made at one week interval, starting from anthesis, for plants grown under three spacings, i. e., 80×10, 80×20 and 80×40cm. The double-sampling method was employed. For the destructive part of sampling, the length, width, area and dry weight of each individual leaf were measured. For the nondestructive part of sampling, only the length and width of individual leaf were measured in the field. By statistical analysis of the data from destructive samplings, the most correlated leaf characteristics and its regression relationship with individual leaf area were determined. By combining the data of destructive and nondestructive samplings, similar infomation on total leaf area per plant was obtained.

The product of leaf length and width was found to be the most correlated characteristics with individual leaf area ($r=0.9822$). The regression coefficients obtained from various density treatments and sampling stages were statistically the same. It was thus suggested that the average regression coefficient of 0.76 would be efficient for estimating individual leaf area.

The area of the 8th leaf(counted from the top)was the most correlated characteristics with total plant leaf area and the regression coefficients obtained from various density treatments and sampling stages were not significantly different, therefore the average regression coefficient of 9.27 would be efficient for estimating the total plant leaf area.

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Effects of Nitrate and Ammonium Nitrogen Sources on the Seedling Growth and Root Phosphoenolpyruvate Carboxylase Activity of Different Crop Species[1]

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Abstacts: Seven crop species, including maize, sorghum, rice, wheat, barley, peanut and soybean, were grown hydroponically with either nitrate or ammonium N at a concentration of 150 ppm. Rates of seedling growth were determined to study the effect of N sources on the performances of carbon metabolism. As ammonium N could enhance the activity of PEP carboxylase in the roots of many crops, attempts had been made to clarify the characteristics of this enzyme in order to provide basis for further studies. Experimental results showed that crops grew better in nitrate N than in ammonium N medium. However, shoots of rice and wheat gained more fresh weight when treated with ammonium N which was different from the results on maize, sorghum and peanut, indicating difference in response to N sources among plant species. Ammonium N could enhance the activity of PEP carboxylase of various crops. The increase was 8—61% in the leaf blade and as high as 4.5 folds in the root. PEP carboxylase extracted from the roots of Tainung 67 rice with the methods employed in this experiment was extremely stable. No decrease in enzyme activity was observed up to 7 hrs after extraction. Optimal pH for rice root PEP carboxylase was between 8.0 and 8.5 with either Tris-HCl (100 mM) or Hepes-KOH(50 mM) buffer. Kinetic study showed that the response curves of velocity versus substrate (PEP) concentration was hyperbolic at pH 8.0 and sigmoid at pH 7.0. Reciprocal plots of the data was linear, indicatng Michaelis-Menten kinetics. Km value for PEP was calculated to be 0.16 mM at pH 8.0. Preliminary results suggested that the ammonium-N responsive root PEP carboxylase was similar to the same enzyme from leaf blades of C3 plants in many aspects. As PEP plays a very important role in carbon metabolism, and in view that ammonium-N at a very low concentration (e. g., 1 ppm) could enhance significantly the activity of root PEP carboxylase, the finding of this experiment could be utilized for further study on the interaction of N and carbon metabolism in plants.

Introduction

It is generally recognized that although plant may take up nitrogen in various forms, the main sources in natural conditions are nitrate and ammonium ions[12]. The physiological responses of plants to these ions are very different[1] and the ability of

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plants to absorb and metabolize them varies greatly [4,8,9]. It has been well noticed that the source and availability of nitrogen can affect carbon metabolism in the plants [4,7]. Its implication on plant productivity has also been assessed [2]. Many research projects focused on the influences of nitrate and ammonium nutrition on photosynthesis and related reactions have been executed and published in recent years. However, the growth responses to different nitrogen sources vary considerably according to plant species and plant age [21,24], and the interactions between nitrogen and carbon metabolism remain obscure [7,20]. The initial aim of this study was to examine the influences of nitrate and ammonium nitrogen on the growth of seedlings of various crops under controlled environment and to compare the resulted changes in photosynthetic and respiratory activities. During the course of this study, it was found that the activity of PEP carboxylase, the key enzyme for photosynthesis in C4 plants, increased markedly in the roots as a result of ammonium-N influences. Hence, efforts had been made to study the characteristics of this enzyme under the experimental conditions employed.

PEP plays a very important role in plant metabolism. For example, it can bind to at least six enzymes which catalyze the allylic replacement of phosphate [22]. PEP is also the control points of glycolysis, gluconeogenesis and in the control of cellular pH [3]. It is therefore worthy to understand the possible importance of PEP as a function of the interrelationship between nitrogen and carbon. As for the enzyme PEP carboxylase, it occurs in all plants and plays particularly important roles in carbon fixation in C4 plants and in CAM plants. However, it has also been reported that PEP carboxylase extracted from the roots of several C4 plants was distinct from the leaf form [17,26]. Hence, the characteristic of this enzyme extracted from roots of C3 plants, especially rice, under ammonium nutrition was studied with the purpose of providing information to future investigation related to this aspect.

Materials and Methods

Plant species used in this experiment included a japonica rice (*Oryza sativa* L.) variety, Tainung 67, two indica rice varieties, Taichung sen 3 and Pegonil (the latter has 1,000-grain-weight ca. 40g), a wheat (*Triticum aestivum* L.) variety, Taichung S. 2, a six-row barley (*Hordeum vulgare* L.) variety introduced from CIMMYT, CB-1, a single-cross maize (*Zea mays* L.) variety, Tainung 351, a single-cross sorghum (*Sorghum vulgare* Per.) variety, Taichung 3, a soybean (*Glycine max* (L.) Mer.) variety, Shih-Shih and a peanut (*Arachis hypogaea* L.) variety, Tainan S. 9. Rice, especially Tainung 67, was used extensively for the study of PEPC activities.

Seeds were soaked in aerated water for 12 hr (soybean and peanut), 24 hr (wheat and barley), 48 hr (maize and sorghum) or 72 hr (rice) followed by germinating in vermiculate for 7 to 10 days. Germination was kept in a growth chamber (Conviron PGW-36, Controlled Environments Co.) at day/night (14/10 hr) temperature of 28/20 C. Uniform seedlings were transplanted to plastic pots and grown hydroponically with the basic salts of Hoagland formulation. One or two seedlings were grown in a pot with 600 ml of culture solution. Nutrient solution was aerated vigorously by using a small compres-

sor connected via perforated polyethylene tubings and was renewed at a two-day interval. Plants were kept in the same growth chamber under a daily regime of 14-hr light at 28°C and 10-hr dark at 20°C. Illumination was provided with fluorescent and tungsten lamps giving 450 uE/m²/sec of irradiation at the top of the plants.

The culture solution contained the following essential elements with the specified concentrations (mg/l): K^+: 234, Ca^{2+}: 200, Mg^{2+}: 48, PO_4^{3-}: 95, SO_4^{2-}: 192, BO_3^{3-}: 2.9, Fe (Fe-EDTA): 2.2, Mn^{2+}: 0.27, Zn^{2+}: 0.13, Cu^{2+}: 0.13 and MnO_4^{2-}: 0.07. Nitrogen was supplied as either NO_3 or NH_4-N with a concentration of 150 ppm, unless otherwise specified. The pH of the freshly prepared solution was 4.85—4.90.

For the extraction of PEP carboxylase (EC 4.1.1.31), immediately after harvesting the leaves and roots under irradiation extracts were made in 4—5 volumes of buffer containing 100 mM Tris-HCl (pH 7.6), 20% (v/v) glycerol, 1 mM EDTA, 5 mM DTE, 1mM $MgCl_2$ and 2% (w/v) insoluble polyvinylpolypyrrolidone (PVP), using a chilled mortar and pestle. An aliquot was taken for chlorophyll determination prior to filtering through one layer of miracloth and centrifuging at 20,000 g for 10 min. PEP carboxylase was assayed spectrophotometrically at 30 C in a total volume of 1 ml by coupling oxaloacetae formation with malate dehydrogenase as described by Uedan and Sugiyama [27]. The assay mixture contained 50 mM Hepes-KOH, pH 8.0 (or 100 mM Tris-HCl, pH 8.0), 5 mM $MgCl_2$, 5 mM DTE, 0.2 mM NADH, 10 mM $NaHCO_3$, 2 mM PEP and 5 units of malic dehydrogenase. The reaction was initiated by the addition of PEP to the assay medium. A Hitachi 150—20 double beam spectrophotometer was used to record the decrease of absorbance at 340 nm.

Results

Vigorous growth of plants in the nitrate medium (initial pH 4.9) caused rapid drift towards alkalinity (pH 7 after two days of culture) in the culture solution, conversely with the ammonium medium plant growth caused an acid drift (pH 3.5;data not shown). The extent of pH drift was correlated with plant age and size. Older and larger plant, especially larger root system, caused more prominent pH drift than the younger and smaller plants. The drift occurred mainly in the first day after renewal of cultural solution.

Seedlings of all crop species tested grew better in the nitrate that in the ammonium culture solution in terms of fresh weight gain of the whole seedling (Table 1). The most significant case was found in maize where total fresh weight per seedling after 9 days in culture was 13.6 g for nitrate and 6.4 g for ammonium treatment, or a 2.14-fold difference was observed. The least difference was in rice where the fresh weight per seedling grown in nitrate solution was only 5—8% higher than that grown in ammonium solution.

It is worthy to note that for maize, sorghum, peanut and soybean, both the shoot and root gained more fresh weight when grown in nitrate than in ammonium medium (Table 1). For example, the ratio of fresh weight between nitrate and ammonium was 2.38 for shoot and 1.77 for root in maize. On the contrary, shoot of rice and wheat

Table 1. Fresh weight (mg/plant) of various crops under hydroponic culture as affected by different N sources[a]

Crop	Days in Culture	NO_3^-			NH_4^+		
		Shoot	Root	Total	Shoot	Root	Total
Corn	0	1,086± 59	793± 22	1,979± 46			
	9	9,311±257	4,330±126	13,641±209	3,922±214	2,440±186	6,362±199
	CGR[b]	914	393	1,307	315	183	498
Sorghum	0	314± 21	219± 34	533± 32			
	7	1,060±144	1,057±189	2,117±163	780±113	933±141	1,713±138
	CGR	107	120	227	67	102	169
Rice Tainung 67	0	77± 11	43± 13	120± 11			
	13	488± 51	442± 27	930± 41	561± 32	304± 28	865± 31
	CGR	32	31	63	37	20	57
Rice Pegonil	0	139± 27	119± 18	258± 23			
	13	945± 36	666± 42	1,611± 40	1,058± 49	477± 39	1,535± 46
	CGR	62	42	104	71	28	99
Wheat	0	205± 17	211± 27	416± 24			
	9	697± 22	764± 43	1,461± 39	783± 53	392± 33	1,175± 46
	CGR	54	61	115	64	20	84
Barley	0	224± 29	200± 16	424± 24			
	9	744± 31	495± 22	1,239± 29	715± 46	350± 32	1,065± 40
	CGR	58	33	91	55	17	72
Peanut	0	1,938±116	1,555±148	3,493±133			
	7	4,035±257	3,721±226	7,756±244	3,027±188	2,689±238	5,716±211
	CGR	230	309	539	156	162	318
Soybean	0	937±111	775±138	1,712±130			
	7	2,517±162	2,231±246	4,748±214	2,001±166	1,845±211	3,846±191
	CGR	226	208	434	152	153	305

[a]Fresh weight at 0 day was similar for both N treatments.

[b]Crop growth rate expressed in mg fresh weight per day in culture.

grew better in the ammonium than in the nitrate solution, whereas the opposite was true in root. Shoot fresh weight of nitrate treatment was only 87—89% of that of ammonium treatment and the value of 140—195% was recorded in root.

Crop growth rate (CGR, expressed in mg fresh weight gained per seedling per day) was also higher for the nitrate-grown plants than for the ammonium-grown plants (Table 1). Highest CGR was found in maize (1,307 mg for nitrate and 498 mg for ammonium treatment) followed by peanut and soybean. CGR of the small-grain crops, i. e., sorghum, rice, wheat and barley was lower when compared with othr crops tested.

Comparison between Tainung 67, a medium-grain rice variety, and Pegonil, a large-grain variety (grain weight of 25 and 40mg, respectively) showed no difference in response to different N sources. However, the large-grain Pegonil grew much faster than Tainung 67. After 13 days in culture, seedling fresh weights were 1,535-1,611 mg

for the former and only 865-930 mg for the latter. Again, CGR of of Pegonil was significantly higher than that of Tainung 67 (99-104 mg vs. 57-63mg).

During biochemical analysis of plants subjected to different N-source treatments, it was found that root PEP carboxylase was enhanced significantly by ammonium-N. Therefore, efforts have been made to explore its characters as well as its role in carbon metabolism as influenced by N supply. Table 2 showed PEP carboxylase activities in the leaf blade and root of various crops grown in either 150 ppm nitrate or 150 ppm ammonia. With the exception of wheat and barley, ammonium-N increased the activity of this enzyme in the leaf blade of the tested crop species although the increase was moderate. It was 8% for rice Tainung 67 and Taichung sen 3, 26—31 % for maize and sorghum and 61% for Pegonil rice. Absolute values, expressed in the unit of decrease in absorbance at 340 nm per gram fresh weight per minute, were highest for sorghum (262 for nitrate vs. 343 for ammonium-N) followed by maize (141 vs. 178). Rice had higher PEP carboxylase activities than both wheat and barley. On the other hand, PEP carboxylase activity in the leaf blade of wheat and barley decreased (32—40%) as a result of ammonium influence (Table 2).

Table 2. PEP carboxylase activities ($\triangle A_{340}$ gfw^{-1} min^{-1}) in the leaf blade and root of various crops as affected by different N sources

Crop	Days in Culture	Leaf Blade			Root		
		NO_3^-	NH_4^+	NH_4^+/NO_3	NO_3^-	NH_4^+	NH_4^+/NO_3
Cron	9	140.92	177.56	1.26	3.79	11.25	2.97
Sorghum	7	262.61	342.86	1.31	4.67	7.91	1.69
Rice Tainung 67	13	7.92	8.53	1.08	4.20	9.63	2.29
Rice Pegonil	13	13.65	21.98	1.61	3.95	8.41	2.13
Rice Taichung sen 3	15	9.34	10.11	1.08	5.92	10.78	1.82
Wheat	9	6.53	4.43	0.68	3.22	14.85	4.61
Barley	9	3.87	2.31	0.60	2.33	7.50	3.22
Peanut	7	nd[a]	nd	nd	0.52	2.49	4.79
Soybean	7	nd	nd	nd	3.03	2.77	0.91

[a]Not determined.

Very pronounced increases of PEP carboxylase activity were found in the root system of almost every crop tested (the only exception was soybean) as a function of ammonium treatment. The increases were from 3.79 to 11.25 units for maize (2.97-fold increase), 4.20 to 9.63 units for rice variety Tainung 67 (2.29-fold), 3.95 to 8.41 units for rice variety Pegonil (2.13-fold), 3.22 to 14.85 units for wheat (4.61-fold), 2.33 to 7.50 units for barley (3.22-fold) and 0.52 to 2.49 units for peanut (4.79-fold.) A slightly decrease in PEP carborylase activity was found in soybean grown in ammonium-N source. It was from 3.03 to 2.77 units (Table 2).

