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中華民國植物病理學會一百零五年度年會論文摘要

Abstract of 2016 Annual Meeting of Taiwan Phytopathological Society

專題演講 Keynote speech

KS01 健康果園的永續經營－蔡東纂 (國立中興大學植物病理學系)

Sustainable management of a Health orchard－Tsay, T. T. (Dept. Plant pa-thology, National Chung Hsing Univer-sity)

果樹永續栽培法，首先得去除園區內主要病害病原菌及蟲害等有害生物，這是短程 (short term) 的作為，可以拮抗 (微) 生物或化學藥物清園。改善水分管理措施和設施，如排水溝及給水管路施設。再者，培育草生植被，草生植物在果園內有減少土壤沖蝕，增加土壤通透性及有機質含量，活化土壤內大、中型及微生物之族群社會生態，調節園區內溫度及水分，促進天敵之孳生等功能。草被植物的選擇以低矮型、地表固著性佳、無攀緣性、無刺、與其他雜草競爭性強、不開大型花，以免薊馬繁生、非豆科植物，減少蟎類孳生、非真菌，細菌，線蟲之寄主等性狀為佳，以禾本科及繖草科多年生草本植物為宜。草被植生，是果園內很有效土壤有機碳貯積 (soil organic carbon pools) 的工廠，更是自營 (autotrophic) 及異營 (heterotrophic)微生物棲息的基地。其三，以強腐生性微生物製造液態肥料，每月二次澆灌，除供果樹生育所需外，也供給土壤益生菌營養。強腐生菌須環境競爭優勢的拮抗微生物，俾利長久存活於果園中，也扮演植株健康之防護角色，這才是永續管理的長程 (long term) 作為。

通常，有機和永續的作物保護作為，以生物防治為主體。微生物對病害的防治分為特別與一般抑制(specific and general suppression)，前者靠微生物的寄生 (hyperparasitism) 及產生抗生物質 (antibiotics) 奏效，其效果較短，不穩定，花費高；不如化學藥劑的效果迅速，速率高且價格便宜。所以，針對主要病及害蟲，可便給施藥。一般抑制作為是長程施作，微生物發揮競爭生長空間，排拒或淨空有害病菌入侵及生長繁衍的機會，另外的功能是誘發植物體產生系統性抗病 (systemic resistance) 與促進植物生長 (plant-growth promotion)。就如人體罹病須即時服藥或手術切除患部，再者得調養生息，繼之養生頤天年，也有短長程療癒程序。

二十一世紀的農業，著重在生態建立，有健全的生態環境才有有機和永續農業存在的價值。根系的健康及壽命決定果樹的產值年限，歸根究底，就是培育及維持健康的抑病土 (suppressive soil)。一般衰弱的果園復健須三至五年時間，熱、亞熱帶常綠果樹，周年生長，休眠期短或不明顯，永續作為施作效果顯著。二十多年來，台灣的柑桔、梨、甜柿、番石榴、棗、酪梨、茶園等有許多成功案例，農友們得充實農業知識，果樹健康診斷正確，作好果園周年施作流程設計，善用資材，必有好收成。

KS02 Overviewing the estimates of plant disease severity: history, current development and future prospects－Chiang, Kuo-Szu (Department of Agronomy, National Chung Hsing Uni-versity, Taichung, Taiwan, 402) and Clive H. Bock (USDA-ARS-SEFTNRL, 21 Dunbar Road, Byron, GA 31008, USA)

Plant pathologists have been at-tempting to obtain quantitative infor-mation on disease severity (the area or proportion of plant tissues shown symp-toms) through visual assessment for over a century. Disease severity data is widely used for predicting yield loss, moni-toring and forecasting epidemics, for assessing crop germplasm for disease resistance, and for understanding fun-damental biological processes including coevolution. The disease severity data must be both accurate and reliable. If inaccurate or unreliable disease assess-ments are obtained, this could easily lead to faulty conclusions being drawn from the data, which in turn might lead to incorrect actions being taken in disease management. Thus, this lecture focuses on current developments in determining the ways to best minimize biases. We consider the effects of three significant factors – assessment method, rater bias, and experimental design on disease severity estimation.

First, what interval characteristics will be more appropriate than others for obtaining accurate, precise data that will minimize the risk of errors? Our results indicated that an amended 10% category scale with additional grades at low se-verities can be considered a good choice for assessing disease severity when use of a scale is preferred. Moreover, cate-gory intervals in the mid-range should not exceed

10% for disease severity ≤50%.

Second, the ramifications of rater bias effects on hypothesis testing demonstrated that the power of the hy-pothesis test is greatest when estimates are unbiased. Furthermore, rater bias decreases the power of the hypothesis test; the effect of rater bias was even greater than the decrease in the power of the test due to use of certain assessment methods, including some category scales.

Third, when we considered the im-pact of experimental design on disease severity estimation with regard to hy-pothesis testing, our results demonstrat-ed that, for unbiased estimates using nearest percent estimates (NPEs), the recommended number of replicate estimates taken per specimen is two (from a sample of specimens of at least 30), as this conserves resources.

Also, some future prospects on the visual estimates of plant disease severity will be discussed. We hope that by demonstrating the effects of different experimental designs, methods of as-sessment, and the effects of rater bias, we can bring forward useful insights that will allow plant pathologists to better select options in the experimental pro-cess and minimize the risk of errors.

YS01 應用媒介昆蟲與微生物之交互作用防治蟲媒細菌性病害之可行性探討－朱家慶 (國立中興大學植物病理學系)
Exploiting insect-microbe interactions for control of insect-transmitted bacterial pathogens of plants－Chu, C. C. (Department of Plant Pathology, Nation-al Chung-Hsing University, Taiwan)

昆蟲媒介傳播之植物病原性細菌在許多作物皆可引起重要之病害。由於昆蟲繁殖速度快且移動範圍相對較大，其傳播之病害在防治上常較為困難。目前常用於控制蟲媒病害之策略包括移除病株與中間寄主、種植防風林、種植無病種苗、施放媒介昆蟲天敵、或是施用化學藥劑等，然而這些方法在成效上常因地而異或是有產生具抗藥性族群之慮。近年來有許多研究顯示昆蟲與其內生細菌之交互作用或許可用於蟲媒病害之防治。有些內共生菌可影響昆蟲寄主傳播病原物之能力，有些菌株則可誘導昆蟲產生胞質不相容現象 (cytoplasmic incompat-ibility)，即透過對雄性生殖細胞之修改造成蟲卵無法孵化以及生殖隔離之情形，而這些現象或許可用於控制媒介昆蟲族群大小或是阻斷病害傳播。本研究以柑橘黃龍病 (citrus greening) 之病害系統為主題，探討應用柑橘木蝨 (Diaphorina citri) 與其體內內共生菌及柑橘黃龍病菌 (*Candidatus* Li-beribacter asiaticus) 之交互作用防治病害傳播之可行性。研究內容包括應用田間採樣與即時聚合酶鏈式反應 (qPCR) 探討 *D. citri* 中三種內共生細菌與 *Ca.* L. asiaticus 族群量之關聯性以及其他的影響因子、使用多位點序列分型 (multilocus sequence typing) 方式探討世界各地 *D. citri* 族群中 *Wolbachia* 之遺傳多樣性是否與不同 *D. citri* 族群間之親緣性有關 (探討 *Wolbachia* 是否可在 *D. citri* 引起胞質不相容現象)、以及其他蛋白質體試驗。實驗結果顯示有些 *D. citri* 內共生細菌的確具有被應用於病蟲害防治之潛力值得被進一步探討。

YS02 Expression and functional anal-ysis of pectolytic enzyme genes and ge-nome analysis of *Xanthomonas citri* subsp. *citri* Taiwan strains－Chang, Shih-Chieh, Deng, Wen- Ling and Tzeng, Kuo-Ching (Department of Plant Pa-thology, National Chung Hsing Univer-sity, Taichung)

Citrus canker, caused by *Xanthomonas citri* subsp. citri (Xcc), is one of the most destructive diseases of citrus. The pectolytic enzymes produced by phytobacteria are important virulence factors involved in tissue maceration, electrolyte loss and cell death of host plants. In this study, the promoter activ-ity of the pectolytic enzyme genes *pel1*, *pel2*, *pel3*, *pglA*, and *peh-1* were inves-tigated in Xcc strain XW19 using the β-glucuronidase (GUS) gene as a re-porter, and the function of *pel1*, *pel3*, *pglA*, and *peh-1* were investigated using the gene deletion mutagenesis. GUS ac-tivity expressed under the control of the *pel1*, *pel3*, *pglA*, and *peh-1* gene pro-moters positively correlated with bacte-rial growth, and displayed high GUS ac-tivity in the presence of sodium poly-pectate and induced in XVM2 medium and host plant. However, only *pel1* was subjected to catabolite repression by glucose. GUS activity was significantly enhanced in the XW19-derived reporter strains after they were inoculated into the leaves of Mexican lime and grape-fruit, suggesting the involvement of the *pel1*, *pel3*, *pglA*, and *peh-1* genes in XW19 pathogenesis. The *pel3* promoter produced the highest GUS activity under all test conditions, whereas no GUS activity was detected using the *pel2* promoter *in vitro* and *in planta*. In comparison with wild type XW19, the canker lesions elicited by the *pel1*, *pglA* or *peh-1* mutant was the same on the leaves of Mexican lime and grapefruit. Although, the *pel3* mutant displayed re-duced growth and induced smaller can-ker lesions on the leaves of Mexican lime or grapefruit compared with the wild type XW19, we demonstrate that Pel3 of Xcc strain XW19 is a virulence factor, and affects the canker elicitation and bacterial growth in host plant. In addition, the PacBio RSII sequencing platform was used to obtain a complete sequence of Xcc strain XW121, an A^r pathogenic variant in Taiwan. Based on Magnifying Genome (MaGe) Annotation Platform analysis, Xcc strain XW121 has a single, circular chromosome and 2 plasmids. The genome size is 5,299,054 bp and the G+C content averages 64.7%. The Xcc strain XW121 genome contains 5,168 putative CDSs, 2 rRNA operons, 56 tRNAs, and 6 misc_RNAs. There were 3,539 CDSs (68.48%) could be assigned to one or more COG functional classes, whereas there was not enough evidence for 1,629 CDSs to be assigned to any COG category. Phylogenetic analysis revealed that Xcc strain XW121 was grouped into the same cluster

with A pathotype and more closely related with strains isolated from China. We also compared Xcc strain XW121 to the genome of Xcc strains 306, NCPPB 3608, and JJ238-24. Comparative genomic analysis showed 368 CDSs are unique to XW121, of which 334 are hypothetical genes, 3 are transposase, and 31 are singletons with predicted functions. In addition, XynB2, PthA1, PthA2, XopC2, XopAK, and Hpa1 were absent from Xcc strain XW121. These virulence factors might be responsible for the atypical symptom-producing and bacterial growth in host plant.

YS03 Overview of *Citrus exocortis vi-roid* and *Hop stunt viroid* in Taiwan－Lin, C.-Y., Hung, T.-H. (Division of Forest Protection, Taiwan Forestry Re-search Institute; Department of Plant Pathology and Microbiology, National Taiwan University)

Two previously reported citrus vi-roids, ***Citrus exocortis viroid*** (CEVd) and ***Hop stunt viroid*** (HSVd), represent main threats to the citrus industry in Taiwan. However, studies on these citrus viroids remain largely to be explored. To establish study about the current status of the citrus viroids in Taiwan, we fo-cused on development of multiplex de-tection, disease ecology of the citrus vi-roids, and relationship between the two citrus viroids. In multiplex detections, a multiplex RT-PCR and a real-time RT-PCR were developed for detecting of CEVd and HSVd. Our field-survey assay of 689 citrus samples in Taiwan re-vealed that HSVd was slightly more prevalent than CEVd (32.2% vs. 30.4%). Furthermore, CEVd and HSVd com-monly co-existed within the citrus culti-vars (up to 55%). Results of the multi-plex quantitative analysis suggest uneven distributions of both viroids within different plant tissues, of which twig bark appears to be the most appropriate and reliable material for viroid detec-tions, thereby being used for quarantine inspections. The genetic diversity assay appeared the existence of ten and five major mutation sites in CEVd and HSVd in Taiwan, respectively. A phylogenetic analysis revealed that Taiwanese isolates of CEVd and HSVd were grouped in three and two clusters, respectively. In studies of disease ecology and interaction, two viroids showed similar patterns when invading shoot apical meristem (SAM) of tomato and citrus as shown by DIG-labeled ***in situ*** hybridization. The two viroids displayed the ability to invade into SAM and leaf primordia of citrus but only leaf primordia of tomato. The study provided useful information for improving of the experimental protocols designed for obtaining viroid-free meristem tissues for control of viroid diseases. In addition, titers of the two viroids were examined by real-time RT-PCR in 17 citrus plants (including blood orange and Murcott mandarin) every 3 months (spring, summer, fall and winter) from 2011 to 2013. No correlation was found between temperature and titer of each viroid, except with HSVd-infected blood orange. This result suggests that temperature is likely not a critical environmental factor during the life cycles of the two viroids. Based on the results of real-time RT-PCR, statistical methods, significant positive correlations, the two viroids appeared in specific tissues of both orange cultivars, except for blood orange at high temperatures. At the cellular/subcellular levels, the two viroids showed similar localization patterns in four tissues and cells, as shown by ***in situ*** hybridization, fluores-cence microscopy, and transmission electron microscopy. Our results demonstrate that the two viroids have a positive relationship and a similar infec-tion pattern, while displaying titer en-hancement and localization similarity with no symptom aggravation.

論文宣讀 Oral presentation

A01 苦瓜青枯病之病原特性分析－蔡佳欣[1]、黃晉興[1]、黃淑芩[1]、李佳蓉[1]、林照能[2] ([1]行政院農委會農業試驗所植物病理組、[2]行政院農委會農業試驗所鳳山熱帶園藝試驗分所蔬菜系)

Characterization of bacterial wilt patho-gen of bitter gourd－Tsai, C. H. [1], Huang, J. H.[1], Hwang, S. L.1, Li, J. R.[1], and Lin, J. N.[2] ([1]Plant Pathology Divi-sion, Taiwan Agricultural Research In-stitute, COA; [2] Vegetable Crop Depart-ment, Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, COA)

2016年屏東地區一處以南瓜為砧木之苦瓜栽植田植株出現萎凋病徵，莖部褐化蔓延，切取莖部罹病組織於光學顯微鏡下可見細菌大量泳出，疑似細菌性病害，該罹病組織於TTC培養基分離出中間粉紅色，周圍白色流質狀之菌落，與青枯病菌菌落型態相似，該細菌經測試可誘導萬國士煙草葉片產生過敏性反應，屬革蘭氏陰性菌。將該菌回接至苦瓜及南瓜植株，均可產生維管束褐化及萎凋病徵，與田間所見相似，且可回分出相同細菌，證實所分離的細菌可感染南瓜及苦瓜。將該菌以Biolog細菌鑑定系統分析，屬於***Ralstonia solanacearum***。以青枯病菌專一性引子Au759f及Au760r及鑑別青枯病菌演化型之引子對 Nmult21:1F、Nmult21:2F、Numlt23: AF、Nmult22:InF、Numlt22:RR對該菌進行複合式聚合酶連鎖反應 (Mul-tiplex PCR)，增幅出280 bp及144 bp之特異性片段，將其鑑定為第1演化型之青枯病菌。將該菌株進行生理小種測試，亦可感染番茄、馬鈴薯、甜椒、茄子等植物，鑑定為第1生理小種。以7種碳水化合物測試該菌之生化型，結果為第4生化型。綜上所述該次所分離之青枯細菌特性為第1生理小種/第4生化型/第1演化型。

A02 拮抗性鏈黴菌在 *Xanthomonas* 屬所造成甘藍黑腐病與葉斑病之防治應用性初探－吳昱陞、曾德賜 (國立中興大學植物病理系)

A preliminary study on the application of antagonistic ***Streptomyces***

for control-ling bacterial black rot and leaf spot diseases of cabbage caused by *Xanthomonas* spp.－Wu, Y. S., and Tzeng, D. S. (Dept. of Plant Pathology, Nation-al Chung Hsing University)

甘藍為世界性重要蔬菜作物，台灣甘藍種植地區遍布全省，以初秋為最主要栽培品種，田間栽培以穴盤苗移植方式已行之有年，種苗的健康是影響產量與品質的關鍵因素，種苗的生產由於採商業化高密度種植，在台灣特有的溫暖潮溼的氣候環境孕育下極有利於各種病蟲媒的滋生與蔓延，帶病種苗一旦擴散至田間每每造成病蟲害的猖獗危害，諸多病媒中尤以 *Xanthomonas* 屬成員*X. campestris* pv. *campestris* (Xcc) 引起的黑腐病危害最劇，近幾年來*X. campestris* pv. *raphani* (Xcr) 引起的細菌性葉斑病所造成之重大損害也備受業者重視。此類病害的防治目前在台灣有通過登記之藥劑只有維利黴素與嘉賜銅，然一則效果常不如理想，二則重覆甚至加重用藥易於衍生抗藥性及造成環境衝擊等問題，發展對環境友善的微生物製劑為此類病害防治管理上亟待投入的一環。本研究旨在探討本研究室既有拮抗性鏈黴菌菌株在此類病害防治上之應用潛力，鏈黴菌為生物防治應用上已被研究多年的有益微生物，具有產生多種重要水解酵素、二次代謝物以及抗生物質的能力，農業用多種抗生素類殺菌劑已知咸由鏈黴菌發酵量產。本試驗所應用之*Streptomyces* sp. S1, S2, S3, 及S4菌株為中興大學本研究室既有，其在多種真菌性病原防治之應用性已經系列研究證實，部份應用性並已獲台灣與美國專利及進入技轉產業界商品化應用階段。另外供測試之9個 *Xanthomonas* 屬病原為本研究分離自埔里地區育苗場發生嚴重複合感染之甘藍幼苗，所分離菌株經柯霍氏法則回接測試以及DNA序列分子鑑定，確定4株為黑腐病菌，5株為細菌性葉斑病菌。本研究利用Xcr PC-9為標的病原測試，證實S1、S2與S4皆具拮抗活性，其中以S2拮抗活性最強。四個鏈黴菌株經測試細菌性病害防治常見應用藥劑的添加對其生長之個別影響，進而證實其對嘉賜克枯爛(14%WP)、維利黴素(5%SL)與亞汰尼(20%SC) 等在500倍稀釋濃度均具有良好耐受性。另外，利用本研究室已建立之試量產級(pilot)發酵生產系統，經系列培養篩選測試，已證實上述拮抗活性最強之S2菌株可在黃豆粉培養基有最佳生長效果，適量食用油的添加更可顯著提升其拮抗活性，此一菌株量產條件之最適化目前仍在進行中，所獲得菌量與抗生活性具改進性活體菌液搭配既有細菌病害常用藥劑在甘藍 *Xanthomonas* 屬病原危害防治的應用效果，將為本研究未來繼續測試探討之重點。

A03　水楊酸防治馬鈴薯青枯病效果評估－林靜宜、林慧如、楊宏仁、倪蕙芳 (行政院農業委員會農業試驗所嘉義農業試驗分所植物保護系)

Control of potato bacterial wilt with salicylic acid－Lin, C. Y., Lin, H. R. , Yang, H. R., and Ni, H. F. (Department of Plant Protection, Chiayi Agricultural Experi-ment Station, Taiwan Agricultural Research Institute)

青枯病是由*Ralstonia solanacearum*所引起，為台灣馬鈴薯生產栽培的限制因子之一，其病原菌為土壤傳播性細菌，可於土壤中存活，主要經由傷口、受污染的水源、種苗或病土等方式傳播，在防治上具有相當的困難度。因此，本研究利用水楊酸溶液進行馬鈴薯青枯病防治效果之初步評估，以期能提供減少病害發生的防治方法。試驗中使用之水楊酸溶液濃度分別為0.05%及0.01%，每14天施用一次，共施用2次，其中於第一次使用水楊酸溶液後，進行青枯病菌接種，之後再行施用1次水楊酸溶液。結果發現對照組於接種後3週開始產生萎凋病徵，而水楊酸處理組則於4週開始表現萎凋病徵，其中以土壤澆灌0.05%水楊酸之處理組效果較佳，於接種後5週開始發病，由此可知，水楊酸具有延緩青枯病發病之效果。罹病度(disease severity)方面，不論是採用土壤澆灌或葉噴法，皆以施用0.05%水楊酸之防治效果較佳，接種後7周罹病度分別為26.7%及30%；而施用0.01%水楊酸防治效果較不顯著，以土壤澆灌法處理其罹病度為56.7%，若以葉噴法處理則罹病度高達80%。結果顯示，0.05%水楊酸較低濃度0.01%水楊酸防治效果佳，可降低馬鈴薯植株之罹病度。未來將做進一步分析試驗，探討其最適合的使用濃度及可能之抗病機制。

A04　*Bacillus amyloliquefaciens* as bio-control agent of black rot disease on cabbage caused by *Xanthomonas campestris* pv. *campestris*－Liaini, A. S.[1], and Lin, Y. H.[2] ([1]Department of Biological Science and Technology；[2]Department of Plant Medicine, National Pingtung University of Science and Technology, Taiwan)

Black rot disease (BRD) caused by *Xanthomonas campestris* pv. *campestris* (Xcc) is considered the most important disease of crucifers world-wide. This destructive disease causes considerable yield losses up to 50% by premature defoliation. Various strategies have been developed to control BRD on cabbage, including use of an-tagonistic microorganisms. The objec-tive of this study was to assay the ap-plication of Bacillus amyloliquefaciens to control BRD on cabbage. Firstly, we assay the inhibitory effect of *B. amyloliquefaciens* strains, PMB04 and PMB05, against Xcc strains on nutrient agar plate. The inhibitory assay re-vealed that both antagonistic strains were able to inhibit the growth of Xcc. Moreover, PMB04 exhibited stronger inhibitory effect against Xcc strains. Therefore, these two strains of *B. amyloliquefaciens* could be used to evaluate their biocontrol efficacy by seed coating method. As a result, *B. amyloliquefaciens* strains PMB04 and PMB05 were significantly suppress BRD on cabbage. The disease inci-dence and disease severity of plants treated by PMB04 and PMB05 were suppressed while compared to the con-trol treatment. In addition, the popula-tion of Xcc in plants were reduced when PMB04 or PMB05 was applied. We concluded that the application of *B. amyloliquefaciens* on seeds is effective strategy to control BRD on

cabbage.

