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中華民國植物病理學會一百零四年度年會論文摘要

Abstract of 2015 Annual Meeting of Taiwan Phytopathological Society

專題演講 Keynote speech

KS01 推動植物醫師制度之現況與展望—張淑賢、馮海東、陳宏伯、顏辰鳳 (行政院農業委員會動植物防疫檢疫局)

The current status and prospects regard-ing the promoting of the plant physician system—Chang, S. S., Feng, H. T., Chen, H. P., and Yen, C. F. (Bureau of Animal and Plant Health Inspection and Quarantine, COA)

人類自有農業生產開始，病蟲危害之困擾即隨之存在，為避免收成遭受損失農民總是絞盡腦汁進行防治，由於過去我國栽植農作物種類較為單純，病蟲害相亦較為單純，防治技術與資材也較為簡單。而今，隨著貿易自由化及氣候變遷劇大轉變，國內栽植農作物種類驟增，相對病蟲害問題更加多元複雜，加上，時代的演進，國人日益重視健康與食品安全議題，尤其是添加物及農藥殘留等，往往成為國人關注的焦點。因此，為應時代及消費者需要，未來在農業生產過程中，病蟲害之防治，不只是選擇使用低風險安全防治資材及推動整合性防治技術外，更應導入專業人員的輔導與協助，而植物醫師制度的適時建立，亦正符合現代社會之殷切期待，不僅可導正農業生產仰賴化學農藥的概念，更能進一步融合生物性農藥、天敵等非化學農藥的防治方法，研擬整合性防疫措施，搭配合理正確的用藥觀念，輔導農業生產者以現代化作物健康管理模式，友善精準使用生物性防治資材，生產高品質及安全的農產品。目前植物醫師制度規劃係朝法制、專區實作及教育三方面推動，同時就防疫檢疫推展、專業人才培訓與消費大眾溝通等議題進行研議，以落實植物醫師之播種、培育、生根與傳承：一、法制面：草擬植物醫師法，作為推動植物醫師證照制度之法源依據；研擬專技人員需求說明書，爭取植物醫師證照納入國家考試；研議植物醫師執業空間及適用法規，作為推動自願性植物醫師之職業範圍與執業依據；訂定植物醫師考試規則，規範植物醫師應考資格、應試科目及其考試方式。二、專區實作面：籌組植物醫師輔導團，透過植物醫師伴隨式輔導標竿農業專區，提供農友病蟲害與栽培管理客製化建議，並導入防治病蟲害之友善資材及方法，除了輔導農友生產安全農產品外，並可提升其自主管理能力。三、教育面：媒合農企業、農會聘用植物醫師及進行培訓；進行在校學生田間診斷實務經驗的養成工作。

KS02 植物病理在生物經濟產業上扮演的角色—張瑞璋（行政院農業委員會農業試驗所植物病理組）

The role of plant pathology in the de-velopment of the bioeconomy in Tai-wan—Chang, Ruey-Jang (Dept. of Plant Pathology, Taiwan Agricultural Research Institute, COA, Taichung, Taiwan ROC)

為因應氣候變遷的影響，滿足全球人口成長的需求，確保糧食安全及能源的永續供應，先進國家的經濟發展逐漸導向生物經濟產業。生物經濟的定義乃是在生物技術基礎上，發展成產品與服務的一種經濟型態，包括所引導衍生的所有經濟活動，其特性具跨領域、創新、環保、生物資源永續利用等，台灣為地球村的成員，為與國際接軌並跟上全球發展生物經濟產業之趨勢，行政院生技產業策略諮議委員會議於2014年即建議以宏觀的思考，規劃未來生物經濟之發展藍圖，促使行政院於2015年8月正式公布推動「臺灣生物經濟產業發展方案」，將以生物資源及生物技術為基礎，提升我國的產業附加價值，平衡產業發展，促進經濟成長並增進國民健康及生活福祉，且從民眾需求、社會變遷、技術演進等方面思考藥品、醫材、健康照護、食品、農業等臺灣生物經濟相關五領域之發展。台灣生物科技產業為政府推動經濟發展之重點領域之一，已陸續推動「臺灣生技起飛鑽石行動方案」與「臺灣生技產業起飛行動方案」，2014年產值已超過新台幣2,800億元，上市櫃公司快速成長已達91家，民間投資逐年成長，到2014年單年即達458億元，而根據歐盟2012年「永續成長創新：歐洲生物經濟」行動計畫，預估到2025年可產生約450億歐元的產業附加價值。植物病理學緣起於1845年愛爾蘭馬鈴薯晚疫病所造成大飢荒，為解決作物病害危及糧食安全，植物病理學才逐漸受到重視，而植物病理學核心專業的養成訓練，包括病害診斷鑑定、植物病原微生物學、植物病害防治技術、作物生產(菇蕈類)、農藥學、病態生理學等，莫不與生物經濟產業所需技術息息相關，就以生物技術與生物資源運用日趨廣泛的農業、工業及醫藥三大生物經濟產業為例，如：應用分子標記輔助技術抗病育種、

抗病基因篩選、有害生物檢測、農藥殘留管理與監測、微生物發酵技術、環境汙染物監測、食品發酵改良生產技術、菇類栽培技術、微生物農藥、新藥及疫苗開發、轉基因微生物應用開發、奈米生物技術、健康食品機能性分析等相關微生物、生物技術之知識領域，植物病理學都有其發揮之空間。另，植物病理學訓練學生以解決問題為導向的思考邏輯及跨領域的專長培育，有助於未來學生融入生物經濟產業體系。目前我國規劃生物經濟發展方案，除協助推動產業全球運籌行銷外，政府部門應先完備法規與國際接軌，獎勵生技產業投資，結合學術研究單位量能，應用創新前瞻科技以促進創業，導入社會資源與業界活力，才能激發台灣生物經濟未來的潛力。

KS03 The roles of *Pseudomonas cicho-rii*-produced lipopeptides in bacterial midrib rot disease of lettuce— Huang, Chien-Jui (Department of Plant Medi-cine, National Chiayi University, Chiayi)

Pseudomonas cichorii is the causal agent of bacterial midrib rot disease of lettuce, characterized by a dark-brown to green-black discoloration of the infected midrib. Interestingly, the type three secretion system is not essential for path-ogenicity of *P. cichorii* on lettuce. Hence, it was intriguing to explore how *P. cichorii* causes midrib rot disease of let-tuce. Formation of necrotic lesions by several plant-pathogenic pseudomonads is associated with production of phyto-toxic lipopeptides, which contribute to virulence. Thus, a dual approach in-cluding biochemical characterization and genome mining was used to identity *P. cichorii*-produced lipopeptides. Two structurally related compounds, named cichofactin A and B, are linear lipopep-tides with a decanoic and dodecanoic li-pid chain, respectively, connected to the N-terminus of an eight-amino-acid pep-tide moiety. The other two phytotoxic compounds, named cichopeptin A and B, are related cyclic lipopeptides composed of an unsaturated C12-fatty acid chain linked to the N-terminus of a 22 ami-no-acid peptide moiety. Cichopeptin B differs from cichopeptin A only in the last C-terminal amino acid residue, which is probably Val instead of Leu/Ile. In addition to biochemical characterization of lipopeptides, two nonribosomal peptide synthetase gene clusters, *cifAB* and *cipABCDEF*, which are responsible for biosynthesis of cichofactins and cichopeptins, respectively, were identi-fied in the genome of *P. cichorii* SF1-54. To investigate the biological roles of lipopeptides in *P. cichorii* SF1-54, mu-tants deficient in either lipopeptide were constructed. A *cifAB* deletion mutant was completely impaired in swarming motility and also caused significantly less rotten midribs than the wild type. The decreased virulence of the cichofactin- deficient mutant may result from completely abolished swarming motility. Moreover, a *cipA*-deletion mutant no longer produced phytotoxic cichopeptins and exhibited significantly less virulence and rotten midribs than the parental strain upon spray inoculation on lettuce. However, the parental and mutant strains multiplied in lettuce leaves at a similar rate, indicating that cichopeptins contribute to virulence of P. cichorii SF1-54. Together, these results reveal that both cichofactins and cichopeptins play different roles in interaction of *P. cichorii* SF1-54 with lettuce.

KS04 Arabidopsis SMALL RNA- BINDING PROTEIN (AtSRBP) func-tions as a messenger to deliver small-RNAs into target tissues through phloem translocation stream—Hu, Wen-Chi[1,2], Ham, Byung-Kook1, Zeng Jing[1], William, J., Lucas[1], and Yeh, Shyi-Dong[2] ([1]Department of Plant Bi-ology, University of California Davis, U.S; [2]Department of Plant Pathology, National Chung Hsing University, Tai-chung)

In plants, small RNAs function as key regulatory factors in plant develop-ment, metabolism, immunity against bi-otic pathogens invasion, and abiotic stress responses. Long-distance delivery of small RNA through the phloem par-ticipates in systemic regulation over plant development, physiology and de-fense-related mechanisms. For the long-distance movement of sRNAs, the model of protein-RNA complex for-mation to stabilize sRNAs during sys-temic trafficking in phloem has been proposed. In this study, a 15 kDa-protein, cucumber phloem protein was identified from small RNA affinity column named as *Cucumis sativus* SMALL RNA BINDING PROTEIN1 (CsSRBP1). Four Arabidopsis orthologs, AtSRBP1, 2, 3 and 4, are potential components of common sRNA cargo machinery in phloem sieve tube system for sRNA de-livery into target tissues. Protein subcel-lular localization indicated CsSRBP1-GFP and AtSRBP1-GFP lo-cated in cytoplasm. Furthermore, CsSRBP1-GFP and AtSRBP1-GFP were present in cells adjacent to the bombarded cells, suggesting non- cell-autonomous movement abilities. However, AtSRBP4-GFP formed ag-gresome-like complexes in bombarded cells and no cell-to-cell trafficking ability. The results indicated AtSRBP4 functions as a negative regulator of AtSRBP1. Moreover, gene expression pattern of SRBPs was performed using *Arabidopsis pAtSRBPs-GUS* transgenic plants. The pAtSRBPs-GUS activity was present in vasculature in cotyledons, hypocotyl-root junction and new emerged shoot apex, suggested func-tional redundancy of AtSRBPs in those tissues.

KS05 Characterization of orchid mycorrhizal Rhizoctonia fungi and its potential application in bio-control of plant disease caused by pathogenic *Rhi-zoctonia*—Jiang, Jr-Hau[1], Wang, Chang-Sheng[1], Chen, Lung-Chung[2] (Department of Agronomy[1], Department Plant Pathology[2], National Chung-Hsing University, Taichung, Taiwan)

Mycorrhizae are considered to be plant symbioses of importance in pro-moting plant growth and protecting plant from pathogens.

Many researchers have confirmed that the primary mycorrhizal fungi associated with most of green orchids are *Rhizoctonia*, but lit-tle is known about the characterization of mycorrhizal *Rhizoctonia* fungi in plant protection. In this study, *Rhizoctonia* fungi from pelotons in the medicinal herb *Anoectochilus formosanus* are identified. Isolates of AG-6, AG-R, and AG-P greatly increased seed germination and promoted protocorm growth from phases III to VI as compared to asymbiotic treatments. All isolates also formed fungal pelotons in tis-sue-cultured seedlings of *A. formosanus*, which exhibited greater growth than nonmycorrhizal seedlings. Therefore, these fungi were identified as mycorrhizal *Rhizoctonia*. The virulence of mycorrhizal *Rhizoctonia* was evaluated in radish, cucumber, and Chinese mustard. All isolates, except that in AG-R, caused low disease severity in 10-day-old tested seedlings. By contrast, *R. solani* AG-4 killed almost all tested plants with symptoms of collapsed hypocotyl and wilted leaves. Of the 13 mycorrhizal *Rhizoctonia* isolates assessed, two isolates of AG-P provided 91% and 100% protection, respectively, against *R. solani* AG-4 in 26-day-old Chinese mustard. Moreover, our studies also indicated that inoculation of AG-P isolates in rice sig-nificantly alleviate the symptoms of sheath blight caused by *R. solani* AG-1 IA. This study revealed that mycorrhizal *Rhizoctonia* in orchid may have the po-tential application to control damp-ing-off disease in Chinese mustard and sheath blight in rice both caused by *Rhizoctonia* fungi.

論文宣讀 Oral presentation

A01 套袋前藥劑噴濕果串處理對葡萄晚腐病防治效果評估—劉興隆、沈原民、趙佳鴻、黃冬青、吳世偉 (行政院農委會臺中區農業改良場)

Control efficacy of fungicide treatments on grape clusters before bagging in pre-venting grape ripe rot disease—Liu, H. L., Shen, Y. M., Chao, C. H., Huang T. C., and Wu, S. W. (Taichung District Agricultural Research and Extension Station, COA)

自不同葡萄產區分離之79個晚腐病菌菌株(*Colletotrichum* spp.)，測試對12種葡萄晚腐病藥劑之感受性反應，結果25.9%得克利水基乳劑2,000倍、25%撲克拉水基乳劑2,500倍及50%撲克拉錳可濕性粉劑6,000倍等3種藥劑，能完全抑制所有晚腐病菌之菌絲生長。田間試驗結合藥劑及套袋處理，藥劑處理只在套袋前使用1次藥劑，將藥劑均勻噴灑在葡萄果串；套袋處理將袋子套在葡萄果柄，纏繞綁牢。自2013年起先後在5個葡萄產區進行5場次田間試驗，其中3場次於1期作(葡萄栽培期1-7月)進行，2場次於2期作(栽培期6-12月)進行，不同試驗田相同期作之結果一致；1期作葡萄，開花後第4週處理，不論「套袋前有噴藥」或是「套袋前無噴藥」，二者發病皆很低且無顯著差異，但開花後第7週處理，「套袋前有噴藥」皆顯著優於「套袋前無噴藥」；而2期作葡萄，不管是開花後第4週或是第7週處理，「套袋前有噴藥」皆顯著優於「套袋前無噴藥」；藥劑處理不論是使用混合藥劑(得克利＋百克敏)或是單劑(得克利或撲克拉)防病效果皆佳。應用本技術全期僅在套袋前施用1次藥劑，即可有效防治葡萄晚腐病發生，而一般農民慣行栽培全期葡萄晚腐病藥劑則施用10次以上藥劑，本技術展現防治晚腐病的的優異效果，且大幅減少90%晚腐病用藥，每公頃晚腐病防治藥劑費用即可節省9,000元，不只解決葡萄晚腐病困擾農友難題，並可提供消費者安全又高品質的果品。

A02 有益微生物披衣處理玉米種子之病害防治效果初探—蘇士閔、江筱曄、蔡雅竹、邱燕欣、黃玉梅（行政院農業委員會種苗改良繁殖場）

Study of maize seeds coated with effec-tive microorganisms for diseases con-trol—Su, Shih-Min, Chiang, Hsiao- Yeh, Tsai, Ya-Chu, Chiu, Yen-Hsin and Huang, Yu-Mei (Taiwan Seed Im-provement and Propagation Station, C.O.A., Executive Yuan)

玉米露菌病係由*Peronosclerospora sacchari* (T. Miyake) Shirai and Hara所引起，屬於系統性病害，目前多數玉米種子產品多以滅達樂進行種子拌藥處理以預防露菌病之發生。玉米紋枯病係由*Rhizoctonia solani* Kuhn所引起，本病害和水稻紋枯病是同一病原，可相互感染；目前的栽培品種均無抗病性，病原菌可藉由種子傳播。本研究以市售有益微生物產品，包含蕈狀芽孢桿菌、木黴菌、枯草桿菌、莎氏菌，進行玉米種子粉衣處理，觀察其對玉米露菌病與紋枯病發生之影響。試驗結果顯示，經粉衣枯草桿菌之玉米種子能降低露菌病發病率至42%，明顯優於未經處理之對照組(發病率91%)，但尚不及化學藥劑滅達樂處理(發病率17%)之效果。在紋枯病之防治上，同樣以枯草桿菌處理組可降低發病率至60%，明顯優於未經處理之對照組(發病率83%)。在貯藏時間試驗結果，各處理在粉衣兩個月後，仍能觀察到四種有益微生物均穩定存活在種子上且不影響發芽率。此外，各處理之種子經播種後一個月在各處理幼苗之根圈土壤中仍有$1.3x10^4$～$3.1x10^5$ cfu/g soil的有益微生物存活。

A03 梨赤星病之藥劑防治效果—沈原民[1,2]、黃冬青[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)

Control of pear rust disease by fungicide treatments—Shen, Y. M.[1,2], Huang, T. C.[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由*Gymnosporangium asiaticum*引起，有梨與龍柏兩種寄主，每年在臺中東勢等高接梨產區普遍發生，在二月，梨赤星病的冬孢子角在龍柏上產生，到了三月，梨葉陸續出現赤星病的病徵。為了測試施用農藥在梨葉上是否

有效降低梨赤星病，選擇臺中市東勢區梨園進行實驗，將梨樹劃分為施藥處理組與未施藥對照組，施藥組在2011年2月中旬至3月中旬之間施用4次不同種類的殺菌劑，而未施藥組不進行任何處理，在當年度3月1日、15日、29日各進行一次調查，施藥組梨葉的平均罹病率(incidence)在0-2%之間，而未施藥組梨葉的平均罹病率在7-20%之間。另外，為了測試農藥對龍柏上梨赤星病感染源的抑制效果，採集龍柏上的赤星病冬孢子角，在實驗室內測試將冬孢子分別浸泡於9種不同種類的殺菌劑(對照組冬孢子浸泡在水中)，再將冬孢子分散在水瓊脂平板上培養，計算發芽率。實驗結果顯示梨赤星病冬孢子經50%三氟敏水分散性粒劑稀釋6000倍與72%波爾多可濕性粉劑稀釋400倍浸泡後，冬孢子的發芽率低於對照組。

A04　評估內生性液化澱粉芽孢桿菌SPX1菌株防治絲瓜萎凋病之效果— 蔡軼羽[1]、王照仁[1]、潘蕙如[1]、鍾文鑫[1] ([1] 國立中興大學植物病理系)

Evaluation of the efficacy of *Bacillus amyloliquefaciens* SPX1 on controlling Fusarium wilt of luffa—Jen-Yu Tsai[1], Chao-Jen Wang[1], Hui-Ru Pan[1], Wen-Hsin Chung[1] ([1] Dept. of Plant Pa-thology, National Chung Hsing Univer-sity, Taichung)

絲瓜萎凋病由尖鐮胞菌*Fusarium oxysporum* f.sp. *luffae*（Fol）所引起，為台灣絲瓜栽培產業之重要限制因子，目前仍無適當的防治藥劑。前人研究指出，液化澱粉芽孢桿菌（*Bacillus amyloliquefaciens*）對多種作物病害具有防治潛力，適合用於目前尚無推薦藥劑之土傳性病害。本研究以分離自葉用甘藷組織內之液化澱粉芽孢桿菌*B. amyloliquefaciens* SPX1菌株，評估其抑制Fol菌絲生長之效果，進一步於溫室和田間觀察其對絲瓜萎凋病病勢發展之影響。菌株對峙試驗顯示，添加不同氮素源（穀氨酸、氯化銨及硫酸銨）可影響SPX1對Fol菌株之菌絲抑制率，其中又以穀氨酸的效果最佳，可提升SPX1對Fol之菌絲抑制率至56.7-63.8%;而氯化銨與硫酸銨處理組之菌絲抑制率則介於11.6-38.4%。溫室防治試驗結果指出，添加SPX1(濃度為10^8 CFU/ml)之處理組，可延緩萎凋病病勢發展達一週。於南投縣魚池鄉進行田間防治試驗，結果顯示處理SPX1菌株16週後，處理組之發病率介於4.2-16.7%，而對照組之發病率為21.7%，顯示SPX1具有延緩田間絲瓜萎凋病發生之能力。為了解SPX1於絲瓜植體內分布情形，將溫室防治試驗與田間觀察試驗之植株取回分離，並透過ituD與lpa-14等兩組引子對進行PCR檢測。結果顯示，SPX1於苗期導入植株一週後，即可纏據在芽栓（peg）與下胚軸組織中;而16週後更可纏據至150cm處的植物組織內。上述研究結果顯示，SPX1菌株對於絲瓜萎凋病菌有抑制生長的效果，且具延緩萎凋病進展的能力，未來可進一步擴大田間試驗。

A05　台灣草莓萎凋病菌之鑑定與其生物防治試驗—陳冠霖、湯佳蓉、鍾光仁、黃振文 (國立中興大學植物病理系)

Identification for the causal agent of strawberry fusarial wilt from Taiwan and its biocontrol experiments.—Chen, K. L., Tang, J. R., Chung, K. R. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan)