In order to verify that the increase of PEP carboxylase activity was a true response to ammonium-N, 10-day-old seedlings of Tainung 67 rice were transplanted from vermiculate (supplied with tap water only) to culture solutions containing nitrate-N (30 ppm) or ammonium-N of different concentrations (1, 10, 30 and 150 ppm) and PEP carboxylase activity in the root was determined with the advance of seedling growth for up to 15 days (Fig . 1). The initial value was 1.99 units. Activity increased continuously but slightly for plants grown in nitrate-N and was only 4.32 units at the 15th day of culture. For those plants grown in 1 ppm or 10 ppm ammonium-N, the increase in root PEP carboxylase activity was also continuous but at a higher rate. The acti-

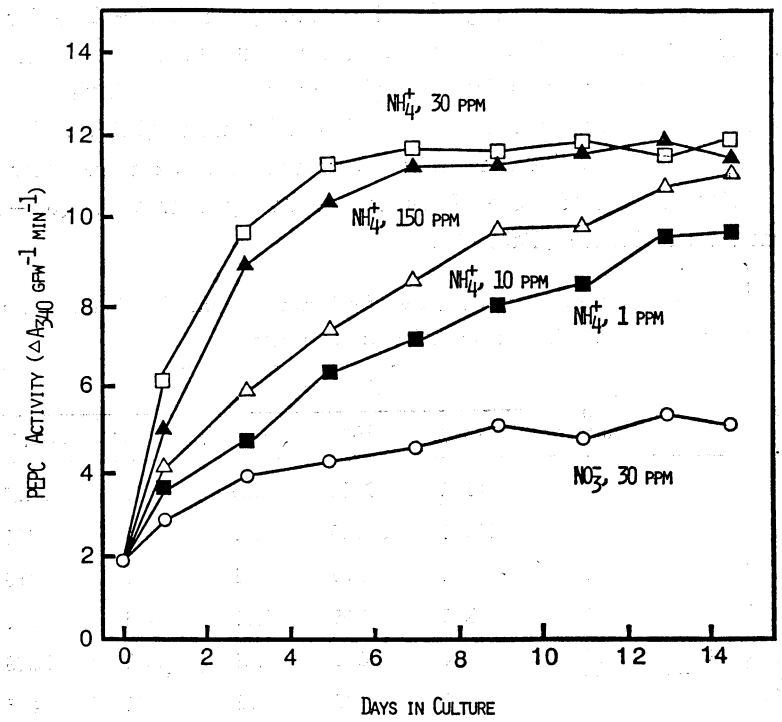


Fig. 1. Responses of root PEP carboxylase activities in developing Tainung 67 rice seedlings to different concentrations of NH_4^+ and NO_3^-.

vities at the 15th day were 9.14 and 10.87 units, respectively. Plants supplied with higher concentrations of ammonia (30 and 150 ppm) showed peak PEP carboxylase activities after 7 days in culture and the values were 2.6 times higher than that of the nitrate treatment. In other words, ammonium concentration of 30 ppm was high enough for the increase of PEP carboxylase activity in the root system of rice and the maximum response was obtained within one week after treatment.

Further studies were made to understand the nature of root PEP carboxylase enhanced by ammonium-N. Fig. 2 showed the diurnal changes of the enzyme activity of rice Tainung 67 grown in 150 ppm ammonium solution. Although there were fluctuations during the 24-hr cycle, the variations were less significant and the response to light

was less clear than those found in the leaf blades of many C4 plants. The activity remained fairly constant from 1 a. m. to 10 a.m. followed by a slightly decrease towards 2 p.m. It increased again until 10 p.m.and then decreased (day time or light time in the growth chamber was from 6 a.m. to 8 p.m.). Since there was no significant difference in PEP carboxylase activity from 8 to 10 a.m., samplings were made during this period for the following experiments in order to obtain results for comparison.

Fig. 2 also showed diurnal changes in soluble protein and malate concentrations in the rice roots. Changes in soluble protein concentration during the 24-hr cycle were in good accordance with those in PEP carboxylase activity. Statistical analysis showed a positive correlation between the two characters ($r=0.941^{**}$). Malate content in the

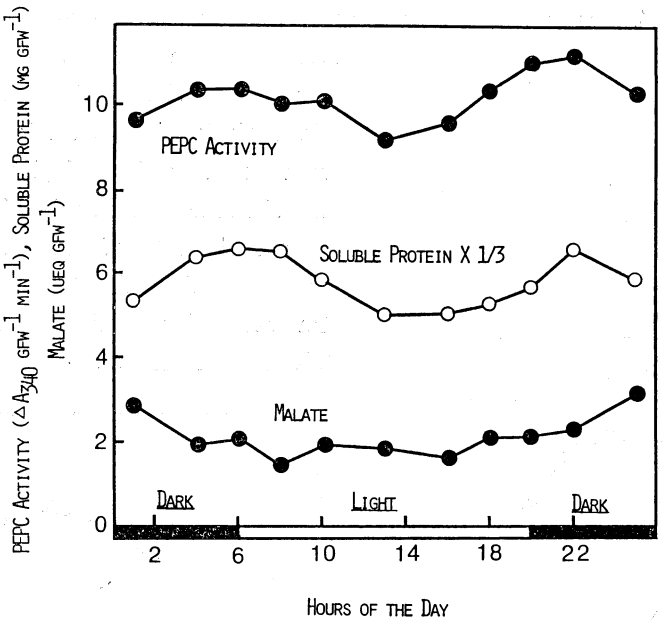


Fig. 2. Diurnal changes of PEP carboxylase activity and contents of soluble protein and malate in Tainung 67 rice roots. 15-day-old seedlings cultured in 150 ppm NH_4^+ solution were used. Data were means of 3-4 measurements.

rice roots was low and the values ranged from 1.72 to 2.86 micro-equivalent per gram fresh weight. There was a tendency that malate content was lower during the day time and higher during the night time although differences were not significant. No correlation was found between PEP carboxylase activities and malate during this diurnal study.

As reports on root PEP carboxylase were very limited, efforts have been made to study the characteristics of this enzyme in rice roots supplied with ammonium-N. Fig. 3A showed rice root PEP carboxylase was very stable with the method employed in this experiment. Activity remined fairly constant for up to 7 hrs after extraction. An 1-6% increase in enzyme activity was recorded during this period which was consi-

dered nonsignificant.

Fig. 3B introduced the responses of rice root PEP carboxylase activity to different pH values. With 100 mM Tris-HCl buffer, maximum enzyme activity was recorded at pH 8.5. The enzyme showed high activities within the pH range of 7.5 to 9.0. Activity decreased sharply towards acidic pH and rather moderately towards alkaline pH. As Hepes-KOH was used extensively in the study of PEP carboxylase by many scholars, it was also included in this experiment to test its effect in the range of 6.0 to 8.5. The results obtained supported those from Tris-HCl study. Maximum activity was recorded at pH 8.0 with slightly decrease in activities at pH 7.5 and 8.5. However, PEP carboxylase activity was much lower at pH 7.0 with Hepes-KOH than with Tris-HCl(Fig.3B).

A comparison was made on PEP carboxylase activity at pH 7 and 8 with varying concentrations of PEP. Response curves of velocity versus PEP concentration were presented in Fig. 4. The maximum activity was substantially higher at pH 8.0 than at 7.0. Response curve was hyperbolic in nature at pH 8.0 and sigmoid at pH 7.0. Kinetic analysis of the data in Fig. 4 was made from double-reciprocal plots of velocity versus substrate concentrations(data not shown). Reciprocal plots of the data were linear, indicating Michaelis-Menten Kinetics, and the Km value for PEP was calculated to be 0.16 mM at pH 8.0.

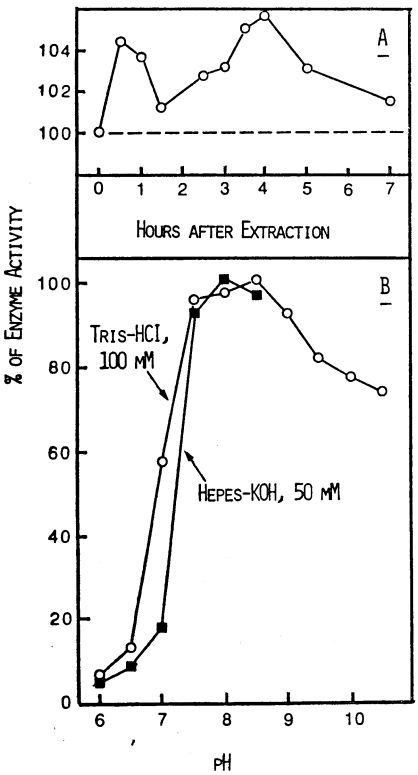


Fig. 3. Influences of (A) time elapsed after extraction and (B) pH value on the activity of PEP carboxylase in Tainung 67 rice roots. 15-day-old seedlings were used. Data were means of duplicate measurements.

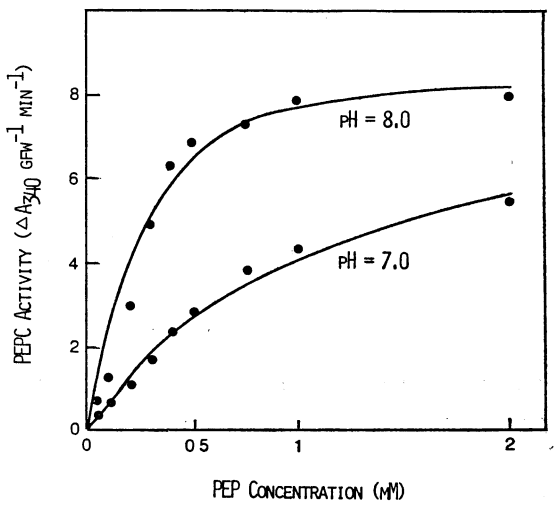


Fig. 4. Effects of substrate(phosphoenolpyruvate) concentrations on the PEP carboxylase activitz in Tainung 67 rice roots. 15-day-old seedlings were used. Data were means of duplicate measurements. Activities at concentrations of 5 and 10 mM decreased at both pH values.

Discussion

Culture medium containing the anionic nitrate became more alkaline and those with cationic ammonia more acidic with the advance of plant growth. The changes in external pH are due to excretion of hydrogen ions by the plant upon ammonium uptake, and hydroxyl ions upon nitrate uptake[22]. In general, lower pH was recorded with larger root volume and higher absorption capability of the plants. No significant difference among the seven crop species was found.

Weight gains by adding ammonia to an all nitrate system have been observed in many plant species[8]. Cox and Reisenauer[1] reported that growth rate and yield of wheat were enhanced under similar condition. The enhancement was suggested to result from the reduced energy requirement in using ammonia in protein synthesis. However, ammonia alone usually possesses a detrimental effect on plant growth. Results of this experiment showed that seedling growth (fresh weight gain) of almost all the crops was inferior when grown under ammonium nutrition (Table 1). Possible causes are the toxicity of ammonium ions to plant metabolism and the lowering of pH in culture medium.

It is well known that nutrient supply affects the biomass distribution in plants, especially with respect to the shoot/root ratio[5] which indicates a change in the effectiveness of shoot or root as sink for assimilates. Rosnitschek-Schimmel[23] reported that in *Urtica dioica*, low N supply favored dry matter formation of the root plus rhizome fraction, whereas high N supply resulted in a preferential growth of the leaf plus stalk fraction. In the current study, seedlings of maize, sorghum, peanut and soybean gained more fresh weight in both shoot and root when grown in nitrate than in ammonium medium. On the contrary, shoot of rice and wheat grew better in the ammonium than in the nitrate solution, whereas the opposite was true in roots (Table 1). It is therefore suggested that not only the extent of N supply but also the N source affect plant growth and N utilization and storage. Variation in responeses to N sources among different plant species was also suggested.

Stimulation of PEP carboxylase in the photosynthetic organs by ammonium N has been reported by De Benedetti *et al.*[4] and Paul *et al.*[19]. Howewer, negative effect of ammonia on the activity of this enzyme has also been realized[13,28]. Results from this study showed that in the leaf blades of maize, sorghum and rice at seedling stage, ammonium-N could increase the activity of PEP carboxylase by 8-61% (Table 2). The influence of such increase on photosynthetic rate remains to be verified. Similar effect was also observed in the roots of almost all the crop species studied. The degree of increasing enzyme activity ranged from 82% to 4.8 folds (Table 2). The hypothesis was further supported by growing rice seedlings in culture solutions containing different concentrations of ammonia (Fig. 1). It was concluded that ammonium concentration as low as 10 ppm colud effectively exhibit the stimulatory function. Results from culture in 1 ppm ammonium also showed distinct difference from those cultured in 30 ppm nitrate. The extraordinarily high activity is not commonly found in the root tissue of

most C3 and C4 plants. It is possible that a considerable amount of carbon dioxide must be fixed by the ammonium-grown plants because earlier report by Ku *et al.* [11] indicated a high total titratable acidity but a low malate content in such plants.

Little information is available on the PEP carboxylase from plant root tissues. In addition, Ting and Osmond [26] and O'Learly [17] stated that PEP carboxylase extracted from the roots of several C4 plants was distinct from the leaf forms. It is therefore necessary to study further the nature of this enzyme, especially under ammonium-N condition, if detailed investigations are to be made. No significant diurnal change in PEP carboxylase activity was found (Fig. 2) . This is understandable as in the dark condition, root PEP carboxylase might not respond to light as leaf PEP carboxylase does. The moderate fluctuation in enzyme activity during the 24-hr cycle corresponded well with the content of soluble protein in the root tissue. In other words, specific activity of this enzyme was fairly constant through the experimental period.

Malate content in the roots of ammonium-grown rice plants was low and was not in correlation with the activity of PEP carboxylase. In generall, oxalacetate formed in the cells can be reduced to malate catalyzed by NAD- or NADP-malate dehydrogenase or converted to aspartate by aspartate aminotransferase. The current experiment did not analyze the activities of these two enzymes. According to earlier and unpublished results of this laboratory, however, wheat plants treated with ammonium-N had extremely high NAD-malate dehydrogenase activity compared to the nitrate-grown plants. Whether this is true in rice is unknown, and the disagreement between malate content and related ezyme activities has yet to be clarified.

The stability of PEP carboxylase after extraction has drawn attentions from many plant biochemists. According to O'Leary [17], activity of the enzyme may change substantially after extraction, especially for CAM plants. For example, if PEP carboxylase in CAM plant leaves was assayed within 1-2 minutes after extraction, the enzyme showed large diurnal variations in activity. However, if extracts were allowed to stand for an hour or more prior to assay, these diurnal variations disappeared. Gavalas *et al.* [6] also indicated that PEP carboxylase in desalted leaf extracts suffered a fast inactivation when activity was assayed at low PEP levels (e. g., below 0.6 mM). Results in Fig. 3A of this experiment showed that PEP carboxylase extracted from rice root with the described method remained fairly constant for up to 7 hrs after extraction. One possible explanation is that 20% glycerol was included in the extraction medium which may stabilize the enzyme [7].

For the assay of PEP carboxylase activity, desalting of the crude extract on a small column of Sephadex G-25 has been practiced extensively as reported by O'Leary [17], Gavalas *et al.* [6] and Karabourniotis *et al.* [10]. Test of desalting on Sephadex G-25 column showed no difference in enzyme activity in this experiment and hence no desalting was practiced in the current study.

PEP carboxylase is usually assayed in the pH range of 7.0—8.5. Studies at single substrate concentration indicate that the enzyme has maximum activity near pH 8 [17]. The affinity of PEP for the maize leaf enzyme decreases above pH 9.5 and below

7.5 [18]. PEP carboxylase extracted from the root of rice plant grown in ammonium nutrition also showed a pH optimum at pH 8.0—8.5 with either Tris-HCl or Hepes-KOH as buffering material (Fig. 3B). This was also in good agreement with the results on C4 plants by Huber and Edwards [9].

At pH 8.0, maize leaf PEP carboxylase showed hyperbolic kinetics with a Km near 1 mM. At pH 7.0, however, the kinetics were sigmoid with a Km that was several fold higher than that at pH 8.0 [17]. The situation with regard to PEP carboxylase from other sources is less clear. In a limited number of reports, Miziorko *et al.* [14] found hyperbolic kinetics with respect to PEP in spinach leaves. Sigmoid kinetics have been reported for enzyme from cotton [15]. In the present study, it was found that for rice root PEP carboxylase, the kinetics were hyperbolic at pH 8.0 and near sigmoid at pH 7.0. Reciprocal plots of the data were linear, indicating Michaelis-Menten kinetics, and the Km value for PEP was calculated to be 0.16 mM at pH 8.0. O'Leary [17] stated that Km value for PEP were about five-fold lower on average for the enzyme from C3 plants than that from C4 plants. In studying the kinetics properties of PEP carboxylase from C3, C4 and C3/C4 intermediate *Flaveria* species, Nakamoto *et al.* [16] reported the response curves were hyperbolic for the C3 and C3/C4 species at either pH 7 or 8 while they were sigmoidal for the C4 species at pH 7 and hyperbolic at pH 8. The Km value for PEP determined from reciprocal plots was the lowest in the C-3 species. Based on the above experimental results and discussion, it is suggested that the ammonium-N resposive root PEP carboxylase from rice roots is similar to the same enzyme from leaf blades of C3 plants in many aspects. More experiments should be done in order to provide a complete understanding of this root enzyme. Results reported here can be utilized as basis for future study in this very important field of plant biochemistry.