A05 運用細菌性軟腐病血清檢定採收後薯球技術－邱燕欣、鍾文全、蘇士閔 (行政院農委會種苗改良繁殖場)

Detection of soft rot Erwinia spp. on seed potatoes by ELISA－Chiu, Y. H., Chung W. C., and Su, S. M. (Taiwan Seed Improvement and Propagation Station, Xinshe, Taichung 426, Taiwan)

馬鈴薯細菌性軟腐病為全球馬鈴薯產地之重要細菌病害，可在馬鈴薯各時期發生，影響地下部之塊莖，造成薯塊之腐爛，在地上部之植株造成莖腐病徵，薯塊上之軟腐病徵包括輕微的維管束褐變到薯球完全腐爛。可造成此病害之病原菌包括 *Pectobacterium carotovorum* subsp. *atroseptica*(Pca)、*P. c.* subsp. *carotovorum*(Pcc)、*P. chrysanthemi*(Pch)，其中以 Pcc 影響範圍最為廣泛，在世界各地之栽培田皆可發現此依病原菌，而 Pca 主要係發生在溫帶地區，Pch 則常見於亞熱帶到溫帶地區。本試驗研究完成*P. c.* subsp. *Carotovorum* (Pcc) 12851 之抗原製備，經免疫反應進行血清製備，獲得50ml之血清，藉由poly A+G 管柱離心法純化完成10ml，回收量為2.76mg/ml之 IgG。專一性測試軟腐病檢定用之 IgG，可以檢定田間所分離之軟腐病菌株，不會對於供試之馬鈴薯細菌性病害如瘡痂病及青枯病，或是馬鈴薯表皮分離之非病原細菌產生呈色訊號，靈敏度分析，可以偵測到10^2cfu/ml之菌體濃度。馬鈴薯感染軟腐病後，若氣候適宜會在地上部造成腐敗軟化的病徵，但是一旦氣候乾燥，菌體仍可於薯球膨大期，潛伏於薯球臍部，可能造成隔年栽種田間初次的感染源，檢定販售之一般食用薯薯球經薯球病原檢定方式取樣檢測，可以檢測到軟腐病的存在（數據未呈現），因此掌握田間檢查時，人員的巡視判斷外，收穫後及栽種前，可以考量於內部管控加入採收後薯球細菌性病害之檢定，把關薯球品質，也可降低田間發病的可能性。

A06 台灣萵苣細菌性葉斑病菌之鑑定與特性分析－黃逸喬[1]、曾貞瑜[1]、吳雅芳[2]、鄧文玲[1] ([1]國立中興大學植物病理系、[2]行政院農業委員會台南區農業改良場)

Identification and characterization of the causal agents of lettuce bacterial leaf spot disease in Taiwan－Huang, Y. C, Tzeng, J. Y. Wu, Y. F. Wu and Deng, W. L. ([1] Department of Plant Pathogen, National Chung Hsing Uni-versity, Taichung; [2] Tainan District Agricultural Research and Extension Station, COA)

2011-2012 年間，於雲林育苗場萵苣幼苗葉部出現斑點型病徵，病徵初期可見水浸狀斑點、病斑隨後轉為深棕色圓形斑點，較嚴重的感染葉片呈現鄰近病斑癒合成黑褐色之壞疽病斑，最後導致全葉萎凋。自兩處育苗苗圃罹病葉組織中共分離得到 12 株菌株，其中 5 株 (菌株編號為 XCVN 1-5) 根據其半選擇性培養基 MMG 上培養之菌落型態、過敏性反應、生理生化測試、病原性測試、脂肪酸圖譜 (fatty acid methyl ester, FAME)、PCR 鑑別引子對、及 16S *rRNA*、internal transcribed spacer sequence (ITS) 與 *gyrB* 核酸序列等分析，鑑定為 *Xanthomonas campestris* pv. *vitians* (Xcv) group B (又名 *X. hortorum* pv. *vitians*)。將純培養後之XCVN 1-5 菌株分別以噴灑方式接種市面上4種常見萵苣商業品種 (農友種苗*Lactuca sativa* L. cv. L143, cv. General, cv. Green Romaine, and cv. Grand Rapid)，細菌可入侵植物葉片、增殖及引發病徵，確定分離菌株皆具有致病力。而從感染之萵苣葉片上分離之其他 7 株菌株 (編號為XC 1-4及 XCVi 1-3) 除可感染上述 4 種萵苣品種、造成斑點型病徵外，其在 MMG 培養基上之菌落型態及ITS序列比對結果均與 XCVN 菌株相似而被歸類為 Xcv，然而此 7 株細菌之生理生化、FAME、PCR 鑑別引子對等分析結果卻與 XCVN 不同。進一步使用 *fusA-gltA-gapA-gyrB-lacF-lepA* 等 6 個基因進行多位點序列分型 (multi-locus sequence typing, MLST) 分析，經近鄰結合法 (neighbor joining) 建立之親緣關係圖得知 XC 1-4 及 XCVi 1-3 被歸類在同一分化枝 (clade)，與 X. pisi 親緣關係接近，但與 XCVN 及 Xcv group B分布於不同的分化枝，顯示台灣萵苣細菌性葉斑病致病菌除了*X. campestris* pv. *vitians* group B外應有其他菌群。

B01 近年葡萄病害調查與防治之初步研究－林筑蘋、蔡志濃、安寶貞、陳品儒 (行政院農業委員會農業試驗所植物病理組)

Investigation and management of grape disease in recent years－Lin, C. P., Tsai, J. N., Ann, P. J., and Chen, P. R. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA)

根據104-105年度調查結果，1. 病害發生嚴重度：其中以露菌病、銹病菌、以及晚腐病菌為全台普遍的重大病原；部分地區會發生房枯、白腐或黑痘病。2. 病原正名：由分子序列分析鑑定蒐集之病原，可發現病原菌種類與以往記載之菌種有異，如晚腐病菌文獻為*Colletotrichum gloeosporioides*，根據本研究結合多重基因序列分析，發現葡萄晚腐病菌應為*C. siamense*引起；銹病菌文獻為*Phakopsora ampelopsidis*，本研究藉ITS序列分析鑑定為*P. euvitis*以及*P. montana*。由此可知，葡萄近年來的病害種類或許有變化，或隨著鑑定技術之進步發展，病原種類之正確性仍值得持續追蹤調查。3. 病原菌殘存調查：*Pestalotiopsis sp.*幾乎密布葡萄枝條或枯葉上，但後續果實並無出現嚴重罹病況；*Colletotrichum sp.*在休眠期枝條與生長期周遭雜草或葡萄枯葉上分離比例高，可能伺機感染果實，藉此調查可做為未來田間衛生管理參考。晚腐病為產業重大限制因子，已有多種化學藥劑供使用，本研究另測試天然植物萃取液或其他安全性高之資材防治效果，結果顯示「肉桂油乳劑」、「石灰硫磺合劑」與「漂白水」對*C. siamense*孢子發芽有良好直接抑制效果，「香茅油混和配方」與「氯化鈣」則否。以隨機完全區集設計實際測試田間果實晚腐病防治效果，結果顯示除了「肉桂油乳劑」外，「香茅油混和配方」與「氯化鈣」等藥劑效果亦佳，可能與形成植物保護膜或誘導植物抗

病性反應有關。

B02 紅龍果濕腐病之生態調查與病害防治技術之開發－林筑蘋、蔡志濃、安寶貞 (行政院農業委員會農業試驗所植物病理組)

Investigation and management of pitaya wet rot diseases－Lin, C. P., Tsai, J. N., and Ann, P. J. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA)

紅龍果濕腐病是目前田間盛產期與果實貯運期最嚴重之病害之一，對紅龍果產業影響重大，係由真菌*Gilbertella persicaria*引起，為台灣新紀錄真菌。另外總和紅龍果濕腐病菌型態、病原性以及序列與標準株之差異，推論紅龍果濕腐病菌可能為*G . persicaria*新的變種，另稱*G. persicaira* var. *pitaya*。【病徵與族群狀態】：除常見之花與果實濕腐病徵外，田間常見紅肉果實出現黑心病徵，經證實亦多由*G. persicaira* var. *pitaya*引起。經調查，目前臺灣之*G. persicaira* var. *pitaya*皆為同一交配型(-)，交配型單一，宜持續監測自其他國家傳入*G. persicaira* (+) 可能性。【病原性探討】：根據試驗結果顯示*G. persicaira*在高溫（28-36度Ｃ）下孢囊孢子發芽率以及菌絲生長高、實際感染果實時大多需要透過傷口，於高溫(最適溫為24-36度C) 與高濕(相對濕度80%以上) 下發病率以及罹病度最高，尤其連續高濕（2 hr以上）的狀況下發病越趨嚴重，而無傷口、低溫（12度Ｃ）或超高溫（高於40度Ｃ）、低濕度（相對濕度70%以下）則大幅降低發病，可作為採收後濕腐病之物理防治建議參考。【藥劑篩選】：結果顯示，賽普護汰寧、扶吉胺、撲克拉錳等，非化學合成藥劑如波爾多液、香茅油製劑，與肉桂油製劑等，具防治田間花期濕腐病潛力；低濃度酒精則在溫室試驗結果顯示對於貯運期濕腐病具良好防治效果，未來有待測試實際應用於產業之成效。

B03 台農7號荔枝炭疽病之發生及現有推薦藥劑室內藥效評估－黃巧雯[1]、倪蕙芳[2]、許淑麗[2]、柯文琪[2]、賴素玉[2]、楊宏仁[2] ([1]行政院農業委員會農業試驗所、[2]行政院農業委員會農業試驗所嘉義農業試驗分所)

Disease incidence and fungicide screen-ing of anthracnose of lychee (Tainung No.7)－Huang, C. W. [1], Ni, H. F. [2], Hsu, S. L. [2], Ko, W. C. [2], Lai, S. Y. [2], and Yang, H. R. [2] ([1]Plant Pathology Division, Agricultural Research Institute, COA; [2]Department of Plant Protection, Chiayi Agricultural Experiment Station, Taiwan Agricultural Research Institute)

近年來，早熟品種－台農7號「早大荔」目前經由田間種植情形，發現此品種對炭疽病極感病，因此本研究針對台農7號之荔枝炭疽病進行發生調查及現有推薦藥劑室內藥效評估，以研擬本病原菌之有效的管理方法。本研究在2016年間，於採收當天在田間果園中進行早大荔荔枝果實炭疽病病害調查，其結果顯示在高雄杉林區、台南楠西區及嘉義農業試驗分所之果實炭疽病罹病率分別為47.0%、34.7%及37.7%。為了進一步瞭解早大荔果實之炭疽病在採後發生情形，於3區果園中分別採集200顆荔枝果實在25℃下儲藏，分別在0天、3天、6天及9天，調查果實於成熟採後炭疽病發病情形，由試驗結果得知，高雄杉林區早大荔果實之炭疽病罹病率依序為54.4%、88.8%、98.8%及98.8%；台南楠西區則分別依序為41.3%、68.1%、94.4%及97.5%；嘉義農業試驗分所則分別依序為56.9%、61.9%、73.8%及80.7%。本試驗亦記錄果實上炭疽病病斑數目(Disease index) 及果實褐化 (Browning index) 情形，由試驗結果所示，高雄杉林區早大荔果實炭疽病罹病度在0天、3天、6天及9天依序分別為20.6%、48.4%、69.5%及69.5%；台南楠西區則分別依序為12.8%、25.2%、49.5%及54.1%；嘉義農業試驗分所則分別依序為22.4%、23.9%、29.9%及33.1%；果實褐化情形在高雄杉林區則分別依序為0、10.3%、33.1%及73.6%；台南楠西區則分別依序為0、1.7%、13.0%及37.5%；嘉義農業試驗分所則分別依序為0、0、6.4%及23.1%。爾後，將現有之荔枝炭疽病推薦藥劑添加於PDA培養基中，測試各藥劑對炭疽病菌絲生長之抑制效果，由結果得知賽普護汰寧、腐絕快得寧、甲基多保淨、百克敏、亞托待克利及得克利對炭疽病菌分離株之菌絲有良好的菌絲生長抑制效果，未來可提供予農友在病害防治上參考依據。

B04 臺中東勢梨赤星病調查－沈原民[1, 2]、林大淵[2]、趙佳鴻[2]、洪挺軒[1] ([1]國立臺灣大學植物病理與微生物學系、[2]行政院農業委員會臺中區農業改良場)

Survey of pear rust disease in Dongshi, Taichung － Shen, Y. M.[1, 2], Lin, D. Y.[2], Chao, C. H.[2], Hung, T. H.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Taichung District Agricultural Research and Extension Station , COA)

臺灣的梨赤星病主要是*Gymnosporangium asiaticum*引起，梨與龍柏為其寄主，調查發現東勢區內計有1,279株龍柏，當地龍柏密度約每平方公里為11株，公營部門的龍柏數量多於其他地點。東勢區內的龍柏在梨赤星病冬孢子成熟期普遍帶有梨赤星病的冬孢子堆。臨近新種植龍柏的梨園，調查距離龍柏5~60 m的範圍內，梨樹與龍柏愈近，梨赤星病的罹病率及每葉平均病斑數愈高。在另一個梨園，4月份台中一號梨罹染梨赤星病的狀況低於台中二號梨，由於台中一號梨的萌芽時間晚，葉片可能因而避開病害感染。於2011~2016年，調查東勢兩個梨園內梨赤星病的罹病率與嚴重度，並換算病害發生進程下之面積(AUDPC)，同一果園內橫山梨與台中二號梨的AUDPC在多數年度差異不顯著，6年當中兩梨園梨赤星病的AUDPC在2013年最低、2016年最高，調查顯示2月至3月是東勢地區梨赤星病大量感染的高風險期，此期間大量降雨可能導致梨赤星病發生嚴重。

B05 開發一系統化之田間香蕉黃葉病罹病組織檢測流程－黃湘珊、林依佳、林盈宏 (國立屏東科技大學植物醫學系)
Development of a systematic protocol for detection of Fusarium wilt in field samples of bananas － Huang, S. S., Lin, Y. J.1, and Lin, Y. H.1 (Dept. of Plant Medicine, National Pingtung Uni-versity of Science and Technology, Pingtung)

香蕉黃葉病 (Fusarium wilt of ba-nana) 屬於維管束病害，此病害發生時會對香蕉 (Banana) 產業造成相當大的損失。為了加速並準確檢測此病害，進行分子檢測技術不失為一個可行的策略之一，然而備俱一套理想的樣品前處理與核酸萃取系統，為建構一套完善分子檢測技術的重要環節。由於目前市售之試劑套組價格昂貴或效果不彰，且操作流程相對繁瑣，本實驗研究目的為，針對核酸萃取 (DNA extraction) 進行試劑改良 (Re-agent improvement) 與流程之開發 (Protocol development)，希望能開發出較良善的核酸萃取試劑及更簡便的萃取流程。本實驗係針對田間香蕉假莖開發出兩種核酸萃取系統，並分別針對此兩套系統，進行對應試劑組之改良，試驗先以香蕉組培苗假莖為檢體，測試出最佳化之試劑與萃取流程。後續分別以此兩種萃取系統，搭配各自最佳試劑，針對田間隨機採樣之不同罹病程度香蕉假莖進行核酸萃取，並以尖鐮孢菌 (*Fusarium oxysporum*) 專一性引子對進行分子檢測。結果顯示，本研究針對不同萃取系統所設計之試劑組，皆可自供試檢體中快速檢測出帶菌檢體。為了進一步開發出更簡便的分子檢測流程，本研究也針對檢體採樣之樣本前處理步驟進行改良，並將上述改良後之方法導入分子檢測流程中，使其更易於進行田間罹病香蕉假莖之分子檢測。目前本研究已開發出三種前處理方式，皆可大幅改進樣本破碎處理方法。綜合以上結果證實，本研究已開發出，能夠取代商用套組之核酸萃取試劑，並已針對萃取流程進行最佳化，同時設計出不需實驗室設備，即可於田間現地進行之簡易核酸萃取方法，未來將能利用此套簡易核酸萃取流程，架構出田間黃葉病罹病蕉株之現地檢測技術平台。

B06 開發台灣香蕉黃葉病菌第四型生理小種之快速診斷平台－楊峻毓、林依佳、林盈宏 (國立屏東科技大學植物醫學系)
Development of a diagnostic system for rapid detection of *Fusarium oxysporum* f. sp. cubense race 4 in Taiwan －Yang, J.Y., Lin, Y.J., and Lin, Y.H. (Depart-ment of Plant Medicine, National Ping-tung University of Science and Tech-nology, Pingtung)