西元2013-2015年間，在苗栗獅潭、大湖等栽培區，發現草莓植株出現矮化、心葉偏上生長、黃化及葉片萎凋乾枯等症狀；若切開病株莖基部則可見到維管束褐變的病徵。由田間取回罹病植株，進行莖基部組織分離可疑病原菌，依柯霍氏法則測試後，確定各分離株的致病毒性後，選取毒力較強之Fofb 01-2與Fofb 4-13兩菌株進行草莓萎凋病菌之鑑定及其生物防治試驗。將Fofb 01-2與Fofb 4-13菌株分別培養於含有1%葡萄糖的Potato Dextrose Agar(PDA)平板上，初期白色菌絲生長綿密，菌落顏色呈白色至灰色，中央有紫色色素沉積，中期菌落上散生有分生孢子褥(sporodochia)呈乳白色至微黃色，偶依附有滴狀分泌物，後期紫色色素沉積加深呈紫黑色，菌落中有墨綠色菌核(sclerotial-like structure)產生；本病原菌的孢子有三種形態，大孢子呈直至彎曲鐮刀狀，無色，大小為19.5-35.6X2.6-5.32 μm (平均25.5X7.1μm)，大多有3個隔膜；小孢子呈現卵圓狀或臘腸狀，無色，大小為4.3-14.1X1.3-5.1μm (平均8.6X3.7μm)，假頭狀排列於單一瓶狀枝之產孢梗上；厚膜孢子呈圓形，大小直徑為4.9-11.5μm。利用Lin等人(2010)針對*Fusarium oxysporum* Schl.所設計的專一性引子對FnSc-1/FnSc-2進行PCR，確定Fofb 01-2與Fofb 4-13兩菌株歸屬於*F. oxysporum*。進一步將病原菌株接種於不同寄主植物，結果僅有草莓植株受感染罹病，隨後以Hirayama等人(2013)針對尖鐮孢菌草莓分化種(*Fusarium oxysporum* Schl. f. sp. *fragarie* Winks & Williams) 所設計的專一性引子對FofraF (CAGACTGGGGTGCTTAAAGTT)及FofraR(AACCGCTAGGGTCGTAACAAA)進行Fofb 01-2與Fofb 4-13的分子生物學分析，並與NCBI資料庫進行比對，確認兩菌株的學名為*F. oxysporum* f.sp. *fragariae*。基於草莓是一種生食的蔬果，需要研發生物防治技術，以確保果品的安全，因此本研究室嘗試由草莓根部分離生物防治的拮抗菌後，經過系列評估測試，選取BM103、BM104及BM105菌株進行溫室防治試驗，首先將三菌株分別施用於草莓穴苗上，連續澆灌3週後，測量草莓植株根長，發現施用各生物防治菌的處理皆較對照未處理組增長2-4公分，證明這三菌株均具有促進草莓植株根部發育之功效，其中尤以BM105菌株促進植株生育的效果最佳。此外，亦以生物防治菌連續澆灌草莓幼苗三週後，再將處理過的草莓植株種植於病菌土中，結果顯示BM103菌株防治草莓萎凋病的效果最佳，大約可降低66%的罹病度。利用生理生化測定及分生技術分析，確定BM103菌株的學名為*Bacillus mycoides*。

A06　液化澱粉芽孢桿菌*Bacillus amy-loliquefaciens* TCBa05及木黴菌*Trichoderma asperelloides* TCTr668對絲瓜萎凋病之防治效果之探討—陳俊位[1]、鄧雅靜[2]、郭建志[1] ([1]行政院農業委員會臺中區農業改良場、[2]朝陽科技大學) Biological control

Fusarium wilt of loofah by *Bacillus amyloliquefaciens* TCBa05 and *Trichoderma asperelloides* TCTr668—Chen, C.W. [1], Teng, Y. C. [2] and Kuo. C.C.[1] ([1]Taichung District Ag-ricultural Research and Extension Sta-tion, COA; [2]Chaoyang University of Technology)

絲瓜萎凋病(Fusarium wilt of loo-fah)為臺灣近年來絲瓜栽培的重要土媒真菌病害之一，其病原菌為*Fusarium oxysporum* f. sp. *luffae* Kawai, Suzuki, and Kawa。病原菌可經由幼苗或土壤侵入植株根莖組織內，致使植株萎凋死亡。本病在中部地區的南投埔里及魚池等地的絲瓜栽培區曾造成嚴重的萎凋型病害，田間發病率高達95%，受害嚴重者甚至廢耕，對絲瓜生產造成很大的衝擊。近幾年在南部地區的高雄大樹及屏東等地的絲瓜產區亦發現萎凋病發生，造成農民嚴重損失。目前本病無適當防治藥劑可用，國內及世界各地多以抗耐病品種或砧木配合綜合管理作為主要預防手段，有效的防治方法仍然闕如。本研究利用本場所篩選對絲瓜萎凋病菌具拮抗能力的菌株液化澱粉芽孢桿菌*Bacillus amyloliquefaciens* TCBa05及木黴菌*Trichoderma asperelloides* TCTr668進行田間防治試驗。應用液化澱粉芽孢桿菌Tcba05與木黴菌TCTr668各100倍稀釋菌液，於絲瓜實生苗種植前採浸泡方式接種木黴菌與芽孢桿菌，分別種植於南投縣國姓鄉絲瓜連作田區及新種植田區後，每週再分別澆灌液化澱粉芽孢桿菌100倍與木黴菌液肥100倍，對照區則施用農友慣行的防治藥劑，在104年5月前初期發病率新種植區在2%以下低於連作區的10%及對照區的20%。在5月豪雨過後，萎凋病發病情形嚴重，但新種植區的發病率2%及連作區的發病率12%皆低於對照區50%的發病率。至9月底採收結束後，處理區的發病率5～15%遠低於對照組的80%。此外，於原種植絲瓜已死亡處之發病田區補植絲瓜苗後，以木黴菌TCTr668 100倍稀釋菌液澆灌補植之絲瓜苗，對照組則依農友慣行用藥處理，一個月後調查發現補植苗處理木黴菌之發病率0%，對照組發病率則高達80%以上。另外，於魚池地區以木黴菌TCTr668 100倍稀釋菌液接種絲瓜嫁接苗後移植田間，對照組則未接種木黴菌。於10月田間調查發病率發現，絲瓜嫁接苗接種木黴菌者發病率在1%以下，對照組發病率則高達70%以上。由試驗結果發現，木黴菌與芽孢桿菌等微生物製劑，不但可有效抑制萎凋病的發生，且對絲瓜產量及品質有極大助益，此於絲瓜病蟲害綜合管理上之應用性值得推薦。

A07 二氧化氯對洋菇褐斑病菌及褐皮病菌之影響—陳錦桐、石信德、鄭吉助(行政院農委會農業試驗所植物病理組). Effect of chlorine dioxide on *Lecanicillium fungicola* and *Myriococ-cum praecox*—Chen, J. T., Shih, H. D. and Cheng, C. C. (Plant Pathology Divi-sion, Agricultural Research Institute, COA)

最近二年在新竹、彰化、南投與雲林等地，許多洋菇栽培場都發現嚴重的病害，罹病的菇體呈現褐色斑點，而後病斑會逐漸擴大凹陷，嚴重時造成菇體畸形，引起經濟嚴重損失。有些栽培場也發現在堆肥製作完成、接種洋菇菌種後，堆肥表層出現白色濃密棉狀菌絲，而後產生大量的孢子並逐漸轉為深褐色，進而佔據菇床，影響洋菇菌絲生長及出菇。由受感染的菇體與堆肥進行病原菌的分離，分別獲得二支菌株，將分離的菌株分別回接於健康菇體與滅菌後的堆肥，三天後出現與分離時相同的病徵。洋菇褐色斑點病菌在顯微鏡下觀察具有無色孢子柄，節間或頂端輪生4-7支的小梗，小梗上著生無色、紡錘狀分生孢子，大小為6-12 x3-5μm，依形態初步判斷為洋菇褐斑病菌 (*Lecanicillium fungicola*)；同時也利用核醣體轉錄外區間序列 (ITS)，增幅解序出一長度為589 bp之序列，經與美國生物訊息中心NCBI 資料庫比對結果顯示該菌株確實為褐斑病菌。感染堆肥的褐色菌株也利用核醣體轉錄外區間序列 (ITS)，增幅解序出一長度為731 bp之序列，經與NCBI 資料庫比對結果顯示此菌株為褐皮病菌 (*Myriococcum praecox*)。將褐皮病菌與洋菇菌株MS、HB、W36、B13及B5等品系進行菌絲對峙培養，發現褐皮病菌與洋菇菌株間沒有拮抗線的產生，但褐皮病菌菌絲卻會抑制菇菌菌落的生長。將不同濃度二氧化氯水溶液噴佈於塗佈有洋菇褐斑病菌孢子懸浮液 (10^3 spores/ml) 的PDA平板後，在24℃培養7天，並以植保手冊推薦用藥撲克拉錳 (50% WP Sporgon) 3000 ppm為對照，結果發現二氧化氯在200 ppm即有抑制孢子發芽效果，而400 ppm時與撲克拉錳3000 ppm同樣具有完全抑制洋菇褐斑病孢子發芽之能力。定量噴灑不同濃度的二氧化氯水溶液於PDA平板上後，再將洋菇褐斑病菌菌絲塊(7 mm)置於平板中央，24℃暗培養6天，並以撲克拉錳3000 ppm作為對照，結果發現二氧化氯水溶液濃度在400 ppm 可抑制洋菇褐斑病菌菌絲之生長，抑制率為71%，濃度在1000 ppm時與撲克拉錳3000 ppm同樣可完全抑制菌絲的生長。進一步將1000 ppm二氧化氯溶液混拌洋菇褐斑病菌之病土(10^5 spores/ml)後靜置12小時，再測試褐斑病菌存活情形，結果發現病土中褐斑病菌孢子已被完全殺死。另外以不同濃度二氧化氯水溶液噴佈在塗佈褐皮病菌10^3 spores/ml孢子的PDA平板上6天後，發現二氧化氯在1000 ppm抑制褐皮病菌的孢子發芽率達99%。以噴佈不同濃度二氧化氯水溶液PDA平板，觀察對褐皮病菌菌絲生長的影響，培養6天後發現二氧化氯800 ppm 即會抑制褐皮病菌菌絲生長，而濃度1000 ppm時則可完全抑制。將二氧化氯溶液混拌褐皮病菌之病土(10^4 spores/ml)，在處理濃度達到1000 ppm，發現具有完全殺滅病土中的褐皮病菌效果。洋菇褐斑病大都由覆土帶菌所引起，現行洋菇或巴西蘑菇栽培場多數以福馬林(Formalin；H_2CO)藥劑消毒覆土基質，但使用福馬林具有危害人體安全之風險，若能使用二氧化氯水溶液進行覆土基質消毒，應較為安全的土壤消毒方式。而洋菇褐皮病菌也可利用二氧化氯有效防治，但其在菇床上的使用方式與消毒時間，仍需作進一步的試驗。

A08 Evaluation of banana cultivars re-sistance to Fusarium wilt disease—Kuan, Cheng-Ping, Chen, Po-Heng, Lin, Meng-Yi and Chen, Han-Wei (Division of Biotechnology, Taiwan Agricultural

Research Institute, COA, Wufeng, Tai-chung)

FOC is the causal pathogen of wilt disease of banana. *Fusarium* wilts caused by FOC is one of the most de-structive diseases of banana worldwide. It is a classic vascular wilt disease in which the fungus gains entry to the wa-ter conducting xylem vessels, then pro-liferates within the vessels causing water blockage. The typical symptoms include wilting and death of the leaves, followed by death of the whole plant. A cost-effective measure of control for this disease is still not available. In this study, for comparing different levels of toler-ance in bananas to Fusarium wilt disease, several cultivars, i.e. 'Pei Chaio', 'For-mosana', 'Tai Chiao No. 5', 'Gros Michel', 'Pisang Awak' and 'Cultivar Rose', which were commonly cultured in Taiwan, were inculcated artificially with *Fusarium* pathogen. Percentages of chlorosis were estimated after culturing two-month-old seedlings of bananas in *Fusaium*-inoculum soil in greenhouse. Besides, we examined browning region of corm sections of Foc-inoculated plants quantitatively and qualitatively and estimate the amount of Foc in each corm section by real-time PCR method. Results showed that 'Cultivars Rose' was the more resistant cultivar among six cultivars; otherwise, 'Gros Michel' was the more susceptible cultivar.

A09 LTH MLs 抗稻熱病基因效益初探與稻熱病菌族群 Avirulence 基因調查—陳繹年、陳美君、陳淑媚、陳美雅、林宗俊、陳純葳 (行政院農委會農業試驗所植物病理組)

Effectiveness evaluation of the rice blast resistance genes in LTH MLs and survey for the avirulence gene of *Pyricularia oryzae* in Taiwan —Y. N. Chen, M. C. Chen, S. M. Chen, M. Y. Chen, T. C. Lin, and C. W. Chen. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA)

為找尋對國內水稻稻熱病菌具抗性之抗稻熱病基因，及分析稻熱病菌族群致病性與分佈狀況，利用自國際稻米研究所 (International Rice Re-search Institute, IRRI, the Philippines) 引進之麗江新團黑穀單基因系 (Liji-anxintuanheigu monogenic lines, LTH MLs) 水稻作為材料，來監測其對國內稻熱病菌族群之抗性表現。2014-2015 年分別檢測 179 及 178 株當年度收集之稻熱病菌株對 31 個 LTH MLs 品系及 20 種國內水稻商業品種之致病性。LTH MLs 中以 IRBL20-IR24、IRBLta2-Re、IRBL11-Zh、IRBLta2-Pi 及 IRBL9-W 等五個品種連續兩年抗病性最佳；IRBLks-F5、IRBLa-C 及 IRBLzt-T 等品種抗病性最差。20 個國內水稻商業品種中，以 TNG 84、TKW 1、TCS 10、TNG 79、TCS 17 及 TT 30 等品種連續兩年抗病性最強；TK 14、TNG 71、TN 11、KH 145、TY 3 及 HL 21 等品種抗病性最差。利用已發表之抗稻熱病基因專一性引子對檢測國內水稻商業品種，在 TNG 84 與 TKW 1 中可增幅出 *Pita* 基因產物；TCS 10 與 TCS 17 中可增幅出 *Pib* 基因產物。針對 2015 年收集之 436 株國內不同區域及寄主來源之稻熱病菌株進行無毒力基因 (avirulence gene) 檢測，PCR 增幅結果顯示，*Avr-Pik*、*Avr-Pita* 及 *Avr-Pib* 普遍存在受測之 417 株水稻菌株中，檢出率分別為 98.8%、95.92% 及 92.81%。根據 LTH MLs 及國內水稻商業品種抗性檢測結果推論，抗稻熱病基因 *Pita2*、*Pib*、*Pik* 與商業品種 TNG 84、TKW 1、TCS 10、TCS 17 良好的抗性表現，可能與國內稻熱病菌族群普遍帶有無毒力基因 *Avr-Pita*、*Avr-Pib* 及 *Avr-Pik* 有關。

A10 以開花期接種探討水稻徒長病菌與稻種的關係—李國維[1]、陳啟予[1]([1] 國立中興大學植物病理學系)

Studies on the association of *Fusarium fujikuroi* and rice seeds by inoculation during flowering stage—Lee, G. W.[1], Chen, C. Y.[1] ([1] Dept. of Plant Pathology, National Chung Hsing University, Tai-chung)

水稻廣泛栽種於世界各地，主要以稻種作為傳播的方式，且稻種的品質會直接影響水稻的生育，進而影響稻米的產量。有多種微生物會藉由稻種攜帶傳播，例如細菌、病毒以及真菌，其中病原真菌常會使稻種品質下降，且病原真菌可以與稻種共存並在水稻的生育階段造成病害，像是由*Fusarium fujikuroi*造成的水稻徒長病。臺灣地區的水稻徒長病好發於一期稻作，於水稻生育期間發病造成部分植株死亡，亦會使成株稻穗充實不完全，造成稻米產量嚴重的損失。已知真菌藉由種子傳播的機制存在著共同演化的關係，然而目前真菌與種子之間的共存關係尚未明確，故本研究欲探討稻種與*F. fujikuroi* 之間的關係且模擬田間*F. fujikuroi*感染水稻的情形。藉由在水稻開花期的稻穗上接種*F. fujikuroi*，並於採收期收集接種處理過的種子，將這些種子以平板法做帶菌率的測試，於未處理表面消毒的種子測得*F. fujikuroi*的帶菌率為16-32%，而在經過表面消毒處理的種子測得*F. fujikuroi*的帶菌率為8-22%，推測*F. fujikuroi*可以藉由開花期於稻穗上接種進入稻種內部，而非僅是表面污染。另外，藉由盆播試驗，比較種衣接種的種子與開花期稻穗接種的種子兩者間發病情形的差異，結果顯示種衣接種處理於苗期有較嚴重的徒長病徵；而開花期稻穗接種的種子，在苗期的病徵較輕微甚至沒有。總而言之，本研究發現*F. fujikuroi*可以藉由開花期侵入水稻種子，並且作為水稻徒長病的初次感染源。

A11 胡麻炭腐病之研究—吳雅芳、吳盈慧、鄭安秀(行政院農委會臺南區農業改良場) Studies on charcoal rot dis-ease of sesame—Wu,Y. F., Wu, Y. H., and Cheng, A. H.（Tainan District Ag-ricultural Research and Extension Sta-tion, COA）

胡麻又稱芝麻、油麻、烏麻及麻仔等，屬胡麻科胡麻屬之一年生草本植物，臺南市為主要產區，佔全臺85%以上，集中在西港、善化、將軍、佳里、安定、七股、安南區等地。以土壤傳播性病害包括疫病、白絹病、萎凋病、炭腐病等為其種植

的最大限制因子。其中最常見也最令栽培者困擾的是炭腐病，主要危害幼嫩或衰老組織，苗期感染可造成幼苗萎凋死亡，開花結莢期由根部或莖基部開始往上變黑，農民稱之為黑腳病。罹病部位密生黑色小點為其柄子殼，表皮下產生小菌核，莖部腐化中空，造成植株萎凋死亡。由罹病組織分離出病原菌進行觀察及病原性測定，並利用ITS4、ITS5引子對進行PCR及基因定序比對，確定病原菌為 *Macrophomina phaseolina*，菌絲生長初期白色，漸轉墨綠色，最後為黑色，最適合生長溫度為30-35℃，於培養基上篩選出10種藥劑可有效抑制 *M. phaseoli* 之菌絲生長，其中四氯異苯腈、免得爛、三得芬、菲克利等藥劑已登記為胡麻用藥。*M. phaseoli* 於人工培養基上產孢量少，為利於後續試驗之進行，本研究利用馬鈴薯培養基與培養土製作 *M. phaseoli* 之接種源，測試不同的培養條件並比較其致病力。為有效防治胡麻之土傳病害，以不同來源之木黴菌與胡麻疫病菌、萎凋病菌、白絹病菌及炭腐病菌，於培養基上進行對峙培養，篩選出可有效抑制病原菌生長之菌株，於溫室內測試其對胡麻種子發芽及生長之影響、防治病害之效果，以利後續田間試驗進行。

A12 雲嘉南地區水稻小粒菌核病發生初步調查與防治藥劑篩選—林國詞、吳雅芳、鄭安秀（行政院農業委員會臺南區農業改良場作物環境課）

Preliminary investigation occurrence and fungicides screening of stem rot of rice in Yunlin, Chiayi, and Tainan—G. C. Lin, Y. F. Wu, and A. S. Cheng. (Division of Crop Environment , Tainan District Agricultural Research and Extension Station ,COA)

稻小粒菌核病病原菌之有性世代為 *Magnaporthe salvinii* (Catt.) Krause & Webster，無性世代為 *Nakataea sigmoidea*,(Cav.) Hara。病原菌侵入水稻後，分解組織使其軟化腐朽，莖稈地基部軟化後，常引起稻株倒伏並提早死亡，可導致50%以上減產。而田間病徵與飛蝨類所引起之「蝨燒」相似，因不易分辨而常互相混淆，大多以下半部莖幹先枯萎組織鬆軟者做為初步判斷。本病害為早期臺灣水稻之風土病之一，隨水稻新品種陸續育成推廣後，本病害銷聲匿跡一段時間，近幾年於田間偶發性出現。104年田間初步調查，分別於一期作與二期作於雲嘉南地區選取15個田區收集田間水稻殘體進行分離，一期作中有6個田區水稻殘體分離出小粒菌核菌，而二期作則無分離得到。利用上述6個菌株以臺南11號水稻進行病原性測試，結果顯示有3個菌株具有病原性，分別分離自雲林縣大埤鄉、嘉義縣太保市、臺南市柳營區。以此3株病原菌進行防治藥劑初步篩選，選取登記於水稻使用之16種不同的作用機制藥劑進行測試，於4天後觀察其抑制率，篩選出有7種藥劑對小粒菌核菌菌絲生長抑制率呈現100%，分別為40%甲基多保淨水懸劑 1000倍、55%貝芬同可濕性粉劑 1000倍、50%福多寧可濕性粉劑 2000倍、50%護粒松乳劑 1000倍、23%菲克利水懸劑 4000倍、20%芬諾尼水懸劑 1500倍與80%鋅猛乃浦可濕性粉劑 500倍。此7種藥劑可作為農民選取防治藥劑之參考。