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硝酸態與銨態氮源對作物幼苗生長與根部 PEP Carboxylase 活性之影響[1]

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摘　要

將水稻與玉米等七種作物培養於含有150 ppm 之硝酸態或銨態氮水耕液中，測定幼苗生長速率，又由於發現銨態氮可顯著提高作物根部 PEP carboxylase 活性，故以水稻爲材料，初步究明其特性，供爲深入探討氮源對以 PEP 爲中心之碳素代謝作用影響的依據。試驗結果顯示各種作物幼苗在含硝酸態氮之水耕液中的生長較爲良好，但水稻與小麥莖葉生長則以銨態氮處理爲佳，亦即作物間的反應具有差異。銨態氮可使各種作物幼苗葉片與根部 PEP carboxylase 活性升高，其幅度在葉片爲 8～61%，在根部最高可達 4.5 倍以上。由本試驗方法萃取所得臺農67號水稻根部 PEP carboxylase 具有極高的穩定性，萃取後 7 小時之活性仍未降低；其最適pH值在使用 Tris-HCl 或 Hepes-KOH 緩衝液時，均爲 8.0 至 8.5。對此一自水稻根部萃取之酵素的Kinetic特性試驗得知，針對 PEP 濃度之反應曲線在 pH 爲 8.0 係屬 hyperbolic ，在 pH 爲 7.0 係屬 sigmoid，經計算 pH 爲 8.0 時之 Km 值爲 0.16 mM。初步判定此一酵素與 C-3 型植物葉片內 PEP carboxylase 之特性相同。由於 PEP 在作物含碳化合物之代謝作用方面極具重要性，而極低之銨態氮濃度（1—10 ppm）即可提升根部 PEP carboxylase 酵素活性，故爲深討氮素對作物生理活性影響之良好資料。

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Oriental Pear Breeding for High Fruit Quality and Adaptation to Subtropical Lowland in Taiwan[1]

Hsin-Tszu Hsu and Shue-Cheng Lin[2]

Abstract: The breeding objective of pear is to develop varieties with high fruit quality but low chilling requirement for table use. Hybridizations of 9 combinations started in 1976 were made from a native, very low-chilling required variety 'Hungshan' and 7 introduced varieties with high-chilling requirement but high fruit quality. Hybrid progenies were planted with a highly dense spacing of 1M×4M at TARI's experimental orchard which is about 100M above sea level. Five hybrid progenies with early maturing, high fruit quality and low-chilling requirement in characters were selected out of more than 1,500 hybrid seedlings after 1 to 5 years juvernile period by the culture of single trunk training and heavy fertilization. Top-working of these selected progenies onto bearing 'Hungshan' trees was followed at various lowlands for preliminary evaluation. Data showed that the genetical deviation of fruit color and size appeared in random, however, fruit quality and date of sprouting and flowering appeared in the middle range of parents. Fruit maturing, on the contrary, earlier than both of parents. The sugar content of slected individuals is superior to their parents. A further evaluation is being carried out for fruit quality, yield and adaptation to various environments in different areas.

Introduction

Taiwan with Tropic of Cancer passing through and surrounded by sea is tropical and subtropical in climate resulted a warm winter which is a detrimental factor for growing deciduous fruits with high quality. Most of the high quality pear cultivars are Japanese and European origin and were planted only in limitted mountain areas with elavation of 1,500—2,300M above sea level. Although the current techniques to produce high quality fruits in lowland by grafting mountain developed flower-bud onto bearing 'Hungshan' trees are very successful, there are still some disadvantages such as

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laborsome annual regrafting, high cost for collection of flower budwoods, high frequency of disease and pest control as well as high level of fertilization [1,4]. The limitted sources and low quality of flower budwoods are also another problems.

For the viewpoints of plant physiology and commercial cultivation, the flower bud grafting practice is supposed only to be an alternative pear producion. Therefore, the pear breeding with objectives to develop varieties with high fruit quality but low chilling requirement for cultivation in lowland was initiated at TARI in 1976.

Materials and Methods

Two groups of pear varieties, low chilling required with poor fruit quality and high chilling requried with high fruit quality, were used as crossing parents in the breeding program. Low-chilling required cultivar 'Hungshan' (*Pyrus pyrifolia*) is naitve and cultivated widely in the lowland of Taiwan. High chilling required cultivars of *Pyrus pyrifolia*: 'Shinseki', 'Nijisseiki', 'Chojuro', 'Kosui', and' Kikukou' as well as cultivars of *Pyrus communis*: 'Bartlett' and 'La France' were used as another group of parents. Reciprocal crosses listed in the note of Table 1 were made between these two groups of parents since 1976 both in lowland and highland orchards. All the combinations of hybrid seeds were collected separately when they were fully matured. Stratification of the seeds was operated with moist vermiculite at 5°C. Germinated seeds with 1cm radicle were transplanted into small polyethylene bags containing vermiculite medium only. Complete nutrient liquid fertilizer applied at an inteval of week during 6 months to one year period in the nursery. Seedlings about 1 to 2M high were transplanted into selection field with a spacing of 1M×4M at TARI orchard. Regular orchard management of pear was routingly practiced. Number of hybrid seedling flowered in each combination were recorded through the first five year period. Fruits harvested from individual seedling were measured and recorded separately with characters of weight, length and diameter. Brix of flesh was measured with hand reflactometer. Texture and flavor of flesh were tasted by 3-person panel. Only fruits with good shape and size, sweet, juicy, crispy and good in flavor were selected for further evaluation. Scionwoods of selected progenies were taken from the upper part of canopy and grafted onto bearing trees of 'Hungshan' for further district evaluation on fruit quality, yield and adaptability in lowland orchards.

Results and Discussion

Juvenile period

It is very difficult to evaluate the juvernile period of pear at present bacause of the polygenic controlling in inheritance [11]. Some segregates started to flower as early as 2.5 years after seeding, the flowering percentages of hybrid seedlings in the first five year was listed in Table 1. Data indicated that the varietal hybrids of *Pyrus pyrifolia* had shorter Juvenile period than that of interspecific hybrids between *P. pyrifolia* and *P. communis*. The reason to cause short juvenile period in *pyrifolia* hybrids was not clear. It might be due to the easier formation of spur in *P. pyrifolia* than

that of *P. communis*. The high density spacing supplemented with single trunk trainning and heavy fartilization used in the experiment also shortened the juvenile period drastically. This method was taken widely in shortening juvernility in deciduous fruits[6].

Table 1. Flowering Percentage of pear hybrid seedling from first flower through five years.

Cross Combinations	SH	CH	HN	HC	HS	HK	LH	HL	HB
% of Flowering	89.1	83.0	79.4	85.4	72.6	61.3	44.4	23.0	27.4

Note : SH : Shinseiki × Hungshan
CH : Chojuro × Hungshan
HN : Hungshan × Nijisseiki
HC : Hungshan × Chojuro
HS : Hungshan × Shinseiki
HK : Hungshan × Kosui
LH : La France × Hungshan
HL : Hungshan × La France
HB : Hungshan × Bartlett

Fruit Quality

Breeding on high fruit quality is the most important goal in our pear breeding program. Fruits with white or light yellow in color, juicy or crispy, and non-browning flesh coupled with sweet and strong flavor are supposed high quality criteria in preliminary selection. Five hybrid progenies, SH—033, SH—078, SH—085, HC—089, and HC—015, were selected from 1,500 hybrid seedlings to meet the above mentioned criteria. The fruit characters of selected hybrid individuals in terms of skin color, shape, sugar content, flesh texture and flavor were measured and listed in Table 2. Sugar content of the selected individuals was significantly higher than their parents.

Table 2. Fruit characters of pear in the breeding parents and selections in 1986

Clutivars and selections	Fruit skin color	Fruit shape[z] Index (L/D)	Average[x] °Brix	Flesh Texture	Flavor
Nijisseiki	Yellow	0.93(0.90—0.97)[yu]	12.2[dwu]	Tender, Juicy	Moderate
Shinseiki (1)[v]	Yellow	0.92(0.85—0.97)	11.4[e]	Tender, Juicy	Mild
Shinseiki (2)	Yellow	0.93(0.90—1.00)	10.9[ef]	Tender, Juicy	Mild
Kikukou	Yellow	0.91(0.85—0.99)	10.4[fg]	Tender, Juicy	Mild
Sinku/Hungshan (1)	Brown	0.91(0.81—1.04)	10.9[ef]	Crispy, Juicy	Mild
Sinku/Hungshan (2)	Brown	0.86(0.82—0.88)	9.6[gh]	Crispy, Juicy	Very Mild
Sinku/Hungshan (3)	Brown	0.82(0.77—0.88)	9.0[hi]	Crispy, Juicy	Very Mild
Hungshan (1)	Brown	0.94(0.90—1.04)	1.0[ef]	Coarse	Moderate, Slightly Sour
Hungshan (2)	Brown	0.94(0.89—1.05)	10.4[fg]	Coarse	Moderate, Slightly Sour
SH—033	Brown	0.97(0.86—1.02)	12.8[cd]	Crispy, Juicy	Strong. Slightly Sour

(續)

Cultivars and selections	Fruit skin color	Fruit shape[z] Index (L/D)	Average[x] °Brix	Flesh Texture	Flavor
SH—085	Yellow	0.94(0.82—1.02)	13.9[ab]	Tender, Juicy	Strong
SH—078	Brown	0.87(0.82—0.92)	14.2[a]	Crispy, Juicy	Moderate
HC—089	Yellow	0.98(0.91—1.04)	14.2[a]	Crispy, Juicy	Moderate
HC—015	Yellow	0.89(0.82—1.03)	13.3[bc]	Tender, juicy	Mild

Notes : z : L/D=fruit length/fruit diameter,
y : Mean value (maximum-minimum)
x : Difference is analysed with Duncan's new multiple range test.
w : Means with the same letter are not significantly different.
v : 1, 2, 3 inside parentheses indicates orchard number.
u : Mean ualue contains 10 fruits.

Adaptability

The TARI selection field, located in central Taiwan with a elevation of 100M above sea level, is very warm in winter. During winter period, the average temperature seldom dropped below 13°C except that the cold front coming down occasionally from Siberia area which would drop temperature lower than 7°C for a few days. Delayed defoliation is a symptom caused by insufficient chilling hours[9,10]. However, our data showed that about 80% of hybrid progenies in the experiment sprouted and flowered normally by April each year without symptoms of delayed defoliation (Table 3). In comparing the flower season of hybrids with parents, we observed that there was 2 to 3 weeks later than 'Hungshan' and 1 to 2 weeks earlier than high chilling parents. However, sprouting date of selected progenies became more narrow to 1 to 2 weeks after topworked onto the bearing 'Hungshan' trees in orchards located at an alitiute from 200 to 500M sea level (data unpublished).

Table 3. Flowering and fruit-maturing seasons of breeding parents and selections

Cultivars and selecoitns	Flowering season	Fruit-matuing season	Locations of orchards (altitude)
Shinseiki	Early April	August	Li-shan(15000—2300M)
Nijisseiki	Early April	August-Early September	Li-shan(15000—2300M)
Chojuro	Early April	Late August	Li-shan(15000—2300M)
Hungshan	February-Early March	September-October	Lowland(100— 900M)
SH—033	March	July -August	Lowland(100— 600M)
SH—078	March	July -August	Lowland(100— 600M)
HC—085	March	July -August	Lowland(100— 600M)
HC—015	March	August	Lowland(100M)

Note : SH : Shinseiki×Hungshan
HC : Hungshan×Chojuro

Fruit Maturity

Flowering and fruit-maturing season of both crossing parents and selected progenies were summarized in Table 3. Fruit maturing was judged by the color of seed which would turn dark brown when fruit matured. It took normally 140 to 160 days after anthesis for the fruits of 'Shinseiki' and 150 to 170 days for 'Nijisseiki' to get matured. But, it took much longer period of 7 to 8 months to reach mature stage on the fruits of 'Hungshan' in Taiwan. However, our data showed that the fruits of selected progenies matured about 2 to 3 months earlier than 'Hungshan' (low-chilling required parent) and slightly earlier than 'Shinseiki' and 'Nijisseiki' (high-chilling required parents) (Table 2). Since the early maturing is rather meaningful to avoid the losses caused by heavy rainfall and typhoon in summer harvesting season. Fruits mature early in selected hybrid is supposed due to the early mature character of high-chilling required parents and warm temperature in lowland.

Conclusion

Pear fruit breeding with an aim of adaptation to warm climates [3,5,7,8] has been a challenge task for those researchers devoted to subtropincal ad tropical horticulture. Although the data collected so far are not yet enough, our data stongly support that to develop new varieties with low-chilling requirement through hybridization is eligible and encouraging. A further evaluation is being carried out for fruit quality, yield and adaptation to various environments in different arsea.

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低海拔高品質梨之雜交育種[1]

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摘　要

爲開發適合低海拔種植之高品質東方梨品系，本所於1976年，以平地之橫山梨與自日本引進之東方梨系：幸水、長十郎、新世紀、二十世紀及西方（洋）梨：拉法蘭西及巴梨高山梨，分別在本所果園及梨山進行互交，共行九個雜組合，得雜交苗逾 1,500株，經行 4 公尺×1 公尺之寬行密植，配合以重肥及單幹整枝，以求快速生長縮短其幼年性，經 6 個月至 1 年之育苗後植於本所果園選拔，植後 1—5 年陸續開花結果，並選出具高糖度，香味濃，質脆多汁，石細胞少不變色及低溫需求量低之後代計有 SH—033, SH—085, SH—078, HC—089, HC—015 等五品系，爾後進行低海拔較成年樹之高接觀察，發現果色、果實形狀及果實大小之變化甚大與親本無顯著相關；果實品質，發芽期與開花期近於兩親之中間；果實成熟度因親本特性及生長期之高溫反較兩親爲早，目前正就選出之優良品系進行區域之適應性觀察。

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水稻不同齡期及品種對稻細菌性穀枯病菌之感病性[1]

簡 錦 忠　張 義 璋

摘要 由 *Pseudomonas glumae*引起之稻細菌性穀枯病，在本省第二期稻作比第一期作發病較爲嚴重，例如據1983年於南部地區調查結果，第二期稻作被害穗率最高達30%，罹病穗常呈聚集狀分佈；第一期稻作之被害穗率一般低於1%，罹病穗則呈零星散佈。稻種以濃度10^8 cfu/ml之*P. glumae*懸浮液接種後，病菌對稻種發芽並無影響，但可引起約98%之秧苗腐敗枯死。孕穗期稻以注射法接種上述濃度之懸浮液於劍葉葉鞘內側，使病原細菌直接與幼穗接觸，其葉鞘及幼穗皆被感染，而病變呈深褐色，同時期如以噴霧法接種，病原細菌並未感染葉鞘，但會引起60%以上之穀粒被感染而未能飽實。抽穗期稻以噴霧法接種上述濃度之病菌懸浮液，亦未見葉鞘病變之現象；稻穗對B24及01兩個菌株，在抽穗前4日至抽穗後2日期間，皆呈極感病；稻穗對B15及B23兩個菌株，在抽穗前4日至抽穗後4日期間，皆呈極感病。在田間種植21種水稻優良推廣品種，於抽穗前10日，以人工噴霧接種結果，顯示菌株間之病原性或稻品種間之感病性皆呈顯著差異。其中以菌株B89之病原性最強，其次爲B90及B43，而B88之病原性最弱。稻品種中以臺南6號最爲感病，其次爲新竹糯4號及臺東27號，以高雄孤7號最爲抗病。由品種與菌株間之交感作用分析結果顯示，稻細菌性穀枯病菌似有不同病原型之存在。

1982年第二期稻作，在本省中南部地區之粳稻（主要爲臺農67號及臺南5號），發現在臺灣尚未被記載之病害。其病徵爲齊穗後被害穀粒初呈蒼白色，漸變爲灰白色或淡黃褐色至深褐色，穗軸及枝梗則健全呈綠色；罹病穀粒多成秕而不稔實，若能稔實則由胚乳基部呈灰白色病變，再轉爲黃褐色，患部與健全部界限明顯常呈深褐色帶狀，糙米終呈萎凋畸形。當時自各地採集病穀並分離病原，經過以柯氏法則（Koch's Postulates）測試，證實其中13個細菌菌株具病原性，又選3個菌株供形態觀察及生理試驗。綜合各項結果，病徵與1955年後藤等在日本發現的「稻籾枯性細菌病」(Bacterial grain rot) 相同，病原形態及生理與1967年栗田及田部井報告 *Pseudomonas glumae* Kurita et Tabei之形態及生理相同，因而取中名爲「稻細菌性穀枯病」[16,17,24]。

1983年筆者等調查屏東地區第一、二期稻作，本病在田間發生情形，發現第一期作發病較少，發病田之被害穗率僅爲0.1～1.0%，而第二期作發病則頗爲普遍，幾乎每塊田皆有發生，其發病田之被害穗呈零星散佈時，被害穗率約爲0.1～5.0%，而每一被害稻穗之罹病穀粒爲10～20粒，但少數被害穗具有一半以上之穀粒枯死；如果被害穗在田間呈聚集狀分佈時，每一聚集點大小約1～60叢，其被害穗率可高達30.0%之譜，每一被害稻穗亦較爲嚴重，一半以上或全穗穀粒均枯死，被害穗常呈直立