香蕉 (Musa sp.) 為一世界上重要果樹，被大量種植於全球熱帶地區，是許多開發中國家的經濟命脈。而由香蕉黃葉病菌 (*Fusarium oxysporum* f. sp. *cubense*, Foc) 所引起之香蕉黃葉病 (Fusarium wilt of banana)，為香蕉產業發展之主要限制因子之一，其中尤以第四型生理小種 (Race 4) 中的熱帶第四型 (Tropical race 4, TR4) 威脅最甚。此病原菌能危害華蕉品系 (Cav-endish) 在內之大部分香蕉栽培種，且傳播方式相當多元，使此病害在防治上更為困難，因此即早進行診斷，達到避病目的，為防治此病害之可行策略之一，本實驗旨在開發出一個快速檢測香蕉黃葉病菌第四型生理小種檢測平台，來延緩病原菌擴散，降低經濟損失。本研究以 Race 4 及 TR4 具專一性之引子組為標的，分別進行該兩組引子，對台灣菌株測試其專一性，並以 Polymerase Chain Reaction (PCR)、Real-time Quantitative PCR (Real time PCR) 兩檢測系統，對 Foc 之不同檢體，測試此兩組引子之靈敏度。試驗證實，此兩組檢測用引子皆具特異性及高靈敏性。而後將此檢測技術用於田間進行帶菌香蕉組織分子檢測，試驗中也同步以病原菌分離純化法，確認檢測系統之可靠性及發展潛力。由試驗結果得知，此檢測系統除了檢出正確率極高外，與病原菌分離純化法相比，也較為省工省時，未來擬將本系統導入香蕉黃葉病之田間現地檢測平台，希望能防止此病原菌之蔓延，從而達到降低香蕉產業經濟損失之最終目標。

B07 十字花科炭疽病菌之一個具有bzip domain轉錄因子的功能分析－陳宏岳、王智立 (國立中興大學植物病理學系)
Functional analysis of a bzip do-main-containing transcription factor in *Colletotrichum higginsianum*－Chen, H. Y., and Wang, C. L. (Department of Plant Pathology, National Chung Hsing University, Taichung)

炭疽病菌 (*Colletotrichum* spp.) 造成全球許多重要的經濟作物生產上的損害，特別是位於熱帶和亞熱帶的國家，作物生長環境更適合炭疽病害的發生。在臺灣，*Colletotrichum higginsianum*在有機田中感染各種十字花科蔬菜，造成產量的減損。研究指出，當病原菌入侵寄主植物時，會誘導寄主產生防禦反應，並藉由不同的生理調控機制，來形成各種逆境環境以降低病原菌入侵的適應性，進而限制病害的發生，而當病原菌接收到逆境訊息後，也會誘導轉錄因子的表現來增加對逆境環境的適應性，以順利進行入侵。由前人研究十字花科炭疽病入侵阿拉伯芥的transcriptome結果，得知*C. higginsianum*有一個功能未知的轉錄因子，其具有一個basic re-gion-leucine zipper (bzip) domain，在入侵植物時有較高的表現量，且本實驗室在小白菜的研究結果也顯示該基因在植物體上 (in planta) 具有較高的表現量。此外，無論在離子型或非離子型的滲透壓逆境培養下，*C. higginsianum* 內該基因的表現皆較對照組顯著低弱，顯示其參與病原菌入侵時的逆境調控。進一步研究指出，該基因與酵母菌的Yeast Activator Protein 1 (YAP1)及其他7個YAP基因，同屬於bzip family的一個亞羣，該亞羣的基因主要參與氧化逆境、滲透壓逆境、離子代謝及DNA damage時之生理調控。為進一步了解此基因於*C. higginsianum*中的功能，透過雙載體方式構築split marker的基因剔除載體，並使用農桿菌媒介轉殖系統[*Agrobacterium tumefaciens* mediated transformation (ATMT)] 取得基因剔除菌株 (gene knock strain) 後，以PCR和南方墨點法 (Southern blot) 來確認基因剔除菌株，在獲得正確的基因剔除菌株後，進行其表現型 (phenotype)的分析試驗，在in

vitro的試驗中，基因剔除菌株之菌落生長速率相對較野生型菌株緩慢，在滲透壓逆境反應的試驗中顯示，基因剔除菌株在離子型滲透壓逆境下，抵抗能力亦較野生型菌株低。在接種鳳山白菜的in planta試驗中，發現基因剔除菌株病徵的發展較野生型菌株延遲。從以上的初步分析結果中顯示剔除該基因會影響病原菌的致病力、菌絲生長情形及對逆境的調控，因此未來將持續對該基因在不同的逆境環境進行分析與測試，來探究該基因的功能為何。

B08 *Paenibacillus polymyxa* as a bio-control agent against strawberry an-thracnose－Chen, Po-Liang, Lin, Chia-Hua, and Chen, Chao-Ying. (De-partment of Plant Pathology and Micro-biology, National Taiwan University, Taipei)

Strawberry production is severely affected by anthracnose caused by *Colletotrichum* spp. *Paenibacillus polymyxa* strain TP3 isolated from strawberry plant has antagonistic activity against *Botrytis cinerea* and exhibits biocontrol activity against gray mold disease by foliar application. In this study, we examined the direct protection and inducing plant systemic resistance ability of *P. polymyxa* TP3 on strawberry anthracnose. *In planta* assay revealed the pretreatment of *P. polymyxa* TP3 on strawberry leaves, petioles and crowns where *C. gloeosporioides* was inoculated, sig-nificantly suppressed anthracnose symptom development. *In vitro* assay showed a distinct antifungal activity of *P. polymyxa* TP3 against the mycelial growth and spore germination of *C. gloeosporioides*. These results suggested that *P. polymyxa* TP3 could suppress *C. gloeosporioides* infection directly. On the other hand, *P. polymyxa* TP3 could protect strawberry from *Colletotrichum* attack while pre-applied on the leaves or roots. The systemic resistance induced by *P. polymyxa* TP3 was found to last at least five days and minimum effective concentration was determined to be 2×10^7 CFU/mL. Additionally, de-fense-related callose deposition and re-active oxygen species accumulation were observed in *C. gleosporioides*-challenged leaves of *P. polymyxa*-treated plants but not in the control leaves. The callose deposition was more prominent when *P. polymyxa* TP3 was applied at 2-4 days before fungal inoculation. In conclusions, *P. polymyxa* TP3 could control strawberry anthracnose *via* directly suppressing *Colletotrichum* growth or indirectly inducing systemic resistance of the plant.

B09 *Myrothecium roridum*對白菜立枯病菌與甘藍立枯病菌之拮抗能力分析－陳炫宇[1]、熊浩哲[1]、徐崧鈞[1]、王升陽[2]、余聰安[1]、江主惠[1] ([1]大葉大學分子生物科技學系、[2]國立中興大學森林系)

Analysis of antagonistic ability of *My-rothecium roridum* to *Rhizoctonia* solani AG - 4 and *Rhizoctonia solani* Kuhn－Chen, H. Y[1]., Xiong, H. Z. [1], Hsu, S. C. [1], Wang, S. Y. [2], Yu, T. A., Chiang, C. H. [1] ([1]Dept. of Molecular Biotechnology, Da-Yeh University, Changhua; [2]Dept. of Forestry, National Chung Hsing Univer-sity, Taichung)

目前農業病蟲害防治主要依賴化學農藥，可能造成生態污染及農產品安全等疑慮，利用微生物之生物防治為可能之替代方式。本試驗由土壤根圈分離編號「9號」之菌株，經對峙培養測試，發現其對多種植物病原菌，包括香蕉炭疽病 (*Colletotrichum mu-sa*)、褐根病菌 (*Phellinus noxius*)、白菜立枯病菌 (*Rhizoctonia solani* AG － 4) 及甘藍立枯病菌 (*Rhizoctonia solani* Kuhn) 等均具有拮抗能力，另外，利用玻璃紙抗生法進行拮抗測試時，可觀察到「9號」菌株對白菜立枯病菌和甘藍立枯病菌之抑制效果更佳。為了鑑定出「9號」菌株，本試驗設計對應真菌18S核醣體核酸之引子對並進行聚合酶連鎖反應及DNA選殖，發現其為*Myrothecium*屬之真菌，進一步再針對核糖體核酸基因間隔區 (ITS region) 進行DNA選殖，發現其與露濕漆斑菌 (*M. roridum*) 之核苷酸序列有99%之相同度。本研究將「9號」菌培養濾液進行冷凍乾燥後添加到PDA培養基，發現當添加濃度達0.1 mg/ml時，對白菜立枯病菌和甘藍立枯病菌分別具有54%及60%之抑制效果，而當添加濃度達到1 mg/ml時，對兩種立枯病菌皆有8成以上之抑制率。利用「9號」菌培養濾液進行穴盤白菜幼苗對立枯病菌之防治測試時發現，培養濾液稀釋到300倍時，可降低15~17%的白菜幼苗死亡率，如果培養濾液稀釋到100倍時，則可降低34~37%的白菜幼苗死亡率。利用液相-液相萃取方式將9號菌培養濾液進行成份分離，結果發現主要抑菌成分存在於乙酸乙酯層，將此層之成份添加到PDA濃度達0.5 mg/ml時，發現對白菜立枯病菌及甘藍立枯病菌分別有92%與96%之抑制效果。乙酸乙酯層之收集液再以矽膠管柱層析與薄層分析，發現利用正己烷：乙酸乙酯(60:40及50：50)所得之沖提液，調配成0.1 mg/ ml濃度時，對白菜立枯病菌及甘藍立枯病菌有最佳之抑制效果。本研究由土壤分離之「9號」菌株為露濕漆斑菌，其確實對白菜立枯病及甘藍立枯病具防治效果，本試驗將再進一步找出其有效之作用成分，期待此菌株能進一步開發成微生物製劑應用於植物保護。

B10 Study on the control ability to pea-nut southern blight disease using *Trichoderma harzianum* － Tsai, Meng-Lu, Chang, Hao-Han and Cheng, An-Hsiu. (Tainan District Agricultural Research and Extension Station, COA, Taiwan, R.O.C.)

Peanut (*Arachis hypogaea* L.) is one of the important dryland crops in Taiwan, predisposed to southern blight disease, which caused by *Sclerotium rolfsii*, in hot and humid environment, chemical fungicides are still the main method for disease control currently. *Trichoderma harzianum* T3 isolate was applied in this study for seed treatment (10g/kg seeds) before culturing and spray

treatment (100-fold) during flow-ering in 8 experimental fields. Result shows that the average severity of southern blight disease on peanut plants treated by *T. harzianum* T3 was lower than control group in all fields, and the severity in 7 of 8 fields have statistically significant difference between experi-mental group and control group, sup-porting that *T. harzianum* T3 treatment can be an effective method to suppress the occurrence of southern blight disease in peanut field.

B11 番茄灰斑病病原菌之特性及防治探討－吳雅芳、吳盈慧、鄭安秀 (行政院農委會臺南區農業改良場)

Characteristics and control of tomato gray leaf spot disease caused by *Stemphylium* sp.－Wu, Y. F. , Wu, Y. H., and Cheng, A. H. (Tainan District Agricul-tural Research and Extension Station, COA)

番茄灰斑病由*Stemphylium* sp.引起，罹病初期於葉片上可見褐色不規則小斑點，小病斑癒合形成片狀壞疽斑，嚴重時造成葉片及葉柄黃化枯萎，近幾年於番茄田間發生率漸增，於氣候適宜時發病速度極快，常令農民措手不及，本試驗擬針對病原菌的特性及防治藥劑方面探討其發病生態及防治措施。2016-2017年收集病葉分離病原菌，完成柯霍式法則後，進行基本特性及藥劑篩選試驗。結果發現*Stemphylium* sp.的孢子塗佈於WA培養基上，經30分鐘便開始發芽，1小時內發芽率可達100%。於PDA培養基上菌絲適生長溫度為25-28℃，40℃與45℃菌絲則停止生長。從番茄的登記用藥中選出29種藥劑進行藥劑篩選試驗，其中23.7%依普同水懸劑、24.9%待克利水懸劑、81.3%嘉賜銅可濕性粉劑、37.5%氫氧化銅水懸劑、70%甲基鋅乃浦可濕性粉劑、33%鋅錳乃浦水懸劑、72%鋅錳克絕可濕性粉劑、80%免得爛水分散性粒劑、62.5%賽普護汰寧水分散性粒劑的菌絲生長抑制率可達80%以上。而75%四氯異苯腈可濕性粉劑、70%甲基鋅乃浦可濕性粉劑、33%鋅錳乃浦水懸劑、72%鋅錳克絕可濕性粉劑、80%免得爛水分散性粒劑、62.5%賽普護汰寧水分散性粒劑可以抑制*Stemphylium* sp.的孢子發芽。

B12 造成2016年秋季埔里百香果大減產的病因探討－黃晉興、林獻哲 (行政院農業委員會農業試驗所植物病理組)

A study on the cause of significant re-duction in production of passion fruit in Puli in the autumn of 2016－Huang, J.-H. and Lin, H.-C. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA)

臺灣的百香果 (passion fruit, *Passiflora edulis*) 主要產區在南投縣埔里鎮，栽培面積佔全臺一半以上，而台灣百香果疫病 (*Phytophthora* blight) 於1976年首次被報告，但一直不是嚴重的病害。2016年10月在埔里百香果栽培區爆發一種嚴重病害，估計發生病害的田區約300公頃，造成嚴重的減產。病徵主要出現在果實、幼葉與新稍，病徵初期出現水浸狀病斑，爾後提早落果、落葉以及新稍枝條呈現褐化、枯死，造成平鋪棚架上的百香果茂密枝葉層出現缺口；在少數的植株主莖幹上出現褐化的壞疽斑，最後可造成植株死亡。2016年10月在南投縣埔里鎮19處百香果栽培田取回病組織分離病原菌，分離之部位有葉片、枝條與莖部組織，以及取自南投縣魚池鄉的一處百香果育苗場出現莖基部腐敗疑似疫病的幼苗，共獲得52株疫病菌 (*Phytophthora* sp.) 菌株。這些菌株在培養基上的菌落形態皆相同，挑選從不同百香果植株部位所分離之14株疫病菌進行形態觀察，孢囊為橢圓形、卵圓形或洋梨形，不易脫落，長寬比約1.3，大小為32.6－62.49×26.38－47.05 μm (avg. 47.53×36.43 μm)，具乳突 (papillate)，並可產生菌絲膨大體 (hyphal swellings）及厚膜孢子 (chlamydospore)，菌絲可在8－36℃生長，最適溫為28℃，菌絲生長速度約為11 mm/day。利用引子增幅出此14株疫病菌核醣體內轉錄區間DNA (ribosomal DNA internal transcribed spacer, rDNA ITS)、β-微管蛋白基因 (β-tubulin) 以及線粒體細胞色素氧化酶mitochondrial cytochrome oxidase I (Cox I) 基因序列並進行比對，其序列與NCBI網路登入的數條*Phytophthora nicotianae*之序列有99-100%的相似度，序列再經進行neighbor-joining分析並畫出演化樹，亦與*P. nicotianae*成為一群。由形態與分子生物資料，這些菌株被鑑定為*P. nicotianae*。以孢子懸浮液接種於百香果果實上，結果顯示幼果、成熟果以及無論有沒有傷口處理接種5天後皆可出現與田間相同的病徵，再經接種在嫁接苗上，接種7天後開始出現幼葉水浸狀病斑與枝條褐化之病徵，與田間病徵相符。本研究顯示，2016年秋季造成埔里百香果大減產的病害是由疫病菌*P. nicotianae*所引起，將進一步探討此病原菌在田間的生態與防治方法。

B13 利用亞磷酸二氫鉀防治胡瓜疫病菌之研究－黃晉興、丁柏瑜、安寶貞 (行政院農業委員會農業試驗所植物病理組)

Utilizing potassium dihydrogen phos-phite to control cucumber Phytophthora blight caused by *Phytophthora melonis*.－Huang, J. H., Ting, P. Y., and Ann, P.-J. (Plant Pathology Division, Taiwan Ag-ricultural Research Institute, COA)

疫病是臺灣夏秋雨季最重要的瓜類作物病害，常在數日的降雨或颱風過後造成大量植株死亡。胡瓜疫病的病原菌中以*Phytophthora melonis*為主要病原菌，然而在鑑定過程中與台灣產孢囊無乳突之疫病菌 *P. drechsleri* 與 *P. cryptogea* 形態易混淆，經由該些菌株的核醣體內轉錄區間DNA (ribosomal DNA internal transcribed spacer, rDNA ITS) 、β-微管蛋白基因 (β-tubulin) 以及線粒體細胞色素氧化酶mitochondrial cytochrome oxidase I (Cox I) 基因序列之neighbor-joining演化樹的結果顯示，3種菌確實可被明確的區分為3群，有助於未來的瓜類作物疫病菌鑑定。以 *P. melonis* 游走子懸浮液噴佈葉部或澆灌莖基部的方式接種於盆栽胡瓜， 3天後分別可使植株葉片出現葉部水浸狀斑點，或莖基部亦出現水浸狀病斑，爾後病斑

擴大，7天後所有接種植株均枯死。胡瓜植株接種 *P. melonis* 游走子懸浮液其發病度隨著接種源濃度提高而升高，7天後調查發病度，葉部與莖基部接種之發病度對游走子接種源濃度之迴歸曲線方程式分別為y=101.224(1-e-0.6497x)，R2=0.9985與y=98.7326(1-e-0.0927x)，R2=0.9764，造成發病度50%之接種源濃度分別為1.02與7.61 zoospore/ml。盆栽胡瓜在接種前3天噴佈亞磷酸二氫鉀350倍 (24 mM)、700倍 (12 mM)、1000倍 (8.4 mM) 或1400倍 (6 mM) 稀釋液，再於葉片或莖基部接種病原菌游走子懸浮液，結果顯示，所有亞磷酸二氫鉀稀釋液的處理對於葉部噴佈接種皆無防治效果、但350倍與700倍稀釋液之處理對莖基部接種後有防治效果，發病度分別為0%與10.0%，與對照組之55.0%有顯著差異，將進一步評估應用此資材在田間防治胡瓜疫病的效果。

C01 Screening root knot nematode- *Meloidogyne graminicola* resistant lines from Wild Rices germplasms－Lin, Yuh-Tsen[1] , Li, Charng-Pei[2] and Chen, Peichen[1] ([1]Department of Plant Pathol-ogy, National Chung Hsing University, Taichung; [2]Taiwan Agricultural Research Institute, COA, Wufeng, Taichung)

Meloidogyne graminicola (Mg), commonly named as rice RKN, is a ma-jor threat to rice agriculture, particularly in Asia. Infected rice in a nursery show-ing stunted growth with apparent chlo-rosis, distorted new leaves and early matured flower. The infected root sys-tems showing typical hook-shaped root galls. *M. graminicola* can be controlled by several tactics including genetically modified plant and resistance cultivars. Previous studies demonstrated the Afri-can cultivated rice (*Oryza glaberrima*) can inhibit J2 penetration, the reproduc-tion and development of giant-cell. Wild rice is a very important germplasms source for genetic improvement of cul-tivated rice, in terms of breeding re-sistance to insects and diseases. Twenty wild rice introgression lines were in-cluded in our first screening test, 200 galls were mixed in 1.5 L Akadama soil to make the diseased soil. The experi-ment was performed three times and each line with 4 replicates. Root degree, gall numbers, gall percentage and galling index were recorded 21 days later. According to the data, lines with repli-cates that was 0 galling index, and 2 lines RI-346 & RI-421 that has the highest gallin index – 3, were selected. A total of 10 lines either has resistant or extreme susceptible trait were processed for the next screening tests. In the secondary screening, 2 weeks rice plants were inoculated with fifty J2, and addi-tional thirty J2 were inoculated 10 days later. The data was taken 28 days after inoculation. The gall percentage and sample numbers was presented as normal distribution and rice line RI-364 and RI-421 were identified as the potential resistance lines.