A13 北部地區甘藷基腐病發生調查及病害田間防治試驗—張為斌、吳信郁、施錫彬 (行政院農業委員會桃園區農業改場)

Investigate incidence of sweet potato foot rot in northern Taiwan and field test of disease control.—Chang, W. B., Wu, H. Y., Shih, S. P. (Taoyuan District Ag-ricultural Research and Extension Sta-tion, COA, Taoyuan)

甘藷基腐病是由*Phomopsis destruens*所引起的甘藷病害，罹病甘藷莖基部乾枯、黑褐化影響藷塊生長，更甚者在後期造成藷塊褐化腐敗，本病害可由風雨與扦插藷苗傳播，並藉由罹病植體殘存田間，嚴重時罹病率可達88%，嚴重影響產值。本試驗於甘藷種植期間，調查新北市金山區或萬里區甘藷田區病害發生情形，藉以瞭解田區病害發生時程，另藉由土壤處理試驗、藥劑處理試驗、產量調查與甘藷品種間罹病差異試驗，瞭解各防治技術之效果，以期提供病害管理方法擬定參考。在田區病害發生調查中，每2週調查一次，各田區採收前病害罹病率介於4-46%，調查期間，病害發生率於7月上旬急劇上升，可作為防治時機之參考。土壤處理試驗，於種植前1個月以邁隆400公斤/公頃處理種植土壤與對照組有顯著差異，平均防治率為52%。藥劑處理試驗，於種植後45日開始進行處理，每週施藥一次，共處理三次，其中以腐絕或貝芬替處理效果較佳，至採收前平均防治率為60.3%，平均產量較對照組分別增加91%及99%；以亞磷酸及氫氧化鉀合劑500倍澆灌處理，約可維持30天的保護效果，具有做為非農藥防治資材的潛力。甘藷品種間罹病差異試驗選擇本場推廣品種桃園1號、桃園2號、桃園3號及慣行品種臺農57號、臺農66號及臺農10號共6種進行接種試驗，最終結果顯示，各品種間雖然罹病率有所差異，以桃園3號及臺農66號較低，但植株平均罹病率仍高達73%及62%，無法單以品種選擇做為防治策略。

A14 除草劑對於草莓炭疽病菌產孢的影響—高宏遠、黃振文 (國立中興大學植物病理學系)

Effect of Herbicides on Sporulation of Strawberry Anthracnose Fungus—Kao, H. Y. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing Uni-versity, Taichung 40227, Taiwan)

除草劑是現代農耕用於管理雜草的重要手段，不當施用會毒傷作物外，尚且會促進病害的發生。黃氏等(1995)指出草脫淨、拉草、丁基拉草及三福林可引起豌豆植株莖部及根部組織褐化壞死，並誘發立枯絲核病菌危害植株。Gindrat、Pezet、Biggs及Sinclair氏(1994)指出巴拉刈可毒傷植物體外，還可促進 *Alternaria, Botrytis, Cercospora, Colletotrichum*及*Phomopsis*等病原真菌的菌絲生長與產孢。本研究主要目的在於探討嘉磷塞、巴拉刈及固殺草等除草劑對於草莓炭疽病菌*Colletotrichum gloeospori-oides* D-2與E-3菌株產孢的影響。試驗研究結果發現25 ppm嘉磷塞、5 ppm巴拉刈及5 ppm固殺草分別可促進61.2%、

53%及59.6%草莓炭疽病菌的孢子發芽；10 ppm嘉磷塞、5 ppm巴拉刈及2 ppm固殺草分別提高炭疽病菌的發芽管增長31%、75%及20.4%。以查氏培養基(Czapek solution agar)分別加入這三種不同濃度的除草劑，製成平板培養*C. gloeosporioides* D-2與E-3菌株，結果發現250 ppm嘉磷塞與3 ppm巴拉刈分別促進炭疽病菌D-2菌株的產孢達254.5%與157.4%，同時這兩種藥劑的劑量也分別可促進E-3菌株產孢達92.8%與111.3%。進一步，利用3 ppm巴拉刈處理草莓植株後，隨即接種炭疽病菌D-2菌株，結果證明巴拉刈水溶液可提升20%草莓植株遭受炭疽病菌的危害。

A15 台灣產尾子菌類真菌之多樣性研究—江冠寰[1]、陳啟予[1] ([1]國立中興大學植物病理學系)

Studies on the diversity of cercosporoid fungi in Taiwan. —Chiang, K. H.[1], Chen, C. Y.[1] ([1] Dept. of Plant Pathology, Na-tional Chung Hsing University, Tai-chung)

本研究目的在於調查台灣的尾子菌類(cercosporoid)真菌之多樣性，尾子菌類真菌是指有性態可以對應到*Mycosphaerella*屬的深色絲孢菌；依據現行之分類概念，尾子菌類真菌涵蓋了許多屬，常見的屬包括*Cercospora*、*Pseudocercospora*、*Ramularia*及*Passalora*。尾子菌類皆為植物病原菌，且具有明顯的寄主專一性，在寄主上造成葉斑。本研究從野外採集病葉，透過病原形態、寄主來達到鑑定目的。目前共鑑定出5種新種(3 *Pseudocercospora*、2 *Cercospora*)、4種新紀錄種(*Pseudocercospora pouzol-ziae*、*Pseudocercospora guianensis*、*Ramularia pratensis*、*Ramularia pleu-ropteri*)；研究過程中，亦透過ITS片段來進行親緣分析，所建構之親緣關係圖譜雖然無法順利區隔種的分類階層，但是在屬之界定上仍然為重要之輔助依據。實驗過程並發展促進尾子菌類真菌產生孢子之培養方式，以作為實驗接種之來源。

A16 台灣玫瑰疫病初報—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)

First report of root and basal stem rot of rose caused by Phytophthora spp. in Taiwan—Huang, J. H. and Ting, P. Y., Ann, P. J. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA)

2015年9月在南投縣埔里鎮數處溫室內土耕栽培的玫瑰(*Rosa rugosa*) 發生一種新的病害，罹病植株由下位葉往上開始呈現黃化而無壞疽病斑，葉片於病害後期易褐化脫落，少數植株部分枝條急速萎凋，大部分的植株出現根腐及莖基部黑褐化的病徵。於不同栽培田取回罹病植株，經組織分離可由根部與莖基部獲得兩種疫病菌—*Phytophthora* sp. A及*Phytophthora* sp. B，將分離得到的疫病菌利用菌絲塊接種於苗齡40-50天的'埔里之星'與'萬年紅'玫瑰品種扦插苗，於溫度30℃及光照12小時之條件下，*Phytophthora* sp. A 於14天即可造成上述2玫瑰品種葉片黃化、莖基部褐化，28天後造成植株枯死，而*Phytophthora* sp. B只感染'埔里之星'造成發病，上述發病的組織均可再分離得到原接種的疫病菌，完成柯霍氏法則。*Phytophthora* sp. A孢囊橢圓形或卵圓形，長寬比為1.26，大小為23.5－65.5×21.1－56.9 μm (avg. 42.3×33.7 μm)，不具乳突，孢囊具有內展再生(internally extended proliferation) 或內巢再生 (internally nested proliferation) 之現象，並可產生菌絲膨大體 (hyphal swellings) 及厚膜孢子 (chlamydospore)，有性世代為同絲型 (homthallism)，其藏精器以側著 (paragynous) 為主，少部分為底著(amphigynous)，大小為11.0－22.7 ×9.1－19.0 μm (avg. 16.7×14.6 μm)，藏卵器 (oogonia) 球形，大小為29.6－45.5 μm (avg. 38.0 μm)，卵孢子 (oospore) 球形，大小為23.1－40.9 μm (avg. 31.5 μm)，菌絲生長溫度為8－33℃，最適溫為28℃，菌絲生長速度為7.0 mm/day；Phytophthora sp. B孢囊為卵圓形或倒梨形，長寬比為1.57，大小為24.9－43.2×19.7－27.9 μm (avg. 35.4×22.6 μm)，具半乳突，其有性世代亦屬於同絲型，藏精器為側著，大小為7.1－14.8×7.0－11.0 μm (avg. 9.8×8.5 μm)，藏卵器球形，大小為23.5－34.5 μm (avg. 28.7 μm)，卵孢子球形，大小為19.7－32.6 μm (avg. 26.2 μm)，生長溫度為16－32℃，最適溫為28℃，菌絲生長速度為12.0 mm/day。利用核醣體內轉錄區間 (ITS) DNA序列比對，*Phytophthora* sp. A屬於Clade7而*Phytophthora* sp. B屬於Clade2，經由形態外觀及DNA序列分析初步鑑定為台灣疫病菌之新紀錄種。本文為台灣首次報導玫瑰疫病。

A17 亞磷酸鹽防治胡瓜露菌病之機制初探—黃晉興、洪雪香（行政院農業委員會農業試驗所）

Preliminary study on the mechanism of phosphite against cucumber downy mil-dew— Huang J.-H. and Hung H.-H. (Plant Pathology Division, Taiwan Ag-ricultural Research Institute, COA)

本研究初步探討含有亞磷酸根離子的溶液，如中和亞磷酸（等量的亞磷酸與氫氧化鉀）、亞磷酸二氫鉀與亞磷酸氫二鉀，防治胡瓜露菌病之機制。這3種溶液對胡瓜露菌（*Pseudoperonospora cubensis*）抑制孢囊發芽與降低病害之結果相近，在12mM亞磷酸根溶液浸漬處理之孢囊發芽率18－72%，而48 mM處理之孢囊發芽率0－8%，其中以亞磷酸氫二鉀對孢囊發芽抑制較差。以葉圓片接種法測試其防治胡瓜露菌病的效果，在上述3種溶液0.19 mM濃度之處理，葉圓片之發病度皆顯著降低，0.75 mM或以上之處理則完全不發病；進一步將上述3種溶液噴佈於盆栽胡瓜7天後再接種露菌，結果顯示3 mM之處理即可顯著降低露菌病發病度，而12 mM之處理可使植株發病度降至0－1%。亞磷酸根溶液噴在第二位葉於當天起即可防治該位葉的露菌病，7－21天後再接種則第二位葉仍有很好防治效果，但僅能輕微降低其他位葉的病害，而噴灑亞磷酸根溶液在葉片上表面當天或7天後再接種病原菌於葉背，皆可防治病害。先於盆栽植株葉片接種露菌0－1天再噴亞磷酸根溶液則完全不發病，接種3－5天後再噴亞磷酸根溶液則有輕

微發病，但顯著較對照組顯著低，而孢囊產量少且不發芽，而接種7天後再噴亞磷酸根溶液則發病度與對較組無差異，但孢囊產量低且發芽率亦低，皆與對照組有顯著差異。綜合以上試驗結果顯示在低於抑制孢囊發芽的亞磷酸根離子濃度即有防治病害的效果；亞磷酸根離子於胡瓜葉片滲透性好，但移行效果不佳；亞磷酸根離子不僅有預防胡瓜露菌病的效果，也有治療的效果。

A18 荔枝露疫病菌殘存研究－黃巧雯[1]、倪蕙芳[1]、林靜宜、柯文琪、楊宏仁[1] ([1]行政院農業委員會農業試驗所嘉義農業試驗分所)

Study on the survival of *Peronophythora litchii*—Huang, C. W. [1], Ni, H. F. [1], Lin, C. Y.1, Ko, W. C.[1], and Yang, H. R. [1] ([1]Department of Plant Protection, Chiayi Agricultural Experiment Station, Taiwan Agricultural Research Institute)

露疫病為荔枝主要病害之一，但到目前為止，露疫病的發病生態尚不完全明瞭，無法妥善控制病情，一旦發生常使農友於短時間造成大量的損失，嚴重影響收益。因此本研究初步探討露疫病菌在果園中殘存情形。以人工接種方式將孢囊懸浮液 (10^5 sporangia/ml) 直接噴佈於荔枝嫩葉上，於接種後經套袋保濕3天後拆袋，之後每5天自田間取回已接種露疫病菌之葉片，並以半選擇性培養基進行病原菌偵測，由試驗結果得知，在接種後226天可偵測到病原菌存活，顯示病原菌可於葉片上殘存時間約半年以上。而在露疫病菌仿落葉上殘存情形試驗，將孢囊懸浮液 (10^5 sporangia/ml) 直接噴佈於荔枝嫩葉上，於接種後經套袋保濕3天後將已接種完成之葉片放置於樹下，每5天採樣一次，並以半選擇性培養基進行病原菌偵測，由試驗結果得知，僅能於接種後10天之罹病落葉偵測到病原菌存活，顯示落葉應不適合此病原菌之長期殘存。另外，進行土壤濕度與荔枝露疫病發生關係試驗，分別於土表面、土表下10公分、20公分及30公分處加入50 ml荔枝露疫病菌孢囊懸浮液 (105 sporangia/ml)，土壤水分持續地維持過飽和狀態，由試驗結果得知，土表面在1天內即可觀察在嫩葉上產生褐色斑點病徵，土表下10公分在57小時其葉片上有褐色斑點產生，於第四天在葉片上偵測到露疫病菌；土表下30公分在81小時於葉片上觀察到褐色斑點病徵，於第五天才可偵測到露疫病菌。由本研究得知荔枝露疫病菌可在樹上之葉片上殘存至少半年以上，但若是落葉狀態不適合此病原菌殘存場所，至於露疫病菌以何種組織結構殘存於樹上葉片或土壤中仍需進一步探討。

A19 以聚合酶連鎖反應偵測食品之山葵成分－許皓婷[1]、王妙媛[2]、余聰安[1]、江主惠[1] ([1]大葉大學分子生物科技學系、[2]國立彰化師範大學生物學系)

Polymerase chain reaction method for the detection of wasabi in foods—Hsu, H. T. [1], Wang, N. M.[2], Yu, T. A. [1], and Chiang C. H.[2] ([1] Dept. of Molecular Bi-otechnology, Da-Yeh University, Changhua; [2] Dept. of Biology, National Changhua University of Education, Changhua)

山葵為傳統日本料理經常使用的調味料，其特殊辛辣氣味特別受饕客喜愛，此外，山葵中所含有的硫氰酸鹽等化合物，具有抑制微生物、線蟲、昆蟲、抗發炎及抗癌症的能力。由於山葵種植面積日漸減少，而需求量卻與日俱增，因此市面所販售之山葵大部分為具相似味道的十字花科植物和綠色食用色素混合的仿製品。因此，本研究利用PCR技術，以山葵芥子酶（myrosinase）基因與其他十字花科植物之myrosinase基因具差異性之特質，設計山葵專一性引子，進行山葵樣品檢測。本試驗首先利用RT-PCR選殖出對應山葵 myrosinase mRNA之序列，共含1644 個核苷酸，對應548個胺基酸，由核苷酸序列比對與演化親緣性分析可發現，山葵myrosinase基因親緣性介於*Brassicacea*作物與*Arabidopsis*植物之間。另外，為了找出十字花科植物myrosinase DNA序列的變異區，以用來設計山葵專一引子，本試驗也同時選殖芝麻葉、小白菜、青江菜、花椰菜及白蘿蔔中的myrosinase DNA片段進行分析與比對，最後以山葵專一引子對配合適當的黏合溫度進行PCR，可偵測出山葵myrosinase之DNA片段，而其他十字花科植物，包括青江菜、花椰菜、白蘿蔔、芝麻葉、芥菜、高麗菜及小白菜之myrosinase DNA幾乎是未檢出，顯示所使用之山葵引子具相當高之專一性。另外，在測試PCR的靈敏度時可發現，山葵總DNA濃度僅為0.01 ng/μl時，仍可偵測到myrosinase之DNA條帶，顯示本系統可偵測極微量之DNA。本試驗之結果將進一步應用在real-time PCR之山葵成份定量，此將可應用於檢驗商品標示之真實性，期待能重新建立國人對食品標示之信任度，恢復我國食品生產之信譽。

B01 利用核鞘基因序列保留區段設計簡併引子對組進行西瓜銀斑病毒血清群種類之鑑定—劉魯垣[1]、陳宗祺[2]、陳滄海[3] ([1]屏東科技大學農園生產系博士班、[2]亞洲大學生物科技學系、[3]屏東科技大學植物醫學系)

Development of degenerate primer sets for identification of *Tospovirus* species in the WSMoV serogroup—Lu-Yuan Liu[1], Tsung-Chi Chen[2], Tsang-Hai Chen[3] ([1]Department of Plant Industry, NPUST, Pingtung, Taiwan; [2]Department of Biotechnology, Asia University, Wufeng, Taichung, Taiwan; [3]Department of Plant Medicine, NPUST, Pingtung, Taiwan)

*Tospovirus*病毒屬為世界性重要經濟危害的植物病毒之一，寄主範圍廣泛，能感染82科900種以上的重要經濟作物。主要造成寄主作物斑點(spot)、黃化(chlorosis)、斑駁(mottle)、矮化(stunting)、萎凋(wilting)及壞疽(necrosis)等重要的病徵。本病毒屬重要的兩個血清群分別是危害歐洲地區的番茄斑點萎凋病毒血清群(TSWV serogroup)及亞洲地區的西瓜銀斑病毒血清群(WSMoV serogroup)，危害我國重要作物的Tospovirus

屬病毒多屬WSMoV血清群，透過媒介昆蟲薊馬的傳播，常造成茄科及瓜類嚴重的經濟損失，甚至絕收的情勢出現。WSMoV血清群有西瓜銀斑病毒(*Watermelon silver mottle virus*, WSMoV)、花生頂芽壞疽病毒(*Groundnut bud necrosis virus*, GBNV)、西瓜頂芽壞疽病毒(*Watermelon bud necrosis virus*, WBNV)、番椒黃化病毒(*Capsicum chlorosis virus*, CaCV)、彩芋黃化斑點病毒(*Calla lily chlorotic spot virus*, CCSV)、甜瓜黃斑病毒(*Melon yellow spot virus*, MYSV)、桑葉脈條斑病毒(Mulberry vein banding virus, MuVBV)、番茄環斑病毒(*Tomato zon-ate spot virus*, TZSV)、番茄壞疽環斑病毒 (*Tomato necrotic ringspot virus*, TNRV)、番茄壞疽斑點關聯病毒(Tomato necrotic spot-associated virus, TNSaV)及胡椒黃化斑點(*Pepper chlo-rotic spot virus*, PCSV)等至少十一種病毒，目前持續發現增列中。本研究利用比對該病毒血清群核鞘(nucleocapsid, N)基因序列並分析其基因序列內的保留區域(conserved region)設計簡併引子(degenerate primer)，分別為正向引子FW1、FW2、FW3、FW4、FW、FW6及反向引子rw1、rw2、rw3、rw4，彼此分別交叉配對，成為24組簡併引子對，針對WSMoV、GBNV、WBNV、CaCV、CCSV、MYSV及TCSV等七種西瓜銀斑病毒血清群病毒的N基因選殖株質體進行PCR測試，除了FW4/rt1、FW2/rt2、FW3/rt3及FW4/rt4完全沒任何反應外，其餘的引子對組在測試各病毒種時，各引子對會分別出現不同有無反應的PCR產物而呈現特定組成的電泳圖譜，其引子對放大的PCR產物分別為200~800 bp區間不同大小的片段。將上述反應的引子對，剔除FW4/rt1、FW2/rt2、FW3/rt3及FW4/rt4，與感染WSMoV及MYSV菸草萃取的總量RNA進行RT-PCR測試，結果顯示與N基因選殖株質體所呈現的電泳圖譜一致，並成功地區分此二病毒，這顯示該簡併引子對組，未來應用於實際感染WSMoV血清群之病毒種鑑定上，具有很好的潛力。本次研究的目的主要探討病毒N基因保留區域應用於病毒種鑑定的可行性，所設計的簡併引子對組，其PCR產物出現的有無反應及電泳呈現的特定圖譜的建立，未來可做為該血清群鑑定病毒種的參考依據。

B02　利用微矩陣生物晶片技術同步鑑定番茄斑點萎凋病毒血清群病毒之種類—劉魯垣[1]、陳宗祺[2]、陳滄海[3] ([1]屏東科技大學農園生產系博士班、[2]亞洲大學生物科技學系、[3]屏東科技大學植物醫學系)

Using microarray for simultaneous iden-tification of *Tospovirus* species in the TSWV serogroup —Lu-Yuan Liu[1], Tsung-Chi Chen[2], Tsang-Hai Chen[3] ([1]Department of Plant Industry,NPUST, Pingtung, Taiwan; [2]Department of Bio-technology, Asia University, Wufeng, Taichung, Taiwan; [3]Department of Plant Medicine, NPUST, Pingtung, Taiwan)