1.臺灣省農業試驗所　研究報告第 1363 號。本研究承行政院農業委員會補助（73—農建—4.1—產—80）謹此申謝。

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本病害可經由稻種傳播 [6,7,12,21]，因此目前機械插秧所需之箱式育苗，頗有利於病害之發生 [7,10,11,12,22]，同時亦會引起箱育秧苗腐敗症，影響農業機械化甚大 [4,13,18,19,20,23]。本病發生嚴重時可直接引起減產，稻穀粒後期被感染時雖對其重量影響不大，但對米質影響頗大 [10]。實有探討本病發生生態之必要，本研究主要對本病疾病環（Disease cycle）中主要三個時期，即稻秧苗期、孕穗期及抽穗期等測試其感病性，並由較受歡迎之水稻品種中選出21個品種，測定各品種對本病之感病性，以供防治對策之參考。

材料與方法

一、供試病原細菌之來源：自臺中及屏東地區採得之罹病稻穀粒，以1%次氯酸鈉溶液行表面消毒1分鐘後，以無菌水漂洗3次，再以無菌濾紙吸乾穀粒表面水分，並利用無菌操作剝開穀殼，取其糙米平放於NA（Nutrient agar）平板上。上述平板置於室溫（26±2°C）下培養48小時，挑取長出之乳白色細菌菌落，以無菌水稀釋後在NA平板上劃線純化之。經連續三次純化後之菌株，以NA斜面培養72小時後，以無菌水製成 10^8 cfu/ml 之懸浮液，再噴霧接種於抽穗期中之水稻上，以測定其病原性。自具有病原性之菌株中，選取不同來源之 B15、B23、B24 及 O1 等4個菌株，供測定秧苗期，孕穗期及抽穗期之感病性。另選 B43、B88、B89 及 B90 作爲測定稻品種間之感病性（表1）。

表一、稻細菌性穀枯病菌（*Pseudomonas glumae*）供試菌株之來源

Table 1. Sources of the isolates of *Pseudomonas glumae*

菌株 Isolate	稻品種 Rice variety	採集地點 Locality of collection	採集日期 Collection date
B 15	臺農67號	臺中縣	10/13 1982
B 23	臺南5號	臺中縣	10/13 1982
B 24	臺農67號	南投縣	10/21 1982
O 1	臺農67號	屏東縣	9/27 1982
B 43	高雄140號	屏東縣	5/11 1983
B 88	臺農67號	臺中縣	10/21 1983
B 89	臺農67號	臺中縣	10/21 1983
B 90	臺農67號	臺中縣	10/21 1983

二、稻細菌性穀枯病菌對秧苗期水稻之致病性：供試臺農67號的健全穀粒，以1%次氯酸鈉溶液消毒10分鐘，再以自來水浸洗，每30分鐘換水一次共3次。將消毒過之稻種以 10^8 cfu/ml 之病原細菌懸浮液浸漬接種30分鐘，取出陰乾。陰乾後之稻穀再經浸種及催芽，萌芽後播種於育苗箱（30×60×3公分），每箱播種量爲 250公克，其他管理均與一般栽培方式相同。本試驗設3重複，另以非病原細菌接種爲對照處理。播種後第5日調查水稻發芽率，第20日調查健全苗數。

三、稻細菌性穀枯病菌對孕穗期水稻之致病性：供試臺農67號健全穀粒，以試驗二同法消毒後直接浸種及催芽，萌芽後以水秧田方式育苗。待秧苗長達4～5葉齡時，移植於本田，並按一般栽培管理方法耕種之。待稻生長至孕穗中期，即幼穗長度約 10cm 時，分別以劍葉內側注射法及噴霧法接種病原細菌。病原細菌懸浮液之準備均與前述同法。劍葉內側注射法係以注射筒將病原細菌懸浮液，自

劍葉葉鞘下部位打入，使該液與幼穗直接地接觸，待懸浮液自葉鞘上端處溢出爲止。另設注射非病原細菌爲對照。每處理設5重複。噴霧法即以小型噴霧器將細菌懸浮液均勻地噴灑於稻植株上，每叢平均接種量爲 15ml，每一菌株接種144（12×12）叢，另以同量之無菌水接種作爲對照。接種後隨時觀察其病變，並於成熟期調查稻穗之稔實率。

四、稻細菌性穀枯病菌對抽穗期水稻之致病性：供試臺農67號水稻之栽培管理與試驗三同法。至孕穗後期將水稻劃訂 144叢爲一試驗小區，並自開始抽穗日（每穗自劍葉葉鞘抽出一半時作爲標準），每日以標籤標示每穗之抽穗日期。開始抽穗後第7天，以上述之噴霧接種法接種病原細菌懸浮液。接種後隨時觀察稻穗之病變，至稻成熟期調查每穗之稔實率及未飽實穀粒百分率。

五、稻品種對細菌性穀枯病菌之感病性：選取在本省栽培面積 1,000公頃以上之21個推廣品種，種植於田間並按一般栽培管理方法耕種之。待生長至抽穗前約10天時，將病原細菌B43、B88、B89及B90 等 4 菌株以前述方法培養，並製成上述濃度之懸浮液，再分別以噴霧接種法接種，另設接種非病原細菌作爲對照，接種後至乳熟期調查各處理被害度。調查時依照各穗罹病穀粒之百分率，將各穗被害程度分爲5級，即0（未感染），1（輕微～20%），2（20～40%），3（40～60%），4（60～80%）及5（80%以上），再換算成被害率。

試驗結果

一、稻細菌性穀枯病菌對秧苗期水稻之致病性：

稻種以 *Pseudomonas glumae* 懸浮液接種，播種後第5天調查結果，顯示4個供試菌株 B15、B23、B24及 O1，對稻種之發芽皆無影響，發芽率與接種非病原細菌之對照區差異不顯著（表二）。但接種病原細菌區之秧苗或停止或緩慢生育並逐漸萎凋，其子葉均呈現褐色病變。至播種後第10日，接種病原細菌區與非病原對照區之差異已極爲顯著，接種病原細菌區之秧苗大部份已枯死，未死者亦陸續發病，後期發病苗均由心葉基部腐敗後枯萎，根部亦已腐死。至播種後20日，接種病原細菌區之秧苗大部份發病枯萎，只有少數健苗存在，但其子葉亦皆呈現褐變，非病原細菌區之秧苗則健壯而僅少數秧苗生長不良（如表三）。

二、稻細菌性穀枯病菌對孕穗期水稻之致病性：

表二、稻細菌性穀枯病菌對秧苗期水稻之致病性*

Table 2. The pathogenicity of *Pseudomonas glumae* to rice plants at seedling stage

菌株 Isolate	稻發芽百分率 % of rice germination	健康秧苗百分率 % of healthy seedlings
B 15	87.9	2.4 b**
B 23	88.4	2.0 b
B 24	89.2	2.3 b
O 1	88.0	1.9 b
CK	88.6	98.5 a
5% LSD值	n. s.	1.9

* ：供試稻品種爲臺農67號。
The variety of rice tested was Tainung 67.

**：英文字母相同者爲 FLSD 測試，5%基準差異不顯著。
Numbers followed by the same letter are insignicantly different at the 5% level by FLSD test.

供試稻生長達孕穗中期，即幼穗長度約 10cm時，以劍葉葉鞘內側注射法接種 *P. glumae*後，第2天葉鞘就呈現水浸狀病變，第3天病變組織轉爲黃綠色，第5天即出現雲狀之病斑，約第10天該病斑轉爲黃褐色並開始枯死。稻穗常無法完全抽出，穗上穀粒皆無法稔實，穀粒外穎均呈褐色病變，極少數穀粒尙能開花授粉，但亦隨之被感染而枯死。接種腐生細菌之對照區則無病變現象，穀粒稔實率與未接種稻差異不顯著（表三）。

表三、稻細菌性穀枯病菌以注射法接種對孕穗期水稻之致病性*

Table 3. The pathogenicity of *Pseudomonas glumae* to rice plants inoculated at booting stage by injection method*

菌株 Isolate	飽實穀粒百分率 % of full grains	未飽實穀粒百分率 % of unfull grains		
		半飽穀粒 Half-full grains	空穀 Empty grains	小計 Subtotal
B 15	0 b**	2.5	97.5 b	100 b
B 23	0 b	3.1	96.9 b	100 b
B 24	0 b	3.8	96.2 b	100 b
O 1	0 b	2.7	97.3 b	100 b
CK	83.5 a	5.9	10.6 a	16.5 a
5% LSD 值	0.6	n. s.	3.3	0.6

* 本表數據係在成熟期調查之結果
The data were recorded at mature stage.
**見表二注脚，See footnote on table 2.

表四、稻細菌性穀枯病菌以噴霧法接種對孕穗期水稻之致病性*

Table 4. The pathologenicity of *Pseudomonas glumae* to rice plants inoculated at booting stage by spraying method

菌株 Isolate	飽實穀粒百分率 % of full grains	未飽實穀粒百分率 % of unfull grains		
		半飽穀粒 Half-full grain	空穀 Empty grains	小計 Subtotal
B 15	28.9 d**	44.4 b	26.7 bc	71.1 d
B 23	37.0 bc	34.3 b	28.7 bc	63.0 bc
B 24	32.4 bcd	35.6 b	32.0 c	67.6 bcd
O 1	39.3 b	39.1 b	21.6 b	60.7 b
CK	86.1 a	4.6 a	9.3 a	13.9 a
5%LSD值	7.5	12.5	8.5	7.5

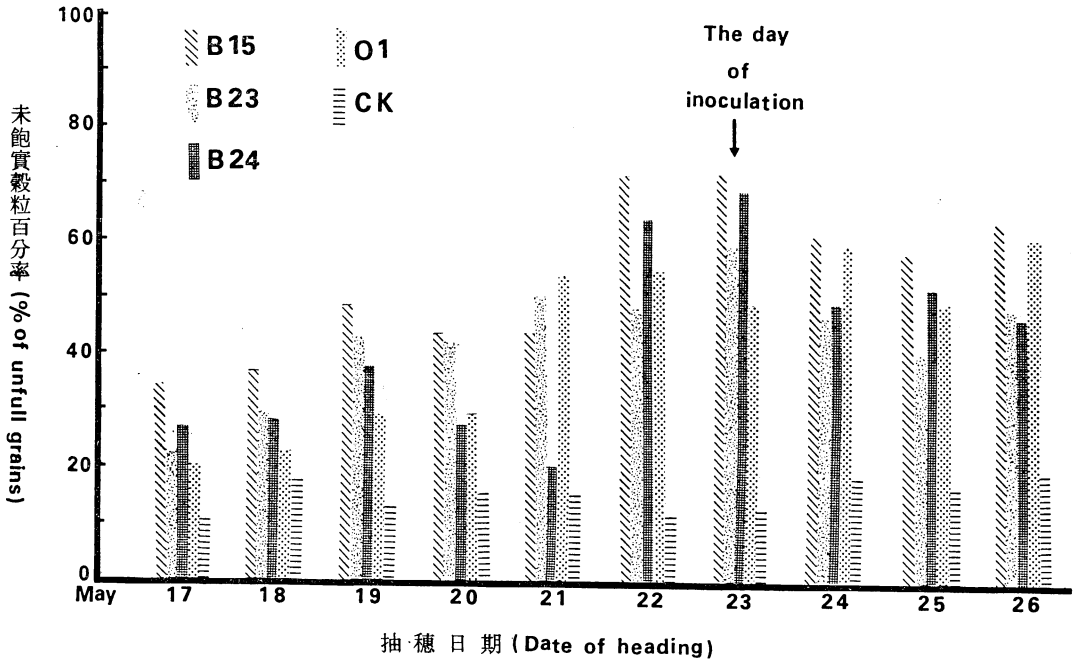
* 本表數據係在成熟期調查之結果。
The data were recorded at mature stage.
**見表二注脚，See footnote on table 2.

在稻孕穗期利用噴霧法接種，對穀粒亦可得到很强之致病力。稻葉鞘則未見有感染現象。只有劍葉之葉舌部位被感染而呈褐變現象。接種病原細菌區，稻抽穗後部份穀粒不能開花而枯死，有的雖能

開花授粉，但亦未能充分飽實而枯死。病穗與田間自然發病田之嚴重病穗相似，呈直立狀不易彎曲。被害穀粒亦與自然發病者相似，其穗軸及枝梗均未被感染跡象，呈青綠色。由每處理隨機採取10叢，每叢調查15穗之稔實穀粒及未飽實穀粒數目，分析結果供試4個菌株 B15、B23、B24及 O1等均引起稻穗有60%以上之穀粒未能飽實，而接種非病原細菌區之稔實率爲86.1%，差異極顯著（表四）。

三、稻細菌性穀枯病菌對抽穗期水稻之致病性：

稻抽穗期以噴霧法接種*P. glumae*懸浮液後，稻葉鞘未見病變之現象，至稻成熟期調查罹病穀率，每處理（接種同一菌株及抽穗日期相同之稻穗）調查50穗，每穗記錄其飽實穀粒及未飽實穀粒數目。結果顯示，在稻抽穗期前至抽穗後2日之間最爲感病。但菌株 B15及 B23兩菌株對抽穗後第4日之穗亦可分別引起48.8及43.1%之未飽實穀粒（圖一）。開始抽穗後第10日， B15及 B24兩菌株接種區業已全部抽出，因此第11日只有 B23、O1及 CK等3接種區尚在抽穗，但抽穗穗數皆未達50穗，故未將資料表示於圖內。3個接種區之未飽實穀粒百分率， B23接種區只有2穗，未飽實穀粒率平均43.0%，O1區有30穗平均爲69.1%，對照區有10穗平均爲10.9%。



圖一、抽穗期水稻接種*Pseudomonas glumae*後未飽實穀粒之百分率

Fig. 1. The percentage of unfull grains from rice plants after inoculated with *Pseudomonas glumae* at heading stage.

四、稻品種對細菌性穀枯病菌之感病性：

供試21品種，每品種栽植30叢（5×6行），並於抽穗前10日行人工噴霧接種後，於乳熟期調查中央12叢，記錄每穗之罹病等級，再換算成被害率。結果如表五所示，菌株間之病原性或品種間之感病性均呈顯著差異。病原細菌以菌株B89之病原性最強，供試稻品種平均被害率 86.3%；其次爲B90及B43分別爲51.5及50.9%；菌株 B88之病原性最弱，僅爲 31.9%。稻品種中以臺南6號平均被害率64.6%最爲感病；其次爲新竹糯4號及臺東27號，分別爲61.7及59.6%。高雄秈7號最爲抗病，平均被害率僅爲28.2%；豐錦及蘿早生則次之，平均被害率分別爲30.4及35.4%。稻品種與病原菌株間之交感作用亦呈顯著性，菌株B43 對臺南6號及新竹糯4號之病原性最強，分別爲87.4及83.2%；菌株

表5. 稻品種對細菌性穀枯病菌之感病性

Table 5. The susceptibilities of 21 commercial rice varieties to *Pseudomonas glumae*

品種 Variety	菌株 Isolate					平均 Average
	B43	B88	B89	B90	CK	
Toyonishiki	38.2*	28.0	48.9	29.6	7.5	30.4
Todorokiwase	38.0	23.6	78.9	25.5	10.9	35.4
Hsinchu A. C. C.	62.7	46.9	82.4	59.6	20.1	54.3
Hsinchu G. 4	83.2	35.7	95.5	79.7	14.7	61.7
Taitung 27	80.2	33.9	94.6	74.8	14.5	59.6
Kaohsiung 139	57.5	30.5	92.3	37.3	17.7	47.1
Tainan 6	87.4	37.3	95.8	86.4	16.2	64.6
Taipei 306	48.2	27.4	96.6	39.7	15.7	45.5
Taipei 309	53.5	24.9	96.4	38.8	12.4	45.2
Taichung 65	64.2	24.9	93.3	47.8	15.8	49.2
Taichung S. 10	39.6	24.0	76.5	34.8	9.1	36.8
Taichung N. 1	55.3	47.4	95.4	54.1	20.0	54.4
Kaohsiung S. 1	37.5	34.3	79.1	58.2	13.8	44.6
Kaohsiung 141	42.5	25.2	93.6	48.0	14.4	44.8
Hsinchu 56	36.4	26.0	99.0	65.8	15.2	48.5
Taitung 29	52.9	31.4	96.9	64.5	12.5	51.6
Taichung G. 46	49.0	28.5	99.0	64.5	17.2	51.7
Tainan 5	38.7	30.4	83.9	60.0	15.9	45.8
Tainung 67	32.9	48.4	91.1	61.6	15.8	50.0
Kaohsiung S. 7	26.8	27.6	50.9	22.0	13.6	28.2
Taichung S. 3	43.5	32.8	73.2	29.6	6.5	37.1
Average	50.9	31.9	86.3	51.5	14.3	47.0
5% LSD	Interaction: 7.04, Mean of variety: 3.15, Mean of pathogen: 1.54					