C02 利用農桿菌媒介茄科作物感染類病毒之生物特性初探－鄭櫻慧、王昭萍、蔡筱婷、林雅雯 (行政院農委會農業試驗所植物病理組)

A brief Study on the biological charac-teristics following Agrobacte-rium-mediated inoculation of solanaceae crops with viroids－Cheng, Y. H., Wang, J. P., Tsai, S. T., and Lin, A. W. (Plant Pathology Division, Agricultural Re-search Institute, COA)

類病毒是目前已知最小病原體之一，由裸露的環狀單股RNA組成，組成類病毒的RNA多數約250-400 bases左右，具有高度互補的穩定二級結構。感染茄科作物類病毒種類有馬鈴薯紡錘形塊莖類病毒(*Potato spindle tuber viroid*, PSTVd)、番茄黃化萎縮類病毒 (*Tomato chlorotic dwarf viroid*, TCDVd)、番椒小果類病毒 (*Pepper chat fruit viroid*, PCFVd)、番茄莖頂矮化類病毒 (*Tomato apical stunt viroid*, TASVd)、番茄雄化類病毒 (*Tomato planta macho viroid*, TPMVd)、金魚藤潛隱類病毒 (*Columnea latent viroid*, CLVd)等6種，可藉由種子種苗傳播，已被列為輸出種子特定病原。台灣尚未有上述6種類病毒的紀錄，利用基因合成於Ti質體構築PSTVd、TCDVd、CLVd、PCFVd、TPMVd與TASVd的infectious clone，轉殖於 *Agrobacterium tumefaciens* 4404後，以*A. tumefaciens*菌液穿刺接種於番茄、甜椒、馬鈴薯與煙草(*Nicotiana benthamiana*)，3星期後以RT-PCR測試類病毒感染與否。番茄、甜椒、馬鈴薯、茄子與煙草都測到PSTVd感染。TASVd不感染番椒，只感染番茄、馬鈴薯、茄子與煙草，煙草上只感染未觀察到病徵，接種感染的馬鈴薯植株正常生長，但在數塊上出現痂皮，感染的番茄植株嚴重矮化，葉片捲縮，果實變小。TCDVd不感染番椒，只感染番茄、馬鈴薯與茄子。CLVd感染番茄、馬鈴薯與煙草。PCFVd感染番茄、甜椒、馬鈴薯與茄子，馬鈴薯薯塊造成紡錘形病徵。TPMVd在接種的植物中，只測到感染番茄與煙草。類病毒感染與否與病徵嚴重程度常因類病毒株系不同或感染寄主植物品種而有差異，對國內常見茄科作物的影響，仍待更多接種實驗證實。

C03 開發感染番茄類病毒的核酸檢測方法－鄭櫻慧、蔡筱婷、林雅雯 (行政院農委會農業試驗所植物病理組)

Development of methods for detection of pospiviroids on Tomato－Cheng, Y. H., Tsai, S. T., and Lin, A. W. (Plant Pa-thology Division, Agricultural Research Institute, COA)

類病毒由裸露的環狀單股RNA組成，具有高度互補的穩定二級結構，沒有鞘蛋白保護，已知的檢測方法包含生物檢定法、核酸雜合法、反轉錄-聚合酶連鎖反應、即時聚合酶連鎖反應、定量即時聚合酶連鎖反應及恆溫環狀擴增反應等。近年來以反轉錄-聚合酶連鎖反應(reverse transcrip-tion-polymerase chain reaction, RT-PCR) 與即時聚合酶連鎖反應於類病毒檢測上應用最多。感染番茄的類病毒種類有馬鈴薯紡錘形塊莖類病毒(*Potato spindle tuber viroid*, PSTVd)、番茄黃化萎縮類病毒(*Tomato chlorotic dwarf vi-roid*, TCDVd)、番椒小果類病毒

(*Pepper chat fruit viroid*, PCFVd)、番茄莖頂矮化類病毒(*Tomato apical stunt viroid*, TASVd)、番茄雄化類病毒(*Tomato planta macho viroid*, TPMVd)、金魚藤潛隱類病毒(*Columnea latent viroid,* CLVd)等6種，都可以經由種子傳播，因此建立其種子萃取與RT-PCR檢測方法。核酸萃取方法以種子或葉片樣本以2種核酸萃取試劑組、市售快萃液、文獻發表類病毒快萃液與改良式自行研發快萃液進行萃取，2種核酸萃取試劑組與改良式自行研發快萃液進行萃取可順利進行RT-PCR，其餘快萃液無法穩定增幅預期條帶。引子設計自GenBank搜尋6種類病毒核酸資料，設計正反向2組簡併式引子對，與6組專一性引子對進行測試，結果8組引子對都以遞減RT-PCR結果最穩定。試驗中並比較引子對與目前常用RT-PCR之檢測靈敏度與實驗結果再現性。

C04 玉米褪綠斑駁病毒可經由種子傳播之證據－周建銘[1]、簡伊萱[1]、蔡錦慧[1]、陳君弢[2]、謝光照[3]、陳裕儒[3]、彭冠甄[1]、鄧汀欽[1]([1]行政院農業委員會農業試驗所植物病理組、[2]行政院農業委員會動植物防疫檢疫局新竹分局、[3]行政院農業委員會農業試驗所作物組)

Evidence of seed-transmissibility of ***Maize chlorotic mottle virus***－C. M. Chou[1], Y. H. Chien[1], C. H. Tsai[1], C. T. Chen[2], G. J. Hsieh[3], Y. R. Chen[3], K. C. Peng[1] and T. C. Deng[1] ([1]Plant Pathology Division, Agricultural Research Institute, COA; [2]Hsinchu Branch, Bureau of An-imal and Plant Health Inspection and Quarantine, COA; [3]Crop Division, Tai-wan Agricultural Research Institute, COA)

玉米褪綠斑駁病毒(*Maize chlorotic mottle virus*, MCMV)傳播方式多樣，可藉由玉米薊馬作為傳播媒介進行短距離的擴散，亦能透過種子傳毒的方式進行長距離的傳播，本病毒於2011年首次於台灣報導發生，推測病毒來源最先應伴隨帶毒種子移入台灣，雖文獻記載MCMV種子傳毒率極低，約為萬分之四至千分之五之間，但仍具有相當風險，然而針對MCMV高發病地區農民使用玉米種子以群體測試採樣檢測玉米種子帶毒情形，結果顯示皆未測得帶MCMV病毒之種子，後經以本實驗室自製之MCMV抗血清，以群體測試採樣檢查進口玉米種子共105批種子(72,450粒種子)，亦皆未測得種子帶毒情形，為釐清MCMV種子帶毒情形，本研究針對MCMV種子傳毒情形進行探討，採樣經檢測感染MCMV玉米植株包括玉美珍、彩珍、台南22號3種玉米品種，鮮穗在38℃條件下烘乾一週後脫粒並進行長出測試(Grow out test)，長出玉米苗可測得台南22號品種種子傳毒率2.1%(1/47)，另取經檢測感染MCMV之玉美珍、彩珍、台南22號玉米及台農1號植株成熟果穗烘乾脫粒並進行長出測試，結果僅台農1號測得種子傳毒率0.67%(1/149)，另以自行接種MCMV之台南22號及玉美珍玉米植株收集成熟種子進行長出測試，可測得玉美珍種子帶毒率為0.67%(1/150)，之後又於夏季高溫期接種MCMV之白龍王品種植株，採收後種子進行長出測試，但未測得種子傳毒情形(0/600)，由上述結果可知，在確認植株罹染MCMV下收穫之種子，其種子傳毒率介於0-2.1%之間；因此為了解MCMV病毒確實可在感染的玉米穗及種子上殘存，以自製之MCMV抗血清進行組織轉漬(Tissue blotting)分析新鮮玉米果穗及玉米種子內的MCMV分布情形，結果顯示在果軸及穗頂不充實處病毒累積量高，玉米種子內則可見MCMV明顯累積於胚根(Radicle)、幼葉(Plumule)及黑離層(Black layer)，此一證據顯示MCMV病毒可透過感染種子胚組織而使其能藉由帶毒種子傳播。

C05 應用分子標誌篩檢抗矮南瓜黃化嵌紋病毒之胡瓜品種－周建銘[1]、林子凱[2]、蔡錦慧[1]、林羿廷[1]、林玫珠[3]、寧方俞[4]、鄧汀欽[1]([1]行政院農業委員會農業試驗所植物病理組、[2]行政院農業委員會農業試驗所作物組、[3]行政院農業委員會農業試驗所鳳山熱帶園藝試驗分所植物保護系、4行政院農業委員會茶業改良場茶作技術課)

Application of molecular markers to screening of cucumber cultivars resistant to ***Zucchini yellow mosaic virus***－C. M. Chou[1], T. K. Lin[2], C. H. Tsai[1], Y. T. Lin[1], M. J. Lin[3], F. Y. Ning[4] and T. C. Deng[1] ([1]Plant Pathology Division, Taiwan Ag-ricultural Research Institute, COA; [2]Crop Division, Taiwan Agricultural Re-search Institute, COA; [3]Plant Protec-tion Department, Fengshan Tropical Horticultural Experiment Branch, Tai-wan Agricultural Research Institute, COA; [4] Tea Agronomy Section, Tea Re-search and Extension Station, COA)

矮南瓜黃化嵌紋病毒(*Zucchini yellow mosaic virus*, ZYMV)在台灣於1985年首次報導可感染胡瓜，嚴重時可造成葉片皺縮嵌紋、植株矮化及果實畸形等病徵，為防治此病害可透過抗病育種方式育成抗性胡瓜品種，然而傳統抗病篩選過程中，可能因氣候、病毒株差異及人為操作等因素影響，導致抗病品系篩選進度緩慢且準確度不佳，透過分子標誌輔助育種(marker assisted breeding, MAB) 可改善此一問題；目前已有多對抗ZYMV分子標誌被發展出來，為確認其是否適用於篩選現有胡瓜品系，本研究針對抗ZYMV之胡瓜分子標誌可應用性進行探討，共選擇YCZ-SCAR-2、YCZ-CAP1、YCZ-CAP2、SSR07248、SSR07884、UW080856、UW080853及T1等八對與ZYMV抗性基因緊密連鎖之分子標誌，以及依據抗性基因候選 (Resistant gene candidate) VPS4蛋白 (Vacuolar protein sortingassociat-edProtein-4) 蛋白SNP位點所設計出之CAPS-T86C及dCAPS-G99A兩對分子標誌測試，並與接種ZYMV病毒株31B-05進行抗感病性表現型(phenotype)之驗證，測試結果顯示CAPS-T86C及dCAPS-G99A分析之抗性基因型與表現型吻合，分析其抗性品系12-120之SNP位點，結果與Amano等人發表之ZYMV VPS4蛋白SNP位點相同，將該ZYMV分子標誌應用於驗證抗性胡瓜親本與F2子代之抗性，結果顯示具抗性分子片段者皆具抗ZYMV病毒株31B-05之表現型，顯見該分子標誌具應用於胡瓜抗ZYMV篩選之潛力。將此分子標誌應用於檢測胡瓜商業品種長青、阿信、新玉、秀玉、萬綠、彩綠二號及萬吉等品

種結果顯示，長青、新玉、秀玉及彩綠二號具抗ZYMV片段，但並非每一單株表現型皆與基因型一致，此一結果是否因缺乏其他與抗ZYMV相關之微效基因或因ZYMV病毒株毒力不同所致，則需後續研究探討。

C06 唐棉嵌紋病之病因鑑定－張庭愷[1]、陳煜焜[2,*]、吳雅芳[3] (台中市[1]國立中興大學植物醫學暨安全農業碩士學位學程；[2]國立中興大學植物病理學系；[3]台南市台南區農業改良場；[*]聯絡作者)

Etiology of the mosaic disease of *Gomphocarpus* spp.－T. K. Chang[1], Y.K. Chen [2,*], and Y.F. Wu [3] ([1]Master Pro-gram for Plant Medicine and Good Ag-ricultural Practice, and [2]Department of Plant Pathology, National Chung Hsing University, Taichung, and [3]Tainan Dis-trict Agricultural Research and Exten-sion Station, Tainan, [*]correspondent)

唐棉 (*Gomphocarpus* spp. syn. *Asclepias* spp.)為夾竹桃科(family Apocynaceae)蘿藦亞科(subfamily As-clepiadoideae)多年生草本植物，原產於非洲。1980年間由日本引進台灣，作為園藝觀賞零星栽培，其葉可藥用。2015年11月間，由台南區農業改良場送驗唐棉病株樣本。罹病植株之葉片呈嵌紋、葉面凹凸、葉片窄小、葉緣波浪狀等疑似病毒感染之病徵。以電子顯微鏡鏡檢罹病組織粗汁液，可觀察到大小約750-850 x 12 nm之長絲狀病毒顆粒；病組織細胞之超薄切片亦可觀察到Potyvirus感染所引起之風車狀 (pinwheel)及板層狀 (laminated)內含體 (inclusion bodies)。將唐棉病葉粗汁液機械接種於奎藜 (*Chenoposium quinoa*)及圓葉菸草(*Nicotiana benthamiana*)，約5 - 7天後在奎藜接種葉出現黃化局部病斑及在圓葉菸草引起系統性嵌紋病徵。以奎藜進行三次單斑分離後，得到之病毒分離株，暫以CM532為代稱。將病毒株CM532以機械接種法分別回接至健康大粒種唐棉(*Gomphocarpus physocarpus* syn. *Asclepias phycocarpa*)和小粒種唐棉 (*G. fruticosus* syn. *A. fructicosa*) 實生苗，約25-28天後兩種唐棉均產生葉片黃化、嵌紋、葉片變窄、及葉緣呈波浪狀捲曲等系統性病徵。接種之病株組織粗汁液含Potyvirus狀的病毒顆粒，亦可用RT-PCR自接種之病株樣本增幅出預期之Potyvirus條帶，證明病毒株CM532對唐棉的病原性。萃取唐棉罹病葉組織之總量RNA，搭配Potyvirus簡併式引子對 (PNIbF1: GGBAAYAATAGTGGNCAACC; Ol-igo-T)進行反轉錄聚合酶鏈鎖反應 (RT-PCR)，可增幅出一條約1.7 Kb涵蓋Potyvirus NIb基因3'半部、鞘蛋白基因及3'非轉譯區(3'-UTR)的cDNA片段。經選殖、解序及BLAST比對後，該cDNA片段與隔山消嵌紋病毒(*Keunjorong mosaic virus*, KjMV) (ac-cession number JF838187)之核酸序列相似度達72%。以KjMV基因序列為藍本設計引子對，增幅並整合各相關片段，目前已完成病毒株CM532全長度基因組序列之解析。病毒株CM532之基因體總長度為9,998個核苷酸 (不含PolyA尾端)，具有典型Potyvirus的基因架構，包括5'-UTR (174 nt)、一個大蛋白開放轉譯框架 [an open reading frame (ORF) of polyprotein] (9588 nt)、以及3'-UTR (236 nt)。大蛋白(poly-protein)可切出P1 (1311 nt)、HC-Pro (1365 nt)、P3 (1038 nt)、6K1 (156 nt)、CI (1902 nt)、6K2 (159 nt)、NIa-Vpg (567 nt)、NIa-Pro (738 nt)、NIb (1551 nt)、CP (801 nt)等10個功能性蛋白。另在P3蛋白區域內有一獨立的ORF，可轉譯一段由90個胺基酸所組成的PIPO蛋白。將病毒株CM532全長度基因體核苷酸序列於NCBI資料庫與18種potyviruses進行BLAST比對後，顯示相似度介於54.2－70.4%之間，而與KjMV最近似 (70.4%)、其次分別為*Peanut mottle virus* (PeMoV, NC002600)(59.1%)、*Beet mosaic virus* (BtMV,AY206394)(58.8%)、*Watermelon mosaic virus* (WMV, KM597071)(58.2%)。外鞘蛋白基因 (CP gene)之胺基酸序列除與KjMV (85.0%)和*Rhopalanthe virus Y* (RhoVY, AF185956)(79.9%)較近似外，與其餘Potyvirus者均低於80%的門檻 (58.6 – 69.3%)。依國際病毒分類委員會 (ICTV)之規範，引起唐棉病毒病害之病毒株CM532可能是Potyvirus 屬的一個新種(new species)病毒，暫以唐棉嵌紋病毒 (*Gomphocarpus mosaic virus*, GoMV)稱之。GoMV的生物、血清、及其他分子特性有待進一步分析確認。

C07 Molecular detection of a be-gomovirus associated with sida yellow vein disease in Thailand－Xie, Xing-Yun[1], Tsai, Wen-Shi[1] (Department of Plant Medicine, National Chiayi University, Chiayi City)

Sida leaf curl virus (SiLCV) is a monopartite, circular, ssDNA be-gomovirus. SiLCV has been reported to cause yellow vein disease on Abutilon and Sida plants in Vietnam and China. The virus-associated ssDNA satellites, DNA-β and DNA-1, are also commonly detected in the diseased plants. In Feb-ruary 2017, the Sida weed with symp-tom of yellow vein was observed fre-quently in Nakhon Ratchasima, East Thailand. Four symptomatic samples were collected and tested presence of begomovirus by PCR using general pri-mer pair-PAL1v1978B/PAR1c715H. The amplified PCR products in 1.4 kb were sequenced and revealed high nu-cleotide sequence identity (94%) to SiLCV isolates from China and Vietnam. Consequently, abutting primers were de-signed to amplify full-length DNA which ranged 2,757-2,758 nucleotides in length and having high nucleotide se-quence identity (>95%). However, the begomoviral DNA-B was not detected. Both virus associated satellites DNA-β and DNA-1 were also tested using the abutting primer pairs Beta01/Beta02 and UN101/UN102, respectively. The pre-dicted amplicons of complete DNA-β and DNA-1 were obtained in all samples, except one sample is detected negative to DNA-1. The DNA-β is ranged 1,362-1,364 nucleotides in length and revealing high nucleotide sequence identity (>92%). The full-length of DNA-1 are ranged 1,385-1,387 nucleo-tides and having >87% nucleotide se-quence identity. The DNA-β

and DNA-1 have the highest nucleotide identity to SiLCV associated DNA-β (>91%) (AM050733 and AM050732), and the associated DNA-1 (>94%) (DQ641717 and AM050735), respectively. To our knowledge, this is the first detection of SiLCV and its associated satellites from Sida plants showing yellow vein disease in Thailand, which implicating the disease distribution is expanding in South-East Asia.

C08 Development of a Multiplex Bead-Based Assay for Detection of cu-curbit Viruses—Kuan, Cheng-Ping[1], Lin, Yu-Chang[1], Cheng, Ying-Huey2 and Deng, Tin-Chin[2], Yang, Tso-Chi[1] ([1]Division of Biotechnology; [2]Division of Plant Pathology, Taiwan Agricultural Research Institute, COA, Wufeng, Tai-chung)

Simultaneous detection of three cucurbit viruses, ***Zucchini yellow mosaic virus***, ***Cucumber green mottle mosaic virus***, and ***Cucumber mosaic virus***, were carried out using a multiplex bead-based assay, a novel detection technique that combines RT-PCR with the florescent detection. On the basis of the establish-ment of the optimal PCR and reverse transcription (RT)-PCR for the detection of a single virus, a quadruplex RT-PCR method that employed virus-specific primers was developed for the detection and differentiation of all the three virus-es in melon or squash plants. The bead-captured virus probe was detected without electrophoresis analysis and ef-fective removal of RT-PCR inhibitors. The developed bead-based assay showed a more higher detection limit comparable to the RT-PCR reaction. The assay was then validated using melon and squash samples infected with one or more viruses collected from fields. The system offers a sensitive, high through-put and rapid detection method for cu-curbit viruses.