應用快速鑑定的技術方法在*Tospovirus*屬病毒種的鑑定，一直是很重要的研究課題，透過鑑定的結果，可以分析病毒的來源、分佈、寄主種類和媒介感染昆蟲等重要資訊的整合，對於在檢疫及防疫策略的擬定，一直扮演重要的角色。*Tospovirus*屬主要有三個重要的血清群，常對於作物經濟栽培造成嚴重的經濟損失，分別是TSWV、WSMoV及IYSV等，其中TSWV血清群我國重要的檢疫病毒之一，本病毒血清群主要有番茄斑點萎凋病毒(*Tomato spotted wilt virus*, TSWV)、鳳仙花壞疽斑點病毒(*Impatiens necrotic spot virus*, INSV)、水仙百合壞疽條紋病毒(Alstroemeria necrotic streak virus, ANSV)、菊花莖部壞疽病毒(Chrysanthemum stem necrosis virus, CSNV)、番茄黃化斑點病毒(*Tomato chlorotic spot virus*, TCSV)、甜瓜嚴重嵌紋病毒(Melon severe mosaic virus, MeSMV)、花生輪斑病毒(*Groundnut ringspot virus*, GRSV)、辣椒壞疽斑點病毒 (Pepper necrotic spot virus, PNSV)、矮南瓜致死黃化病毒(*Zucchini lethal chlorosis virus*, ZLCV)。本研究分析該病毒血清群核鞘(nucleocapsid, N)基因序列內的變異區域(variation region)進行專一性探針(specific probe)的設計，分別為TSWV：tswv101、tswv401；INSV：insv101、insv401；ANSV：ansv101、ansv401；CSNV：csnv101、csnv401；TCSV：tcsv101、tcsv401；MeSMV：mesmv101、mesmv401；GRSV：grsv101、grsv401；PNSV：pnsv101、401及ZLCV：zlcv101、zlcv401，分別將探針以0.8焦耳UV處理固定在PVC晶片表面後，以TSWV、ANSV、INSV、TCSV、GRSV、CSNV及MeSMV等七種病毒N基因選殖株質體所增幅標誌生物素的PCR專一性產物，在50℃反應條件下，進行雜合反應測試，並以Streptavidine-alkaline phosphatase及NBT/BCIP呈色系統進行訊號放大，結果顯示，所設計出來的專一性探針在六小時單一時間檢測內，可以同時鑑定並區分TSWV血清群內的病毒種，將此晶片平台與RT-PCR的檢測系統結合，以萃取感染TSWV及INSV菸草的總量RNA進行測試，結果顯示與質體測試的結果無異，另外針對不同TSWV分離株的測試方面，結果也相符合，顯示本技術未來在應用於實際感染TSWV血清群之病毒種*Tospovirus*屬類鑑定上具有很好的潛力。本次研究的目的主要探討病毒N基因變異區域應用於病毒種類鑑定的可行性，所設計的專一性探針，未來可做為該血清群鑑定病毒種類的快速鑑定工具。

B03　設計具有同時檢測WSMoV及IYSV血清群專一性的簡併引子對以快速偵測檢疫的*Tospovirus*屬病毒—劉魯垣[1]、陳宗祺[2]、陳滄海[3] ([1]屏東科技大學農園生產系博士班、[2]亞洲大學生物科技學系、[3]屏東科技大學植物醫學系)

Design of WSMoV and IYSV serogroups-specific primer pairs for prompt detection of quarantine *Tospovirus* species —Lu-Yuan Liu[1], Tsung-Chi Chen[2], Tsang-Hai Chen[3] ([1]Department of Plant Industry, NPUST, Pingtung, Taiwan; [2]Department of Bio-technology, Asia University, Wufeng, Taichung, Taiwan; [3]Department of Plant Medicine, NPUST, Pingtung, Taiwan)

*Tospovirus*病毒屬之西瓜銀斑病毒血清群(WSMoV

serogroup)為我國重要造成作物經濟損害的病毒，主要包括西瓜銀斑病毒(*Watermelon silver mottle virus*, WSMoV)、花生頂芽壞疽病毒(*Groundnut bud necrosis virus*, GBNV)、西瓜頂芽壞疽病毒(*Watermelon bud necrosis virus*, WBNV)、番椒黃化病毒(*Capsicum chlorosis virus*, CaCV)、彩芋黃化斑點病毒(*Calla lily chlorotic spot virus*, CCSV)、甜瓜黃斑病毒(*Melon yellow spot virus*, MYSV)、桑葉脈條斑病毒(Mulberry vein banding virus, MuVBV)、番茄環斑病毒(*Tomato zon-ate spot virus*, TZSV)、番茄壞疽環斑病毒(*Tomato necrotic ringspot virus*, TNRV)、番茄壞疽斑點關聯病毒(Tomato necrotic spot-associated virus, TNSaV)及胡椒黃化斑點(*Pepper chlo-rotic spot virus*, PCSV)等十一種病毒，其中WSMoV及MYSV迄今仍是瓜類嚴重減產的重要禍首之一，以洋香瓜為例，2008年MYSV在我國中南部，造成洋香瓜重要的經濟損害；而鳶尾花黃斑病毒血清群(IYSV serogroup)則為我國重要的檢疫病毒，主要包括鳶尾花黃化斑點病毒(*Iris yellow spot vi-rus*, IYSV)、番茄黃輪病毒(*Tomato yellow ring virus*, TYRV)、蓼屬輪斑病毒(*Polygonum ringspot virus*, PolRSV)、孤挺花黃化輪斑病毒(*Hippeastrum chlorotic ringspot virus*, HCRV)等四種病毒。本研究同時比對WSMoV與IYSV血清群病毒核鞘(nucleocapsid, N)基因序列內的保留性區域(conserved region)，成功地設計出可檢測出WSMoV血清群FW3/rw1、FW3/rw2專一性的簡併引子對(degenerate primer pairs)，分別增幅出約300 bp及400 bp專一性PCR產物片段；IYSV血清群則有FW4/rw1、FW4/rw2、FW4/rw3、FW4/rw4、FW6/rw3及FW6/rw4專一性的簡併引子對，分別增幅出200 bp、300 bp、400 bp及500 bp大小的專一性PCR產物片段。在同時檢出WSMoV及IYSV血清群的部分，則有FW1/rw2及FW5/rw2兩組專一性的簡併引子對，分別增幅600 bp及300 bp等不同大小的專一性片段。上述結果皆由攜帶不同病毒的N基因選殖株質體進行PCR測試。選取上述專一性引子對，FW3/rw1：專一性檢測WSMoV血清群；FW4/rw3：專一性檢測IYSV血清群；FW5/rw2：同時檢測出WSMoV和IYSV血清群，分別以感染WSMoV及IYSV的菸草萃取分離的總量RNA進行RT-PCR測試，結果顯示，與質體測試的結果相符，另外進行定序比對發現與現有病毒種的N基因序列亦相符合。上述結果顯示，該簡併引子對組未來可分別應用在WSMoV及IYSV血清群的檢測上，對於種苗及檢疫的篩檢上提供新的檢測技術方向。

B04 木瓜捲葉廣東病毒(PaLCuGdV)引起的洋桔梗(*Eustoma grandiflorum*)新病害—趙鴻宇[1]、石珮蓉[1]、蔡宛育[2]、趙佳鴻[2]、陳煜焜[1] ([1], 台中市 國立中興大學植物病理學系；[2], 彰化大村台中區農業改良場)

New viral disease of lisianthus (*Eustoma grandiflorum*) caused by Papaya leaf curl Guangdong virus (PaLCuGdV) —Chao, H.Y.[1], P.R. Shih[1], W.Y. Tsai[2], C.H. Chao[2], and Y.K. Chen[1] ([1], Depart-ment of Plant Pathology, National Chung Hsing University, Taichung; [2], Taichung District Agricultural Research and Extension Station, Changhua)

洋桔梗(*Eustoma grandiflorum*)為重要的花卉經濟作物，已知有多種病原可感染之，其中以病毒為大宗。目前已知能感染洋桔梗的病毒至少有22種，其中本國有紀錄者即佔一半，分別為*Begomovirus*屬的藿香薊黃脈病毒(*Ageratum yellow vein virus*, AYVV)、*Carmovirus*屬的康乃馨斑駁病毒(*Carnation mottle virus*, CarMV)、*Cucumovirus*屬的胡瓜嵌紋病毒(*Cucumber mosaic virus*, CMV)、*Fabavirus*屬的蠶豆萎凋病毒(*Broad bean wilt virus*, BBWV)、*Tobamovirus*屬的番茄嵌紋病毒(*Tomato mosaic vi-rus*, ToMV)、*Tombusvirus*屬的洋桔梗壞疽病毒(Lisianthus necrosis virus, LNV)、*Aurensvirus*屬的黃金葛潛隱病毒(*Pothos latent virus*, PoLV)、以及*Potyvirus*屬之菜豆黃化嵌紋病毒(*Bean yellow mosaic virus*, BYMV)、蕪菁嵌紋病毒(*Turnip mosaic virus*, TuMV)、番椒葉脈斑駁病毒(*Pepper veinal mottle vi-rus*, PVMV)和鬼針草斑駁病毒(*Bidens mottle virus*, BiMoV)等11種。2015年九月分別於彰化芳苑與嘉義北港採得植株矮化、上位葉片捲曲、葉背與花瓣具畸脈贅生(enation)等病徵的洋桔梗病株。由於病徵類似AYVV感染者，推測為Begomovirus屬病毒所感染。萃取罹病植株總量DNA，以Begomovirus屬簡併式引子對(5'-GCATCTGCAGGCCCA- CATYG TCTTYCCNGT-3'與5'-GATTTCTGC AGTTDATRTTYTCRTCCATCCA-3')進行PCR檢測，均可自罹病樣本增幅出1.5 kb的cDNA片段。經選殖、定序及BLAST比對後，此cDNA片段與*Papaya leaf curl Guangdong virus* (PaLCuGdV)及*Malvastrum leaf curl Guangdong virus* (MLCuGdV)有95-96%的相似度。解析病毒全長度基因體，得知其只具有DNA-A (accession number LC089013、LC089014、LC089766)。三個病毒株的全長度DNA-A均含2732個核甘酸，對應6個開放轉譯架(open reading frame, ORF)，分別為AV1(774 nt)、AV2(351 nt)、AC1(1194 nt)、AC2(408 nt)、AC3(405 nt)及AC4(258 nt)。與NCBI資料庫進行BLAST比對後，顯示三個病毒株彼此的基因體DNA序列相似度達99%以上，且與*Begomovirus*病毒屬的*Papaya leaf curl Guangdong virus* (PaLCuGdV)最為近似(相似度達93.3%)。洋桔梗病毒DNA-A序列以滾環式擴增法(rolling circle amplification, RCA)進行增幅，並以限制酵素*Bam*HI酶切以獲取2倍全長之病毒序列。將2倍全長病毒序列選殖於載體pCAMBIA 1304與pCAMBIA0380，並轉殖於農桿菌。以Agro-inoculation法將體外感染載體(infectious clone)注射接種於洋桔梗和圓葉菸草(*Nicotiana benthamiana*)進行病毒致病性測試。結果顯示圓葉菸草於接種後21天、洋桔梗於接種後90天，均在新葉出現捲葉及畸脈贅生等和田間病株相似的病徵。診斷鑑定的結果顯示芳苑與北港等地區的洋桔梗，應是受到*Begomovirus*屬的PaLCuGdV感染。PaLCuGdV於我國已在百香果上發生，在洋桔梗上則為新發現的病原。

B05 Development of a probe assay for the detection of banana

viruses—Kuan, Cheng-Ping[1], Chang, Yi-Lin[1], Chen, Han-Wei1 and Yeh, Hsin-Hung[2] ([1]Division of Biotechnology, Taiwan Agricultural Research Institute, COA, Wufeng, Taichung; [2]Agricultural Biotechnology Research Center, Academia Sinica, Taipei)

An effective management strategy for virus-free banana plantlets produc-tion is dependent on rapid detection of infected plants so that potential source plants of various viral pathogens can be destroyed promptly. Simultaneous de-tection of three banana viruses, Banana bunchy top virus (BBTV), Banana steak virus (BSV) and Cucumber mosaic cu-cumovirus (CMV), were carried out using a multiplex bead-based assay, a nov-el detection technique that combines RT-PCR with the florescent detection. On the basis of the establishment of the optimal PCR and reverse transcription (RT)-PCR for the detection of these ba-nana viruses, a RT-PCR method that employed virus-specific primers was developed for the detection and differ-entiation of viruses in bananas. 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 relative similar detection limit comparable to the RT-PCR reaction. The assay was then validated using banana samples infected with one or more viruses collected from fields and tissue-culture banana industries. The system offers a sensitive, high throughput and rapid detection method for banana viruses.

B06 Molecular detection of potyviruses based on genomic amplification—Kuan, Cheng-Ping[1], Chiu, Guan-Zhi[1], Tsai, Shang-Yu[1] and Cheng, Ying-Huey[2] ([1]Division of Biotechnology, Taiwan Agricultural Research Institute, COA, Wufeng, Taichung; [2]Division of Plant Pathology, Taiwan Agricultural Research Institute, COA, Wufeng, Taichung)

Potyviruses disease, one of the most important diseases of solanaceous plants in the world. Many potyviruses cause economically important yield losses in potato, pepper and tomato crops throughout the world. Potato virus Y (PVY) or Pepper mottle virus (Pep-MoV) infected plants in fields serve as the one of main source of inoculum for aphid-mediated transmission. Therefore, planting of virus-free plantlets is the most effective way to reduce PVY or PepMoV infection and prevent further early current season spread of the virus by aphids. An accurate estimation of PVY or PepMoV incidence in seedling plants plays an important role in the control and management of PVY or PepMoV in tomato and sweet pepper production systems. Simultaneous quan-titation of two potyviruses, PVY and PepMoV were carried out using the TaqMan real-time PCR, that combine multiplex PCR and using one primer pair with the power of fluorescent detection. The assay would also reduce risks of cross contamination and showed higher sensitivity between samples as compared with conventional RT-PCR. This quantitative detection assay will be a valuable tool for promote the selection of virus-free planting material, thus aid-ing in the control of the disease and di-agnosis.

B07 提升番茄對捲葉病抗性物質之探討—鄭櫻慧[1]、王昭萍[1]、蔡筱婷[1]、詹富智[2] ([1] 行政院農委會農業試驗所植物病理組、[2] 國立中興大學植物病理系)

Study of substances enhanced resistance to tomato leaf curl disease—Cheng, Y. H. [1], Wang, J. P. [1], Tsay, S. T. [1], and Jan, F. J. [2] ([1] Plant Pathology Division, Agricul-tural Research Institute, COA; [2] Dept. of Plant Pathology, National Chung Hsing University)

番茄捲葉病害在1960年代發生於以色列及約旦地區，目前已廣泛分佈於世界各地，在台灣番茄捲葉病也成為番茄栽培上最嚴重病害之一。台灣感染番茄造成捲葉病的begomoviruses 計有*Tomato leaf curl Taiwan virus* (ToLCTWV)、*Tomato leaf curl Hsinchu virus* (ToLCHsV)、*Tomato yellow leaf curl Thailand virus* (TYLCTHV)、*Ageratum yellow vein Hualien virus* (AYVHuV)和*Ageratum yellow vein vi-rus* (AYVV) 5種。抗病育種，不論是傳統抗病育種或是基因轉殖，是防治病毒病害最有效的方法，在番茄捲葉病的防治上，以亞洲蔬菜中心育成帶有抗病基因抗病親本，在世界各地也育成商業用雜交品種上市為主。除了抗病品種之外，降低媒介昆蟲粉蝨族群也為重要防治策略。但田間常因病毒在番茄上複合感染、基因重組，使其變異增大，造成抗病品種效果逐年遞減。非農藥資材防治方法之研究以真菌病害最多，防治植物病毒病害較為少見，用於病毒病害之資材以牛奶之研究最早，牛奶中的乳清蛋白有效抑制TYLCTHV在番茄植株之濃度。本實驗測試國內已商品化具有誘導對真菌抗病力之*Bacillus subtilis*、*B. mycoides*與具誘導抗病毒能力之物質如亞磷酸，茉莉酸、聚胺、醛酸、殼寡糖、乳清蛋白、奶粉等資材每週葉片噴佈處理*Nicotiana benthamiana*，再機械接種TYLCTHV或農桿菌穿刺接種ToLCTWV，初步篩選結果以乳清蛋白、脫脂奶粉與全脂奶粉效果最好，接種未處理對照組發病率達90%時，3種處理發病率各為72%、48%與20%，其他物質則無顯著提升抗病效果。以奶粉分別處理0次、1次、2次與3次，並於接種後2天再進行1次處理後，觀察病徵表現1個月，未處理對照組90%發病的接種條件下，4處理組發病率20-30%。接種後0、3、5、7、10天開始處理，之後每週處理1次，接種後10天再處理與未處理對照發病率一樣。分別處理全株、3片下位葉與未處理3組，接種前處理1次，接種後每週處理1次。接種後12天未處理組植株開始發病，發病率66.6%，處理全株與下位葉之植株觀察1個月後，均未發病。以奶粉處理*N. benthamiana*，接種後處理較接種前處理更為重要。試驗證實奶粉處理可以提升*N. benthamiana*對捲葉病毒的抗病力，而非誘導產生抗病力，其抗病機制尚待更多探討。

B08 Molecular characterization of a dis-tinct begomovirus associated with gold-en mosaic symptom of velvet bean (***Mucuna pruriens***) in Taiwan —Kang, Yun-Ting[1], Lai, Hsin-Shun[2] and Tsai, Wen-Shi[1], ([1]Department of Plant Medi-cine, National Chiayi University, Chiayi; [2]Fengshan Tropical Horticultural Ex-periment Branch, Taiwan Agricultural Research Institute, COA, Kaohsiung)

Velvet bean (***Mucuna pruriens***) is an important green manure in Taiwan. In 2014, a virus-like golden mosaic symp-tom were observed velvet bean fields which showed 50% incidence in Hualien area, Taiwan and seven diseased velvet bean samples were collected from two fields. All of the collected samples were detected positive for begomovirus by PCR using begomovirus detection general primers PAL1v1978B/PAL1c715H. However, virus DNA-B and associated beta satellite were not detected. All 1.5 kb PCR amplified viral DNAs showed high nucleotide sequence identity (> 98%). Based on the partial viral se-quences, the specific primer pair BGV-FV/BGV-FC was designed to am-plify full length viral genomic DNAs. Viral DNAs of two virus isolates from each field were completed sequenced and revealed high nucleotide sequence identity (> 99%). Based on the species demarcation of International Committee on Taxonomy of Viruses and less than 81% nucleotide sequence identity with other begomoviruses, the newly identified velvet bean-infecting begomovirus should be considered as a new species and tentatively named as "Velvet bean golden mosaic virus, VbGMV". Based on our knowledge, this is the first report of a bean-infecting begomovirus in Taiwan.