* 被害百分率

* Percentage of disease incidence

B88 病原性較弱，對臺農67號及臺中在來 1 號之病原性較對其他品種之病原性稍強，分別爲48.4及47.4%；菌株 B89 對各品種之病原性均甚強；菌株 B90 對各稻品種之病原性，差異較大，對臺南 6 號引起86.4%被害率，對高雄秈 7 號引起22.0%。由此交感作用結果顯示，本病原細菌可能有不同病原型之存在，有待今後之探討。

討論與結論

本省自1982年於南部首次發現稻細菌性穀枯病，爾後經田間調查，得知該病害已遍佈本省西部之

稻田。而且第二期稻作比第一期作發生較爲普遍且嚴重，前者常呈聚集狀發生，其被害率可高達30%之譜，被害稻穗之穀粒多半不能稔實或呈半飽實而萎縮，因此稻穗呈直立不易彎曲，對產量及米質之影響頗大。

據日本報告，稻細菌性穀枯病可經由稻種傳播[6,7,12,21]，在箱育苗時會引起極爲嚴重的秧苗腐敗症[4,13,18,19,20,21]，影響箱育秧苗之工作及耽誤農時，爲稻作栽培上嚴重的新問題。爲瞭解本病在田間的消長情形，後藤、渡邊[7]及後藤[12]等將稻種接種病原細菌後播種，育成秧苗並移植於田間，追綜其發生生態，結果發現幼苗期稻株上的病原細菌濃度很高，但插秧時稻株上幾乎分不到病原，直到分蘗最盛期以後，稻株上病原密度才漸次提高，病原在各齡期稻株上變化的機制至今尚未被明瞭。十河[2]將被害稻穀埋在田間越冬，下期作種植秧苗時，由被害稻穀釋出之病原細菌，可感染稻株發病。本研究將B15、B23、B24及O1等4個菌株，經人工接種於稻種，播種後調查稻種發芽率及秧苗腐敗症之發生，結果發現稻種發芽雖未受影響，但播種後5天秧苗子葉已被害呈現病徵，10天後處理間之差異甚爲明顯。與吉村、植松[4]及後藤等[13]報告頗爲相似，證實稻種上帶有本病菌，播種後可引起嚴重的腐敗症，爲今後箱育秧苗的新問題。

水稻孕穗期以注射法將細菌性穀枯病菌的懸浮液，接種於劍葉葉鞘內側時，稻穗嚴重受害，除極少數半飽實穀粒外，均呈空穀粃粒。病原細菌亦可由注射針之傷口，侵入葉鞘組織引起褐色病變。據久原等[3]報告，稻如受害蟲啃害，所留下傷口有利病原細菌侵入，引起葉鞘病變，本研究結果亦可再予印證。同時期以噴霧法接種，亦可引起稻穗穀粒嚴重受害，其穀粒飽實率僅有29～39%，與對照組之86%呈極顯著差異。接種區穀粒雖會受粉，但被害而呈半飽實者，佔34～44%之間，與對照區之4.6%亦呈極顯著差異。相對地，葉鞘均未見病變褐化，僅部分劍葉之葉舌被感染而褐變。據後藤[14]報告指出，稻劍葉之葉舌被感染後，病原細菌在葉舌上繁殖，當稻穗抽出時接觸葉舌，病原細菌極易附著稻穗上，而感染稻穀引起發病。本研究於孕穗期以噴霧法接種病原，得到甚高之發病率，葉舌被感染再傳給稻穗，可能爲其主要原因。

稻抽穗期對細菌性穀枯病菌之感病性測定結果，顯示抽穗前至抽穗後2日內之期間最爲感病。抽穗前接種者，未受粉呈空粃穀較多；抽穗後接種者，則以受粉後病變呈半飽實粃穀較空粃爲多。遠藤等[21]以人工接種抽穗期之日本晴品種水稻，結果在抽穗前4日至抽穗後1日之不稔穀粒最多。安永等[5]研究稻抽穗開花日與感染之關連性，結果指出，無降雨時，開花1～2天前之穀粒最爲感病，又開花順序與感病程度有關，即感染時期與抽穗開花時期有關。另十河等[1]指出，稻於抽穗期被感染時，可影響穀粒之稔實率及成熟率，罹病穀粒率與產量指數之間有 —0.974之負相關係數，對產量之減收影響頗大。綜合前人研究及本研究之結果，對於本病之防治應注意孕穗期及抽穗期兩個時期，尤其葉舌上病原之控制更不可忽視。

後藤等[8,9]及後藤[15]分別搜集日本國內外共267個水稻品種，在抽穗開花期行人工接種，結果所有品種均發病，但品種間之病勢進展差異極爲顯著，其差異性與稻品種之早晚熟並無相關性，不同來源之菌株對稻品種間之致病性亦有顯著差異。本研究供試21個水稻推廣品種，於抽穗前約10日時行人工接種，結果顯示稻品種間之感病性呈顯著差異；其中以臺南6號最爲感病，其次爲新竹糯4號及臺東27號，而高雄秈7號最爲抗病。病原細菌菌株間之致病性亦呈顯著差異。稻品種與菌株間之交感作用呈顯著差異，推測本病之病原細菌可能具有不同病原型（Pathotype）之存在，有關這方面有待今後之探討。

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The Susceptibility of Rice Plants at Different Growth Stages and of 21 Commercial Rice Varieties to *Pseudomonas glumae*[1]

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Summary

The results showed that the pathogen did'nt affect the germination of rice seeds but caused seedlings to die about 98% when rice seeds were dipped in a water suspension of *Pseudomonas glumae* (10^8 cfu/ml) for 30 minutes prior to sowing. Rice plants inoculated with the suspension of *P. glumae* (10^8 cfu/ml) at booting stage by injection method, the panicles and sheaths of rice plants were all infected and discolored. However, the pathogen didn't infect the sheaths but the panicles when rice plants were inoculated at either botting or heading stage by spraying method. It was estimated that more than 60% of unfull grains were recorded when rice plants inoculated at booting stage by spraying method. The rice was more susceptible to isolate B24 or O1 from 4 days before heading to 2 days after heading, and to isolate B15 or B23 from 4 days before heading to 4 days after heading if inoculation was made at heading stage.

Rice plants with a total of 21 commercial varieties were separately inoculated with 4 isolates of *P. glumae* for testing their susceptibilities to bacterial grain rot 10 days before heading. The results indicated that isolate B89 was the most virulent while the isolate B88 was the least. Among 21 rice varieties tested, Tainan 6 was the most susceptible variety which was reached 64.6% of the disease incidence, but Kaohsiung S. 7 was the most resistant variety, the disease incidence was averaged only 28.2%. Based on the significant interaction between rice varieties and pathogen isolates, the pathogen might exist differential strains in nature.

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瓜類露菌病室內接種試驗[1]

蔡 武 雄[2]

摘要：於試驗室內利用瓜類之摘葉培養瓜類露菌病菌（*Pseudopenospora cubensis*），由於摘葉之葉柄可以生出鬚根，吸收水分，延長摘葉之保鮮時間，使病原菌保存在摘葉一個月以上。一般在胡瓜摘葉後7—9天，洋香瓜7—13天即可生出鬚根。瓜類露菌病菌在15°C，20°C及25°C接種於瓜類葉片4—5天後即可產生病斑，而5—6天後即可產胞。瓜類葉片正背兩面在產生病斑及產胞上無差異。病原菌胞子囊發芽以10°C及15°C最適，在6小時後發芽率達95.18%及97.52%，高溫時發芽率降低。胞子囊釋放游走子之數目以7個最多，3個及11個者最少，平均爲6.7個。室內瓜類露菌病之接種步驟可參考本文圖2方法行之。

瓜類露菌病係由 *Pseudoperonospora cubensis* 引起之病害，在本省於胡瓜、洋香瓜及其他瓜類產區發生普遍而且嚴重。目前對於本病之防治唯賴施用農藥，但施用農藥亦常不能達到預期的防治效果，故是否病原菌產生抗藥性[4,5]或有其他原因均待探討。由於本病原菌係絕對寄生菌，不能在人工合成之培養基上培養，致使有些室內研究工作受到限制。筆者[1]發現利用瓜類葉片之摘葉在培養皿內培養時，可由葉柄處生出鬚根，吸收水分，延長保鮮期間，有利於病原菌之接種及保存。現僅就本病在室內接種方法及步驟寫成報告，供作參考。

材料與方法

1.供試植株培育

胡瓜（豐中種苗5號）及洋香瓜（日陞）種子浸於水中，24小時後取出放在培養皿內之濾紙上，濾紙加無菌水，使其保持濕度。然後放在25°C之定溫箱中，經72小時後，種子發芽，取出種於直徑10公分之植缽中。植株置於25°C之室內，每天早上6時到下午6時以40W的植物生長燈照射，經20—25天即可供摘葉使用。生長期間需注意白粉病（Powdery mildew）之防治及徒長的抑制。白粉病以25%蠣離丹（Morestan）可濕性粉劑1000倍每隔15天噴灑一次，徒長之抑制以生長抑制劑CCC（2-chlorethyltrimethyl-ammonium chloride）2000倍每隔10天噴灑一次。植株培育60天以後即淘汰。

2.葉序生根試驗

從植株頂端展開葉由葉柄處向下剪下第1，第2及第3葉片，在葉柄包以吸滿無菌水之棉花塊，然後放在直徑10公分培養皿內之濾紙上，濾紙加無菌水，以保持濕度，最後培養皿置於25°C之定溫箱中，每葉序3葉片，重複3次。

3.摘葉葉柄生根與溫度之關係

由植株頂端向下第2葉片，由葉柄處剪下，在葉柄處包以吸滿無菌水之棉花塊，然後放在直徑10

1.臺灣省農業試驗所　研究報告第1364號。

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公分之培養皿內之濾紙上，濾紙加無菌水，以保持濕度，最後將培養皿放在 10，15，20，25，30 及 35°C 之定溫箱中，每不同溫度 3 葉片，重複 3 次。

4.瓜類露菌病病斑形成及產胞與溫度之關係

由田間採回之瓜類露菌病葉片以洗滌瓶用無菌水將葉片表面之胞柄及胞子囊冲洗掉，然後將葉片放在培養皿內之濕濾紙上，置於 25°C 之定溫箱中，經24小時後取出病葉，將新生成胞子囊之葉片切成 1 公分正方之小片放在試管之無菌水中振盪，做成胞子懸浮液（4×10^4/ml）以小型噴霧器噴於葉片之背面，接種後之葉片放在培養皿內之濾紙上，加入無菌水，以保持濕度，最後培養皿置於10，15，20，25，30及 35°C 之定溫箱中，觀察病斑形成及產胞所需日數，每不同溫度每次 3 葉片，重複 2 次。

5.摘葉葉片正面及背面病斑形成及產胞所需日數

如試驗 4 之方法，將胞子懸浮液噴於摘葉葉片之正面及背面，接種後置於 20°C 之定溫箱中，觀察病斑形成及產胞所需日數，每處理 5 葉片，重複 2 次。

6.瓜類露菌病菌胞子囊在不同溫度下發芽之情形

將接種後 1 週之洋香瓜病葉上之胞子囊以洗滌瓶裝無菌水冲掉以後，放置在 25°C 之定溫箱 1 天後，以刀片切成 1 公分正方之病葉及胞子囊，置於試管之無菌水中，然後放在振盪器上，洗下胞子囊，做成胞子懸浮液，以吸管吸取少量之胞子懸浮液置於凹形之載玻片上，再分別放在10，15，20，25，30及 35°C 之定溫箱中，經 2，4 及 6 小時後取出，在150 倍之顯微鏡下觀察，每玻片隨機鏡檢 3 視野，記錄胞子囊發芽率。本試驗每溫度玻片 3 片，每次18片，重複 3 次。

7.露菌病菌胞子囊釋放游走子之數目

以吸管吸取胞子懸浮液滴在凹形載玻片上，以蓋玻片蓋上，放在 25°C 之定溫箱中 1 小時後取出在150倍之顯微鏡下觀察單個胞子囊釋放游走子之數目。

結　　果

1.葉序生根試驗

胡瓜葉片經 7 — 9 天，洋香瓜經 7 —13天即由葉柄處長出鬚根，鬚根可在濾紙上生長蔓延，胡瓜葉片在培養皿內可保持新鮮綠色 3 — 6 週，而洋香瓜可保持 2 — 4 週，葉序第 1 、第 2 及第 3 葉片在生根及保持綠色無甚差異（表 1）。

表1. 瓜類摘葉葉柄生根及保持綠色之日數 (25°C)[1]

Table 1. Days for root formation and greenish color maintained on the detached leaves of cucurbits.

葉序 Leaf position from top	胡瓜 *Cucumis sativus* L.	洋香瓜 *Cucumis melo* L.
1	7—9[2] (21—42)[3]	7—13 (14—30)
2	7—9 (20—40)	7—13 (15—32)
3	7—9 (22—45)	7—13 (13—30)

1 每處理 3 葉片，重複 3 次。Three leaves for each treatment with three replications.

2 生根所需日數。Days for root formation.

3 保持綠色期間（⅔葉片綠色，⅓葉片變黃）。Duration for greenish color maintained (2/3 of leaf remained in green and 1/3 of leaf was yellowing).

2.摘葉葉柄生根與溫度之關係

不同溫度對生根的影響，胡瓜及洋香瓜均以 30°C 最快，其次爲20及 25°C，胡瓜在 15°C生根最慢。胡瓜在10及 35°C 不生根，洋香瓜在10，15及 35°C 均不生根（表 2）。

表2. 溫度對瓜類摘葉葉片之生根與保持綠色之影響

Table 2. Effect of temperature on the root formation and green maintenance of detached leaves of cucurbits.

溫度 Temperature (°C)	生根所需日數 Days for root formation		綠色維持日數[2] Days of green maintenance	
	胡瓜 *C. sativus* L.	洋香瓜 *C. melo* L.	胡瓜 *C. sativus* L.	洋香瓜 *C. melo* L.
10	—[1]	—	10 — 19	14 — 19
15	14 — 20	—	21 — 30	10 — 15
20	7 — 9	8 — 13	22 — 47	15 — 30
25	7 — 9	7 — 13	21 — 45	15 — 30
30	5 — 7	5 — 10	18 — 25	14 — 21
35	—	—	5 — 6	5 — 6

1 不生根。No root formation.

2 綠色標準：⅔以上之葉面積爲綠色。Green was based on 2/3 or more of the leaf area was green.

3. 瓜類露菌病病斑形成及產胞與溫度之關係

胡瓜或洋香瓜之葉片在15，20及 25°C 接種後 4 — 5 天可形成病斑，在15及 20°C 經 5 — 6 天，在 25°C 經 6 — 8 天後產胞，但在10，30及 35°C 不產生病斑，亦不產胞（表 3）。

表3. 瓜類露菌病菌 (*Pseudoperonospora cubensis*) 接種在瓜類病斑形成與產胞所需日數與溫度之關係[1]

Table 3. Effect of temperature on lesion formation and sporulation of *Pseudoperonospora cubensis* on the detached leaves of cucurbits

溫度 Temperature (°C)	病斑形成所需日數 Days for lesion formation	產胞所需日數 Days for sporulation
10	—[2]	—
15	4 — 5	5 — 6
20	4 — 5	5 — 6
25	4 — 5	6 — 8
30	—	—
35	—	—

1 正處理 3 葉片，重複 2 次。Three leaves for each treatment with two repliicatons.

2 不形成病斑及不產胞。No lesion formation and no sporulation.

4. 摘葉葉片正面及背面病斑形成及產胞所需日數

葉正面及葉背面在 20°C 下病斑形成所需日數均爲 4 — 5 天，產胞所需日數亦均爲 5 — 6 天，故葉正面及背面在病斑形成及產胞均無差異。

5. 瓜類露菌病菌胞子囊在不同溫度下發芽之情形

胞子囊在不同溫度下之發芽以 10及 15°C 發芽率最高，在 10°C 時經 2 小時即有73.68%發芽，

6小時後發芽率達95.18%，在15°C經2小時有59.68%發芽，6小時後發芽率達97.52%，但在20°C下經6小時僅24.35%發芽，溫度越高，發芽率越低（表4）。

表4. 瓜類露菌病菌胞囊在不同溫度下之發芽率（%）*

Table 4. Percent germination of sporangium under different temperatures.