C09 Improving Resistance to Fusarium Wilt Disease in Transgenic Banana Plants expressing PFLP—Kuan, Cheng-Ping, Chen, Po-Heng, and Chen Han-Wei, Yang, Tso-Chi (Division of Biotechnology, Taiwan Agricultural Research Institute, COA, Wufeng, Tai-chung)

Fusarium wilt disease, caused by ***Fusarium oxysporium*** f. sp. ***cubense*** (Foc), is considered as one of the most important banana diseases in the world and no chemical control or fungicides are available so far. To decrease the damages of Fusarium wilt on banana production, planting resistant cultivars or culturing transgenic bananas expressing pathogen resistant genes are best options could be applied in the Foc-infected field. Here, we developed a foliar rating system to assess the progress of Fusarium wilt in several transgenic banana plants expressing the fer-redoxin-like protein (***PFLP***) gene. The responses of the ***PFLP*** transgenic lines and non-transgenic banana cultivars were evaluated on their susceptiblility or tolerance to the pathogen in pots in the greenhouse. In order to clarify the re-sistance of transgenic banana to Fusarium pathogen, the existence of the ***PFLP*** gene in transgenic bananas were examined by PCR. Several transgenic lines of plantlets expressing the ***PFLP***, were challenged with the Foc. Leaves yellowing or wilting symptoms of tissue cultured banana seedlings appeared over 54 dpi after Foc inoculation. Results showed that "MCPER 3-4" was the more resistant line among seven lines. Based on these results, a bioassay that is fast and space-effective could be used to evaluate as screening of transgenic ba-nanas against the disease.

C10 Evaluation of the efficacy of phloem specific promoters in application of grafting transmissible RNA interfer-ence on plant protection against tomato yellow leaf curl disease in ***Solanaceae***—Ho-Hsiung Chang[1] and Hsin-Hung Yeh[2] ([1]Dept. of Plant Pathology, National Chung Hsing University, Taichung; [2]Agricultural Biotechnology Research Center, Academia Sinica, Taipei)

Tomato yellow leaf curl virus (TYLCV) cause devastating disease on ***Solanaceae*** crops globally. Breeding of resistant cultivars or transgenic RNAi approach can provides resistance to TYLCV. However, how to efficiently transfer the resistance loci or introduce RNAi to highly diverse commercial ***Solanaceae*** cultivars remains a problem. Graft-transmissible RNA interference (gtRNAi) allow the RNAi signal trans-ferred through grafting, which may al-low the transfer of TYLCV RNAi re-sistance to different cultivars by grafting. Previously reports indicate that phloem specific promoter can induce better gtRNAi, Thus, we try to identify pro-moters from phloem associated viruses including ***Banana bunchy top virus***, ***Coconut foliar decay virus***, ***Commelina yellow mottle virus*** (CoYMV) and ***Rice tungro bacilliform virus*** (RTBV) for triggering better gtRNAi. The selected promoters were fused with firefly lucif-erase, green fluorescent protein and par-tial fragment of hair-pin gluta-mate-1-semialdehyde aminotransferase (hpGSA) gene for promoter activity, lo-calization and generating transgenic plants, respectively. Our analysis indi-cates that promoters derived from CoYMV and RTBV showed strong promoter activity, phloem specificity, and induce better RNAi (more T0 plants showing GSA silencing phenotype). In addition, grafting experiment showed that transgenic rootstock with CoYMV and RTBV promoters deliver better gtRNAi than transgenic rootstock with 35S promoter. It indicates that CoYMV and RTBV promoters performed better in inducing RNAi and delivered better gtRNAi. We generated transgenic plants with CoYMV, RTBV and 35S promoters to express hpRNA targeting to ***Tomato yellow leaf curl Thailand virus*** (TY-LCTHV). Consistently, our results indi-cate more transgenic progenies with CoYMV and RTBV promoters were immune to TYLCTHV.

C11 百合*Plantago asiatica mosaic virus*人工接種之寄主種類初探－陳金枝、陳美雅、江芬蘭 (行政院農業委員會農業試驗所植物病理組)

Preliminary studies on the artificially inoculated hosts of *Plantago asiatica mosaic virus*－Chen, C. C., Chen, M. Y., and Chiang, F. L. (Division of Plant Pa-thology, Taiwan Agricultural Research Institute, COA, Taiwan ROC)

國內種植之百合種球主要由國外進口，進行切花生產，年產值近15億元。近年來國際間之百合種球繁殖生產，深受車前草嵌紋病毒 (*Plantago asiatica mosaic virus*，簡稱 PlAMV) 之危害，影響百合種球品質及其切花產值甚鉅。PlAMV為*Potexvirus*屬病毒成員，可藉由汁液機械傳播予健康株，或帶毒種球繁殖行銷而於國際間傳播。PlAMV已被台灣規範為百合重要檢疫病毒，並實施嚴密的進口種球病毒監測。本研究由進口種球攔截到之PlAMV，藉由其可用罹病組織液以機械傳播方式接種於奎藜 (*Chenopodium quinoa* Wild.) 而形成單斑之特性，於隔離溫室控管下，以奎藜單斑罹病組織作為接種源進行PlAMV之寄主測試，測試植株與栽培介質試驗完均經由高溫高壓滅菌處理。以RT-PCR (Reverse transcription polymerase chain reaction) 和西方墨點法 (western blotting) 檢測植株經病毒接種後再新生之葉片，以釐清是否有受PlAMV感染。由受測的十字花科、葫蘆科、豆科及其他科屬植物共23種作物中，番杏、蠶豆、蘿蔔、山萵苣和蕎麥接種後之取樣組織，均可於RT-PCR反應中與PlAMV的引子對 (PlAMV-CPu/PlAMV-CPw) 產生預估之核酸片段。此5種植物接種後，番杏和蠶豆葉片出現黃化斑點；蘿蔔、山萵苣和蕎麥則無病徵呈現。而番杏、蠶豆和山萵苣葉片組織更可於西方墨點法中，與PlAMV多元抗體產生預期之蛋白質反應條帶。本研究依據RT-PCR反應中，可與PlAMV引子對產生預期反應條帶之結果，判斷上述五種作物為PlAMV之實驗室接種寄主作物。番杏為文獻上已報導之PlAMV寄主，而蠶豆、蘿蔔、山萵苣和蕎麥為本研究新發現可經由汁液機械接種而被PlAMV感染者。本研究另外將PlAMV接種於車前草上，並未出現明顯病徵與檢出PlAMV，目前經由人工方式以汁液機械接種法尚未能具體證明分離自百合之PlAMV可回接到車前草上，有待探討接種方式或PlAMV分離株種類對其接種效果之影響。本研究目前有關PlAMV人工接種寄主之結果，可作為田間作物栽培種類對PlAMV風險管理之參考依據。

C12 金花石蒜實生苗未檢出*Lycoris mild mottle virus* (LyMMoV)、*Narcissus degeneration virus* (NDV)和*Nerine latent virus* (NeLV) 3種病毒之研究－陳金枝、江芬蘭、黃美容 (行政院農業委員會農業試驗所植物病理組)

Study on the three viruses, *Lycoris mild mottle virus* (LyMMoV), *Narcissus degeneration virus* (NDV) and *Nerine la-tent virus* (NeLV), were not detected from the seedlings of golden spider lily －Chen, C. C., Chiang, F. L., and Huang, M. R. (Division of Plant Pathology, Taiwan Agricultural Research Institute, COA,Taiwan ROC)

金花石蒜 (*Lycoris aurea* Herb) 為台灣本土原生的球根花卉，在分類上屬於石蒜科 (Amaryllidaceae) 石蒜屬多年生植物，台灣以北部淡水地區為最大產區。目前記錄上感染金花石蒜之病毒種類包括*Potyvirus*屬的*Lycoris mild mottle virus* (LyMMoV)和*Narcissus degeneration virus* (NDV)、*Carlavirus*屬的*Nerine latent virus* (NeLV) 及*Cucumovirus*屬的*Cucumber mosaic virus* (CMV)等。其中LyMMoV和NDV常複合感染金花石蒜，罹病植株葉片出現嵌紋、斑紋或黃褐條斑等病徵；NeLV則常見於金花石蒜上且不引起明顯病徵。本研究探討受LyMMoV/NDV、或LyMMoV/NDV/NeLV病毒複合感染的金花石蒜罹病株，其種子播種後之實生苗後代是否仍帶有病毒。試驗以上述複合感染病毒之母本株，於開花期進行人工授粉後，採集種子播種於栽培介質，放置於隔離溫室中進行栽培與觀察。採取種子播種後新生葉片組織進行indirect-ELISA (Indirect en-zyme-linked immunosorbent assay) 和RT-PCR (Reverse transcription poly-merase chain reaction) 檢測，以分析葉片組織是否帶有上述特定病毒。結果顯示，由22棵LyMMoV/NDV複合感染之母本株所收集之種子播種後，採集208個苗株之葉片樣品，於兩種檢測法中均未檢出LyMMoV和NDV；由10棵LyMMoV/NDV/NeLV複合感染之母本株所收集之種子播種後，採集74個苗株之葉片樣品，於兩種檢測法中均未檢出LyMMoV、NDV和NeLV。由本研究結果初步發現，LyMMoV、NDV和NeLV並未隨著金花石蒜種子而傳播到子代。NeLV目前並未有種子傳毒之紀錄，本研究在金花石蒜上也發現相同之現象；而LyMMoV和NDV感染金花石蒜後不經由種子帶毒傳播為首次發現。本研究之結果可作為金花石蒜無此3種病毒之健康種苗繁殖選育的參考。

學生論文宣讀比賽
Student oral presentation contest

SA01 Diversity, characterization and application of bacterial endophytes from banana in copper-polluted fields in Tai-wan－Lin, Bo-Wen[1], Wang, Chao-Jen[1], Chen, Yi-Jeng[1] and Chung, Wen-Hsin[1, 2] ([1]Department of Plant Pathology, Na-tional Chung Hsing University, Taichung; [2]Agricultural Products Approval and Certification Center, National Chung Hsing University, Taichung)

Endophytes have been known for plant protection, including disease con-trol, overstress, growth promotion, etc. In this study, the bacterial endophytes from banana grew in paddy fields with or without copper pollution in Homei, Changhua County, were carried out their diversity, characteristic and applicability. The banana grew

in 3 copper-polluted and 3 unpolluted fields were collected for bacterial endophytes isolation. In this study, a total of 386 strains were ob-tained and half of them were isolated from banana grew in polluted or unpol-luted fields. The isolation ratio showed that bacteria from banana shoot in pol-luted fields (36.8%) is higher than un-polluted fields (21.2%). According to the 16S rDNA sequence, the Bacillaceae was the major family in both fields. The Shannon index and Simpson index re-vealed that diversity in unpolluted fields (Shannon index=1.588; Simpson in-dex=3.390) were higher than polluted fields (Shannon index=1.420; Simpson index=4.428). Moreover, only *Entero-bacter*, *Erwinia* and *Tsukamurella* could be isolated from polluted fields, whereas *Microbispora*, *Dyella* and *Pseudomonas* were only in unpolluted fields. The copper-tolerant test indicated that the 63% and 37% strains from polluted and unpolluted fields could grow in the me-dia with 120 ppm of copper (CuSO4), respectively. Consequently, the abilities of strains against copper were unassoci-ated with the isolation part of banana. Among the copper-tolerant strains, 35 strains have the ability to promote the growth of rape (*Brassica rapa* L.) and rice (*Oryza sativa* L.), and *Micromonospora* sp. R6-22, *Paenibacillus* sp. PS6-4, *Rhizobium* sp. R6-6-1, *Herbaspirillum* sp. P5-6 and *Lysobacter* sp. R5-43 showed the higher relative vigor index than others. In addition, two strains of former five endophytic bacteria, R6-6-1 and PS6-4, could promote the rice-seedling growth to overcome the stunting symptom under copper stress. The phytohormone examination demon-strated that R6-6-1 and PS6-4 could produce Indole-3-acetic acid (IAA).

SA02 The molecular mechanisms of *Bacillus species* to control the tomato bacterial wilt caused by *Ralstonia solanacearum*－Hsieh, Song-He[1], Lin, Yi-Hsien[2], Guo, Woei-Jiun[3] and Huang, Tzu-Pi1 ([1]Department of Plant Pathology, National Chung Hsing University, Tai-chung; [2]Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung; [3]Institute of Tropical Plant Sciences, National Cheng Kung University, Tainan)

Tomato bacterial wilt caused by *Ralstonia solanacearum* is one of the devastating diseases in tomatoes. Plant growth promoting rhizobacteria such as *Bacillus species* have been shown to produce lipopeptides and antagonize against various pathogenic fungi and bacteria including *R. solanacearum*, thus their potential and mechanisms in dis-ease suppression were investigated. Two *Bacillus subtilis* strains 151B1, GAP-B2 and one *Bacillus amyloliquefaciens* strain PMB05 native in Taiwan were shown to exhibit differential activity against various bacterial and fungal pathogens and were potential biocontrol agents for various plant disease. The main objectives of my research were to compare the antagonistic activity of the three *Bacillus* strains against *R. solanacearum*; to assess the biofilm for-mation and root colonization by the three Bacillus strains and to determine whether the above phenotypes are asso-ciated with their efficacy in controlling tomato bacterial wilt; to determine if sugar transporters in tomato and Ara-bidopsis plants may involve in biofilm formation and colonization by *Bacillus species* and by *R. solanacearum*; and to evaluate the activity of plant growth promotion by the *Bacillus* strains and the putative mechanisms. Our results in-dicated that the *B. subtilis* 151B1, GAP-B2 and *B. amyloliquefaciens* PMB05 all showed similar antagonistic activity against *R. solanacearum* PS152 which was isolated from a diseased to-mato plant. To determine if the types of sugars may affect biofilm formation by the *Bacillus* strains, the carbon source glycerin in MSgg (*Minimal* Salts Gluta-mate Glycerol) medium were replaced with sucrose, fructose or glucose and biofilm formation by three *Bacillus species* strains was assessed in 24 wells mi-croplates. The biofilm formation by the strains PMB05 and GAP-B2 was signif-icantly higher than strain 151B1 in the MSgg medium. All three strains formed less biofilms in sucrose containing media than in other sugars. Among three *Bacillus* strains, 151B1 exhibited the highest activity of producing in-dole-3-acetic acid and 2, 3-butanediol for plant growth promotion. Other ob-jectives of my study which would reveal the role of sugar transporters in biocon-trol bacteria, or bacterial wilt pathogen and tomato plants interactions, and its association with biocontrol efficacy was under investigation.

SA03 改良式芽孢桿菌屬細菌轉型及再生效率提升技術－邵宇恆[1]、鄧文玲[1]、黃振文[1]、吳哲嘉[1]、周浩平[2] ([1]國立中興大學植物病理系、[2]行政院農業委員會高雄區農業改良場)

An improved method for efficient trans-formation and regeneration of diverse *Bacillu*s species－Shao, Y. H.[1], Deng, W. L.[1], Huang, J. W.[1], Wu, J. J.[1], and Chou H. P.[2] ([1] Dept. of Plant Pathology, National Chung Hsing University, Tai-chung; [2] Kaohsiung District Agricultural Research and Extension Station, COA)

芽孢桿菌屬菌株為革蘭氏陽性菌，可在環境逆境下產生內生孢子而存活。此屬細菌目前有廣泛的農業及與工業應用價值。由前人研究得知具有生物防治能力的芽孢桿菌屬菌株在系統分類學上多分屬於 *subtilis* group，然而只有少數的馴化菌株可進行遺傳分析。為能有效分析野生型芽孢桿菌屬細菌功能性基因體，提高轉型效率，本研究使用三株已獲得全基因體序列之生物防治菌株 (*Bacillus mycoides* BM02、*B. pumilus* PMB102、及 *B. amyloliquefaciens* PMB01) 為實驗材料，使用改良之 PEG 原生質體轉型法 (protoplast transformation) (Chang and Cohen, 1979) 進行逢機基因突變。在優化的原生質體轉型法中，細菌以終濃度 2 ppm 之 lysozyme 與 lysostaphin 溶菌酶混合液處理2 至 3 小時可有效提高原生質體形成率，後續以 PEG6000 進行質體 DNA轉型時，額外添加終濃度為 3.3 mM

filter-sterilized ATP，再將轉型菌株培養於含抗生素之DM3固態培養基 [洋菜濃度為 2% (w/v)] 則可提昇轉型及原生質體再生效率。以優化之原生質體轉型法將帶有跳躍子 Tn-YLB-1 之 pMarB 質體送入 BmBM02、BpPMB102 與 BaPMB01 中，所得之逢機突變菌株庫，以 96 孔微孔盤建立快速篩選平台，可測試跳躍子隨機插入作用的突變效率及穩定性；下一步可製作專一性基因突變菌株，用以分析二次代謝物生合成基因與細菌生物防治能力的相關性。另外，運用優化之轉型法將綠螢光蛋白 (GFP) 表現質體 pAD43-25 送入 BmBM02 及 BpPMB102 中，可於螢光顯微鏡下觀察表現綠色螢光之轉型株，後續可用於原位 (in situ) 觀察細菌在植物根系分布情形。

SA04　羽毛分解細菌的分離與鑑定及其於白菜健康管理之應用－林庭緯、黃振文(國立中興大學植物病理學系)

Isolation and Identification of Feath-er-degrading Bacteria and Their Appli-cation for Managing Health of Chinese Cabbage－Lin, T. W. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan)

西元2013與2014年，台灣廢棄的羽毛分別高達27,003與27,804公噸，若不妥善處理會有汙染環境之虞。羽毛富含豐富的氮素，其成分係由結構穩定的角蛋白組成，動植物不易直接利用。因此，本研究的主要目的在於篩選與鑑定具有分解羽毛能力的微生物，進而利用分解的羽毛粉研製具有保護白菜健康的製劑產品。首先從台南地區的廢棄雞毛中分離出具有分解羽毛能力的細菌共27菌株，其中PDM0417菌株分解羽毛的效率最高，可達42%以上，其次有10菌株的羽毛分解率介於26-35%左右，其餘分解率均低於25%; 此外，FDB05菌株尚可有效抑制白菜黑斑病菌(*Alternaria brassicicola* ABA31)與白菜黃葉病菌(*Fusarium oxysporum* f. sp. *conglutinans* Foc-JR01)的菌絲生長，且還可抑制孢子發芽與引起發芽管膨大變形。進一步，利用Biolog分析與16S rDNA 序列比對法，將PDML0417與FDB05菌株分別鑑定為*Deinococcus* ficus Lai et al.與 *Bacillus subtilis* (Ehrenberg) Cohn。比較不同緩衝溶液之羽毛培養基對於PDML0417分解羽毛能力的影響，結果顯示添加0.1%之磷酸氫二鉀-磷酸二氫一鉀緩衝溶液可顯著提高羽毛分解率達80%。利用含有磷酸鉀緩衝液之羽毛培養基作為基礎配方，逐一測試不同無機鹽類添加物，發現同時添加鈉、鉀、鎂及鈣鹽可使PDML0417菌株繼代培養數次後，仍可維持其原始的羽毛分解能力。利用PDML0417分解過之羽毛粉作為基質，添加不同碳素源後培養FDB05菌株，結果顯示添加1%(w/v)麥芽精、綠豆粉、玉米粉、馬鈴薯粉或地瓜粉之FDB05發酵液200倍稀釋液可使ABA31發芽管膨脹率皆達80% 以上，其中添加綠豆粉或玉米粉之50倍發酵稀釋液可使Foc-JR01孢子發芽率分別降至16.0 % 與13.3 %。評估不同濃度羽毛粉對白菜生育之影響，結果顯示栽培介質中添加0.5%(w/w)羽毛粉可分別提高白菜鮮重24%與株高9%; 然而添加濃度超過0.5%(w/w)時，即不利於白菜的生長。