B09 檢疫病毒*Arabis mosaic virus* (ArMV)檢測試劑開發及檢疫監測應用—陳金枝[1]、劉星君[1]、江芬蘭[1] ([1]行政院農業委員會農業試驗所植物病理組)

Development of the detection reagents of ***Arabis mosaic virus*** and its applica-tion on the virus quarantine —Chen, C. C. [1] Liu, H. C. [1], and Chiang, F. L. [1] ([1] Division of Plant Pathology, Taiwan Agricultural Research Institute, COA, Tai-chung)

ArMV屬於*Nepovirus*屬之RNA病毒，可藉由汁液機械傳播、種子(球)帶毒及線蟲 (*Xiphinema diversicanda-tum*和*X.coxi*) 傳播。寄主範圍廣，可危害約174屬，215種植物。ArMV被歸列為檢疫病毒，且國內尚無此病毒發生。本研究成功開發ArMV之核酸引子對ArMV-350u /ArMV-350d，可成功檢測市售之ArMV核酸對照品，檢出預期350 bp之核酸片段。本研究另將ArMV鞘蛋白序列3'端約948 bp (預估蛋白大小34.8 kDa)選殖於pET28b表現載體，並於細菌宿主*E. coli* BL21 (Resetta) 中進行病毒鞘蛋白之表現與純化，以純化之表現蛋白進行其多元抗體製備 (代號#ArMV34)；將#ArMV34之免疫球蛋白IgG進行ELISA及西方墨點法檢測，並與市售之ArMV多元抗體(＃ArMV-Agdia，Agdia公司出品)進行檢測比較。結果於ELISA檢測中，兩種抗體均可與ArMV表現蛋白、健康奎藜及百合植物組織產生非專一性之反應，而無法判讀正確結果。而於西方墨點法中，自製之 #ArMV34抗體可與預估34.8 kDa表現蛋白及ArMV正反應對照品於預估分子量位置產生正反應，且不與奎藜及百合健康組織產生反應；然而市售之#ArMV-Agdia抗體會與奎藜或百合健康組織於約55 kDa條帶處產生強烈之非專一性反應。此等結果顯示自製之多元抗體#ArMV34 無法應用於ELISA檢測，而適用於西方墨點法為之，且其效果優於Agdia公司出品之ArMV抗體。本研究顯示ArMV之檢測仍以RT-PCR為精準，因此可應用於強化進口種球對此病毒監測之需，尤其是具經濟重要性之球根花卉(如百合)種球。

B10 宮燈百合*Gloriosa stripe mosaic virus*鑑定之首次記錄及其檢測試劑開發—陳金枝[1]、江芬蘭[1]、高達瑋[2] ([1]行政院農業委員會農業試驗所植物病理組；[2]國立臺灣大學植物病理與微生物學系)

First identification of the ***Gloriosa stripe mosaic virus*** on Christmas Bells (***Sandersonia aurantiaca*** Hook) and develop-ment of virus detection reagents—Chen, C. C. [1] , Chiang, F. L.[1], and Kao, D. W.[2] ([1] Division of Plant Pathology, Tai-wan Agricultural Research Institute, COA, Taichung; [2] Dept. Plant Pathology and Microbiology, National Taiwan University, Taipei)

宮燈百合 (***Sandersonia aurantiaca*** Hook) 原產於南非，而紐西蘭為主要之商業化栽培國；其為國內新興之花卉，種球主要由紐西蘭進口，於國內之清境、埔里及后里有零星栽培。目前國際間已記錄可感染宮燈百合之病毒種類為*Cucumber mosaic virus* (CMV)。本研究於進口種球檢測出一種*Potyvirus*病毒 (分離株代號CB6)，經鞘蛋白胺基酸序列鑑定分析，確認其乃屬於火焰百合上之*Gloriosa stripe mosaic virus* (簡稱GSMV)成員。後續將CB6以RT-PCR法分段增幅其核酸片段，解得其包含全長度大蛋白 (含3052個胺基酸) 之核苷酸序列共9156 bp，相關序列已登錄於GenBank並取得登錄序號為EF427894。對應CB6分離株之鞘蛋白(coat protein, CP) 有798個核苷酸，可轉譯出266個氨基酸，預估分子量約為29.3 kDa。CB6對應Nucleous inclusion b (NIb) 及CP之裂解位置乃位於VYHQ/S胺基酸序列，由CP之N端起始第6個胺基酸位置上有一DAG triplet，推測CB6應具有藉蚜蟲傳播之能力。GSMV為國內外的火焰百合上已有之病毒記錄，但於宮燈百合上為首次發現之記錄，本研究並為首度完成GSMV全長度定序者。本研究另由火焰百合分離得到3個GSMV分離株 (代號GL2、GL3及GL4)，並與宮燈百合GSMV-CB6以及GenBank上由荷蘭學者已登錄之火焰百合GSMV 分離株 (序號EU042761)，以及國內學者(Wang et al.)於2007年已登錄之火焰百合嵌紋病毒 (Glory lily mosaic virus，序號EU250360) 進行比對分析，結果顯示此等分離株間之鞘蛋白胺基酸序列相同高達91%以上，顯示其均應屬於GSMV之不同

系統。本研究另設計一組可增幅GSMV-CB6分離株全長度鞘蛋白核苷酸序列之專一性引子對，經RT-PCR增幅後，將其選殖於表現載體pET28b上，再轉型於*E. coli* Rosetta（DE3)宿主內誘導大量表現蛋白之生成，將預估分子量約29.3 kDa之細菌表現蛋白純化後，經兔免疫注射而獲得對應GSMV-CB6之多元抗體。此抗體可應用於ELISA和西方轉漬法 (Western blotting)，與同源抗原產生專一性反應。本研究另針對GSMV-CB6之鞘蛋白核酸序列所設計之3組簡併式引子對(上游引子分別為GSMV65u及GSMV219u，搭配共同下游GSMV709d引子對)，利用RT-PCR可同時檢測出宮燈百合及火焰百合上之GSMV；但另一組引子對GSMV12u/GSMV709d僅可檢測到宮燈百合之GSMV，對感染火焰百合之GSMV則無預期片段被增幅出，顯示此GSMV12u/GSMV709d引子對可應用於宮燈百合GSMV之鑑別性檢測之需。本研究所製備之GSMV-CB6引子對及多元抗體，可應用於進口宮燈百合種球以及國內栽培者之病毒監測，提升我國對GSMV之自主檢測能力。

B11　感染台灣蔥蒜之蔥屬X病毒屬(*Allexivirus*)的鑑定與親緣分析—林玫珠[1]、林羿廷[1]、簡伊萱[1]、鄧汀欽[1]、周建銘[1] ([1]行政院農業委員會農業試驗所植物病理組)

Identification and phylogenetic analysis of coat protein gene of *Allexivirus* in Taiwan—Lin, M. J.[1], Lin, Y. T.[1], Chien, Y. H.[1], Deng, T. C.[1], and Chou, C. M.[1]. ([1]Division of Plant Pathology, Taiwan Agricultural Research Institute, COA)

感染大蒜的病毒種類廣泛存在於田間，且該病害的發生率極高，交互感染的情形普遍，蒜株一旦罹患病毒病，其全株生長受阻，影響大蒜的品質及產量。蔥屬X病毒屬有大蒜螨媒長絲狀病毒 (*Garlic miteborne filamen-tous* virus, GarMbFV) 及 Garlic virus A (GarV-A)及GarV-B, -C, -D, -E, -X等及 *Shallot virus X*。除GarMbFV外，其餘病毒在台灣未曾被報導過。先前研究，以 Allexivirus 簡併式引子對 Allex-cp (+) (5'- TGGRCNTGCYA CCACAAYGG-3') 及 Allex-NABP(-) (5'-CCYTTCAGCATATAGCTTAGC-3') 對13個青蔥、28個大蒜及16個進口大蒜樣品進行RT-PCR分析，結果顯示有4個青蔥樣品、25個田間樣品及13個進口樣品帶有*Allexivirus*，青蔥的帶毒率為30.8%，而大蒜的帶毒率則高達89%及81%；田間病毒株葉片呈現黃化及嵌紋等病徵。將部份增幅的核酸片段選殖、定序及親緣分析，結果顯示台灣的蒜 '3G'、 '19-2'、 '22'、 '23'、 '50'等病毒株為*Garlic virus* A，其中蒜'3G'、'19-2'、'23'、'50'與中國病毒株 (AJ551472、AJ221477) 較為相近，相同度達到97.5-98.9%，而青蔥'黃加'分離株之核酸定序，經比對後鑑定為*Garlic virus* B，與韓國(AF543829)、捷克(JX682831)及義大利(KC207716)的大蒜分離株最為相近，相似度分別為98.8%、98.3%與95.3%。而病毒株蒜'50'、'SN'與青蔥'HS'鑑定為*Garlic virus* D，其中蒜'SH'與波蘭(KF446188)、伊朗(HQ681944)等相似度達94%，蒜'50'病毒株與俄國(JX682887)和阿根廷(KF550407)之GarV-D相似度僅介於87.4-87.8%之間，青蔥'SH'病毒株則與中國(AJ551497)及巴西(KF955570)的病毒株較為接近，相似度為97.7%。由上述結果得知部分田間樣品由二種以上*Allexivirus*感染，為有效偵測田間感染的蔥屬X病毒屬病毒種類，目前針對GarV-A、GarV-B、GarV-C 及 GarV-D等病毒設計專一性引子對，以簡併式引子對 Allex-cp (+)及Allex-NABP(-)增幅的RT-PCR產物為模板，於56℃或60℃的鍊合溫度下進行nest-PCR，分別可增幅出332 bp、480 bp、608 bp及408 bp的專一性條帶，可作為田間蔥蒜植株檢測之用，結果顯示多數的植株皆複合感染二種以上的*Allexivirus*，其中以複合感染GarV-A及GarV-B的比例較高。

B12　以大蒜中分蔥潛隱病毒的親緣特性回溯蒜頭產地之可行性評估－鄧汀欽[1]、林羿廷[1]、林玫珠[1]、陳君弢[2]、周建銘[1]、劉兆烘[3] ([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]行政院農業委員會科技處)

Feasibility study of backtracking origins of garlic bulbs by phylogenetic charac-teristics of *Shallot latent virus* in the garlic－Deng, T. C.[1], Lin, Y. T.[1], Lin, M. J.[1], Chen, C. T.[2], Chou, C. M.[1] and Liu, C. H.[3] ([1]Division of Plant Pathology, Taiwan Agricultural Research Institute, COA; [2]Hsinchu Branch, Bureau of Animal and Plant Health Inspection and Quarantine, COA, [3]Department of Science and Technology, COA)

利用病毒演化分析及分子流行病學證據，以鑑定帶病毒的個體源自何處，在醫學及獸醫體系已廣泛應用(AIDS 10:S13-S20,1996)，但是在植物病理學上並未普遍。鑒於大蒜市場分流區隔的政策，海關單位對於客觀判別蒜頭產地的技術有殷切的需求，因此本研究試以大蒜中所帶有的分蔥潛隱病毒(*Shallot latent virus*, SLV)之核酸序列特性作為回溯大蒜產地的依據。前曾以GenBank所列及國內已發生之SLV的鞘蛋白基因進行親緣分析，結果顯示SLV可分為3群(植病會刊 24:153, 2015)，今分別設計各群之專一性引子對如下：第一群SLV (G1)為SLVcpG1-up (5'-AACAGAKTG AAGA AYTTGCCC-3') /SLVcpG1-dw (5'-GT CACATTWATGCTAGATAA TT-3')、第二群SLV (G2)為SLVcpG2-up (5'-AGTAACGTGCAGA AMTTGCCG-3')/SLVcpG2-dw (5'-AG CGRGCAGACTTGSCGCAA-3')、與第三群SLVtw (G3)為SLVcp-tw1 (5'-AACTCRGTAASTTGCCGACTCGC-3')/SLVcp-tw2 (5'-ACGGCAA AATTGKCGCAGWGTMG-3') 進行nest-PCR，來檢測所收集的各株SLV的分群屬性，並評估應用在鑑定蒜頭產地的可行性。其方法如下：先以引子對SLV-DF1/SLV-DR1(植保會刊 56: 75-88, 2014)或CAR-V1/CAR-CP3 (Arch. Virol. 143:1093-1107, 1998)進行RT-PCR，確認有SLV感染在供試材料中。再以CAR-V1/CAR-CP3引子對增幅的產物(約940-1140 bp)，分別以各群專一性引子對於55℃的鍊合溫度下進行

nest-PCR，可各增幅出 187 bp (G1)、548 bp (G2) 及 540 bp (G3) 的專一性條帶。結果共檢測15個南韓進口蒜頭，15個都屬G1，其中1個雜有G2；國產的黑葉大蒜18個有16個屬G1，2個未能分群；和美蒜20個都屬G1，其中1個雜有G2；宜蘭白蒜14個都屬G1，其中6個雜有G3；國內青蔥9個樣本都兼有G1及G3；韭菜11個樣本都屬G3，其中9個雜有G1。

B13 Identification and characterization of a new begomovirus with a distinct betasatellite associated with rose mallow exhibiting leaf curl and vein enation—Tai, Chia-Hsing[1], Sharma, Nabin[1], Chao, Chia-Hung[2] and Jan, Fuh-Jyh[1] ([1]Department of Plant Pathol-ogy, National Chung Hsing University, Taichung; [2]Crop Environment Section, Taichung District Agricultural Research and Extension Station, COA, Dacun, Changhua)

In November 2013, rose mallow (*Hibiscus rosa-sinensis* Linn) plants with begomoviral disease-like symptoms of leaf curl and vein enation were collected from Changhua County in central Taiwan. Total DNA was extracted from plant tissues and used for polymerase chain reaction with the primers specifi-cally for detecting begomoviral DNA-A, -B and associated satellite. A 1.5-kb fragment representing begomoviral DNA-A was detected from the sympto-matic leaf tissue using the degenerate primers. The full length viral DNA-A genome was cloned and sequenced after rolling cycle amplification. The com-parison of DNA-A genome sequence with those of the other geminiviruses available in GenBank indicated that the virus has the highest nucleotide identity 88.7% with Malvastrum yellow vein Baoshan virus (FN386460). Primers specific for begomoviral DNA-B failed to amplify any products from diseased samples. In contrast, virus-associated satellite DNA was detected in the leaf tissue with symptoms, confirming that the virus was the same as other monopartite begomoviruses. The se-quence of associated satellite DNA was obtained and identified as a betasatellite DNA with one ORF in the virus com-plementary sense (βC1) and conserved nano-sequence as the identified DNA-A. The comparison of virus-associated be-tasatellite DNA sequence with those of others available in GenBank showed that the virus shared its highest nucleotide identity of 78.6% with *Malvastrum yel-low vein betasatellite* (AJ971708). Both of the infectious DNA-A and betasatel-lite were constructed by cloning tandem repeats of viral genomes into a binary vector. Agroinoculation of infectious vi-ral DNAs to *Nicotiana benthamiana* showed that the virus alone induced mild symptoms, whereas co-inoculation with betasatellite induced severe symp-toms. Because of DNA-A nucleotides have less than 89% identity with other geminiviruses, the virus associated with rose mallow leaf curl and vein enation disease should be considered as a new monopartite begomovirus with a distinct betasatellite and was designated as Hi-biscus vein enation virus.

B14 Identification of two dual zinc fin-ger A20/AN1 domain containing pro-teins involved in salicylic acid depend-ent plant immune signaling pathway— Chang, Ho-Hsiung, Chang, Li, Chang, Jui-Che and Yeh, Hsin-Hung (Agricul-tural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan)

The salicylic acid (SA)-related plant defense response is an important plant immunity pathway against bio-trophic pathogen including viruses. However, the transcriptional regulation of this pathway remains largely to be resolved, and the mechanism involved in virus defense in this pathway is not fully understood. We used a *Cym-bidium mosaic virus* (CymMV)-induced gene silencing sys-tem to screen the putative genes in-volved in this pathway on *Phalaenopsis* orchid, and identified two protein with A20/AN1 dual zinc finger domain, *PhaTF13* and *PhaTF21*, involved in this process. Transient knockdown and overexpression analysis indicated that *PhaTF13* and *PhaTF21* played on PhaNPR1- dependent, PhaNPR1-independent pathways and in CymMV accumulation. PhaTF13 and PhaTF21 localized in nucleolus, conferred self-E3 ligase activity, and can be induced by SA treatment and CymMV infection. The expression level of *PhaTF13* is much higher than *PhaTF21* in orchid leaf tissue. Muta-tion were introduced to A20 and/or AN1 domain of PhaTF13/PhaTF21 for domain functional analysis. We found that A20 domain of PhaTF13 conferred more self-E3 ligase activity and in-volved in regulation of PhaNPR1; however, AN1 domain played im-portant roles in CymMV accumulation. Both A20 and AN1 domain involved in regulation of the SA dependent NPR1-independent marker gene, alter-native oxidase gene (*PhaAOX*). On the contrary, AN1 domain of PhaTF21 conferred more self-E3 ligase activity. Although overexpression of PhaTF21 decreased the accumulation of CymMV and expression of glu-taredoxin gene (*PhaGRX*; another SA dependent NPR1-independent marker gene), respectively, overexpression of A20 and/or AN1 domain mutants did not affect the CymMV accumulation and *phaGRX* expression. It suggested A20 and/or AN1 domains are not im-portant for PhaTF21 regulating CymMV accumulation and *phaGRX* expression.

B15 阿拉伯芥非光合作用型硫鐵蛋白AtFd3對番茄生長、細菌性青枯病與根圈細菌之影響－黃志暄[1]、陳郁雯[1]、姚仲軒[1]、黃祥恩[1]([1]國立台東大學生命科學系)

The Role of Arabidopsis Non -photosynthetic-Type Ferredoxin, AtFd3, effected plant development, *Ralstonia solanacearum* and *Basillus* spp. in *Ly-copersicon esculentum* cv. Micro-Tom — Huang, C. H.[1], Chen, Y. W.[1], YAO, J. H. [1], Huang, H. E.[1] ([1] Dept. of

Life Science, National Taitung University)

植物根部經常會面臨病原微生物或非病原微生物的侵擾，若能藉由改變植物的基礎代謝路徑，增加根圈有益微生物的數量，減少病原微生物的入侵，將可有效提升植物的產量與品質。非光合作用型硫鐵蛋白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。此類蛋白通常存在於植物的儲藏性組織，如根、種籽及果實等細胞的澱粉性質體 (amyloplast) 中。當植物進入開花繁殖期或遭遇病原菌攻擊時，非光合作用型硫鐵蛋白會有增量表現的現象。為了解此類蛋白質對植物的影響，本研究將阿拉伯芥非光合作用型硫鐵蛋白 AtFd3 基因利用 CaMV 35S 啟動子，增量表現於番茄植株 (*Solanum lycopersicum* cv. Micro-Tom)，並測試這些基因轉殖株進入開花繁殖期、對青枯病菌抵抗能力及根圈微生物族群數量維持能力之影響。實驗結果顯示OE 2A-1 及 OE 66-5 為同型合子品系，而 OE 23B-3 為異型合子品系。其中OE 2A-1 品系外源性AtFd3 基因的mRNA及蛋白質表現量遠高於 OE 66-5 及 OE 23B-3品系。而 OE 2A-1 品系的植株高度較野生種矮小，OE 23B-3 及 OE 66-5 則與未轉基因植株無顯著差異。在花朵及果實等繁殖組織總數方面，OE 2A-1 及 OE 23B-3品系會有增加的現象，而 OE 66-5 品系則反而出現減少的結果。接種青枯病菌 *Ralstonia solanacearum* Rd4 七天後，基因轉殖品系 OE 66-5 出現明顯抗病的現象，且微管束內病原菌總數也較未轉基因品系少約 1,000 倍以上。在根圈微生物纏聚能力的維繫能力方面，兩株不同的 *Bacillus* spp. 在 OE 66-5及 OE 23B-3 轉基因品系，都出現可以延長根圈微生物在植株根部的纏據能力。上述結果顯示，非光合作用型硫鐵蛋白可以改變番茄的植株生長及繁殖組織的產生數量，達到增加作物產量的目標，又可幫助植物提升對於細菌性青枯病菌的抵抗能力，延長根圈微生物在植株根部的殘留時間，未來將更進探討 AtFd3 在番茄體內如何同時提升繁殖組織總量、增加青枯病菌抵抗能力及維持土壤微生物的機制，藉此發展出可兼顧產量、抗病能力及根圈微生物族群數量的作物。

B16　馬鈴薯瘡痂病與土壤性質之相關性初探—蔡孟旅、吳盈慧、鄭安秀（行政院農業委員會臺南區農業改良場）

The study of relationship between potato common scab severity and soil proper-ties — Tsai, M. L., Wu, Y. H., Cheng, A. H. (Tainan District Agricultural Research and Extension Station, COA.)