溫度 Temperature (°C)	時間 Time		
	2 小時 (hr.)	4 小時 (hr.)	6 小時 (hr.)
10	73.68	91.98	95.18
15	59.68	94.85	97.52
20	12.33	15.41	24.35
25	7.15	7.89	8.00
30	1.79	4.44	6.74
35	0.34	2.64	4.70

* 三次之平均值 Average of three replications.

6.露菌病菌胞子囊釋放游走子之數目

在所觀察的35個胞子囊所釋放之游走子數目，其中以釋放7個游走子者最多，再爲6個，而以釋放3個及11個者最少，平均爲釋放6.7個（圖1）。

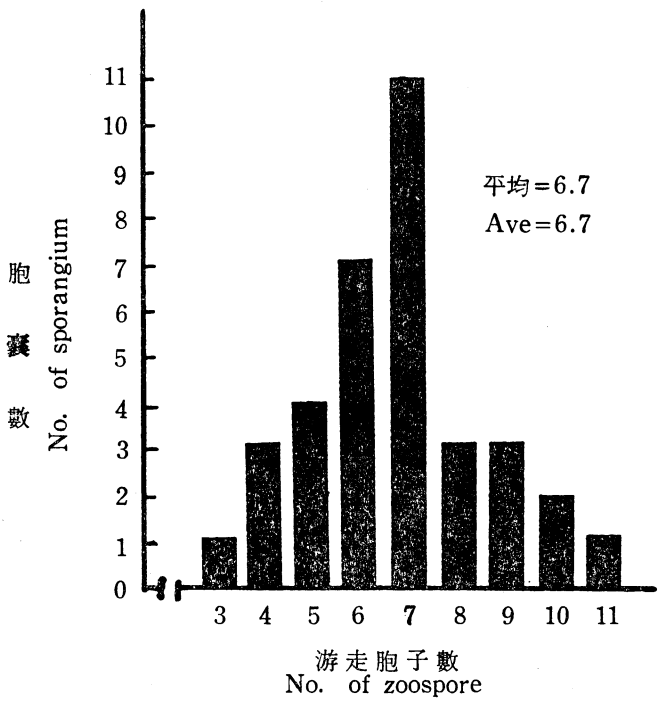


圖1. 游走胞子囊釋放之游走胞子數

Fig. 1. Number of zoospore released by sporangium

討　　論

瓜黑露菌病在本省瓜類栽培地區，如雲林縣水林鄉，嘉義縣太保鄉及臺南縣七股鄉等地，發病普遍而且嚴重，往往導致減產，造成損失。目前防治本病的方法僅靠農藥防治，但農民在使用農藥以後常常抱怨防治效果未達理想。根據推測，或有病原菌產生抗藥性的可能，因爲瓜農在病害發生期間，常縮短施藥間隔，並增加施藥次數，尙不能抑制病勢的進展，此有待進一步探討。有關病原菌的生態已有綜合性的報導[3]，但由於病原菌爲絕對寄生菌，無法在人工合成之培養基上培養，所以室內研究常受到限制。本接種方法甚爲簡單，摘葉在培養皿內可由葉柄生出鬚根，吸收水分，延長保綠期間，使病原菌在摘葉上達一個月以上，然後又可再移植到新葉片上，如此病原菌可長久保存在培養皿內，其接種步驟可綜合如圖2。

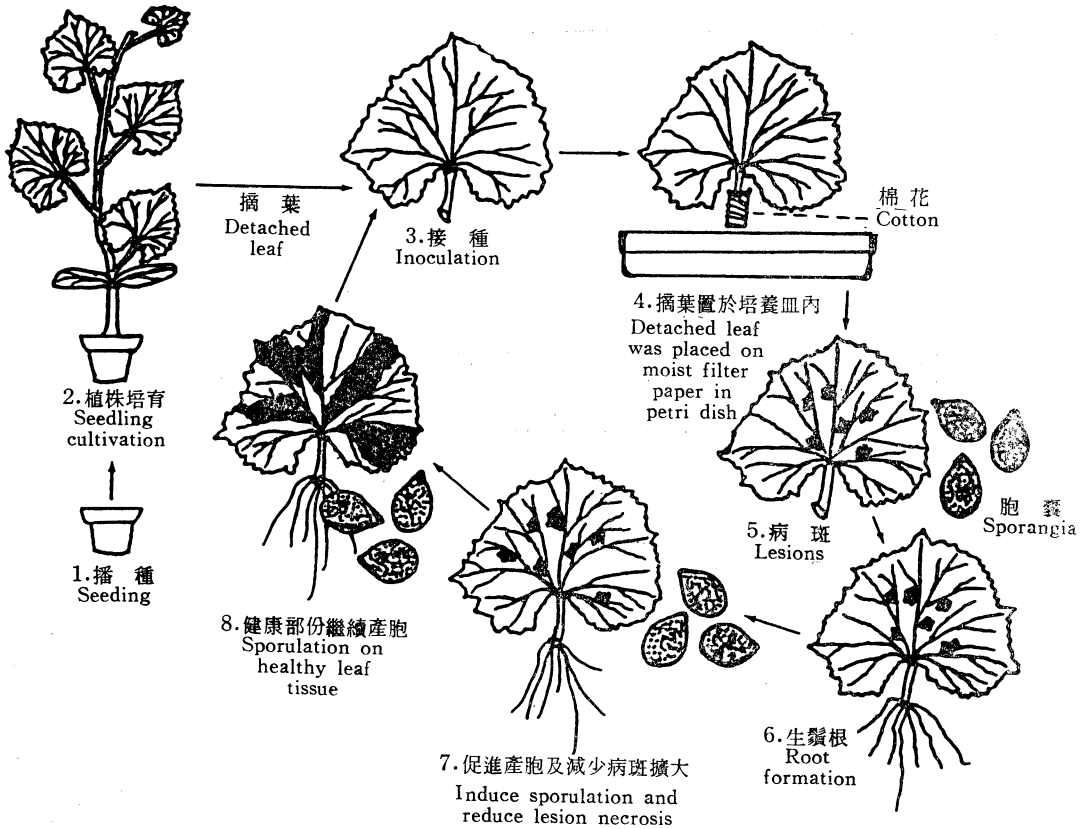


圖2. 瓜類露菌病室內接種步驟

Fig. 2. Procedures for inoculation of downy mildew on cucurbits

1.播種。2.植株培育：於室內 25°C，每天以 40W 植物燈照射12小時，經20～25天可供摘葉。生長期間需注意白粉病防治及徒長的抑制，白粉病防治以25%蝴離丹（Morestan）可濕性粉劑 1,000 倍每隔 15 天噴灑一次，徒長之抑制以生長抑制劑 CCC（2-chloroethyl trimethylammonium chloride）2.000倍每隔10天噴灑一次。植株培育60天即淘汰換新。3.摘葉接種。4.摘葉置於殺菌之培養皿內，葉柄覆蓋棉花塊，放二張濾紙於培養皿內，並加無菌水，以保持濕度。5.於 25°C 下經4～5天產生病斑及胞囊。6.經七天葉柄基部長出鬚根，其功用可延長葉片於培養皿內之保鮮期間。7.移至 15°C 定溫箱內，可防止病斑快速擴大，並可促進產胞。8.於 15°C 定溫箱內移至 25°C 1天可使病斑擴展至健康部分，並繼續產胞。

胡瓜及洋香瓜摘葉葉柄生根之溫度範圍，前者在 15—30°C，後者在 20—30°C，在 10°C 下兩者葉片均有凍傷的現象，在 35°C 下則葉片黃化甚速。產胞能力根據報告 (2) 以 15°C 或日夜爲 20：15°C 時產胞最高，而本試驗結果顯示在 15—20°C 產生病斑及產胞最好，但在10，30及 35°C 則不發病亦不產胞。一般在田間產胞主要在葉背面，但在試驗室行人工接種時葉正面及葉背面在發病及產胞上並無差別。病原菌胞子囊之發芽率以10及 15°C 最好，溫度越高，發芽率越低，胞子囊所釋放之游走子數目以 7 個爲最多，平均爲6.7個。

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Inoculation of Downy Mildew on Cucurbits under Laboratory Conditions [1]

W. H. Tsai [2]

Summary

Inoculation of downy mildew (*Pseudoperonospora cubensis*) on detached leaves of cucurbits were studied. Due to the root formation on the petioles of the detached leaves to absorb water, the detached leaves prolonged the fresh period in the petri dishes. Hence, the pathogen could be maintained on the detached leaves over one month. In general, root formation on the detached leaves was, observed 7—9 days on *cucumis sativus* L. and 7—13 days on *cucumis melo* L. The lesion formed at 4—5 days and sporulation at 5—6 days after seperated inoculation under 15, 20 and 25°C. No difference was observed on lesion formation and sporulation on both upper and lower leaf surfaces Lesion formation and sporulation were observed 4—5 days and 5—6 days after inoculation, respectively. The optimum temperature for sporangium germination were observed at 10°C and 15°C after 6 hours of incubation, the percentage of germination were 95.18 % and 97.52%, respectively. Germination decreased at higher temperature. Most sporangium produced seven zoospores with an average number of 6.7, a few sporangia produced 3 or 11 zoospores. The procedures for inoculation of downy mildew on cucurbits under laboratory conditions are shown on Fig. 2.

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Differential Preference of the Flower-Dwelling Thrip, *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae), to Some Gladiolus Cultivars[1]

Jeng-Shong Chen and Paul Kang-Chen Lo[2]

Abstract: Flower-dwelling thrip, *Thrips hawaiiensis* (M.) appeared to have certain appetite for flowers of some cultivars of gladiolus. Cultivar T512 was the most favorite one for this thrip. White Prosperity and T609 also had good performance, but the other four cultivars, Red Beauty, Albert, Applause and Peter Pears seemed to have little seduction for the thrip. Experiments were conducted both in the field and laboratory. All data were shown as No. of thrips/flower or No. of thrips/spike. The floral scent was proved to be a major factor influencing the thrip's choice of cultivars of gladiolus. In addition, color hue could be another improtant factor. Fully blooming gladiolus flowers had potent attraction to the thrip, and their performance was proportional to the degree of development of flower buds. The conspicuous color contrast, floral scent, floral shape and nectar of an inflorescent gladiolus flowers in concert played important roles in guiding the thrip flying toward them. These attraction factors of gladiolus flowers to the thrip are discussed.

Introduction

Cut flower is one of the exportable produces of Taiwan. The climate of this island is suitable for year-around cultivation of gladiolus (Wang 1982) and the consumption of cut flowers has increased considerably in the past few years. Therefore gladiolus has received more and more attention. Several insect pests on gladiolus including three flower-dwelling thrips, *Thrips hawaiiensis* (M.), *Frankliniella formosae* M. and *Haplothrips chinensis* P. have been reported in Taiwan (Wang 1982).

Color hues (Gibb 1950; Trimble & Brach 1985; Bhuyan & Das 1985; Katsoyannos et al. 1985), brightness (Katsoyannos et al. 1985), scents (Kullenberg et al. 1984; Numata et al. 1985; Hodgson & Elbakhiet 1985; Young 1986), texture (Lovell 1918) and shape

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(Proctor & Yoe 1972; Hodgson & Elbakhiet 1985) of the plants, play important roles as insects seek their hosts for feeding, shelters, and oviposition sites.

Insects would not visit flowers unless some sort of reward is held out to them, and the reward offered by many plants to insect visitors is nectar (Gibbs 1950). Flowers with nectaries are likely to be frequented by the flower-dwelling or visiting insects for their nectar and pollens, while those without would be visited only for pollens. Flowers are also occasionally visited by insects seeking shelter or making mistaken landing (Proctor & Yoe 1972).

The floral scents of anisaldehyde significantly increased the catches of seven species of flower-dwelling thrips (Kirk 1985). This experiment on attraction of *Thrips flavus* by gladiolus of different colors indicated that color was one of the factors influencing *T. flavus*'s choice of flowers. We also found *T. hawaiiensis* (M.) had the tendency to appear more frequently on some cultivars of gladiolus. This paper describes the phenomenon and the distribution of the thrip on gladiolus inflorescences. The ways of attraction are also discussed.

Materials and Methods

Nine cultivars of gladiolus (Table 1) were grown twice in a random manner in TARI experimental field in September and December, 1986. The bulbs were dipped in 1,000X benomyl solution for 1 hour before planting. No pesticide was applied in the growing period. Plants were left to natural infection by *T. hawaiiensis* (M.) in the field Observations were made when the plants began to bloom.

Table 1. Color Hues of the 9 Cultivars of Gladiolus Used

Cultivar	Color Hue
Albert	Crimson with red blotch on the lower petals
Applause	Rosy with red flecks on the lower petals
Blue Isle	Violet with a white band in the middle of each petal
Nymph	White with crimson flecks
Peter Pears	Pale orange-pink with creamy ill-defined blotch on lower petal which were slightly streaked with orange-scarlet
Red Beauty	Blood-red with velvet texture
T512	Pink with slightly rosy flecks in the center of the flower which had white band in the middle of each petal
T609	Lilac with white flecks at the center of the flower
White Prosperity	Snow white with a violet strip on the lower petals

Experiments I, II-1 and III were conducted in the field, II-2 and IV in the laboratory. The performance of attraction is expressed as No. of thrips/flower or No. of thrips/spike. Data were analyzed by Duncan's multiple range test.

〔Experiment I〕 *Distribution of the Thrips on Gladiolus Inflorescence*

Flowers and buds on an infloresence were classified according to the degree of development: (1) full-bloom stage; (2) half-bloom stage, the length of visible petals were about 6 to 8cm; (3) bud stage I, petals from 3 to 6cm were seen; (4) bud stage II, petals from 0 to 3cm could be observed, and (5) bud stage III, no petal was visible. Flowers and buds of the 9 cultivars were picked and the number of adult *T. hawaiiensis* (M.) was counted. These plants were sampled at random in 5 repetitions on different days with sunshine.

〔Experiment II〕 *Cultivar Preference of the Thrips*

II-1. Field Experiment

Some cultivars, Albert, Applause, Peter Pears, Red Beauty, T512, T609 and White Prosperity, were chosen to observe the differential preference of the thrip in the field. Gladiolus flowers at full-bloom and half-bloom stages were picked and dissected to count the number of the thrips. Five repetitions were conducted on sunny days.

II-2. Laboratory Experiment

Spikes of Albert, Applause, Peter Pears, Red Beauty, T512, T609 and White Prosperity were cut when the petals were still in the buds. Those spikes without detectable *T. hawaiiensis* (M.) were randomly put in containers and moved to a closed room with sunlight shining from a window. When they began to bloom, adult thrips from the field were introduced at the distance approximate 5m away. The inflorescent flowers were dissected for the thrip count after 72 hours. Three repetitions were conducted. Room temperature was 24-28°C.

〔Experiment III〕 *Attraction of the Thrips by the Color Hues of the petals of Six Cultivars*

Six cultivars, Albert, Applause, Peter Pears, T512, T609 and White Prosperity were used in this experiment. Thirty spikes per cultivar were sampled in the field at random. Petals of inflorescent flowers were deprived, and only corolla tubes, stamens and pistils were kept. Flowers still in the buds were removed. For the control, petals were kept. Observations were made and number of *T. hawaiiensis* (M.) recorded after 48 hours. Four repetitions were conducted.

〔Experiment IV〕 *Attraction of the Thrips by the Floral Scent of the Cultivars*

Spikes of Albert, Applause, Peter Pears, Red Beauty, T512, T609 and White Prosperity cultivars, obtained as experiment II-2, were put in a dark room. When the gladiolus began to bloom, adult thrips were introduced as in the experiment II-2. After 72 hours, the inflorescent flowers were picked and dissected. Three repetitions were carried out and the room temperature was 25-27°C.

Results

Most of the adult thrips, *T. hawaiiensis* (M.), were found in the inflorescent gladiolus flowers. The more the flower buds developed, the more attractive they were. Inflorescent flowers were noticed to be the most important source of attraction to the thrip, 4.94 thrips/flower was observed, and there was ca. 1 thrip/semi-blooming flower. On average, almost no thrips were found in bud stages. And buds without petals broken

out were the least effective, and none was found (Table 2).

Table 2. Distribuion of Thrips, *T. hawaiiensis* (M.) on the Inflorescence of Gladiolus

Development Stage	No. of Flowers Examined	No. of Thrips[2]	No. of Thrips/Flower
Full-Bloom	711	3,512	4.94a[3]
Half-Bloom	427	410	0.96b
Bud[1] (I)	334	43	0.13c
Bud (II)	453	20	0.04c
Bud (III)	394	0	0 c

1. Classified According to the Length of the Visible Petals: I. 3-6cm; II. 0-3cm; III. Nothing
2. Only Adult Thrips Were Counted
3. Means with the Same Letter Were Not Significantly Different at 5% Level

Half-blooming gladiolus were less attractive to adult *T. hawaiiensis* (Table 3). The result repeated the observation of table 2. The cultivar of T512 appeared to be the most attractive of all, 1.08 thrips/flower. White Prosperity and T609 had some attraction, with 0.84 and 0.62 thrips found per flower, respectively. The thrip seemed to have no appetite for the other cultivars.