SA05　稠李鏈黴菌PMS-702製劑防治胡瓜露菌病的功效－范雅婷、黃振文 (國立中興大學植物病理學系)

Efficacy of a biocontrol product of *Streptomyces padanus* PMS-702 for controlling cucumber downy mildew－Fan, Y. T. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan)

Pseudoperonospora cubensis (Berk. et Curt.) Rostov.可引起胡瓜露菌病，是葫蘆科作物的重要葉部病害之一，在世界各地曾造成嚴重的經濟損失。國內外防治胡瓜露菌病的主要方法是採用殺菌劑與栽植抗病品種；然而隨著抗藥性菌系的出現及抗病品種的育成不易，研發替代的防治策略為當前努力的重要方向。近年來，利用微生物防治植物病害已成為新的趨勢，且已有許多成功的案例。西元2003年石氏等人證明*Streptomyces padanus* (Bal-dacci E et al.) PMS-702菌株可抑制立枯絲核菌，且發現其培養濾液可減輕番茄晚疫病的發生。西元2004年洪氏亦發現PMS-702之培養濾液可有效減少小星辰花苗炭疽病的發生率達53%以上。此外，石氏等人(2003)證明PMS-702菌株是以其產生的治黴色基素(fungichromin)作為主要的抑菌指標成分。西元2007年黃氏等人發現葡萄糖及花生粕作為培養PMS-702菌株的碳氮素源，可顯著提高治黴色基素的產量；吳氏(2006)及陳氏(2008)證明在黃豆粉-葡萄糖培養液中添加油脂或界面活性劑，有助於提升治黴色基素的產量。本研究的主要目的在於研製*S. padanus* PMS-702的最佳製劑配方，期能有效防治胡瓜露菌病的發生。首先將*S. padanus* PMS-702培養在黃豆粉-葡萄糖培養液(SMG)中，五天後取10倍醱酵稀釋液處理*P. cubensis*之孢囊，發現其可完全抑制孢囊的發芽；隨後以胡瓜切離葉評估其防病功效，證明該醱酵稀釋液亦可完全抑制露菌病的病斑產生。進一步，以治黴色基素直接處理於孢囊及胡瓜切離葉上，發現治黴色基素濃度高於10 ppm即可完全抑制孢囊發芽及病斑產生。在SMG中添加不同種類的植物油後，分別培養PMS-702，五天後發現添加1 % (v/v) 椰子油之醱酵液(代號簡稱SMG-C-1)可提升治黴色基素之產量達20倍以上，亦即產量由原始配方之68 mg/L提高到1418 mg/L。利用蒸餾水稀釋SMG-C-1的100倍液，可顯著抑制孢囊發芽及病斑產生；惟於溫室中噴佈SMG-C-1之100倍稀釋液於胡瓜植株上，卻不能有效防治胡瓜露菌病的發生；然而在SMG-C-1醱酵液中再添加Tween80，以蒸餾水稀釋成100倍液噴佈於胡瓜植株上，發現胡瓜露菌病的罹病度可從54%顯著降至16%。(主要引用文獻：Shih et al. 2003. J. Agri. Food. Chem. 51: 95-99 ; Huang, et al. 2007. Can. J. Plant Pathol. 29: 261-267)

SA06　新種*Begomovirus*引起之洋桔梗 (*Eustoma russellianum*) 新病害－石珮蓉、趙鴻宇、陳煜焜* (台中市, 國立中興大學植物病理學系；*聯絡作者)

A new disease of lisianthus (*Eustoma russellianum*) caused by a new species of *Begomovirus*－P.R. Shih, H.Y. Chao, and Y.K. Chen (Department of Plant Pa-thology, National Chung Hsing Univer-sity, Taichung; *,correspondent)

洋桔梗 (lisianthus, *Eustoma russellianum*)因其花型優美、花色鮮麗多變，且耐儲存運輸，向為我國外銷切花的主力之一。已知洋桔梗之病蟲害種類甚多，其中以病毒病害為大宗，有紀錄者至少已有32種，台灣有紀錄者即有12種，其中包括*Begomovirus*屬的藿香薊黃脈病毒(*Ageratum yellow vein virus*, AYVV)及木瓜捲葉廣東病毒(*Papaya leaf curl Guangdong virus*, PaLCuGdV)。2015年八、九月間於彰化芳苑和嘉義北港分別採得有葉片捲曲(leaf curl)、葉背及花瓣有贅脈畸生(enation)、以及植株矮化(stunting)等病徵之洋桔梗植株。因其病徵與AYVV引起之洋桔梗病害相似，於是抽取罹病洋桔梗總量DNA，搭配*Begomovirus*屬DNA-A簡併式引子對(5'-GCATCTGCAGGCCCACAT-YGTCTTYCCNGT-3'與5'-GATTTCTGCAGTTDATRTTYTCRTCCATCCA-3')、DNA-B及DNA-β之簡併式引子對進行PCR。結果僅DNA-A的引子對增幅出一條約1.5 kb之條帶，顯示罹病洋桔梗之病因可能與單基因體(monopartite)之begomoviruses有關。經過選殖、解序並經由NCBI資料庫進行BLAST後，發現一疑似新種的*Begomovirus*。解析該疑似新種*Begomovirus*之全長基因體發現其DNA-A (accession numbers LC091538和LC091539)含有2759個核苷酸，對應六個開放性譯讀框 (open reading frames, ORFs)，分別為V1 (771 nt)、V2 (387 nt)、C1 (1083 nt)、C2 (408 nt)、C3 (405 nt)及C4 (291 nt)。將此疑似新種病毒之DNA-A全長度核苷酸序列與NCBI資料庫之單基因體*Begomovirus*之DNA-A序列比對後，發現其相似度介於 74.4 – 86.6%之間，而與番茄黃化捲葉泰國病毒(*Tomato yellow leaf curl Thailand virus*, TYLCTHV)最為近似(84.8 – 86.6%)；與感染洋桔梗之AYVV (JN703794)及PaLCuGdV (LC089013)之全長度DNA-A核苷酸序列相似度分別為74.6% 和77.7%。根據ICTV對於*Begomovirus*屬病毒之新種界定，全長度DNA-A之核苷酸序列相似度小於91%可定義為新種，因此暫以洋桔梗贅脈捲葉病毒 (*Lisianthus enation leaf curl virus*, LisELCV) 稱之。由親緣樹分析結果顯示LisELCV與感染洋桔梗之AYVV和PaLCuGdV分屬於不同分化支(clade)。針對AYVV、LisELCV、和PaLCuGdV設計專一性引子對， 應用於同時檢測並鑑別感染洋桔梗之begomoviruses。利用所設計之專一性引子對檢測田間病株，可知芳苑地區之洋桔梗病株均為PaLCuGdV所感染(8/8)，而北港地區的洋桔梗病株則分別有LisELCV (1/9)或PaLCuGdV (5/9)單獨感染，以及LisELCV和PaLCuGdV兩病毒複合感染(3/9)之現象。以rolling circle amplification (RCA)增幅並選殖兩倍全長度LisELCV DNA-A感染力選殖株(infectious clone)於*Agrobacterium tumefaciens* LBA4404。以農桿菌注射法(Agro-infiltration)接種圓葉菸草(*Nicotiana benthamiana*) 和洋桔梗。圓葉菸草於接種22-26天後，出現新葉葉緣上捲致葉片成杯狀、葉背葉脈贅生，老葉葉片褪綠、葉脈綠化等病徵。PCR亦可自接種葉及系統病葉中檢測出LisELCV專一性條帶，證實LisELCV之病原性。LisELCV對洋桔梗之致病性目前仍在觀察中。

SA07 除草劑促進草莓炭疽病發生的副效應－高宏遠、黃振文 (國立中興大學植物病理學系)

Side-effect of Herbicides on Enhancing Occurrence of Strawberry Anthracnose－Kao, H. Y. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan)

除草劑是現代農耕栽培過程用於管理雜草的重要手段，一旦施用不當，除了會毒傷作物之外，尚且會促進作物病害的發生。本研究主要目的在於探討巴拉刈、嘉磷塞及固殺草等除草劑對於草莓炭疽病菌(*Colletotrichum gloeosporioides* (Penz.) Sacc.) MD-02與ME-03菌株在基質上生長繁殖與感染草莓植株的影響，期有助於瞭解田間施用除草劑是否會影響草莓炭疽病的發生。本試驗研究發現病原菌處理過25 ppm嘉磷塞、5 ppm巴拉刈及5 ppm固殺草後4小時，分別可促進61.2%、53.0%及59.6%草莓炭疽病菌的孢子發芽。在查氏培養基(Czapek's medium)分別添加不同濃度的除草劑後製成平板後，接種炭疽病菌菌絲塊的處理，經過5天，發現2 ppm巴拉刈、25 ppm嘉磷塞及1.5 ppm固殺草皆可促進菌絲生長約9%；以分生孢子懸浮液塗佈的方式，經過3天後，5 ppm巴拉刈與250 ppm嘉磷塞則分別可促進炭疽病菌產孢達198.6%與419.5%。在含有炭疽病菌分生孢子的土壤中分別添加巴拉刈、嘉磷塞或固殺草後5天，分析炭疽病菌的菌量變化，發現土壤中添加2500 ppm巴拉刈或12500 ppm嘉磷塞，炭疽病菌族群量分別可提升832.3%或1223.5%；至於添加固殺草的處理組則與對照組相仿，結果呈現逐日遞減的趨勢。土壤添加巴拉刈、嘉磷塞或固殺草後，分析土壤中細菌、放線菌、真菌及酵母菌的族群量變化，結果顯示2500 ppm嘉磷塞可提升真菌與酵母菌的族群量約1205.3%及363.1%；至於添加500 ppm巴拉刈及675 ppm固殺草的處理組與對照組間無顯著差異。進一步以10 ppm巴拉刈與50 ppm嘉磷塞噴施草莓植株後，翌日接種草莓炭疽病菌，發現處理過巴拉刈及嘉磷塞可顯著提高植株的罹病度達305%及117%。此外，先將巴拉刈或嘉磷塞添加於土壤中再種植草莓植株，結果發現巴拉刈濃度超過320 ppm或嘉磷塞濃度超過40 ppm時，草莓的新葉會出現黃化的症狀；若處理巴拉刈濃度在240 ppm或嘉磷塞20 ppm時，草莓植株外觀雖無異樣，然而接種炭疽病菌後，植株炭疽病罹病度卻可提高61.6～64.1%左右。

SA08 十字花科根瘤病菌田間雜草寄主及生態角色之探討－潘劉至元、劉峴恩、沈偉強 (國立臺灣大學植物病理與微生物學系)

Study on the weed host plants of *Plasmodiophora brassicae* and

their role on disease occurrence－Pan-Liu, C. Y., Liu, T. M.-E., and Shen, W. C. (Department of Plant Pathology and Microbiology, National Taiwan University)

十字花科根瘤病菌 (*Plasmodiophora brassicae* Woronin)，感染十字花科植物會造成寄主植物根部組織不規則腫大的根瘤病徵，進而影響根部輸水功能，嚴重時植株黃化萎凋死亡。根瘤病是全球性且相當古老的病害，好發於低溫高濕的土壤環境，對於溫帶地區及國家，或是亞熱帶及熱帶氣溫較低的高山地區十字花科蔬菜的生產，影響甚鉅。根瘤病於臺灣夏季時，好發於台中福壽山、南投仁愛鄉等高冷蔬菜產區；冬季則多出現在北部平地冬季蔬菜生產區。根瘤病菌為絕對寄生性黏菌，寄主範圍相當廣泛，除了可以感染大多數的十字花科作物外，目前已知亦可感染數種非十字花科植物，不過只有在含有特殊的硫配醣體 (glucosinolate) 的十字花科寄主植物 (host) 上，會產生根部腫大的根瘤病徵，在非寄主植物 (nonhost) 上，則不表現根瘤病徵，因此非寄主植物對於田間根瘤病的發生及管理，具有相當的重要性。本研究蒐集來自南投台大梅峰山地實驗農場甘藍田區中常見的9種雜草，透過根部組織染色、專一PCR引子對偵測，及接種實驗，確認這些雜草是否為根瘤病菌的非寄主植物，並模擬田間情況，探討其在根瘤病菌生活史中所扮演的角色。期望透過本研究，了解臺灣田間常見雜草，對於根瘤病發病生態的角色，並作為田間病害管理的重要參考。

SA09　利用乳酸菌*Lactobacillus paracasei*發酵液提升植物生長及逆境抵抗能力－許育仁、陳俊任、許鍾毓、黃祥恩 (國立台東大學生命科學系)

Increasing plant growth and resistance to abiotic stress and pathogen by fermented broth of *Lactobacillus paracasei*－Hsu, Y. J., Chan, J. R. , Hsu, J. Y. , Huang, H. E.(Department of Life Science, Nation-al Taitung University, Taitung 950, Tai-wan)

作物在田間耕作環境中，常常無法兼顧產量與逆境抵抗能力的提升，本實驗利用一般環境及食用安全微生物 (Generally Recognized as Safe (GRAS) 乳酸菌*Lactobacillus paracasei*發酵液 (No-2) 施用於農作物。並檢測其對於逆境抵抗能力、根圈微生物族群數量、病原菌抵抗能力及作物生長的影響。實驗結果顯示，No-2能夠有效提升番茄體內過氧化酵素 (peroxidase, POD) 的活性、過氧化氫 (Hydrogen peroxide, H_2O_2) 的累積量，同時也會增加細胞膜上的離子滲漏度，提升JA 抗性相關標記基因 *21* 基因的表現量，若將No-2經高溫處理後 (Heat-No2)，則能同時誘導 JA 及 SA 相關抗性標記基因 *LeCOI1* 與 *LePR1* 的表現量。此外 No-2 也能直接抑制炭疽病菌 *Colletotrichum gloeosporioides* 等病原菌菌絲生長及孢子發芽能力，增強番茄對於炭疽病菌 *C. gloeosporioides* 的抵抗能力。提升番茄幼苗對於 UV-C、高溫及缺水逆境的耐受能力，No-2 也可以幫助番茄根部維持土壤根圈細菌 *Bacillus thuringiensis* (HS1) 及 *B. amylo-liquefaciens* (HS3) 的族群數量。在田間試驗，結果發現單獨處裡No-2能促進番茄植株高度及葉片數 ，而Heat-No2只能增加植株高度。也可以增加水稻分蘖數、稻梗長度和稻梗數、洋香瓜的花朵及果實數及提升番荔枝果實的產量。但是單獨處裡No-2對於番荔枝的田間病害防治效果卻不明顯，但是如果混合 *B. thuringiensis* (HS1) 及 *B. amyloliquefaciens* (HS3) 及鈣離子之後，則不但具有降低病害的能力，同時依然可以維持增加釋迦果實產量的能力。綜合上訴的研究結果顯示，乳酸菌發酵液 No-2，能經由促進植物體內 H_2O_2 含量及 POD 活性，誘導植物的抗性基因表現，增加根部微生物族群數量，借由多重方式來達到同時保護植物及提升作物產量的雙重目標。

SA10　初探線蟲於菌蠹蟲防治之潛力－張祁舜[1]、余冠毅[1]、施欣慧[2]、林清山[3]、陸聲山[2]、楊爵因[1] ([1]國立台灣大學植物病理與微生物學系、[2]行政院農業委員會林業試驗所森林保護組、[3]臺中市大里區草湖國民小學)

Investigation of the nematode biologi-cal-control potential against ambrosia beetles－Chang, C. S.[1], Yu, G. Y.[1], Shih, H. H.[2], Lin, C. S.[3] , Lu, S. S.[2] and Yang, J. I.[1] ([1] Department of Plant Pathology and Microbiology, National Taiwan Univer-sity, Taipei; [2] Division of Forest Protec-tion, Taiwan Forestry Research Institute, Taipei; [3] Tsau-hu Elementary School, Taichung)

在自然界中存在許多昆蟲寄生性線蟲，此類線蟲帶有對昆蟲有致死力之共生細菌，在入侵昆蟲體內後即會將其釋放而殺害昆蟲。菌蠹蟲(ambrosia beetles)危害眾多森林樹種及經濟作物，其特化的儲菌器(mycangia)構造可攜帶含有多種植物病原之真菌，作為菌蠹蟲侵染樹木後於蛀蝕孔道中之穩定糧食來源。數種源於亞洲之菌蠹蟲傳播入美國後，造成當地酪梨產業嚴重經濟損失。研究已知危害最鉅的菌蠹蟲為*Euwallacea*屬，其所攜帶的共生真菌主要為具有植物寄生性的鐮孢菌*Fusarium*屬，受侵染的樹木往往受到昆蟲與真菌的雙重危害，卻苦無有效防治管理措施。本研究調查台灣境內與菌蠹蟲相關的線蟲族群，於各地蒐集菌蠹蟲及其取食之木材，分離線蟲並以分子方法鑑定種類，最終透過基因相關性分析進行初步篩選出具有防治潛力的物種。在台灣的11個地區所採集菌蠹蟲蟲體及蛀蝕木材之403個樣本中，計有4屬線蟲族群：*Acrostichus* sp., *Deladenus* sp., *Koerneria* sp. 及*Sphaerularia* sp.與6屬菌蠹蟲 *Cnestus* sp., *Eccoptopterus* sp., *Euwallacea* sp., *Scolytoplatypus* sp., *Xyleborus* sp.與 *Xylosandrus* sp.具有相關性。未來擬挑選具昆蟲寄生性之線蟲對菌蠹蟲做寄主感染測試並建立培養線蟲之系統，以期於田間防治策略提供新的方法。

SA11　水稻白尖病與徒長病之二合一快速檢測技術之建立及應用－余冠毅、楊爵因 (國立台灣大學植物病理與微生物學系)

Development and application of a rapid detection assay White-tip disease and Bakanae disease on rice－Yu, G. Y., and Yang, J. I. (Dept. of Plant Pathol-ogy and Microbiology, National Taiwan

University, Taipei)

水稻是亞洲重要糧食作物之一，而水稻白尖病以及水稻徒長病為重要水稻種子傳播性病害，嚴重影響稻米產量。由於病原 *Aphelenchoides besseyi* 及 *Fusarium fujikuroi* 皆寄生於水稻種子內，故若能於水稻育苗時期進行種苗篩選，則能有效管理此二病害於田間之發生。本研究利用恆溫圈環形核酸增幅法 (Loop-mediated isothermal amplification, LAMP) 建立兩病害之快速檢測技術，並將其最佳化而得應用於水稻種苗檢測。針對水稻白尖病病原線蟲*A. besseyi*粒線體DNA之COI gene，設計專一性LAMP引子組AB-ID37，在其最適反應溫度63℃進行靈敏度以及專一性測試。結果顯示，反應時間為 60分鐘可偵測出的最低濃度為104 copies/ul，且確實避免其他地上部植食性線蟲所造成之偽陽性反應。在接種有*A. besseyi*的水稻苗進行測試，其偵測之靈敏度則可以達到每株苗5隻。水稻徒長病的偵測則針對病原真菌*Fusarium fujikuroi*之FUM1 gene，設計專一性LAMP引子組FF-ID14。在專一性試驗中，證實可以有效排除*Fusarium*屬中的其他相近種*F. proliferatum*和*F. verticillioides*的檢出。此兩組LAMP引子組之最佳化條件將搭配Hydroxy naphthol blue (HNB)和Lateral-flow strip之應用，於偵測終端快速顯色，達到快速以及同時偵測二病原之目標。