馬鈴薯瘡痂病是遍及全球的病害，近年來於雲嘉南馬鈴薯主要產區發現瘡痂病在不同田區之發病嚴重程度差異頗大，因此103-104年於雲林、嘉義地區共31個採樣點調查罹病度，同時於馬鈴薯生長中期進行根圈土壤性質之檢測，包含 pH值、電導度(dS/m)、有機值含量(%)、有效性磷、鉀、鈣、鎂(mg/kg)之含量等，調查結果瘡痂病罹病度介於 5% - 65 % 之間，將土壤性質與罹病度數據進行線性迴歸分析，結果顯示土壤中的有機質含量與馬鈴薯瘡痂病罹病度間具有顯著的負相關性(P=0.0004,R2=0.36)，有機質含量越高的田區，其瘡痂病罹病度越低，而其餘土壤性質對瘡痂病罹病度的影響在本次調查中均不具顯著相關性。

B17　利用亞磷酸防治馬鈴薯青枯病之效果初步評估—林靜宜[1]、黃巧雯[1]、陳幸葵[1]、楊宏仁[1]、倪蕙芳[1] ([1]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 phosphorous acid salt—Lin, C. Y. [1], Huang, C. W.[1], Chen, S. K.[1], Yang, H. R.[1], and Ni, H. F.[1] ([1]Department of Plant Protection, Chiayi Agricultural Experiment Station, Taiwan Agricultural Research Institute)

青枯病是由*Ralstonia solanacea-rum*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，本研究利用亞磷酸中和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。試驗中使用之亞磷酸中和液濃度分別為0.05%及0.08%，每7天施用一次，共施用4次，其中於第一次使用亞磷酸中和液後，進行青枯病菌接種，之後再行施用3次亞磷酸中和液。結果顯示使用0.05%濃度之亞磷酸中和液，以葉面施用法防治馬鈴薯青枯病，植株發病率達100%，罹病度(disease severity)為96%，若利用土壤澆灌法施用，發病率則為80%，罹病度為68%。而使用0.08%濃度之亞磷酸中和液進行試驗，結果則發現以葉面施用法之植株發病率仍達100%，罹病度為92%，但若利用土壤澆灌法施用亞磷酸中和液，發病率則為60%，罹病度分別為40%。以上結果顯示依不同的施用方法，其防治效果具有差異性：利用土壤澆灌法施用亞磷酸較葉面施用法更能降低馬鈴薯青枯病菌引起之萎凋率及罹病度；而使用濃度方面則以濃度較高之0.08%亞磷酸中和液防治效果較佳。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。

B18　具拮抗能力水稻葉表細菌之篩選與其防治水稻白葉枯病之效果評估 — 陳以錚[1]、王玉瑤[1]、林易辰[2]、周浩平[1]、曾敏南[1]、黃德昌[1] ([1]高雄區農業改良場、2國立屏東科技大學植物醫學系)

Screening of antagonistic foliar bacteria from rice and assessment of their effi-cacy on controlling bacterial leaf blight of rice — Chen, Y. J., Wang, Y. Y., Lin, Y. C., Chou, H. P., Tseng, M. N., Huang, T. C. ([1] Kaohsiung District Agricultural Research and Extension Station, COA, Pingtung; [2] Dept. of Plant Medicine, Na-tional Pingtung University of Science and Technology, Pingtung)

由*Xanthomans oryzae* pv. *oryzae*引起的白葉枯病為水稻重要葉部細菌性病害，在台灣每年發病面積超過20,000公頃，造成嚴重損失。白葉枯病目前防治方法以化學方法和抗病育種

為主；然化學方法易引起環境及食品安全疑慮，且病原菌有產生抗藥性之風險，抗病育種則耗時費久；因此生物防治方法愈來愈受到重視。本研究嘗試自水稻葉表分離對白葉枯病菌具拮抗效果之細菌，並評估其防治白葉枯病之潛力。2015年間自雲林、高雄及屏東地區田間水稻葉表分離得到143株細菌並經拮抗活性測試，結果有22株細菌可在NA培養基上抑制白葉枯病菌 (*X. oryzae* pv. *oryzae* E13) 生長，其中以分離自東港之DG1A-2、港東之GD1A-2、海豐之HF1A-1、高樹之KSLOVE及南州之ND5B-1等菌對白葉枯病菌抑制圈半徑超過3公分為最佳；另於PDA、LB、OMA、CMA和YDC等5種培養基上評估上述5菌株拮抗白葉枯病菌之能力，結果顯示上述菌株在5種培養基中皆可抑制白葉枯病菌的生長，抑制圈半徑介於1.5 - 3.7公分之間。進一步將上述5株微生物與其他*Xanthomonas*屬植物病原細菌包括十字花科黑腐病菌 (*X. cam-pestris* pv. *campestris* Xcc86) 及檬果黑斑病菌 (*X. campestris* pv. *manigifer-aeindicae* CK05) 等；水稻主要病原真菌包括稻熱病菌 (*Pyricularia oryzae* MO15012)、胡麻葉枯病菌 (*Bipolaris oryzae* DJ402)、徒長病菌 (*Fusarium fujikuroi* Ico1)及紋枯病菌 (*Rhizoctonia solani* 13087) 等進行對峙培養，結果顯示5株細菌皆可在PDA培養基上抑制稻熱病菌及胡麻葉枯病菌之生長，抑制圈半徑分別在1.5 - 2.5和1.8 - 2.7公分之間。將先培養於NA培養基上0、24、48、72及96小時的5株葉表細菌用以對峙白葉枯病菌，結果顯示5株細菌皆可在接種0小時之處理下產生抑制圈，然抑制圈大小隨先培養時間不同而增加。發酵濾液活性測試則表明HF1A-1、DG1A-2、ND5B-1等3菌株之發酵液經過濾後對白葉枯病菌仍具有抑制效果，抑制圈半徑介於0.6 - 0.8公分之間。進一步於溫室中測試5株葉表細菌對白葉枯病的防治效果，結果顯示DG1A-2處理 (10^8 cfu/ml) 於第14天時白葉枯病罹病度為2.2 % 相對於對照組38.2 %差異顯著 ($p \leq 0.05$)。此研究證實水稻葉表微生物DG1A-2具有防治白葉枯病之潛力。

B19 比較東方鏈黴菌在不同醱酵配方與孢子產能提升之探討—<u>許玉霖</u>[1]、劉魯垣[2]、朱子逸[1]、王惠亮[3]、陳滄海[4]（[1]屏東科技大學植物醫學系碩士班、[2]屏東科技大學園藝生產系博士班、[3]高雄師範大學生物技術系、[4]屏東科技大學植物醫學系）

The research of different fermentation formulas comparison for spore mass production in *Streptomyces orientails* Y31014—<u>Yu-Lin Hsu</u>[1], Lu-Yuan Liu[2], Hui-Liang Wang[3], Zi-Yi Zhu[1], Tsang-Hai Chen[4] ([1]Department of Plant Medicine, NPUST, Pingtung, Taiwan; [2]Department of Plant Indus-try,NPUST, Pingtung, Taiwan; [3]Department of Biotechnology, NKNU, Taiwan; [4]Department of Plant Medicine, NPUST, Pingtung, Taiwan;)

溫室常見之害蟲有蚜蟲、粉蝨類等，其中又以銀葉粉蝨及桃蚜最為常見，銀葉粉蝨與桃蚜皆能傳播植物病毒病害且體型細小且繁殖力強，故長久以來一直是農業上之重要害蟲。*Streptomyces orientalis* Y31014醱酵液經發現對銀葉粉蝨及桃蚜具有致死效果，為極具生物農藥開發潛力之菌株。本研究以Czapek's medium為基礎培養基，於5L醱酵槽測試下，調整溫度、pH、轉速、碳氮源及碳氮比等醱酵參數，得到最佳優化的培養條件及配方，可獲得10^{10} CFU/mL孢子數，相較原有108 CFU/mL產量，可成功地提高到100倍的產能，將修正後的醱酵產物，稀釋成10^8 CFU/mL的孢子濃度進行桃蚜殺蟲效力生物活性試驗，結果顯示三天內其死亡率達到90%，與前人研究所設定的殺蟲效力分析數值均一致，這表示並不因為醱酵參數的調整，而導致殺蟲活性的失去。本研究目前僅以5L的醱酵產能進行模擬量化測試，其相關優化醱酵參數的成果，未來可以作為大規模商業化量產的參考依據。

學生論文宣讀比賽
Student oral presentation contest

SA01 Nucleic acid binding ability of functional motifs of tospoviral NSs pro-tein—<u>Foo, Mung Hsia</u>, Huang, Chung-Hao, and Yeh, Shyi-Dong (De-partment of Plant Pathology, National Chung Hsing University, Taichung)

RNA silencing is a major antiviral mechanism in plants and also plays es-sential roles in host biological processes. Meanwhile, viruses overcome this barrier by evolving RNA silencing suppression (RSS) to antagonize the host defensive reaction. The modes of action of viral RSS target different steps of RNA silencing pathway, such as binding dsRNAs that are processed into miRNA and siRNA duplex by DICER-like pro-teins, sequestration of signaling siRNA, and blocking the function of key pro-teins in RNA silencing pathways such as Dicers, RNA dependent RNA polymer-ase, Argonaute protein and other host factors. Binding of dsRNA is one of the strategies of RNA silencing sup-pressors to thwart the plant defensive system. The NSs protein of *Water-melon silver mottle virus* (WSMoV) has been identified as a RNA silencing sup-pressor and pathogenicity determinant. Previously, our laboratory has identified two novel motifs of WSMoV NSs pro-tein responsible for silencing suppression and pathogenicity. These include H^{113} at the common epitope (CE) (^{109}KFTMHNQIF^{117}) which is highly conserved in Asia type tospoviruses, and Y^{398} which is located at the C-terminal β-sheet motif (^{397}IYFL^{400}). Also, we have found that a putative helix at the C-terminal region of NSs protein is crit-ical for self-interaction of NSs protein. In this study, the dsRNA binding ability of mutated NSs proteins were examined. Mutated NSs proteins were expressed by *Escherichia* coli and the nucleic acid binding ability was tested with gel shift assay using synthesized and radiolabeled small and long dsRNAs. Our results showed that the mutated H113A NSs

protein that loses its suppressor function cannot bind small dsRNA, whereas the mutants Y398A and Y338T/H350P /F353M (denoted as TPM), which affect protein stability and self-interaction re-spectively, are able to bind small dsRNA. Furthermore, when the monoclonal an-tibody targeting the CE of NSs was used to compete with siRNAs, the binding ability of wild type NSs protein was diminished. Our results indicate that the CE of NSs protein is a siRNA binding motif. Taken all together, we proposed that the CE of Asia type tospoviruses is critical for siRNA sequestration, thus resulted in RNA silencing suppression. On the other hand, the TPM mutation that affects protein stability and self-interaction of the NSs protein does not interfere with the nucleic acid binding ability.

SA02 Generation of mild virus strains by modification of pathogenicity factor HC-Pro of ***Papaya ringspot virus*** for control by cross protection in Taiwan— Lin, Tzu-Tung and Yeh, Shyi-Dong (Department of Plant Pathology, Nation-al Chung Hsing University, Taichung)

Generation of mild virus strains by modification of pathogenicity factor HC-Pro of ***Papaya ringspot virus*** for control by cross protection in Taiwan — Lin, Tzu-Tung and Yeh, Shyi-Dong (Department of Plant Pathology, National Chung Hsing University, Taichung)

Aphid-borne Papaya ringspot virus seriously limits papaya production in tropical and subtropical areas. A ni-trous-acid induced mild strain HA 5-1, derived from Hawaii severe strain HA, has been widely used in Taiwan and Hawaii for control of PRSV by cross protection since 1985. However, cross protection is highly strain-specific, thus rendered this control measure drawn back in Taiwan and difficult to be ap-plied in different geographic regions. Based on our previous analyses, we have explored that four conserved residues of HC-Pro gene, Phe7, Arg181, Phe206 and Asp397, are essential motifs respon-sible for potyviral pathogenicity. Ac-cordingly, we attempted to modify these residues of PRSV HC-Pro gene for gen-eration of attenuated mutants from a Taiwan severe strain PRSV-YK. Through the manipulation of the in vivo YK infectious clone, site-directed mu-tagenesis was performed on the four conserved residues. Seven mutants were infectious on papaya plants, and among them single mutants of R181I, F206L and D397N induced attenuated symptoms but still prominent on infected plants. The other single mutant F7I and double mutants F7I+R181I, F7I+F206L and F7I+D397N displayed mild symptoms followed with recovery on inoculated plants of papaya. Ac-cumulation of F7I and F7I+F206L in papaya plants showed a typical zigzag pattern, an indication for triggering RNA silencing from the host and effec-tive silencing suppression by the mutants. Transient analysis of individual mutated HC-Pros by agroinfiltration in *N. benthamiana* plants revealed that F7I and F7I+F206L mutated HC-Pros have lower RNA silencing suppression capa-bilities, 35% and 55% as that of wild type HC-Pro, respectively. Cross pro-tection tests under greenhouse condi-tions showed that these two mutants provided complete cross-protection (100%), from three independent trials with a total of 30 papaya plants chal-lenged with YK 3 wk after the protective inoculation, as compared to that of 10% provided by HA 5-1. Our resulted indicate that the two mutants have a great potential for control of PRSV in Taiwan. Their long-term stability and feasibility for cross protection are being investigated under isolated field conditions.

SA03 The fifth residue (Leu) of the coat protein of ***Turnip mosaic virus*** responsi-ble for inability of systemic translocation in a local lesion host and loss of aphid transmissibility in a systemic host— Tsai, Jui-Chi[1], Hu, Wen-Chi[1], Chen, Chin-Chih[2]. and Yeh, Shyi-Dong[1]([1]Dept. of Plant Pathology, National Chung Hsing University, Taichung; [2]Plant Pathology Division, Agricultural Research Institute, COA, Wufeng, Taichung)

Previous studies revealed that HC-Pro and CP genes of a potyvirus fa-cilitate cell to cell of movement and in-volve in systemic movement. In addi-tion, the interaction between HC-Pro and CP is mandatory for aphid transmis-sion. Previously, we constructed an in vivo Turnip mosaic virus (TuMV) infec-tious clone YC5D, however, its derived virus cannot move systemically in Che-nopodium quinoa plants and lost aphid transmissibility in Nicotiana benthamiana plants. In this study, the HC-Pro and CP regions of YC5D infectious clone were replaced with the corresponding cDNA fragments amplified from the original virus isolate YC5. Our results showed that the newly replaced wild type CP region restored the ability for systemic movement in inoculated C. quinoa plants. Sequencing analysis indicated that leucine (L) at position 5 and threonine (T) at position 262 of the wild type CP were changed to proline (P) and alanine (A), respectively in YC5D virus. Interestingly, the L5P mutation was found adjacent to the ^{6}DAG^{8} motif, which is involved in aphid transmissibility. Analysis by site -directed mutagenesis, our results indi-cated that P5L, but bot A262T, is critical to permit systemic spread of the YC5 virus in C. quinoa plants and the aphid transmissibility of YC5D was also re-stored by the P5L recombinant. GFP-tagged TuMVs with or without changes in the two aa residues were constructed for investigation of long distance movement of TuMV within C. quinoa plants. The results of fluores-cence microscopy revealed that GFP signal can be observed in petioles of in-oculated leaves and upper leaves of C. quinoa plants infected by YC5D-P5L, while that of YC5D-A262T was ob-served only in petioles of inoculated leaves. Taken together, our results suggest

that amino acid substitution from leucine to proline (L5P) in the CP of TuMV hampers the virus to enter the vascular tissue of the main stem of C. quinoa plants. Aphid transmission test of YC5D-P5L recombinant virus showed that its aphid transmissibility was restored. Taken our results together, we suggest that the residue L5 at the N-terminal region, adjacent to the ^{6}DAG^{8} motif, is important for the inter-action between HC-Pro and CP, which leads to the correct formation of viral complex systemically transported in the vascular system of the local lesion host C. quinoa plants. Meanwhile this correct complex is essential for aphid transmissibility in a systemic host.

SA04 A Candidatus Phytoplasma aster-is-related strain, purple woodnettle witches'-broom phytoplasma, repre -senting a new subgroup of 16SrI group in Taiwan—Tseng, Yi-Wen[1], Chang, Chung-Jan[1,2], Su, Chiou-Chu[3], Deng, Wen-Ling[1], and Jan, Fuh-Jyh[1]. ([1]Department of Plant Pathology, Na-tional Chung Hsing University, Tai-chung; [2]Department of Plant Pathology, University of Georgia, Griffin; [3]Pesticide Application Division, Taiwan agricultural Chemicals and Toxic Sub-stances Research Institute, COA, Tai-chung)

In October 2013, a new disease af-fecting purple woodnettle (*Oreocnide pedunculata*) plants was found in Miaoli County, Taiwan. Diseased plants ex-hib-ited leaf yellowing and witch-es'-broom symptoms. Molecular diag-nostic tools and electron microscopic cell observation were used to investigate the possible cause of the disease with a specific focus on phytoplasmas. The re-sult of PCR with universal primer pairs indicated that phytoplasmas were strongly associated with the symptomat-ic purple woodnettles. The virtual re-striction fragment length polymorphism (RFLP) patterns and phylogenetic anal-ysis based on 16S rDNA and ribosomal protein, *rplV-rpsC* region revealed that purple woodnettle witches-broom phy-toplasma (PWWB) belongs to a new subgroup of 16SrI and rpI group and was designated as 16SrI-AH and rpI-Q, respectively, herein. RFLP analysis based on tuf gene region revealed that the PWWB belongs to tufI-B, but phy-logenetic analysis suggested that PWWB should be deline-ated to a new subgroup under the *tufI* group. Taken together, our analyses based on *16S rRNA* and *rplV-rpsC* region gave a finer differentiation while classifying the subgroup of aster yellows group phyto-plasmas. To our knowledge, this is the first report of a *Candidatus* Phytoplasma asteris-related strain in 16SrI-AH, rpI-Q and tufI-B subgroup affecting purple woodnettle, and of an official documen-tation of purple woodnettle as being a new host of phytoplasmas.

SA05 Growth enhancing effect on *Bo-trytis elliptica* driven by the N-terminal region of antifungal protein LsGRP1— Liu, Fang-Wei[1], Lin, Chia-Hua[1], Chen, Chao-Ying[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

LsGRP1 is a pathogenic fungus *Botrytis elliptica*-inducible plant class II glycine-rich defense protein located in the cell surface of lily leaf tissues, and could protect plant from infection *via* the antimicrobial activity conferred by its C-terminal region, LsGRP1^{C}. *In vitro* and *in planta* assays revealed that LsGRP1 exhibited greater inhibitory activity on *B. elliptica* than LsGRP1^{C} did, suggesting the other composition regions in LsGRP1 may play helper roles to facilitate the anti-*B. elliptica* effect conducted by LsGRP1^{C}. Because *in vitro* treatment of the N-terminal region, LsGRP1^{N} slightly enhanced the spore germination of *B. elliptica* but inhibited or did not alternate the spore germination of other tested pathogenic fungi, some specific interaction between LsGRP1^{N} and *B. elliptica* might present and accordingly involve in the sensitivity of *B. elliptica* to LsGRP1^{C}. Thus, in this study, we intended to explore the function of LsGRP1^{N} and its effect on *B. elliptica*. At first, SUMO-LsGRP1^{N} fusion protein was infiltrated into lily leaves before inoculation with *B. elliptica* to verify *in planta* effect of LsGRP1^{N} on *B. elliptica*, and earlier spore germination, larger fungal biomass and severer host cell death occurred as compared with the control treatment of SUMO partner of fusion protein. Since further assay revealed that treatment of SUMO-LsGRP1^{N} did not trigger plant cell death under un-inoculated condition, the enhanced growth of *B. elliptica* triggered by LsGRP1^{N} was likely a cause of severer plant cell death. In addition, the *in vitro* treatment of an equal-length peptide with the identical amino acid composition and different sequence of LsGRP1^{N} did not affect spore germination and hyphal growth of *B. elliptica*, the possibility of LsGRP1^{N} as a nutrient supplement was excluded. Nevertheless, slightly germinating spores of *B. elliptica* were found more sensitive to LsGRP1C as compared with that of un-germinated ones. A combination of LsGRP1N and LsGRP1^{C} was proven slightly driving spore germination of *B. elliptica* and conducting stronger inhibitory effect than LsGRP1^{C} did. Thus, germination of of *B. elliptica* spores triggered by LsGRP1^{N} became vulnerable to LsGRP1^{C} was presumed.

SA06 Degenerate primer design for rapid diagnosis of tospoviruses in one-step real-time reverse transcrip-tion-polymerase chain reaction — Sun, Jing-Hua and Chen, Tsung-Chi (De-partment of Biotechnology, Asia Uni-versity, Wufeng, Taichung 41354, Tai-wan)

The thrips-borne *Tospovirus* is the only plant-infecting genus of the family *Bunyaviridae* that possesses qua-si-spherical enveloped particles of 80-120 nm in diameter and a segmented

tripartite single-stranded (ss) RNA ge-nome, named L, M and S according to the molecular sizes. The S RNA-encoded nucleocapsid protein (NP) is the most important target for identifi-cation and classification of tospoviruses. Based on the serological and phyloge-netic relationships of NPs, the 29 current *Tospovirus* species are clustered into six serogroups (or clades). In general, tospoviruses clustered in a serogroup are serologically indistinguishable when an-tisera against the NPs are used; however, they can be identified by reverse tran-scription-polymerase chain reaction (RT-PCR) with the N gene-specific pri-mer pairs. The degenerate primers de-signed from the consensus sequences of genomic RNAs are useful for rapid di-agnosis of tospoviruses. In this study, two forward degenerate primers (dTospoF1 and dTospoF2) and two re-verse degenerate primers (dTospoR1 and dTospoR2) were newly designed from the conserved regions of the available tospoviral L RNA sequences to test the ability of tospovirus detection in the SYBR Green I-based one-step real-time RT-PCR method. Total RNAs extract-ed from *Chenopodium quinoa* leaves in-dividually inoculated with 20 *Tospovirus* species were used for assays. Apparent signals were amplified from all samples of tested tospoviruses when the two primer pairs dTospoF2/dTospoR1 and dTospoF2/dTospoR2 were used, and the specific PCR products were validated by melting curve assay and agarose gel electrophoresis. No signals were found in the outgroup controls. Furthermore, the primer pairs were used to diagnose tospovirus infections in field cowpea and pepper samples, and the amplicons were directly sequenced to identify the causal agents are Groundnut chlorotic fan-spot virus and *Watermelon silver mottle virus, respectively*. Our results showed that the new degenerate primer pairs are *Tospovirus* genus-specific and can be applied in one-step real-time RT-PCR to explore tospoviruses.