Table 3. Cultivar Preference of Thrips, *T. hawaiiensis* (M.) on Gladiolus in the Field

Cultivar	Half-Bloom			Full-Bloom		
	No. of Flowers Examined	No. of Thrips[1]	Rate[2]	No. of Flowers Examined	No. of Thrips	Rate
Albert	136	19	0.14e[3]	152	135	0.89c
Applause	233	49	0.21de	168	99	0.59c
Peter Pears	195	27	0.14e	459	152	0.33c
Red Beauty	149	56	0.38d	158	66	0.42c
T512	204	221	1.08a	195	2,012	10.32a
T609	129	80	0.62c	122	467	3.83b
White Prosperity	188	158	0.84b	145	289	1.99c

1. Adult Thrips Were Recorded Only
2. No. of Thrips/Flower
3. Means with the Same Letter Were Not Significantly Different at 5% Level

Inflorescent flowers of cultivar T512 again showed strong attraction, 10.32 thrips/flower, to adult *T. hawaiiensis* among the records of the seven cultivars in the field test (Table 3). Swarms of the thrips gushed out when the flowers of T512 were being dissected. Besides, T609 also showed intermediate attraction. Cultivar White Prosperity was a little more inviting to the thrips than the other four, yet the difference was not significant.

The fascination of gladiolus cultivars to the thrips in the laboratory was considerably less than that in the field (Table 4). But cultivar T512 was still the most seductive one, T609 the next and White Prosperity the third. This result was quite similar to that obtained in the field experiment (Table 3).

Depriving the petals from gladiolus flowers decreased the attracting effect by 8-33 folds (Table 5). Corolla without petals of T512, T609 and White Prosperity were still potent to attract the thrips, while Albert, Applause and Peter Pears became less attractive to the thrips.

In the dark, White Prosperity and T512 were equally more attractive to the thrips than the other remaining 5 cultivars. T609 had medium effect but the other four gave poor performance (Table 6).

Table 4. Cultivar Preference of Thrips, *T. hawaiiensis* (M.) on Gladiolus in the Laboratory

Cultivar	No. of Flowers[1] Examined	No. of Thrips[2]	No. of Thrips/Flower
Albert	132	24	0.18cd[3]
Applause	119	12	0.10de
Peter Pears	108	18	0.17d
Red Beauty	127	9	0.07e
T512	136	84	0.62a
T609	120	45	0.38b
White Prosperity	135	36	0.27c

1. Full-Bloom
2. Only Adult Thrips Were Recorded
3. Means with the Same Letter Were Not Significantly Different at 5% Level

Table 5. Attraction of Thrips, *T. hawaiiensis* (M.) by the Color Hues of the Petals of Six Cultivars

Cultivar	Without Petals			With Petals		
	No. of Spikes[1] Examined	No. of Thrips[2]	Rate[3]	No. of Spikes Examined	No. of Thrips	Rate
Albert	120	31	0.26bc[4]	120	378	3.15c
Applause	120	20	0.17c	120	342	2.85c
Peter Pears	120	36	0.30b	120	296	2.47c
T512	120	75	0.63a	120	2,481	20.68a
T609	120	81	0.68a	120	1,953	16.28b
White Prosperity	120	76	0.63a	120	2,127	17.73b

1. Flowers in Bud Stages Were Removed
2. Only Adult Thrips Were Record
3. No. of Thrips/Spike
3. Means with the Same Letter Were Not Significantly Different at 5% Level

Table 6. Attraction of Thrips, *T. hawaiiensis* (M.) by the Floral Scent of the Cultivars in a Dark Room

Cultivar	Flowers[1] No.	Thrips[2] No.	Rate (Thrips/Flower)
Albert	113	7	0.06c[3]
Applause	147	18	0.12c
Peter Pears	130	13	0.10c
Red Beauty	116	8	0.07c
T512	126	42	0.33a
T609	102	24	0.24b
White Prosperity	116	43	0.37a

1. Full-Bloom
2. Only Adult Thrips Were Recorded
3. Means with the Same Letter Were Not Significantly Different at 5% Level

Discussion

During this investigation, many insects have been found on gladiolus inflorescences, such as thrips, aphids, leaf beetles and mirid bugs, with *T. hawaiiensis* (M.)as the major flower-dwelling insect of gladiolus. Insects usually gather around the center of the corolla, many are even drowned in the nectar. The basal part of the corolla tubes of the flowers seemed to be the most favorite site for *T. hawaiiensis* (M.), the stamens and pistils the next, and the sepals the least. Most of the immature thrips dwell in the space between the sepals and the petals. Our observation (Table 2) on the distribution of the thrip indicated that adult *T. hawaiiensis* (M.) were inclined to visit fully blooming gladiolus flowers, and disliked flowers in bud stages. Swarms of flower-visiting insects are generally present in inflorescent gladiolus flowers and thus pollinate the flowers. Thrips have been reported to be pollinators of beet (Imms 1924), *Calluna vulgaris*, *Erica tetralix* and *Hypochaeris radicata* (Hagerup 1950). *Taeniothrips ericae* has been reported to complete its whole life-cycle in the flowers of *Calluna* and often cause pollination (Hagerup 1950). In this respect, thrips may be considered as beneficial insects on the other hand.

Animal-pollinated plants generally provide a convenient alighting place, food, conspicuous coloring and scent to attract the pollinators. The size, shape and arrangement of flowers are involved in insect attraction (Proctor & Yoe 1972). Nectar is one of the important factors of attraction as many insect-pollinated flowers contain nectaries (Wilson & Loomis 1967). Carbohydrates, the main constituents of nectar, are the principal food requirement of adult winged-insects (Proctor & Yoe 1972). The rate of nectar-production and the sugar concentration sometimes vary with the age of the flowers (Jaeger 1957), and those flowers which were highest on a plant or in an inflorescence, produce the least nectar (Proctor & Yoe 1972). According to our preliminary observation in this study, large amount of nectar was found in the inflorescent gladiolus flowers,

much less in the semi-blooming ones, and none in the buds. This could be one of the reasons why the performance of attraction was proportional to the degree of development of the flower buds.

Insect-pollinated flowers are usually brightly colored or scented, sometimes both. Some experiments have confirmed the general belief that insects are attracted to flowers by colors and scents (Wilson & Loomis 1967). According to a well-known principle of physics , a color viewed in the presence of other hues will be more brilliantly than it is viewed alone (Lovell 1918). Studies on effect of background contrast on host-seeking of *Tabanus nigrovittatus* M. pointed out that the attraction of *T. nigroviatttus* M. to gray test panels was significantly greater when contrast against this gray background panel was high (Allen & Stoffolans, Jr. 1986). There were more than one color in an inflorescent gladiolus flower. The brilliant spots or bands near the center of the corolla (Table 1) made the flower more conspicuous than the non-fully blooming ones and thus might seduce more thrips. Inflorescent gladiolus flowers might release some special scents which attract the thrips to fly toward them. Some species of flowers, for example, *Caryophyllacece*, produce scent to attract insects only when their flowers are open (Proctor & Yoe 1972). Shape response of apterous *Myzus persicae* is reported to determine its flying orientation (Hodgson & Elbakhiet 1985). The floral shape is said to be a critical factor for the host-seeking of some tropical bees (Proctor & Yoe 1972). The thrips might also be able to perceive the shape of a fully blooming gladiolus flower.

Flowers usually owe their conspicuousness to a bright-colored corolla or calyx. The bright hues of flowers serve as signals or flags to attract the attention of insects living on pollens and nectar (Lovell 1918). Some flower-visiting insects manifest a preference for certain colors, as honey-bees for blue, butterflies for red, hoverflies for yellow, and carrion-flies for lurid purple (Lovell 1918). *Thrips flavus* like bright red gladiolus flowers most, and white ones the least (Lal & Chandra 1972-74). *T. hawaiiensis* (M.) seemed to like the color hues of violet, lilac, pink and white as they visited the gladiolus cultivars with these colors more frequently (Table 3 & 4). All the efforts to investigate the importance of the color hues alone failed due to the interference of the floral scent. Crepe-paper-made flowers were used to attract the thrip, but it was hard to imitate the combiation of color hues in gladiolus flowers, and the attraction was poor. Therefore, not enough direct evidence was obtained to define the role of color hues in the favorite-flower-seeking of the thrip. As it was not easy to determine which factor was involved in thrip attraction in wide open fields, we started laboratory experiments. The attraction of *T. hawaiiensis* (M.) in the laboratory was far less than that in the field (Table 4). Lack of air flow could be an obstacle to the difusion of floral scent. The sunlight came in from one side only could be another artificial factor which disturbed the moving action of the thrips. Some thrips were found on windows and walls probably because they had been confused. But their preference for certain cultivars was nevertheless similar to what was observed in the field. Experiment III was an effort to judge the importance of combined effect of color hues, floral scent and shape. The gladiolus flowers which had lost their petals lost their seduction to the thrips (Table 5). Similar

phenomena were observed on honey-bees, which ceased to visit the flowers of common pear when their petals were deprived (Lovell 1918). As those petal-deprived-flowers still kept their scent and nectar, the decrease of attraction to the thrip should have something to do with the lack of color index and floral shape. But still few thrips could find the gladiolus flowers without petals. Floral scent should be an important factor as well.

Proctor & Yoe (1972) reported that scent was the means of guidance to beetles approaching flowers of *Arum* from a distance. Attractions were recognized as playing an important part in host-plant selection by *Eurema hecabe mandarina* larvae (Numata et al. 1985). Table 6 suggested that floral scent had a role in attraction. With nothing visible to the thrips, the floral color and shape became useless signals at all. Yet as some thrips still could locate the flowers of gladiolus, and even chose the favorite ones, indicating that the floral scent played an important role in favorite-cultivar-seeking of the thrip. Young (1986) reported that beetles flew to the inflorescence in darkness, suggesting that floral scents were attractants. Kirk (1985) reported that the floral scents significantly increased the attraction of some thrips. The floral scent of gladiolus was hard to detect by human nose, but certain insects were indeed capable of detecting very faint scents (Gibbs 1950). The cultivars T512, T609 and White Prosperity might have some special scents different from the other cultivars, which could guide more thrips flying toward them in darkness (Table 6), even in the abscence of floral petals (Table 5). Hoverflies, *Helophilus pendulus* and *Syrphus balteatus* approached the flowers of *Parnassia palustris* by visual preception, but at very short range, the floral scent induced the insects to alight (Daumann 1932, 1935). It was possible that color hues and their contrast, and floral shape of gladiolus might serve as visual signals guiding the thrips flying toward the inflorescence, and then the floral scent at a closer range attracted the thrips to alight on their favorite corolla.

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花薊馬對數種唐菖蒲品系偏好性之研究[1]

陳政雄　羅幹成[2]

摘　要

花薊馬，*Thrips hawaiiensis*（M.）對某些唐菖蒲品系之花朵有特殊的偏好性。無論是田間或者實驗室試驗均顯示花薊馬最喜歡T512品種。White Prosperity和T609亦有良好的引誘效果，但其它品系，如：Red Beauty, Applause, Albert和Peter Pears 的引誘效果不佳；結果以引誘率，薊馬蟲數／花或薊馬蟲數／花穗，表示之。當花薊馬在挑選某些唐菖蒲品系時。花的氣味是其判斷的主要因素之一。顏色可能也是重要因素。在唐菖蒲的花序中，盛開的花朵對花薊馬有强烈的引誘效果，但半開者和花苞則不太有吸引力。盛開花朵的形狀、鮮明的顏色對比、特別的氣味與花蜜可能是吸引大量的花薊馬飛向它們的主因。本文著重於探討這些引誘因素和花薊馬間的關係。

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星天牛為害印度棗調查及其防治試驗[1]

溫　宏　治[2]

摘要：南部地區印度棗遭星天牛〔*Anoplphora maculata* (Thompson)〕為害，隨樹齡之增長而增加，被害株率介於 4.23～18.75%之間，其中 5 ～ 7 年生棗樹被害率低於10%，10～13年生者介於 10～15%，15～20 年生高於 15%，樹幹遭星天牛幼蟲為害部位以離地面 45 cm 以下最多，樹齡愈長，被害部擴及枝幹。於 4 ～ 8 月間，每個月噴施一次 Furadan 40.64% F 稀釋100倍液於株幹上，可有效防止成蟲產卵為害。

印度棗（*Zizlphus mauritiana* L.）之栽培面積在本省約有1,000公頃，以南部地區較多，其中以高縣之大社、燕巢及田寮較為集中，民國73年初筆者至該地區調查番石榴害蟲時，發現附近棗園遭星天牛為害不輕，次年又經彼等鄉農會推廣股之說明及農民在農業技術諮詢座談會中，提出該蟲之為害及造成支幹枯萎現象已相當普遍，尤其於10～12月期間，凡遭星天牛為害之植株會造成嚴重落果，給農民增添許多困擾，目前農民除了用螺絲起子及利器穿刺幼蟲外，別無他法，如不予有效防治，再經數年，恐該地區棗園將深受其害。星天牛幼蟲俗稱「箍蟲」主要分佈於臺灣、中國大陸、海南島、韓國、琉球及日本。為害作物有柑桔、荔枝、木麻黃、柳、紫荇麻、樹豆、苦楝、黃枝花、無花果、桑、櫸等。(2) 以往本省南部以木麻黃及苦楝受害最重，最近幾年楊桃及荔枝受害亦相當普遍，其中於荔枝栽植 5 年以後開始被害，10 年生受害最為厲害，20 年以上之大荔枝樹有為害中段基幹之習性(1)。依此推測近年來棗樹紛紛遭天牛為害，與棗樹樹齡之增長有關，況且當地棗樹樹齡大部分已超過15年，星天牛為害似有嚴重之趨勢，防治工作實刻不容緩。本試驗期間配合當地受害果農協助調查蟲穴，並用螺絲起子挖刺幼蟲，減少為害，並初步進行藥劑防治試驗，茲將調查及試驗結果報告如下，俾供參考。

材料及方法

一、星天牛為害棗樹調查

於民國74年10至12月間，分別至大社鄉、燕巢鄉及田寮鄉等地，抽樣 2 — 3 處棗園調查，每處調查45～89株不等，凡株幹上有星天牛幼蟲為害之蟲穴（排出木屑），或已被農友用螺絲起子挖開者，均列為被害株。

二、星天牛為害部位調查

民國74年10～12月間於大社鄉選定 8 處棗園，調查包括 5 — 7 、10—13及15—20年生被害株各25株，每株由基幹離地45、46～100、101～150及151公分以上不同部位，分別計算星天牛幼蟲為害蟲穴，以資比較。

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三、藥劑防治試驗

本試驗於民國75年 4～8 月間於大社鄉神農村選定兩個約15年生相鄰棗園進行，試驗藥劑爲 Furadan 40.64% FP，分 100及 200倍二種濃度，每種濃度複分 1 個月及 2 個月各處理一次，與不施藥處理共 5 處理。每處理每小區 5 株（每株均選定未受天牛爲害株），重覆 4 次，按逢機完全區組排列，然後於 4 月 2 日、5 月 7 日、6 月13日、7 月 3 日及 8 月 2 日按各處理施藥，將藥液噴於株幹全身。於10月 3 日調查其防治效果，調查時凡株幹上有星天牛幼蟲爲害孔或豆粒大的瘤，並排出少許細小的木屑，均列爲害株。

結果及討論

一、星天牛爲害棗樹調查

有關南部棗樹遭星天牛爲害，以往發生輕微，而近年來却有增加的現象，被害株率介於 4.23～18.75%（表一），樹齡約 5－7 年生者，被害株率低於10%，而 10—13年生者被害株率介於 10～15%，15—20年生者被害株率高於15%，是故樹齡愈長，樹幹愈粗，遭星天牛攻擊之機會愈大，況且該地區棗樹樹齡大都超出15年，故星天牛之爲害有逐漸增加之趨勢，如不及時遏止，爲害必繼續蔓延增加。

表一、高雄地區星天牛爲害印度棗調查（74年10～12月）

Table 1. The investigation on infestation of *Anoplphora maculata* on indian jujube in Kaohsiung area (Oct. -Dec. 1985)