SA12　小麥赤黴病生物防治及新月毒素生合成偵測平台之建立－楊翰祁[1]、林宗俊[2]、王智立[1] ([1]國立中興大學植物病理系、[2]行政院農委會農業試驗所植物病理組)

Developing the biocontrol and con-structing a platform for detection of trichothecene production of Fusarium head blight of wheat－Yang, H. C.[1], Lin, T. C.2 and Wang, C. L.[1] ([1] Dept. of Plant Pathology, National Chung Hsing University, Taichung; [2] Plant Pathol-ogy Division, Taiwan Agricultural Research Institute, COA)

小麥赤黴病在世界各地的產區造成嚴重的危害，好發於溫暖及潮溼的環境，除了造成直接的經濟損失外，赤黴病菌另會產生新月毒素造成穀粒的污染，哺乳動物攝取毒素後會產生消化系統中毒的症狀。許多台灣農民以友善環境的方式耕作小麥，但缺乏防治小麥赤黴病的有效方法，本研究欲開發生物防治方法及建立篩選降低新月毒素產生之拮抗微生物的平台，期融入台灣小麥的生產體系。本研究經初步篩選後，具拮抗能力的菌株分別來自農試所及小麥花藥分離株，上述菌株依菌落型態分為兩類群，32個菌株為*Bacillus*類，另13個菌株在NA培養基上形成圓形黃色菌落。為測試適合兩類拮抗菌株生長的基礎培養基，隨機挑選菌株，以常用商業化培養基作為基礎培養基進行篩選，*Bacillus*類之菌株培養於TSB及LB培養基的拮抗效果較佳，圓形黃色菌落之菌株則培養於PDB及TSB培養基時的拮抗能力較佳。將兩類菌株分別以其適合之基礎培養基進行菌株拮抗能力篩選，*Bacillus*類菌株中，I-2-9菌株的抑制效果最佳；圓形黃色菌落之菌株中，則以I-1-4菌株的抑制效果最佳。由於此兩菌分別有較佳的拮抗能力，且均分離自小麥花藥 (赤黴病田間感染點)，故選擇此兩菌株進行配方的篩選及改良。為尋找工業生產之替代原料， I-2-9菌株以LB進行成分的替換，將蛋白胰以各類氮素營養源替換，酵母萃取物以酵母粉替代，另進行碳源以及植物油的篩選後，以脫脂奶粉、酵母粉、蔗糖與氯化鈉組成之配方有較佳的拮抗能力。至於I-1-4菌株經碳源篩選後，進行馬鈴薯萃取物的替換及植物油的測試，結果顯示以蔗糖、馬鈴薯煎汁與葵花油組成的配方有較佳的拮抗效果。進一步測試兩菌株的適當培養環境，以不同pH值及溫度進行測試，I-2-9菌株於pH 8及24℃的條件培養3天有較佳的抑制率； 至於I-1-4菌株培養在各pH值差異不大，但其培養於24℃與3天的條件下有較佳的抑制率。為瞭解各成分對其菌量與其對病原菌孢子抑制率的影響，以兩水準因子試驗設計進行分析，並額外添加其他前述試驗中可能的有效成分，在I-2-9菌株的配方中，綠豆粉具顯著影響；而I-1-4菌株配方之酵母萃取物與馬鈴薯煎汁具顯著影響，將此分析抑制率最佳的配方進行溫室試驗，施用I-2-9菌株發酵液的10倍稀釋液，可降低57%的罹病度以及40%的罹病率，而施用I-1-4菌株發酵液的25倍稀釋則可降低68%的罹病度以及67%的罹病率，顯示其具有開發為生物製劑之潛力。在建立篩選偵測新月毒素生合成平台方面，利用新月毒素生合成路徑第一個酵素*Tri5*的啟動子與*GFP*基因結合後之載體，以PEG媒介基因轉殖產生9個轉殖株，再以PCR及南方墨點法進行基因型的確認，結果顯示9個菌株均成功插入*Tri5*的基因座內，已知*Tri5*基因於低pH誘導下可大量表現，將各轉殖株於pH 3.5之培養基中進行誘導，均可見到明顯的GFP表現，而在pH 7的情況下，GFP表現較弱，結果與前人研究相符，期所建立之轉殖株未來可用來篩選降低新月毒素之拮抗微生物。

SA13　製備不同比率銀銅薄膜對於炭疽病病原菌*Colletotrichum gloeosporioides*之抗菌效果初探－沈子謙[1]、林依佳[1]、黃詠鈞[2]、林鉉凱[2]、林盈宏[1] ([1]國立屏東科技大學植物醫學系、[2]國立屏東科技大學材料工程研究所)

Preliminary evaluation of antifungal ac-tivity of silver-copper thin films against *Colletotrichum gloeosporioides*－Shen, T. C.[1], Lin, Y. J.[1], Huang, Y. J.[2], Lin, H. K.[2], and Lin, Y. H.[1] ([1]Dept. of Plant Medicine, National Pingtung University of Science and Technology, Pingtung; [2]Inst. of Materials Engineering, National Pingtung University of Science and Technology, Pingtung)

檬果為最重要的熱帶水果之一，檬果炭疽病其病原菌為*Colletotrichum gloeosporioides*，此為嚴重影響檬果儲藏價值之病原真菌，能造成檬果產業的大量損失，本研究目的在於了解銀及銅對於此植物病原菌的抗菌效果及其原理，藉由熱蒸鍍的方式於玻璃試片鍍上不同比例之銀銅薄膜，並使病原菌分生孢子接觸一段時間後，觀察其抑菌效果。在測試抗菌效果前以掃描式電子顯微鏡 (Scanning Electron Microscope, SEM) 及能量色散x射線光譜 (Energy Dispersive X-Ray Spec-troscopy, EDX) 分析

薄膜之厚度、元素比例及表面結構。實驗發現銀銅薄膜確實具有對 *C. gloeosporioides* 抑制的能力，且經由測試發現，隨著銀含量的增加對於 *C. gloeosporioides* 抑制的能力也呈上升趨勢，顯示 *C. gloeosporioides* 對於銀的感受性遠高於銅。

SB01 Functional analyses of candidate effector proteins in ***Colletotrichum acutatum*** strain 524－Chiang, Chen-Lin[1], Lin, Meng-Yi [1], Chuang, Shu-Cheng[2], Shih, Ming-Che[2] and Lee, Miin-Huey[1] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; [2]Agricultural Biotechnology Research Center, Aca-demia Sinica, Taipei 115, Taiwan)

Chili pepper anthracnose is one of the most important diseases that dra-matically diminishes yield of chili pep-pers. According to the investigation of The World Vegetable Center (AVRDC), ***Colletotrichum acutatum*** is the dominant pathogen of chili pepper anthracnose in Taiwan. The pathogen employs a multistage hemibiotrophic infection strategy to invade host plants but its pathogenicity remains largely unknown. Recently, the genome of ***C. acutatum*** isolate Coll-524 has been sequenced and the transcriptomes during Coll-524 in-fection have been established and ana-lyzed. These data provide the chance to study Coll-524 pathogenicity at the ge-netic level. There is a group of genes encoding small and secreted proteins in Coll-524, called candidate effector pro-teins (CEPs), expressed specifically during infection processes. The objective of this study is to study the function of two small and secreted proteins, CEP002 and CEP012. CEP002 was pre-dicted as a hypothetical protein and CEP012 contains two conserved LysM domains which shown to bind pepti-doglycan in bacteria and chitin in eu-karyotes. After obtaining gene knockout mutants by *Agrobacterium tumefaciens*-mediated transformation, myce-lium growth, spore germination and pathogenicity of wild-type and mutants were examined. Results showed no sig-nificant differences between wild-type and ΔCEP012 in all tests, suggesting that CEP012 might not participate in mycelium growth, spore germination and virulence of Coll-524. However, although ΔCEP002 compared with wild-type showed no significant differ-ences in in vitro tests, results of patho-genicity tests showed significant differ-ences, indicating that CEP002 might in-fluence virulence of Coll-524. Moreover, preliminary tests of heterologous ex-pression of GFP fused full length or mature effectors in *Nicotiana benthamiana* showed that the two effectors could not cause hypersensitive reaction and localized differently in planta. In the future, how CEP002 affects virulence of the pathogen and what roles of CEP012 plays during infection process are need-ed to be investigated.

SB02 芒果炭疽病 (***Colletotrichum gloeosporioides***) 之組胺酸激酶 (His-tidine kinase) 基因CgHK6功能性分析－陳原諄、林獻哲、李敏惠 (國立中興大學植物病理學系)

Functional analyses of histidine kinase gene CgHK6 in Colletotrichum gloeo-sporioides－Chen, Y. J、Lin, H. C.、Lee, M. H. (Department of Plant Pathology, National Chung Hsing University, Tai-chung, Taiwan)

芒果炭疽病菌(***Colletotrichum gloeosporioides***)在田間造成芒果炭疽病 (mango anthracnose) 進而使芒果產量下降，貯藏期縮短，造成經濟上極大的損失。組胺酸激酶 (Histidine ki-nase) 可於細菌、古細菌、黏菌以及植物中被發現，然而動物並沒有組胺酸激酶傳訊系統。組胺酸激酶可感知來自細胞外與細胞內的訊號，進一步調控基因表現來對外界產生反應，根據蛋白質序列可將真菌組胺酸激酶分成11群。本研究之目的為探討芒果炭疽病菌之第三群組胺酸激酶基因CgHK6。利用農桿菌轉殖法 (ATMT；*Agrobacterium tumefaciens* -mediated transformation) 對目標基因進行剔除，獲得兩轉殖株ΔCgHK6A1 與 ΔCgHK6A2 做為後續之供試菌株，以野生型菌株TYC-2為對照進行進一步的測試。於致病性測試中，兩轉殖株之毒力與TYC-2並沒有名顯差異。於MS培養基上ΔCgHK6A1 與 ΔCgHK6A2有較快的生長速率。於PDA培養機上兩轉殖株有較深色的菌落型態。於滲透壓逆境中，兩轉殖株在添加甘油的環境下生長較TYC-2快速，然而在含有山梨糖醇的培養基則有明顯生長減緩的現象。於氧化逆境中，轉殖株具有較高的抗性。將轉殖株培養於含有依普同培養基上可發現轉殖株生長並不受到依普同的抑制。根據以上結果CgHK6參與滲透壓以及氧化逆境，此外CgHK6所轉譯之蛋白可能為依普同之作用點，因此將CgHK6剔除之轉殖株對依普同具有抗性。由PDA培養基上產生較深色菌落之現象推斷CgHK6可能參與黑色素之生合成。

SB03 以農桿菌轉殖法探討西瓜蔓割病菌的致病性或毒力－李玟儀[1]、張碧芳[1, 2] ([1] 國立中興大學植物病理學系；[2] 國立中興大學農業生物科技中心)

Studies on the pathogenicity or virulence of ***Fusarium oxysporum*** f. sp. ***niveum*** by ***Agrobacterium tumefaciens***-mediated transformation method－Li, W. Y.[1]，Chang, P. F. L.[1, 2] ([1] Department of Plant Pathology, National Chung Hsing Uni-versity, Taichung; [2] Agricultural Bio-technology Center, National Chung Hsing University, Taichung)

西瓜蔓割病 (Fusarium wilt of watermelon)為土壤傳播型病害，其病原菌為***Fusarium oxysporum*** f. sp. ***niveum*** (E. F. Smith) Snyder& Hansen，可經由種子帶菌或土壤中殘存的孢子發芽侵入植株根莖組織，切開罹病株的維管束可見明顯褐化病徵，致使植株萎凋死亡，其厚膜孢子可在土壤中殘存數年仍可發芽，嚴重影響西瓜連作地區的產量。目前西瓜蔓割病菌的致病過程還未完整研究，已知相關的致病機制包括致病過程相關基因(pathogenicity related genes)、毒質的多寡、MAPK (mitogen-

activated protein kinase)訊息傳遞路徑等等。本研究將西瓜蔓割病菌*F. oxysporum* f. sp. *niveum* H0103 菌株以農桿菌轉殖法(*Agrobacterium tumefaciens*-mediated transformation)獲得帶有綠螢光蛋白(green fluorescent protein)基因插入的轉殖菌株，在經過接種測試後，得知轉殖菌株Y1對感病品種藍寶西瓜(Grand Baby)的致病能力幾乎喪失，其他轉殖菌株致病能力則與H0103菌株相近或毒力稍微降低或上升。所有轉殖菌株在產孢能力上與H0103菌株並無明顯差異，Y1菌株在減糖PDA培養基上之氣生菌絲生長減少且色素沉積減少。利用共軛焦顯微鏡觀察低毒力Y1菌株和與H0103菌株毒力相近之X3菌株於感病品種蜜寶西瓜(Sugar Baby，SB)與抗病品系JSB西瓜根部之侵入情形，得知在接種初期菌絲侵入與發芽能力無明顯差異，但接種初期SB西瓜根部內的菌絲數量較JSB多，接種後第三天即可見到X3菌絲進入維管束中柱進行快速纏聚，而Y1菌絲侵入速度較慢；X3菌株於接種後第八天可觀察到厚膜孢子的產生，但是Y1菌株在侵入後第十二天仍無法產生厚膜孢子，惟後期Y1菌株在根部的菌絲量已經與X3菌株相近。本研究已用thermal asymmetric interlaced PCR (TAIL-PCR)技術分析轉殖菌株的T-DNA插入區位，將針對其插入基因或鄰近基因之表現與其對致病性或毒力之影響進行後續研究。(本研究感謝NSC 101-2313-B-005-028-MY3、MOST 104-2313-B-005-026、MOST 105-2313-B-005-018等計畫支持)

SB04 台農82號誘變系與台灣良質米抗稻熱病基因座之定位－施昱全[1]、廖大經[2]、吳永培[2]、沈偉強[1]、鍾嘉綾[1] ([1] 國立臺灣大學植物病理與微生物學系、[2]行政院農業委員會農業試驗所嘉義農業試驗分所)

Identification of resistance genes to rice blast in Tainung 82 mutant lines and Taiwan's high-quality rice varieties－Shih, Y.-C.[1], Liao, D.-J.[2], Wu, Y.-P.[2], Shen, W.-C.[1], and Chung, C.-L[1]. ([1] Dept. of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2] Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, COA)

稻熱病普遍發生於全球各水稻產區，可嚴重危害水稻生產，而種植抗病品種被認為是最經濟、有效且對環境友善的防治方式。本研究以誘變育種法結合次世代定序技術開拓新抗性種原，並以連鎖定位法分析臺灣既有水稻品種所含抗病基因，以提供抗病品種使用與輪替之必要資訊。台農82號經連續5代之疊氮化鈉誘變處理及病圃篩選後，獲得44個具優異抗性之誘變系；11個「台農82 x 台農82誘變系」雜交組合之F2族群 (共6300植株) 已於105年二期作在農試所嘉義分所稻熱病圃完成抗性檢定，其中台農82號 x WM1363 之F2族群中，抗感病個體比例為408:162，符合單基因遺傳分離率3:1，目前正以MutMap analysis探討極抗F2族群個體與台農82號親本間具差異之SNP位點，期盼找出新穎抗性基因。台稉8號、台稉14號、台中192號及台東30號為國內種植面積廣且在不同地區均呈現中等抗性的良質米品種，而*Pi2/Pi9/Piz/Piz-t*、*Pik/Pik-h/Pik-m/Pik-p/Pik-s/Pi1*、*Pi20*與*Pita-2*基因座上的抗性等位基因對臺灣稻熱病菌群普遍較具抗性；本研究將四個良質米品種與感病品種LTH雜交，建立F2族群，透過人工接種評估抗性表現，及新設計多型性分子標誌進行基因型分析，初步確認台稉14號、台稉8號及台東30號分別在*Pi2/Pi9/Piz/Piz-t*、*Pik/Pik-h/Pik-m/Pik-p/Pik-s/Pi1*及*Pita-2*基因座帶有抗性等位基因，相關成果可供稻熱病防治及育種參考。

SB05 Cloning three acetylcholinesterase genes from two *Aphelenchoides besseyi* with different nemati-cide-susceptibilities—Hsu, Jung-Kai[1], Chen, Pei-Chen[1], Tsay, Tung-Tsuan[1] ([1]Department of Plant Pathology, Na-tional Chung Hsing University, Tai-chung)

Using pesticides is a common tactic to control harmful pests, however, more and more cases of pesticide resistances had been reported. Several studies showed that target site insensitivity is the main resistance mechanism to or-ganophosphates (OPs) and carbamates pesticides. The OPs and carbamates are acetylcholinesterase inhibitor and non-fumigate nematicides are mostly in these two categories. Single nucleotide polymorphisms (SNPs) is found to be responsible for the drug target site insensitivity in many insecticide studies. The aim of the study is to clarify the as-sociation between nematicides and acetylcholinesterases in *Aphelenchoides besseyi* at gene and protein levels. Two isolates of A. besseyi were used, the Fm isolate was originated from bird's nest fern and Rl isolate from rice. Three nematicides including fenamiphos, carbo-furan and oxamyl were used to treat these two nematode isolates. The LD50 of Fm isolate when treated with fenam-iphos, carbofuran and oxamyl are 103.6 ppm, 226.7 ppm, and 116.2 ppm, re-spectively. The LD50 of Rl isolate when treated with fenamiphos, carbofuran and oxamyl are 167.2 ppm, 2087.2 ppm, and 1166.7 ppm, respectively. The results showed that two isolates had different susceptibilities to these three nematicides. Three Acetylcholinesterase (Ace) genes, *ace-1*, *ace-2* and *ace-3*, of the Fm and Rl isolates were cloned and sequenced, and was the first report in this study. *Ace-1* of Fm and Rl isolates encodes an acetylcholinesterase 1 (AChE1) protein, both of two genes comprise a calculated 628 amino acid (aa) residues. *Ace-2* of Fm and Rl isolates encodes an acetylcholinesterase 2 (AChE2) protein comprising calculated 635aa and 500aa residues, respectively. *Ace-3* of Fm and Rl isolates encodes an acetylcholines-terase 3 (AChE3) protein, both of two genes comprise a calculated 628 aa res-idues. At the amino acid level, *ace-1* and *ace-3* from Fm isolate showed some differences from the two Rl isolate genes, both genes are of 94% identities between isolates. The Fm isolate *ace-2* only had 77% coverage to the Rl isolate *ace-2*, and the overlapping part

had 93% identity. Protein structure prediction showed the AChE2 of two isolates are different. Our results suggested the dif-ferent nematicide-susceptibilities of these two isolates might due to their *ace-2* gene differences.