SA07 辣椒炭疽病菌 (*Colletotrichum acutatum*) 生長與致病過程相關之基因功能性分析—郭家琪、林詠筑、李敏惠、鍾光仁 (國立中興大學植物病理學系)

Functional analysis of genes involved in vegetative growth and virulence of chili pepper pathogen *Colletotrichum acutatum*—Kuo, C. C., Lin, Y. C., Lee, M. H. and Chung, K. R. (Department of Plant Pathology, National Chung Hsing Uni-versity, Taichung, Taiwan)

辣椒 (*Capsicum* spp.) 為世界重要的辛香料作物之一，台灣位於亞熱帶地區，終年高溫多濕於田間易有辣椒炭疽病發生，主要危害果實造成農民嚴重經濟損失，台灣辣椒炭疽病主要由*Colletotrichum acutatum*造成。為了要更加了解*C. acutatum* 的致病機制，本實驗室以農桿菌轉殖法 (ATMT；*Agrobacterium tumefaciens* -mediated transformation) 建立*C. acutatum*突變菌株庫，並從中篩選出單一T-DNA插入造成生長性狀或致病能力有差異之轉殖株 B7與B84。轉殖株B7對於硝酸態氮利用能力顯著下降，轉殖株B84為嚴重毒力下降之菌株。轉殖株B7之T-DNA插入位置為非轉譯區，進一步分析發現T-DNA插入造成基因體大片段DNA序列 (19-kb) 剔除，共三個基因 (GPI-anchored protein (GPI-Ap)、Nitrate transporter (NTP) 和 RecQ family ATP-dependent DNA helicase) 受影響；轉植株B84之T-DNA則插在RNA polymerase II mediator complex subunit 18 (med18) 與 Auxiliary activity family 9 (AA9) 兩基因之間。為研究B7 與B84轉殖株造成生物性狀改變之主要基因為何，本篇將可能影響轉殖株B7與B84之生物性狀的5個基因包括Nitrate transporter、hypothetical protein、GPI-Ap 、med18及AA9 依次進行基因剔除(knockout)，並進一步分析其功能，目前已獲得前4個基因剔除之基因突變株，其中Nitrate transporter基因剔除突變株對於硝酸態氮利用能力顯著下降與轉殖株B7性狀相似，應為B7生物性狀變異之主要影響基因。med18的基因剔除轉殖株生長速度較慢且毒力有下降情形，但其生長能力及病原性之缺失狀況並不如B84嚴重，顯示med18 僅為B84生物性狀缺失因子之一，另一受T-DNA 插入影響之基因AA9也可能造成B84生物性狀缺失，AA9基因剔除正在進行中。

SA08 探討番茄萎凋病菌鈣調磷酸酶於菌絲生長及致病力所扮演之角色—王宣富[1]、徐立航[1]、陳穎練[1] ([1] 國立臺灣大學植物病理與微生物學系)

The roles of calcineurin in hyphal growth and virulence of *Fusarium oxysporum* f. sp. *lycopersici*—Hsuan-Fu Wang[1], Li-Hang Hsu[1], and Ying-Lien Chen[1] ([1] Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan)

鐮孢菌(*Fusarium*)為絲狀真菌，其包含許多重要的植物病原菌、真菌毒素(mycotoxin)生產者以及潛在的人類病原。尖鐮孢菌(*F. oxysporum*)可感染超過100種不同的作物且會造成嚴重危害，因此闡明尖鐮孢菌的致病機制將有助於找到此病害之防治策略。鈣調磷酸酶 (calcineurin)對於病原真菌的生長及致病力扮演重要功能，但在不同的真菌，如人類病原真菌*Candida albicans*及*Cryptococcus neoformans*，或是植物病原真菌*Magnaporthe oryzae*和*Ustilago maydis*則有調控上的差異，例如造成對不同藥物和環境因子(溫度和酸鹼值)的敏感性差異。鈣調磷酸酶由catalytic (Cna1)和regulatory (Cnb1) subunits組成，且為heterodimeric calmodulin-dependent的蛋白磷酸酶，其可調控病原真菌的Ca^{2+} 訊息傳遞、型態發育、環境壓力反應及致病力。然而鈣調磷酸酶在尖鐮孢菌所扮演的角色仍不清楚。本研究以番茄萎凋病菌(*F. oxysporum* f. sp. *lycopersici*)為主軸，根據序列比對多種真菌的鈣調磷酸酶，我們發現Cna1 5' catalytic domain、Cnb1-binding helix與Calmodulin-binding domain序列具高度保守性，但serine-proline rich region (SPRR)為真菌Cna1所特有之序列。Cnb1次單元則具有4個Ca^{2+} binding EF-hand motifs可與Ca^{2+}結合。目前研究結果顯示突變株 *cnb1*(LHS2)和 *cnb1* (HFW3)的蛋白磷酸酶活性測試(phosphatase activity test)

分別為110.09±56.01和90.17±7.34，顯著低於野生株645.08±49.34 nmoles・min^{-1}・mg^{-1}，另外*cna1*、*cnb1*突變株菌絲生長速率分別為3.87±1.03 m/hr和3.49±0.68 m/hr，皆比野生株28.95±8.79 m/hr慢約7倍，而菌絲之隔膜間距分別為67.87±15.51m和72.69±14.01m為野生株隔膜間距167.95±47.76的一半，暗示隔膜之形成可能影響突變株菌絲生長速率。我們進一步測量*cna1*、*cnb1*突變株於25℃之孢子萌芽時間分別為10.8±0.5 hr和10.8±0.5 hr皆比野生株6.3 ± 0.7慢約4小時。另外*cna1*與*cnb1*突變株於100 ml PDB液態培養七天之產孢數量約為3.55±0.17×10^6 CFU及5.83±0.2×10^6 CFU比野生株產孢量1.65±0.69×10^8 CFU少約50倍。值得注意的是我們發現兩突變株於液態培養時會有凝聚現象的發生，而兩突變株之回復株*cna1+CNA1* (HFW2)和*cnb1+CNB1* (HFW10)皆回復近似野生株之表現型。另外也發現*cna1*對番茄農友301品系喪失致病力。未來研究將完成型態分析包括厚膜孢子、入侵菌絲的穿透能力以及環境壓力和藥劑敏感性等測試，並期望針對尖鐮孢菌鈣調磷酸酶調控菌絲生長及致病機制提供更進一步的闡明。

SA09 芒果炭疽病菌 (*Colletotrichum gloeosporioides*) 之組氨酸激酶 (His-tidine kinase) 基因群分析與*CgHK1*基因功能性分析—林獻哲[1]、謝岱庚[2,3]、施明哲[2]、鍾光仁[1]、李敏惠[1] ([1]國立中興大學植物病理學系；[2]中央研究院農業生物科技研究中心；[3]中興大學與中央研究院微生物基因體博士學位學程)

Characterization and phylogenetic anal-ysis of histidine kinase genes in mango pathogen *Colletotrichum gloeosporioides* and functional analysis of *CgHK1*—Lin, H. C.[1], Hsieh, T. K. [2], Shih, M.C. [2], Chung, K. R.[1] and Lee, M. H.[1] ([1]Department of Plant Pathology, National Chung Hsing University, Tai-chung, Taiwan; [2]Agricultural Biotech-nology Research Center Academia Sinica ; [3]Ph.D. Program in Microbial Genomics, National Chung Hsing Uni-versity and Academia Sinica, Taiwan)

芒果 (*Mangifera indica* L.) 為漆樹科之熱帶果樹，是世界上年產量第五大之重要水果。在芒果病害中以*Colletotrichum gloeosporioides*所造成的炭疽病害 (anthracnose) 危害最為嚴重，在芒果的葉片、花穗、枝條上皆能造成褐色病斑，使得植株生長受到影響產果量下降，也能潛伏感染於果實內，於儲藏期時發病，造成產業上極大的經濟損失。組氨酸激酶 (Histidine kinase；HK) 之功能為感知外界環境刺激後調控下游反應而達到對環境變化之適應，依所帶有之蛋白功能區真菌之HK可以分成11群，其功能為主要包括參與生長調控、滲透壓逆境、殺菌劑感受性、氧化還原逆境、重金屬耐受性、亞硝酸鹽利用以及病原性等。本研究藉由全基因體解序與同源基因比對發現芒果炭疽病菌中有12個HK基因。蛋白功能區分析發現只有CgHK2p具穿膜蛋白功能區；親緣性分析得知芒果炭疽病菌之HK以第I群分布最多，但缺少第II群、第VI群以及第VII群之HK。除此之外，本篇發現光照可以誘導*CgHK1*之表現量上升，為了瞭解其功能利用ATMT結合Split maker技術成功獲得*CgHK1*基因剔除之轉殖株，轉殖株之生長與發育相關特性、環境壓力耐受性及致病能力等已完成分析，將一併討論。

SA10 臺灣良質米品種改良之抗稻熱病基因座分析與分子標誌開發—陳韋綸[1]、沈偉強[1]、張芳瑜[2]、張為斌[3]、余宗學[1]、賴明信[4]、廖睿瑜[1]、吳志文[2]、鍾嘉綾[1] ([1]國立臺灣大學植物病理與微生物學系；[2]行政院農業委員會高雄區農業改良場；[3]行政院農業委員會桃園區農業改良場；[4]行政院農業委員會農業試驗所作物組)

Analysis of blast resistance genes and molecular markers development for the improvement of Taiwan high-quality rice varieties—Chen, W. L.[1], Shen, W. C.[1], Chang, F. Y.[2], Chang, W. B.[3], Yu, T. H.[1], Lai, M. H.[4], Liao, J. Y.[1], Wu, C. W.[2], Chung, C. L.[1]([1] Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2] Crop Im-provement Section, Kaohsiung District Agricultural Research and Extension Station, Pingtung; [3] Crop Environment Section, Taoyuan District Agricultural Research and Extension Station, Taoyuan; [4] Crop Science Division, Tai-wan Agricultural Research Institute, Council of Agriculture, Taichung)

稻熱病為台灣一期稻作之主要流行病害，使用抗病品種為已知有效且對環境友善之防治方法，當前新興的分子標誌輔助育種技術 (mark-er-assisted selection, MAS)，可有效節省育種所需時程，因此成為水稻抗性改良之發展趨勢。本研究以來自國際水稻研究中心、帶有已知抗性基因之 31 個 IRBLs，接種 18 株從雲林、嘉義、臺南、屏東、花蓮、臺東分離之稻熱病菌菌株，發現位於*Pi2/9*、*Pik*、*Pita/Pita-2*基因座上的抗性等位基因對較多菌株呈現抗病性。接著對於抗幅較廣之 13 個 IRBLs 及 20 個臺灣良質米品種，分別蒐集前人文獻及利用genotyping-by-sequencing (GBS) 定序資料，設計分子標誌並進行多型性測試，建立 11 對 R gene 分子標誌與 96 對背景分子標誌。為了瞭解本土良質米對稻熱病抗性，以12YL-TT4-1及13TN-HB1-3兩菌株進行接種測試，結果顯示，台粳4號、台粳9號、台粳10號、台粳14號、台粳15號、台農71號、桃園3號、台南11號、高雄145號、高雄147號等10個品種對兩菌株均呈現感病，表示不帶有 *Pi9* (IRBL9-W)、*Piz5* (IRBLz5-CA、IRBLz5-CA (R))、*Piz* (IRBLz-Fu)、*Pi1* (IRBL1-CL)、*Pi7* (IRBL7-M)、*Pik* (IRBLk-Ka)、*Pik-p* (IRBLkp-K60)、*Pik-m* (IRBLkm-Ts)、*Pik-h* (IRBLkh-K3)、*Pi20* (IRBL20-IR24) 及*Pitz-2* (IRBLta2-Pi、IRBLta2-Re) 等對台灣稻熱病菌有抗性之等位基因，適合以 13 IRBLs 及新開發之多型性分子標誌進行抗性改良。本研究同時運用此分子標誌平臺實際將 10 個 IRBLs 之抗性基因導入高雄145號中，期望選育出高雄145號之抗稻熱病多系品種 (multiline variety)。

SA11 利用病毒誘導基因靜默技術探討調控日日春PR1a之轉錄因子的表現對日日春葉片黃化病的影響—林稜雅[1]、林長平

[1]、陳仁治[2] ([1]國立台灣大學植物病理與微生物學系、[2]國立台灣大學生物科技研究所)

Analysis of the effects on symptom de-velopment of periwinkle leaf yellowing after knockdown of ***CrPR1a*** regulatory transcription factors using VIGS—Lin, L. Y.[1], Lin, C. P.[1], Chen, J. C[2]. ([1] Dept. of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Institute of Biotechnology, National Taiwan University, Taipei)

植物菌質體 (phytoplasma) 為寄生於植物篩管內無細胞壁的絕對寄生性原核生物。主要透過媒介昆蟲進行傳播，可引起葉片黃化、簇葉、花器綠化等病徵，並在世界各地造成多種經濟作物的損失。從日日春 (*Catharanthus roseus*) 的研究中發現，植物抗植物菌質體的機制可能與系統性抗病(systemic acquired resistance，SAR) 有關。先前的研究發現，當抑制可能參與系統性抗病的*CrNPR1*的表現，不但使*CrPR1a*在罹病植株中的表現量下降，亦加速由植物菌質體造成的日日春葉片黃化病 (periwinkle leaf yellowing, PLY) 之病程發展。因此推測調控*CrPR1a*表現的轉錄因子，可能參與在植物對抗植物菌質體的抗病途徑，進而影響日日春葉片黃化病之病程發展。先前我們已利用病毒誘導基因靜默技術（virus-induced gene silencing, VIGS） 對307個轉錄因子進行篩選可能影響*CrPR1a*表現的轉錄因子。結果篩選出3個ARF，1個Aux/IAA，以及1個bZIP類別之轉錄因子。因此，本研究將進一步探討以VIGS靜默這些轉錄因子的表現是否會對日日春葉片黃化病的病徵發展造成影響。在初步的兩次試驗中發現靜默與阿拉伯芥*ARF17*接近的ARF轉錄因子 *CrARF17* 能降低 *CrPR1a* 的表現量並也造成日日春葉片黃化病之病程發展加速。此結果與先前發現靜默*CrARF17*抑制*CrPR1a*表現的結果一致。由於在阿拉伯芥的研究中發現ARF一類的轉錄因子多參與在植物生長素的訊息傳遞上，且植物生長素可能透過壓抑植物中水楊酸 (salicylic acid，SA) 的累積進而影響植物對病原菌的抗性。往後我們將針對CrARF17在植物對日日春葉片黃化病的抗性上可能扮演的角色作更深入的探討。看其角色是否與生長素的訊息傳遞及生長素與水楊酸交互作用相關。

SA12　利用阿拉伯芥進行日日春 *NPR1* 與 *NPR3* 基因之功能分析—陳鈺凌[1]、宋宜璋[1]、林長平[1]、陳仁治[2] (國立台灣大學植物病理與微生物學系[1]、國立台灣大學生物科技研究所[2])

Functionalanalysis of ***CrNPR1*** and ***CrNPR3*** in ***Arabidopsis thaliana***—Chen, Y. L.[1] , Sung, Y. C.[1], Lin, C. P.[1], and Chen, J. C.[2] ([1]Dept. of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Institute of Bio-technology, National Taiwan University, Taipei)

植物菌質體 (phytoplasma) 為一不具細胞壁寄生於植物韌皮部之細菌。它能透過媒介昆蟲傳播，感染多種經濟作物，造成農作物損失。當日日春受日日春葉片黃化病 (periwinkle leaf yellowing, PLY) 植物菌質體感染時，有病徵及無病徵枝條中*PR1*基因的表現皆被誘導，因此推測系統性抗病反應可能對植物抗植物菌質體之感染扮演重要角色。系統抗病 (Systemic-acquired resistance, SAR) 屬於誘導性的植物抗病機制，當局部植物組織遭受病原感染時，會釋放訊號刺激未受感染部位水楊酸 (salicylic acid,SA) 的累積並表現 pathogenesis-related (PR) 基因來增強植物抵抗下一次病原感染時的能力。*NPR1*與*NPR3*為系統抗病途徑之重要基因。受PLY植物菌質體感染的日日春，其*CrNPR1*基因表現趨勢與*CrPR1*相似，且基因靜默*CrNPR1*會使得*CrPR1*基因表現量下降，亦加速受植物菌質體感染之日日春病程發展；在*CrNPR3*靜默之植株則病程發展較為遲緩。因此CrNPR1與CrNPR3可能在日日春對抗植物菌質體入侵中扮演重要的調控因子。*CrNPR1*的表現僅會些微受到SA的誘導，且當*CrNPR1*靜默時，*CrPR1*基因表現量下降。這些結果與阿拉伯芥中NPR1參與在系統性抗病的研究結果相似。因此，本研究欲利用阿拉伯芥的Col-0、*NPR1*突變株*npr1-1*及水楊酸含量低的*NahG* (SA hydroxylase) 轉殖株分別大量表現*AtNPR1*、*CrNPR1*與*CrNPR3*，並感染*Pseudomonas syringae*以誘導系統性抗病反應，最後分析阿拉伯芥PR1是否被誘導表現以及觀察病徵的發展情況。目前已得到共九種轉殖株進行第二次*Pseudomonas syringae*接種試驗，然在菌量累積的分析上由於誤差較大，尚無法確認CrNPR1及CrNPR3的功能是否能回復*npr1-1*的抗病反應。未來將再多次進行接種試驗以確認結果，並探討 CrNPR1與CrNPR3是否與AtNPR1功能相同皆須透過SA的誘導進入細胞核中作用。希望藉此釐清日日春抗植物菌質體之感染所誘導的抗病反應。

SA13　非光合作用型硫鐵蛋白基因 AtFd3 對阿拉伯芥發育及青枯病菌抗病能力之影響－陳郁雯[1]、蔡兒倩[1]、許育仁[1]、黃祥恩[1] ([1]國立台東大學生命科學系)

Non-photosynthetic-Type Ferredoxin, AtFd3, regulated plant development and disease resitance to ***Ralstonia solanacearum*** in Arabidopsis — Chen, Y.W.[1], Tsai, E.C., Hsu Y. J.[1], Huang, H. E.[1] ([1] Dept. of Life Science, National Taitung University)

硫鐵蛋白 (Ferredoxin, Fd) 是一群帶有二鐵二硫 (2Fe-2S) 官能基的氧化還原蛋白，此類蛋白可藉由電子的傳遞來調控植物的基礎代謝反應，包括生長發育與抗病能力。通常存在綠色組織中的 Fd 被歸類為光合作用型硫鐵蛋白 (photosynthetic type Fd, PT-Fd)，而存在非綠色儲藏組織的Fd則被歸類為非光合作用型硫鐵蛋白 (non-photosynthetic type Fd, nPT-Fd)。植物在進入開花繁殖期時，會開始累積 nPT-Fd，藉此將光合作用所獲得的能量轉換至儲存性組織，而微生物的感染也會影響植物體內 nPT-Fd的含量。為了探討 nPT-Fd 對於植物發育及對抗病原菌的角色，本實驗利用花椰菜崁紋病毒 (CaMV) 的 35S 啟動子，增量表現阿拉伯芥 nPT-Fd 基因 AtFd3的轉殖品系 (OE line)。另外藉由 RNA 干擾技術 (RNA interference, RNAi) 來降低阿

拉伯芥內源性nPT-Fd含量 (RNAi line)，觀察 OE line 與 RNAi line 在生長、開花及對青枯病菌 *Ralstonia solanacearum* Rd4 抗病能力之影響。實驗結果顯示，OE line 的營養生長有明顯下降的現象，但是卻會提早進入開花繁殖期，增加花苞、果莢、側葉、側枝的數量。利用水淹處理 *R. solanacearum* Rd4 與 Rd15可以誘導AtFd3及AtFd4基因的表現，並引起過敏性反應 (Hypersensitive Response, HR)。 OE line 上，Rd4 引起的 HR 會造成較高的離子滲漏率及水楊酸抗性路徑標記基因 *AtPR1* 基因的表現，同時茉莉酸相關抗性途徑的標記基因 *AtPDF 1.2* 的表現則有被抑制的現象。此外 harpin 誘導的 HR 也顯示相同的結果。然而 AtFd3 OE line 卻出現對於 ROS 誘導物質 H_2O_2、MV 反應較為嚴重的現象，而對 DCMU 的反應則無明顯差異。青枯病菌的剪根接種試驗則發現，AtFd3 OE line 會對 *R.solanacearum*有較高的抗性，但是在炭疽病菌 *Colletotrichum* sp. 的接種試驗中則發現， AtFd3 OE line 會出現更加嚴重的感染。另一方面 AtFd3 RNAi line 則出現促進營養生長，並且延遲進入開花繁殖的時間，同時果莢數也有顯著的減少。但是在水淹青枯病菌、harpin、H_2O_2、MV、DCMU 處理時，AtFd3 RNAi line 的細胞膜離子滲漏率並無差異，而在 *Colletotrichum* sp. 也沒有差異，但是在AtPR1 的表現量則有被抑制的現象。綜合以上結果我們推測 nPT-Fd 可以改變植物進入開花繁殖期的時間與繁殖組織的數量，同時可以增強植物對於過敏性誘導物質的反應，並藉由水楊酸相關的抗性路徑，引發對於青枯病菌的抗性，但是卻會降低對於茉莉酸相關抗性路徑的抗病能力。