地點 Locality	日期 Date	樹齡 Age of plant (yr)	調查株數 Number of plant	爲害株數 Plant infested	被害株率 % of plants infested
大社 (Ta-she)	2/X	15—17	45	8	17.78
	3/XI	10—12	77	9	11.69
	15/XI	15—20	64	12	18.75
燕巢 (Yane-chaw)	15/XI	10—13	89	10	11.24
	18/XII	5—6	65	6	9.23
田寮 (Ten-liaw)	I3/XII	5—7	71	3	4.23
	30/XII	10—13	74	9	12.16

二、星天牛爲害棗樹部位調查

星天牛在本省一年發生一世代，其成蟲出現於 4～9 月間，其中以 5～7 月最多[2,6]，此時棗樹接枝不久，大部分母幹暴露於外，目標顯著，易引來天牛產卵。星天牛雌成蟲產卵時，係先將樹幹的樹皮用口器咬成一T字形之裂縫，然後調轉方向，以產卵管伸入裂縫處產卵，卵孵化後幼蟲隨即侵入棗樹幹的皮部爲害。幼蟲在樹皮內侵蛀勒皮部時，被侵蛀之樹皮，雖已枯死，但外觀上難予判斷。待幼蟲鑽入木質部時排出木屑，即可判定其爲害。目前一般棗樹主幹均粗大，幼蟲在樹皮內侵蛀情形，很少有環繞一圓環，而使植株致死現象，唯影響樹液輸送流動，降低棗樹之生長勢。一般而言以主幹較小之棗樹，被「箍」死之機會較多。幼蟲爲害部位如表二所示，以樹幹離地 45cm 以下最多，5－7 年生左右最顯著，10—13年生以後爲害部位擴及上部，至15年生以上，151cm 高之支幹仍發現被害。造成爲害部位上移現象，與成蟲產卵習性有關，而成蟲似乎有判斷力，知道樹幹可負荷供幼蟲寄生之能力，而選粗大之樹幹，供爲產卵處所，由於棗樹於採收後，均須剪除枝幹，只剩母幹，故其主幹較易粗大，而10～15年生之樹幹中段已相當粗大，足够其幼蟲食害，故成蟲產卵部位延伸向上發展，

致使後來幼蟲爲害擴及樹幹上部。

表二、星天牛幼蟲爲害棗樹部位調查（74年10～12月）

Table 2. The infested position of indian jujube by *Anoplphora maculata* (Oct. -Dec. 1985)

樹齡 Age of tree	孔數／25株 (Holes/25plants)	爲害率 (%) Infested rate (%) 樹幹離地部位 (cm) The position of branch from ground			
		0～45	46～100	101～150	151～
5—7年生 (5—7 years)	31	83.87	16.12	0	—
10—13年生 (10—13 years)	46	67.39	19.56	13.04	0
15年生以上 (Over 15 years)	58	65.52	24.14	8.62	1.72

三、星天牛藥劑防治試驗

有關星天牛之防治方法甚多，大致可分爲①捕殺成蟲②預防成蟲飛到樹幹產卵③殺死樹幹之幼蟲[1,7,8,9]，其中以噴藥預防成蟲飛來樹幹產卵效果較好，以往在其他作物上防治該蟲均用劇毒之 DDT 及阿特靈等有機氯劑[5,10,11]，自該等藥劑禁用後，目前僅在柑桔及木麻黃推廣 Furadan 40.64% F 防治該蟲。而本試驗係噴藥於全株幹防止成蟲飛來產卵，既或飛來產卵，於咬破樹皮時，亦會中毒而亡，試驗結果如表三，以 Furadan 40.64% F 100倍每個月施藥一次效果最佳，防治率達100%，200倍每個月施藥一次效果次之，無論100或200倍者每二個月施一次者效果均差，唯各施藥處理間差異不顯著。

表三、印度棗星天牛藥劑防治試驗結果（民國75年4～10月）

Table 3. Control of *Anorlphora maculata* on indian jujube by Furadan 40.64% F (Apr. -Oct. 1986)

處理 Treatment	被害株率 (%) % of plant infested[a] 重複 Replication				平均 Mean	防治率 Percent Control
	Ⅰ	Ⅱ	Ⅲ	Ⅳ		
1. Furadan 40.64%F 1：100 (one month interval)	0	0	0	0	0a	100
2. Furadan 40.64%F 1：100 (two month interval)	5	0	5	0	2.5a	83.33
3. Furadan 40.64%F 1：200 (one month interval)	5	0	0	0	1.25a	91.67
4. Furadan 40.64%F 1：200 (two month interval)	0	0	5	5	2.5a	83.33
5. Check	15	20	15	10	15b	—

綜觀上述試驗結果，由於南部印度棗之樹齡大都已逾15年，遭天牛爲害有逐漸加劇之趨勢，其爲害樹幹部位也因樹幹之增大而向上蔓延。防治該蟲可於每年4—8月間，每個月噴 Furadan 40.64% F 100倍一次，唯噴藥時可將樹幹分上、中、下三段，樹幹較小（約5年生左右）者，噴藥下段即可，樹幹中等（約10年生左右）者，噴藥中段及下段，樹幹粗大者（15年以上），則須噴及全株樹幹。

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Field Investigation of White Spotted Longicorn Beetle (*Anoplophora maculata*) on Indian Jujube and Control[1]

Hung-chich Wen[2]

Summary

White spotted longicorn beetle, *Anoplophora maculata* (Thompson) attacked the indian jujube, *Zizyphus mauritiana* L. was a positive correlation with age of trees in southern Taiwan. The percentage of infestations were below 10 % on 5—7 years old trees, 10 to 15% on 10—13 years old trees, and over 15% on 15—20 years old trees, respectively. The positions which the beetle's larvae took place on indian jujube trunks were mostly under 45cm from the ground and rised with the age of plants.

It can be efficiently prevented the adult of white spotted longicorn beetle from laying egg on the trunk of indian jujube, to spray carbofuran 40.64% F. which can be diluted 100 times with water, one a month from April to August.

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微小昆蟲卵之染色觀察法[1]

呂 鳳 鳴[2]

摘要：多種產卵於葉肉組織內之害蟲如潛蠅類、薊馬類其產卵量之觀察不易，本文用1%酸性洋紅液及乳酸酚—酸性洋紅液染色後，可在解剖顯微鏡下清晰觀察雌蟲產卵量。前者無毒且價廉，對埋於葉肉組織（厚、薄葉類）之卵的染色效果很好，其卵呈暗紅色；但其需較長之處理時間。後者配方爲酚：乳酸：蒸餾水：甘油以1：1：1：2之比例配或250ml之溶液，再加入0.25g之酸性洋紅；其染色效果更加明顯，卵呈紫紅色。但因其中酚具毒性及腐蝕性，且價格較昂，當需短時間內得知結果時，可使用水浴加熱法，適用於少量調查或薄葉類染色時，若大量調查或厚葉類染色時，爲恐加熱時間太長有欠安全，故改用浸泡法進行，個人可視情況加以選用。

近年來本省許多微小昆蟲如潛蠅類、薊馬類之經濟重要性逐漸升高，國人亦有研究其基本生活史及生態之報告[1,2,3,4]。但因此類蟲體微小，室內飼育不易，其卵粒又多埋於葉肉組織或花瓣中，致使肉眼不易觀察。以往一般用幼、若蟲之孵化數來推算其原母蟲之產卵量[10,12]，但因有的作物對卵之孵化有抑制作用[14,15]，因而無法確知雌蟲之產卵量。1942年Curtis以甲基紅（methyl red）進行馬鈴薯葉蟬，*Empoasca fabae*（Harris）卵之染色[5]，1967年 Everett及Trahan 將水稻植株浸泡在酒精中的方法來觀察稻象蚧 *Lissorhoprus oryzophilus*（Kuschel）之卵粒[6]，但浸泡時間一長植物組織和卵之顏色十分相近而不易區分，因而誤差很大，耗損工作人員精神及時間。1969年 Gifford和Trahan將1937年Goodey 提出植物根部線蟲之染色法[8]加以應用到稻象蚧及葉蟬卵之染色上[7]，1982年Parrella做蕃茄等潛蠅*Liriomyza tifolii*（Burgess）卵之染色[11]，其均以乳酸酚—酸性洋紅（Lactophenol-acid fuchsin）液做爲染色液，本文係將此法應用於瓜類、蔬菜類及果樹上潛蠅及薊馬類卵之染色；再與1986 Khan 及Saxena以1%酸性洋紅（acid-fuchsin）液來染兩種水稻飛蝨 *Nilaparvata lugens*（Stal.），*Sogatella furcifera*（Horvath）及一種葉蟬 *Nephotettix virescens*（Distant）之卵的方法[9]，加以比較應用。使埋於葉肉組織內害蟲類之卵清晰可見，方便對此類害蟲生活史方面之觀察與研究。

材料和方法

一、染色液種類及配方：

（A）1%酸性洋紅液（acid-fuchsin）

（B）乳酸酚—酸性洋紅液（Lactophenol-acid fuchsin）

酚：乳酸：蒸餾水：甘油＝1：1：1：2，配成250ml溶液後加0.25g之酸性洋紅。

---

1 臺灣省農業試驗所 研究報告第 1367 號。文承李主任錫山斧正試驗期間並承黃益美小姐之協助，謹表謝忱。

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二、方法及步驟：

(a) 薄葉類：

至田間採集有薊馬或潛蠅等爲害之苦瓜（葉、花）、甘藍菜、葱等，直接浸在1%酸性洋紅液或乳酸酚—酸性洋紅液中，前者浸泡1—2天；後者以水浴法加熱3～5分鐘，冲却後以水冲去多餘之染色液，在雙筒解剖顯微鏡（40X）下觀察。

(b) 厚葉類：

採集腹鉤薊馬，*Rhipiphorothrips cruentatus Hood* 之雌、雄成蟲並飼育於固定之夾式壓克力片中，數天後由懸掛於各種果樹（蓮霧、芭樂、楊桃、檬果等）上之壓克力片中取下各可能被產卵之葉片，攜回實驗室，進行染色。各種葉片每次做五片，重複多次。各葉片先以蒸餾水加熱5分鐘，移入95%酒精中泡1—3天，以除去葉綠素等，若酒精液色呈濃綠色則需更換之。以清水冲洗乾淨，再投入乳酸酚—酸性洋紅液浸泡2—3天，兩者方法分別染色後以清水冲去多餘之染色液，再用顯微鏡觀察之。

結果與討論

本試驗是先以乳酸酚—酸性洋紅液來進行葱薊馬 *Thrips tabaci* L. ，甘藍菜潛蠅 *Liriomyza brassica* (Riley) ，瓜類南黃薊馬*Thrips palmi* Karny等卵之染色，其結果見圖1（a、b、c）。

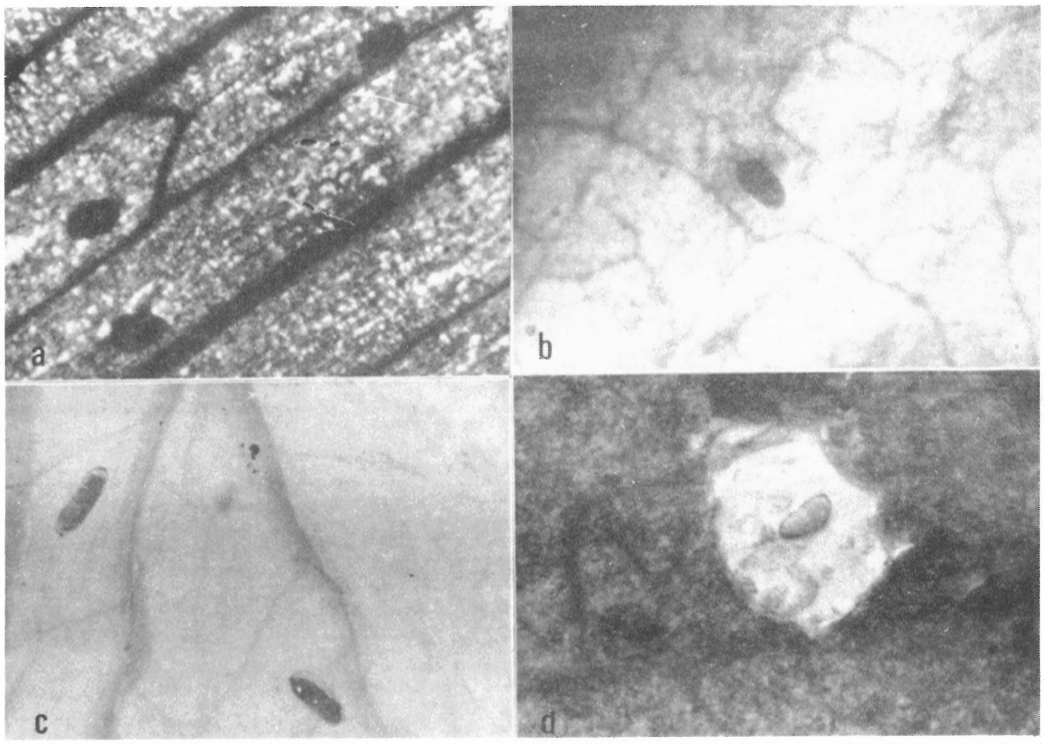


Fig. 1. Stain of different insect eggs imburied in plant tissues.
- a. Eggs of *Thrips tabbci* Lindeman stained with lactophenol acid fuchsin solution.
- b. Eggs of *Liriomyza brassicae* (Riley) stained with lactophenol acid fuchsin solution.
- c. Eggs of *Thrips palmi* Karny stained with lacophenol acid fuchsin solution.
- d. Eggs of *Rhipiphorothrips cruentatus* Hood stained with 1% aqueous acid fuchsin solution.

圖一、葉肉組織內卵染色之觀察。

染色後之葉片用底部打光之解剖顯微鏡（40X）即可輕易辨識各卵粒之位置及數目，若將上層葉肉挑開，更可見鮮明紫紅色之卵粒。薄葉類植物，在染色液中加熱的時間較短，僅3－5分鐘即可。但厚葉類植物如蓮霧等，需經酒精脫色等步驟，且在染色液中加熱時間較長約10—15分鐘，因其中酚具神經毒及腐蝕性[13]，而加熱時異味瀰漫，汚染空氣與環境，若大量調查時，使用加熱法對工作人員有所傷害，故宜選用浸泡法，將葉片投入乳酸酚—酸性洋紅液中浸泡2－3天再予觀察。或改用 Khan 及Saxana 之方法，用1％酸性洋紅液來浸泡2－3天，經此染色後之卵粒爲暗紅色，亦甚明顯，見圖1 d. 兩種染色液之比較見表一，如250ml之1％酸性洋紅液約需10元，而乳酸酚—酸性洋紅液則需180 元左右，兩者差異甚大。但因乳酸酚—酸性洋紅液比1％酸性洋紅液染之色效果更優；當在薄葉類染色時，以乳酸酚—酸性洋紅液加熱處理之時間較快，工作人員接觸之時間較短，欲很快得到清晰之結果，故可選用乳酸酚—酸性洋紅液來染色。若無急需之狀況，則用1％酸性洋紅液亦可。當厚葉類染色時，爲安全之計，若用乳酸酚—酸性洋紅液則用浸泡法爲妥，或用1％酸性洋紅液效果尙優，研究人員得視情況需要而作適當之選擇。此法尙可能適用於其它作物如茄子，花類等潛蠅或薊馬類產卵量之調查上。

表一、兩種卵染色液效果之比較

Table 1. Comparison of the two methods for staining insect eggs.

項目	染色液種類及方法		
	1％酸性洋紅液	乳酸酚—酸性洋紅液	
		水浴加熱法	浸泡法
1.染色效果	卵呈暗紅色	卵呈紫紅色	
2.染色液費用	較便宜N.T.$10元/250ml	較昂貴N.T.$180元/250ml	
3.毒性	無	酚具神經毒及腐蝕性	
4.染色時間	慢 {薄葉類浸1－2天；厚葉類浸2－3天}	快 {薄葉類加熱3－5分鐘；厚葉類加熱10—15分鐘}	慢 {厚葉類浸泡2－3天}
適用範圍	薄、厚葉類之調查或大量調查時用	少量調查及薄葉類調查時用	大量調查及厚葉類調查時用

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Different Staining Methods for Eggs of Diminutive insects

Feng-Ming Lu

Summary

Eggs of many leaf miners and thrips are laid in the plant tissues. We used two staining solutions in order to detect and count the eggs which are 1% aqueous acid fuchsin and lactopenol acid fuchsin. The preceding solution is cheaper and no poison but need long time to stain it. The other solution is more effective but costed higher and also poisoned to the human nervous system. There fore, we usually heat it in water bath for the rapid dipping of the thin leaf, and in immersion method of long period dipping for the thick leaf, it will decrease the damage of the toxic phenol. In conclusion, we select the proper staining method for the special purose. After treatment, the color of eggs became deep red or purple red that were easier to observe.

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