SB06 Comparative Genomics Analysis and Effector Characterization of the Bermuda Grass White Leaf (BGWL) Phytoplasma—Cho, Shu-Ting[1, 2], Lin, Chan-Pin[1], Kuo, Chih-Horng[2]. ([1]Department of Plant Pathology and Microbiology, National Taiwan Univer-sity, Taipei; [2]Institute of Plant and Mi-crobial Biology, Academia Sinica, Taipei)

'*Candidatus* Phytoplasma cynodon-tis' is the causative agent of the turf dis-ease—Bermuda grass white leaf (BGWL), which was first reported in Taiwan in 1972. This phytopathogenic bacterium is transmitted by insects and is restricted to the sieve tubes of infected plants. The symptoms include chlorosis and dwarfism, thus decreasing the qual-ity of sports fields and pasture, as well as lowering the protection against soil erosion. Despite extensive effort, phyto-plasmas have remained uncultivable outside of their hosts, making the inves-tigation of their biology extremely dif-ficult. To circumvent this difficulty, we conducted whole genome sequencing for a diseased plant collected in Guanyin (Taoyuan, Taiwan) to investigate the gene content of this bacterium. The draft genome assembly contains five contigs with a combined size of 502,218 bp and 435 protein-coding genes. To better un-derstand the evolution of gene content within this genus, we selected six other phytoplasma genomes for molecular phylogenetics and comparative genomics analysis. The results indicated that the gene content is highly diversified across different phytoplasma species. Moreover, the BGWL phytoplasma harbors a new type of potential mobile unit (PMU) that has not been described in other phytoplasmas. Because PMUs could promote genome instability and horizontal transfer of effector genes, this finding expands our understanding of phytoplasma genetic diversity. Finally, we found that all of the three character-ized phytoplasma effectors (i.e., SAP11, SAP54/PHYL1, and TENGU) are absent in the BGWL phytoplasma genome, indicating that this bacterium harbors novel effectors. Our bioinformatics prediction identified 45 putative effector genes and our preliminary screening through expression in transgenic plants identified one strong candidate that causes multiple symptoms (e.g., chlorosis, dwarfism, witches' -broom, and sterile flower). Fu-ture functional characterization of these putative effectors could further improve our knowledge of this plant pathogen.

SB07 Development of marker-free transgenic resistance against thrips-borne tospoviruses in tobaccco plants—Kitty Tsang, Shu-Ting Yip, Uthaman Yazhisai, Ya-Ling Huang, Shyi-DongYeh (Dept. of Plant Pathology, National Chung Hsing University, Taichung)

Cultivated *Nicotiana tabacum* plants are the common tobacco for ciga-rettes and cigars. However, thrips-borne tospoviruses including Pepper Chlorotic spot virus (PCSV), a member of *Watermelon silver mottle virus* (WSMoV) serogroup, cause severe damages on to-bacco crop in Yunnan Province, southern China. So far, there are no effective measures to control this virus due to the lack of resistant tobacco varieties. The transgenic resistance based on post transcriptional gene silencing (PTGS) is an effective approach to control viral diseases; however, the maker genes for regeneration selection are concerns for ecology and food safety. Hence, we pre-viously developed a marker-free two-T-DNA-binary vector, which con-tains a selection marker (*nptII*) and a target gene separately constructed in two individual sets of T-DNA borders to generate maker-free transgenic plant. Transgenic *Nicotiana benthamiana* lines transformed with the hairpin construc-tion of pBI2T-HpL/NSs/N, which carry highly conserved tospoviral replicase (L) gene region, NSs coding sequence (NSs) and antisense fragment of N coding se-quence of WSMoV, have been generated to confer broad-spectrum resistance to tospoviruses. In this study, we attempted to use the same construct to develop marker-free transgenic resistance in the real crop of cultivated tobacco variety Yunyan-87. A total of 41 kanamycin positive tobacco lines were obtained and among them 22 lines were transgene positive, as detected by polymerase chain reaction. Enzyme-linked im-munosorbent assay (ELISA) was con-ducted at 14 days post inoculation (dpi) and 25 dpi with PCSV. A total of 11 lines showed delay in symptom devel-opment for 7-11 days, 3 lines showed recovered symptom at 25 dpi, and 3 lines were symptomless and ELISA negative up to 25 dpi. The selected 6 lines with high levels of resistance to PCSV are being challenged with WSMoV and *Tomato spotted wilt virus* to validate the broad-spectrum resistance. Following by self-fertilization of select-ed transgenic lines, marker-free F2 to-bacco progenies with broad-spectrum resistance to different species of tospoviruses are being screened.

SB08 Generation of useful mild strains for cross protection against *Papaya ringspot virus* in Vietnam—Thu-Yen Thi Tran, Zu-Tung Lin, Chung-Ping Chang and Shyi-Dong Yeh (Department of Plant Pathology, National Chung Hsing University, Taichung)

Papaya ringspot virus (PRSV) is a major limiting factor for papaya (*Carica papaya* L.) production worldwide in-cluding Vietnam. Coss protection using HA 5-1, a nitrous acid-induced mild mutant derived from a severe Hawaii strain HA, has been widely applied to control PRSV in Taiwan since 1984. However, the

problem of strain-specific protection renders the application of HA 5-1 in Taiwan and other geographic re-gions difficult. For solving the problem of strain-specific protection in Vietnam, recombinant mild strains using the long-term stable PRSV HA 5-1 as a backbone to carry the coat protein (CP) and 3'UTR regions of major Vietnam PRSV strains were attempted in this study. Isolate M-TG5 with mosaic symptom and isolate W-ST2 with wilting symptom were chosen as the repre-sentative isolates from Southern Vietnam. To engineer the recombinant mild strains, the whole CP region with the complete 3'UTR of the infectious cDNA clone p35S HA 5-1 were replaced with the corresponding cDNA fragment of M-TG5 or W-ST2 to generate p35S HA5-1/M-TG5cp or p35SHA 5-1/W-ST2cp, respectively. The two re-combinants induced mild symptoms on papaya plants similar to those induced by HA 5-1. The cross protection effec-tiveness provided by the two recombi-nants against M-TG5 and W-ST2 were evaluated under greenhouse conditions, by three independent experiments with a total of 30 papaya plants tested. The plants were first inoculated with indi-vidual mild recombinants and challenged with individual severe strains three weeks later. Recorded one month after the challenge inoculation, our results showed that the recombinant HA5-1/M-TG5cp provided 70% and 40% protection against M-TG5 and W-ST2, respectively; HA5-1/W-ST2cp provided 50% and 60% protection against M-TG5 and W-ST2, respectively; compared to HA 5-1which provided on-ly 20% and 10% protection against M-TG5 and W-ST2, respectively. Thus, we conclude that both mild recombi-nants have potential for control of PRSV in Vietnam.

SB09 *In vitro* encapsidation of genomic RNA segments of ***Watermelon silver mottle virus*** with its nucleocapsid protein－Yue-Rong Tan and Shyi-Dong Yeh (Department of Plant Pathology, Nation-al Chung Hsing University, Taichung)

Reverse genetics systems are piv-otal for understanding the replication cycle and pathogenesis of plant RNA viruses. However, successful infectious clones for negative-sense multipartite RNA tospviruses have not been es-tablished. We hypothesized that stable nucleocapsids reconstituted from viral genomic RNAs transcribed from full-length cDNA constructs with tospo-viral nucleocapsid protein (NP) are es-sential to avoid degradation during in-fectivity assay. Thus, the objective of this study was to determine the condi-tion for the in vitro assembly of WSMoV nucleocapsids. A subcellular WSMoV fraction purified from leaf tissues of WSMoV infected ***Chenopodium quinoa*** plants by differential centrifugation and 20% sucrose density pelleting induces numerous local lesions on leaves of ***C. quinoa*** plants, similar to those induced by the wild type WSMoV. The viral L, NSm, NSs, and N proteins were abundantly accumulated in the fraction as detected by western blotting using the corresponding antisera, suggesting that these viral proteins are essential for viral infection. Immuno-trapping by the nu-cleocapsid protein (NP) antiserum, followed by immuno-decoration or immuno-gold-labelling revealed the presence of numerous nucleocapsids in the fraction as observed by transmission electron microscopy. Furthermore, these nucleocapsids were proved resistant to RNAse digestion. The NP expressed by ***Escherichia coli*** were able to encapsidate WSMoV RNAs extracted from the infectious fraction and form vi-ral-like nucleocapsids. Our results in-dicated that the condition for ***in vitro*** encapsidation of WSMoV RNAs is possible. To further confirm the ***in vitro*** RNA encapsidation capability, viral NP purified from the infectious fraction or bacteria-expressed NP are currently being reconstituted with ***in vitro*** tran-scribed artificial viral S-RNA carrying green fluorescence protein (S G-RNA) to form nucleocapsids. The replication capability of encapsidated artificial S G-RNA is currently being conducted.

SB10 病毒誘發奎藜葉片過敏反應之基因調控－李相伶[1]、葉錫東[2]、陳宗祺[1] ([1]亞洲大學生物科技學系、[2]國立中興大學植物病理學系)

Gene regulation of virus-triggered hy-persensitive response in leaves of ***Chenopodium quinoa***－Siang-Ling Li[1], Shyi-Dong Yeh2 and Tsung-Chi Chen[1] ([1]Department of Biotechnology, Asia University, Wufeng, Taichung, Taiwan; [2]Department of Plant Pathology, Nation-al Chung Hsing University, Taichung, Taiwan)

Plants evolve innate immunity to counteract pathogens' attacks. Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are important signals for regulation of plant immunity. ***Chenopodium quinoa*** is a common indicator plant for isolation of plant viruses by inducing local lesions, which is consid-ered a defensive hypersensitive response (HR). RNA-seq was recently con-ducted to obtain the foliar transcriptome of ***C. quinoa***. The transcriptomic con-tigs of ***C. quinoa***, ***de novo*** assembled from the reads sequenced from healthy and virus-infected foliage inoculated with distinct viruses, were annotated by the ***Arabidopsis*** genome and evaluated for differentiate expression using the ContigViews platform (http://www.contigviews.bioagri.ntu.edu.tw). Some HR-related orthologs, in-cluding pathogenesis-related (PR) pro-teins, transcription factors (TFs), mito-gen-activated protein kinases (MAPK) and defense proteins, that are involved in the defense response of ***Arabidopsis*** were assayed for gene modulation of ***C. quinoa*** responsive to virus infection. The total RNAs extracted from mock-inoculated and Groundnut chlo-rotic fan-spot tospovirus (GCFSV)-infected leaves of ***C. quinoa*** at 1 day post-inoculation (dpi) and 4 dpi, denoted the early and late HR stage, re-spectively, were used for quantitative assay

using the real-time reverse tran-scription-polymerase chain reaction (RT-qPCR) method. The expression levels of the assayed contigs were esti-mated from relative quantitation (RQ) by comparison with the endogenous GAPDH transcript. The results showed that the PR protein CqPR1, the TF CqWRKY53 and the defense protein CqPLA2A were up-regulated at both 1 dpi and 4 dpi. The PR proteins CqBG1 and CqPR4, the TFs CqWRKY42 and CqWRKY75, and the MAPKs CqEBF2 and CqMKK1 exhibited down-up regulation. The defense protein CqSOBIR1 showed up-down regulation. The ex-pression of the TF CqERF1 and the defense protein CqFMO1 significantly re-duced after GCFSV infection. According to the results of the RQ assays, we propose an insight into the vi-rus-triggered HR on leaves of ***C. quinoa*** as the consequence of the SA-mediated cell death. The resistance-related genes of ***C. quinoa*** will be explored in future.

SB11　台灣番茄黃化病毒之分子特性探討－康雅琪[1]、王筠棋[2]、夏君銘[2]、蔡文錫[3]、葉錫東[1]、陳宗祺[2] (1國立中興大學植物病理學系、[2]亞洲大學生物科技學系、[3]國立嘉義大學植物醫學系)

Molecular characterization of ***Tomato chlorosis crinivirus*** in Taiwan－Ya-Chi Kang[1], Yun-Chi Wang[2], Chun-Ming Hsia[2], Wen-Shi Tsai[3], Shyi-Dong Yeh[1] and Tsung-Chi Chen[2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; [2]Department of Biotechnology, Asia University, Taichung, Taiwan; [3]Department of Plant Medicine, National Chiayi University, Chiayi, Taiwan)

Members of the genus ***Crinivirus*** in the family ***Closteroviridae*** possess a bi-partite genome, named RNA-1 and RNA-2, and are transmitted by white-flies in a semipersistent manner. RNA-1 encodes one large ORF for two proteins, ORF1a and ORF1a-ORF1b, which are essential for virus replication. The ORF1a-ORF1b is so-called RNA-dependent RNA polymerase (RdRp). RNA-2 contains a "hallmark gene array" consisting of heat shock protein 70 homologue (Hsp70h), a pro-tein around 59-60 kDa (P59 or P60), a major capsid protein (CP) and a minor capsid protein (CPm). A threshold of 75% amino acid (aa) identity of RdRp, Hsp70h and CP is the key criterion for demarcation of a crinivirus species. ***Tomato chlorosis virus*** (ToCV) is one of important criniviruses causing severe yield losses of tomato production worldwide. ToCV infected tomato and zinnia plants were first recorded in Tainan in 1998 and has been prevailing in central Taiwan since 2013. In this study, the whole genome sequence of a tomato isolate of ToCV collected from Xinshe District, Taichung City, denoted XS, was determined and analyzed. The nucleotide (nt) sequences of RNA-1 and RNA-2 of XS share lower identities of 77.8-78 % and 78-78.1%, respectively, with those of the ToCV isolates collect-ed in Brazil, China, Korea, Greece, Spain and the United States. The RdRp, Hsp70h and CP of XS sharing 88.3-96.2% aa identities with those of other ToCV isolates indicated that XS is an isolate of ToCV. Phylogenetic analyses of RdRp, Hsp70h and CP re-vealed that all ToCV isolates are closely related except the XS isolate forms an independent clade. A one-step reverse transcription-polymerase chain reaction method using primers designed from the XS genomic sequence was able to detect ToCV in infected tomatoes plants or in individual whiteflies. A field survey was carried out during 2013 to 2016, resulting in a high ToCV rate of 62.0% from 121 symptomatic samples detected. Our results demonstrate that the ToCV-XS isolate is phylogenetically distinct from other geographic ToCV isolates and the virus has become a se-rious threat for the production of tomato in Taiwan.

SB12　利用光合作用型硫鐵蛋白作為分子平台篩選芽孢桿菌提升辣椒及牛番茄對高溫、紫外線逆境及青枯病菌抵抗能力－黃志暄[1]、張育誠[1]、陳思妤[1]、張雅雲[1]、黃祥恩[1]([1]國立台東大學生命科學系)

Screening ***Bacillus*** to increase plant re-sistance against heat stress, UV damage and ***Ralstonia solanacearum*** by molec-ular marker of photosynthetic type fer-redoxin in ***Capsicum annuum*** Linn. and ***Solanum lycopersicum***－Chih Hsuan Huang[1], Yu-Cheng Chang[1], Szu-Yu Chen, Ya-Yun Zhang[1], Hsiang-En Huang[1] ([1] Dept. of Life Science, Na-tional Taitung University)

植物生長在自然環境中經常會遭遇許多不同逆境的侵擾，若能藉由改變植物的基礎代謝路徑來增加植物對於逆境的抵抗能力，將可有效同時提升植物對這些不同逆境的抵抗能力。在過去的研究顯示光合作用型硫鐵蛋白 (photosynthetic type Ferredoxin, PT-Fd) 為一群可調控基礎代謝路徑相關酵素活性的氧化還原蛋白。利用基因工程的技術增量表現 PT-Fd 可以有效提升植株對於逆境及病原菌的抵抗能力。然而多數人對於基因轉殖作物的食用及環境衝擊存在許多不確定性的疑慮。為減少基因轉殖所帶來的爭議，本研究針對目前已被廣泛作為生物防治的芽孢桿菌屬細菌進行篩選。研究結果挑選出三株具芽孢桿菌特性的土壤分離菌株進行 PT-Fd 誘導能力測試，經由 RT-PCR 、北方墨點法及西方墨點法確認此三株菌皆具有誘導辣椒 (***Capsicum annuum*** Linn.) 及牛番茄 (***Solanum lycopersicum***) 增量表現 PT-Fd 的能力。利用 16S rDNA 定序結果分別為 ***Bacillus thuringiensis*** HS1、***B. subtilis*** HS2 或 ***B. cereu***s HS2 以及 ***B. amyloliquefaciens*** HS3。其中 HS2 具有增加辣椒葉片大小、高度以及根部發育之能力，其於兩株細菌皆無明顯影響植物生長的現象。在誘導植物抗性基因表現方面，將土壤分離細菌 HS1、HS2 及 HS3 處理在 30 天大辣椒及牛番茄植株根部，具有誘導植物表現抗性基因 PR1 的能力。並有增強抵抗青枯病菌 ***Ralstonia solanacearum*** Rd4 發病的效果。在高溫及

紫外線等非生物性逆境抵抗能力方面， HS1 或 HS2 處理後的辣椒植株，分別進行 2 小時 40℃ 的高溫逆境或 0.5 小時的紫外線 (UV-C，19W) 逆境後皆具有較強的抵抗效果。且過氧化物 H2O2 及 MDA 在 HS1 及 HS2 處理後均有上升的情況。此研究成果顯示藉由土壤微生物的添加，可以達到與基因轉殖植株增量表現 PT-Fd 類似的效果。未來此方式可以廣泛應用在類似 PT-Fd 這類透過基礎代謝調控基因功能的案例，解決利用基因轉殖技術知識與實際運用的落差，達到利用植物生理性的分子指標，來篩選土壤保護性微生物的目標。

SB13　仙人掌X病毒與紅龍果X病毒交互作用之探討－黃靖益、張佑瑋、張雅君 (國立臺灣大學植物病理與微生物學系)

Investigation of the interaction between ***Cactus virus X*** and ***Pitaya virus X***－Huang, C. Y., Chang, Y. W., and Chang, Y. C. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

仙人掌X病毒(*Cactus virus X*, CVX)與紅龍X病毒(*Pitaya virus X*, PiVX)皆是為害紅龍果之*Potexvirus*屬絲狀病毒，近年來因紅龍果逐漸在台灣市場興起，而田間調查發現果園裡的紅龍果幾乎全數帶有病毒，因此開始受到重視。CVX於2001年由本系劉瑞芬老師實驗室首先發表可感染紅龍果，並造成輕微斑駁(mild mottling)病徵；而PiVX則由我們實驗室於2008年發現、鑑定並命名，此二病毒於紅龍果上複合感染的現象非常普遍。為了瞭解CVX與PiVX在寄主植物體內是否有相互影響之情形，我們使用CVX與PiVX感染性選殖株為實驗材料，期望從複合感染時病毒複製之情形，以及在植株上分布狀態，這兩層面探討兩病毒之關係。研究CVX與PiVX對彼此複製之影響，我們以圓葉菸草(*Nicotiana benthamiana*)和紅龍果之原生質體(protoplasts)為植物材料，接種由病毒感染性選殖株所製備之RNA轉錄體，分析兩病毒於單獨和複合感染時，病毒RNA累積之情形。經北方雜合分析法，透過專一性RNA探針偵測目標病毒之RNA訊號，發現複合感染時，兩病毒之RNA累積量皆高於單獨感染之組別。此外，我們也設計能區分CVX與PiVX之專一性引子對，以即時聚合酶鏈鎖反應(q-RT-PCR)分析單獨和複合感染時兩者之RNA累積量；得到之結果與北方雜合分析法相符，再次證實複合感染有利於CVX與PiVX之複製。而觀察兩病毒於植物體內分布之實驗，我們則構築能表現螢光蛋白之病毒選殖株：CVX-mCherry、CVX-EGFP及PiVX-EGFP，將其單獨或共同接種至白藜(*Chenopodium quinoa*)植株上；經共軛焦顯微鏡觀察，發現CVX-mCherry與PiVX-EGFP確實可進入同一細胞內，而CVX-mCherry和CVX-EGFP則很少發生感染同一細胞之情形。綜合上述結果，我們推論當CVX與PiVX共同感染植物寄主時，應可進入同一細胞，並共創對彼此之複製有利之環境，如此的協力作用可能是田間紅龍果普遍受此二病毒複合感染的原因之一。而CVX與PiVX協力作用之詳細機制，還有待更多的研究探討釐清。