SA14 兩種仙人掌X病毒感染性選殖株之構築及分析－張佑瑄、張雅君 (國立臺灣大學植物病理與微生物學系)

Construction and analyses of two *Cactus virus X* infectious clones－Chang, Y. W., and Chang, Y. C. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

紅龍果(*Hylocereus* spp.)為臺灣近年新興之熱帶水果，自2001年首次報導仙人掌X病毒(*Cactus virus X*, CVX)起，亦陸續鑑定出紅龍果X病毒(*Pitaya virus X*, PiVX)及蟹爪蘭X病毒(*Zygocactus virus X*, ZyVX)，共三種*Potexvirus*屬病毒可感染紅龍果。因紅龍果種植多採無性扦插之方式繁殖，加之生長期間枝枒修剪頻繁，使田間之病毒感染甚為普遍。經分析比對國內已發表之CVX-Hu及CVX-NTU分離株之全長基因體序列，設計可區分CVX兩種分離株之專一性引子對，針對臺東池上、屏東佳冬及萬丹等三處果園進行檢測，結果顯示田間紅龍果植株複合感染率至少達73%。為研究兩種分離株於植物體內之感染情形，我們採集臺中霧峰及南投集集之罹病紅龍果，從中選殖並構築出兩個CVX感染性選殖株－H015及N015，兩者與CVX-Hu及CVX-NTU全長核酸序列相同度達97%，與其他Potexvirus屬病毒進行親緣演化樹分析，皆歸屬於感染仙人掌科植物之分支。將CVX-N015與CVX-H015分別接種白藜(*Chenopodium quinoa*)，可見CVX-N015於接種葉產生之黃斑病徵較CVX-H015明顯。進一步於圓葉菸草(*Nicotiana benthamiana*)原生質體接種兩選植株的轉錄體，結果顯示CVX-N015的複製效率遠較CVX-H015為佳。經胺基酸序列比對後，選擇對兩者的RdRP及CP基因進行置換分析，期望探究影響兩者複製效率差異的因子。另外欲將CVX-N015與CVX-H015共同接種於植株，利用定量即時反轉錄聚合酶連鎖反應(quantitative real-time RT-PCR)比較病毒量的累積，以分析兩者之交互作用。除此之外，本研究亦嘗試建立紅龍果原生質體系統，測試結果顯示同時使用1%纖維酶(cellulase)、1%果膠酶(macerozyme)及1%蝸牛酶(snailase)製備原生質體，可獲得最佳產量；此原生質體系統對於未來探討CVX感染紅龍果之研究，將提供極有用之研究平台。

SA15 齒舌蘭輪斑病毒與蕙蘭嵌紋病毒感染性選殖株致病力之強化及二者之協力侵染現象探討—郭尚明、張羽萱、張雅君 (國立臺灣大學植物病理與微生物學系)

Improvement of the infectious clones of *Odontoglossum ring spot virus* and *Cymbidium mosaic virus*, and the syner-gistic effect between two viruses—Kuo, S. M., Chang, Y. H., and Chang, Y. C. (Department of Plant Pathology and Mi-crobiology, National Taiwan University, Taipei)

病毒之五端及三端非轉譯區(5'- and 3'-UTR)已被證實參與在病毒侵染寄主之過程，例如：菸草嵌紋病毒(*Tobacco mosaic virus*)之5'-及3'-UTR與病毒基因之轉譯有關，3'-UTR帶有複製負股基因體RNA之必要序列。本實驗室先前已構築由T7 promoter驅動之齒舌蘭輪斑病毒(**Odontoglossum ringspot virus**, ORSV)之感染性選殖株pORSV7；當改用35S promoter驅動時，除構築p35S-ORSV-9外，亦透過外加核酶酵(ribozyme)序列於其3'-UTR的方式，獲得p35S-ORSV-Rzh-9-1。接種結果顯示改良後的p35S-ORSV-Rzh-9-1選殖株可有效感染圓葉菸草(*Nicotiana benthamiana*)。此外，為同時以農桿菌短暫表現法(*Agrobacterium*-mediated transient expression)表現病毒與其他蛋白於植株中，亦將ORSV基因體序列選殖至雙元載體(binary vector)中；以此法接種的結果發現，若將該載體中ORSV 5'-UTR上游之非病毒序列去除後，可大幅提升其感染效率。同樣的現象也在蕙蘭嵌紋病毒(*Cymbidium mosaic virus*, CymMV)之感染性選殖株中發現。以上結果說明了病毒之5'-及3'-UTR的正確性對其感染力有很大的影響。在蘭花的栽培上，ORSV與CymMV常常同時感染同一植株，造成較單獨感染更為嚴重的病徵，此現象稱為協力作用(synergistic effect)，目前對此現象之機制仍不甚明瞭。前人研究中，以病毒RNA接種於石斛蘭之原生質體時，兩種病毒之累積量均較單獨感染為多。本實驗室之研究發現，圓葉菸草之原生質體受到複合感染時，協力作用仍存在；將ORSV與CymMV之triple gene block 1 (TGB1)蛋白，或將CymMV與ORSV之複製酶次單元p126共同表現時，病毒鞘蛋白與RNA均有顯著提升。目前的研究結果顯示，受到複合

感染之圓葉菸草的系統葉除了ORSV典型的嵌紋病徵加劇外，原先只能感染接種葉的CymMV，在有ORSV的複合感染下能夠成功地轉移到系統葉中，但有少部分系統葉僅能偵測到ORSV的存在。將兩種病毒以農桿菌短暫表現法感染圓葉菸草葉片，CymMV之累積量相較於單獨感染為低，但以受病毒感染植物之汁液接種，則結果恰好相反，顯示接種方法可能影響協力作用結果的呈現。

SA16　探討SlSOBIR1交互作用蛋白NbRLP1的特性—施維哲[1]、黃俊慈[1]、王昭雯[2]、劉瑞芬[1] ([1]國立臺灣大學植物病理與微生物學系；[2]中央研究院植物暨微生物學研究所)

Functional characterization of NbRLP1, a putative SlSOBIR1-interacting pro-tein—Shih, W.-C.[1], Huang, C.-T.[1], Wang, C.-W.[2], and Liou, Liou R.-F.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan Univer-sity, Taipei; [2]Institute of Plant and Microbial Biology, Academia Sinica, Tai-pei)

植物因應病原菌侵染所啟動之先天免疫 (innate immunity) 可分為pattern-triggered immunity (PTI) 及effector-triggered immunity (ETI)；前者為植物以pattern-recognition receptor 辨認病原菌之pathogen-associated molecular pattern (PAMP)，並在BAK1及 BIK1等蛋白共同作用下，所引發的基礎防禦反應。目前在疫病菌已被報導的PAMP包括elicitin及Pep-13等，但這些PAMP如何啟動植物防禦反應仍待深入探究。本實驗室先前發現，以TRV-mediated gene silencing靜默*SOBIR1*基因之表現時，不僅削弱植物對疫病菌(*Phytophthora parasitica*)的抗病性，也會抑制*P. parasitica* elicitin ParA1在圓葉菸草(*Nicotiana benthamiana*)引發necrosis的能力。SlSOBIR1為receptor-like kinase，一般分布於植物細胞膜，但以疫病菌感染或以ParA1處理植物時，SlSOBIR1都會藉由endocytosis自細胞膜移動至胞內，顯示其在植物對疫病菌的基礎防禦反應扮演重要角色。為了探討*SlSOBIR1*參與植物基礎防禦反應所涉機制，我們應用免疫沉澱與液相層析串聯質譜分析技術(LC-MS/MS)鑑別與SlSOBIR1交互作用的蛋白，進一步以*Agrobacterium*-mediated overexpression分析的結果，發現其中一個蛋白(命名為NbRLP1)參與ParA1在圓葉菸草引發necrosis的作用。NbRLP1主要分佈於植物細胞膜，但以ParA1 處理植物時，NbRLP1明顯由細胞膜移往細胞質，顯示該蛋白確實參與ParA1在植物的作用。

SB01　引起金線連莖腐病之兩病原型菌株快速檢測技術的開發與田間感染源探討—許子娟[1]、王照仁[1]、鍾文鑫[1] （[1]國立中興大學植物病理學系）

Development of specific PCR primer for identification of the two pathotypes cas-ing stem rot in *Anoectochilus* and dis-tribution of inoculum in field — Tzu-Chuan Hsu[1], Chao-Jen Wang[1], Wen-Hsin Chung[1] ([1] Dept. of Plant Pa-thology, National Chung Hsing University)

尖鐮孢菌 (*Fusarium oxysporum*) 可引起多種作物的萎凋病，目前已報告指出全球有150種植物可受其感染而發病。在台灣，由*F. oxysporum* f. sp. *anoectochili* (*Foa*)所引起之金線連莖腐病，依其菌落形態與核醣體轉錄間隔區間 (IGS) 序列之差異，可區分為兩種病原型(pathotype)，分別為白色菌絲型cottony alba (CA)與分生子座型sporodochial (S)。本研究為能建立快速診斷兩型菌株之平台，開發以IGS序列為基礎之分子標幟。測試結果顯示，依兩型菌株在IGS序列上之差異所設計的兩組專一性引子對，可分別鑑定CA與S 型菌株，所增幅DNA條帶大小分別為618 bp與434 bp。引子對之靈敏度測試結果得知，兩組專一性引子對最低可偵測菌株DNA濃度為10^{-2} ng/ µl；此外，引子對對兩型Foa菌株具高度專一性，無法增幅出其他分化型或非病原性之*F. oxysporum*菌株。進一步利用該兩組引子對，分析溫室環境內之栽培介質、地表沙塵、盆缽、植株及空氣中Foa兩型菌株之可能分佈與優劣勢。結果得知，空氣中懸浮與自植株所得*Foa*菌株以CA 型菌株為主；而來自土壤、盆缽及地面所搜集沙塵之樣品內的*Foa*菌株，則以S型菌株為主。

SB02　評估微奈米鈣對番茄疫病菌與細菌性斑點病菌之生長影響、溫室防治試驗及機制探討—陳念煒[1]、張碧芳[1]、黃振文[1]、鍾文鑫[1] ([1]中興大學植物病理學系)

Evaluation of nano/micro calcium on growth inhibition of *Phytophthora cap-sici* and *Xanthomonas perforans*, control in greenhouse and mechanism—Chen, N.W.[1], Chang, P.F.[1], Huang, J.W.[1], Chung, W.H.[1] ([1] Dept. of Plant Patholo-gy, National Chung Hsing University, Taichung)

前人研究指出，鈣離子 (Ca^{2+}) 可強化植物細胞膜與細胞壁防禦病原，並誘導植物產生抗病原之功效。將物質微奈化後可增加其被植物吸收之功效，本研究探討微奈化鈣對番茄疫病菌 (*Phytophthora capsici*) 與細菌性斑點病菌 (*Xanthomonas perforans*) 生長影響、防治番茄葉部病害之功效及其可能產生之防禦機制。於病原生長抑制結果得知，添加微奈米化鈣之NA培養基可以抑制*X. perforans*的生長；反之，微奈米化鈣卻可促進*P. capsici*之菌絲生長。在溫室防治實驗中，番茄葉部施用微奈米化鈣後，能降低由*X. perforans*所引起之細菌性病害的罹病度達 45.4%。探討可能防治機制，證實番茄施用微奈米化鈣後，體內與抗病相關之PR2與PR3蛋白降低，顯示奈米化鈣無法誘導番茄產生PR2與PR3蛋白。進一步分析與抗病相關酵素，結果指出，先施用奈米化鈣後再接種*X. perforans*，番茄體內超氧化物歧化酶 (superoxidase dismutase, SOD) 和過氧化酵素 (peroxidase, POD) 的生合成在第三天達最高峰；而僅接種病原菌的處理，則分別要到第六與第四天才有明顯提升現象。在過氧化氫酶 (catalase) 分析結果得知，同樣先施用奈米化鈣後再接種*X. perforans*，番茄體內過氧化氫酶含量可提高15%；而分析番茄體內苯丙酸胺裂解酵素 (phenyalanine ammonia lyase,

PAL) 之含量，則顯示與只接種病原之植株無顯著差異。

SB03 分離自進口梨接穗之梨花枯病菌 *Pseudomonas syringae* pv. *syringae* 生理與遺傳特性分析—鄭日新、曾貞瑜、鄧文玲 (國立中興大學植物病理學系)

Physiological and genetic charac-terization of *Pseudomonas syringae* pv. *syringae* isolated from imported pear scions— Cheng, Jih-hsin, Tzeng, Jen-Yu, Deng, Wen-Ling (Department of Plant Pathology, National Chung Hsing Uni-versity, South District, Taichung 402, Taiwan)

Pseudomonas syringae van Hall 1902, originally isolated from lilac (*Syringa vulgaris* L.), is a complex species that infects many plant species under favorable conditions and is classified to different pathovars based on their com-patibility with plants. Generally, *P. syringae* pathovars harbor different viru-lence factors and exhibit differential reactions upon interactions with plants, which cannot be easily revealed by physiological and biochemical tests. The common virulence factors include the type III secretion system (T3SS)-secreted effector proteins and non-ribosomal protein synthetase (NRPS)-synthesized phytotoxins, both are involved in interfering plant metab-olism and defense responses to promote bacterial parasitism. In Taiwan, where the humid subtropical climate is gener-ally considered unsuitable for *P. syringae* infection, a few disease incidences, e.g. bacterial spot of carambola and angular leaf spot of cucurbits, have been recorded in the orchards and nurseries of southern Taiwan. In 2014, Japan-imported pear scions developed typical blossom blast symptom after they were grafted to local pear stocks. Suspect bacterial pathogen was isolated from the symptomatic plant tissues, and the colony morphology on King's B medium, LOPAT tests, and fatty acid methyl ester analysis (Agilent Tech-nologies, Santa Clara, CA) revealed the isolated bacteria shared similar charac-teristics of *P. syringae*. Koch's postu-lates were fulfilled by prick-inoculating the isolated bacteria to pear leaves, and the bacteria showing similar character-istics can be re-isolated from the symp-tomatic tissues of the inoculated plants. To determine the pathovar identity, *P. syringae* pear strains were inoculated to the pods of common bean by syringe infiltration. The elicitation of brown necrosis in 3 days post inoculation was recorded as a typical feature of the pathovar *syringae* in comparison with water-soaked symptom induced by bean-pathogenic strains. Multilocus se-quence typing (MLST) of 7 housekeep-ing genes *(rpoD*, *gyrB*, *acnB*, *cts*, *gap*, *pgi*, and pfk) revealed that the pear strains from the imported pear scions were phylogenetically separated from the other pear-pathogenic *P. syringae* pv. *syringae* found in other countries. Genome-wide comparison of *P. syringae* pv. *syringae* from different sources also showed that these strains contained various virulence factors, suggesting that *P. syringae* pv. *syringae* strains may have co-evolved with their host plants to achieve optimum infection under diverse environments. For accurately and quickly identifying the suspect pathogen from infected plant tissues, the nucleotide sequences of sy-ringomycin biosynthesis genes, a con-served 166-bp genomic locus, and genes coding for the type III secretion system were used to develop standard PCR detection protocol. The application of PCR detection and plant inoculation assay will ensure the correct identifica-tion of *P. syringae* pv. *syringae*.

SB04 Control of strawberry anthracnose by *Bacillus subtilis* strains TKS1-1 and SP4-17—Huang, Hsiang-Yu[1], Chen, Yu-Ssuan[1], Tzeng, Der-Syh[1] and Huang, Tzu-Pi[1] ([1] Department of Plant Pathology, National Chung Hsing Universi-ty,Taichung)

Strawberry anthracnose caused by *Colletotrichum gloeosporioides* severely impacts the strawberry nurseries and greatly reduces yields and quality of strawberry in Taiwan and worldwide. The current commercial control measures for anthracnose disease rely mainly on chemical pesticides. Toward environmental friendly control strategies, we focus on the selection of microbial agents for management of the disease. Bacillus species is representative genera of plant growth promoting rhizobacteria which not only promote plant growth but could also act as biocontrol agents by producing antibiotics, triggering induced systemic resistance. Bacillus subtilis strains TKS1-1 and SP4-17 previously were shown to antagonize against various pathogenic fungi and *Xanthomonas* species and to reduce disease incidence of citrus bacterial canker and rice bacte-rial blight. In this study, we also found that the two strains showed antagonistic activity against anthracnose fungi of strawberry. The colonization and survival ability of *B. subtilis* strains TKS1-1 and SP4-17 on leaves of strawberry plants were determined. Results indicated that at 21 days post-treatment of the two strains,10^5 colony forming units per cm^2 of strains TKS1-1 or SP4-17, were colo-nized on leaves of strawberry plants, on which the numbers of cells colonized were similar to that were inoculated. The disease severity of strawberry anthrac-nose was greatly reduced when treatment with 100-fold diluted endospore formu-lations of *B. subtilis* TKS1-1 and SP4-17. Application of culture filtrates from en-dospore formulations of *B. subtilis* TKS1-1 and SP4-17 inhibited spore ger-mination, caused deformation of the spores, and caused reduction in mito-chondrial membrane potential and energy metabolism of *C. gloeosporioides*. In ad-dition, *B. subtilis* TKS1-1 and SP4-17 both showed the effect on growth pro-motion of strawberry plants. To summa-rize, *B. subtilis* TKS1-1 and SP4-17 are potential biocontrol agents for strawberry anthracnose.

SB05 利用液化澱粉芽孢桿菌Ba01防治馬鈴薯瘡痂病一林芝[1]、吳佳晏[1]、張雅琳[1]、陳碧玉[2]、楊玉良[2]、蔡佳欣[3]、陳穎練[1] ([1]國立台灣大學植物病理與微生物學系、[2]中央研究院農業生物科技研究中心、[3]農業試驗所植物病理組)

Biological control of potato common scab using *Bacillus amyloliquefaciens* Ba01—Lin, C.[1], Wu, C. Y.[1], Chang, Y. L.[1], Chen, B. Y. [2], Yang, Y. L.[2], Tsai, C. H.[3], Chen, Y. L.[1] ([1]Department of Plant Pathology & Microbiology, National Taiwan University, Taipei, Taiwan; [2]Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan; [3]Department of Plant Pathology, Taiwan Agricultural Research Institute, Taichung, Taiwan)

馬鈴薯瘡痂病(Potato common scab)主要由土傳性放線菌(*Streptomyces scabies*)引起，是近年來影響台灣馬鈴薯產業甚鉅的細菌性病害。馬鈴薯瘡痂病菌會造成馬鈴薯表皮破裂、產生褐色瘡痂病斑，嚴重時病斑會成網狀龜裂，使馬鈴薯的經濟價值下降。然而目前對此病害仍無有效防治方法。本研究利用分離自台灣田間之液化澱粉芽孢桿菌(*B. amyloliquefaciens* Ba01)來拮抗馬鈴薯瘡痂病菌。在培養基對峙培養及掃描式電子顯微鏡的實驗中，發現液化澱粉芽孢桿菌Ba01能有效抑制馬鈴薯瘡痂病菌之生長及產孢。經由影像質譜儀分析，發現三種可能的抑菌物質，分別為荷質比介於1046.4～1075.4的Surfactin、1095.6～1123.9的Iturin A和1451.0～1545.3的Fengycin。在溫室的盆栽實驗中，馬鈴薯瘡痂病菌和液化澱粉芽孢桿菌Ba01同時接種和澆灌之處理組，馬鈴薯的罹病嚴重度由55.6%(無澆灌Ba01的處理組)降至5.6% ($P < 0.05$; Tukey's test)。另外在雲林縣斗南鎮執行的田間試驗中，1x109cfu/ml的液化澱粉芽孢桿菌Ba01，其稀釋200倍、100倍和50倍施用在馬鈴薯瘡痂病田，皆能降低馬鈴薯瘡痂病的罹病嚴重度($P < 0.05$; Tukey's test)。此結果驗證液化澱粉芽孢桿菌Ba01能有效減少馬鈴薯瘡痂病菌之危害。

SB06 Biocontrol of bacterial wilt caused by *Ralstonia solanacearum* using *Bacillus subtilis* strains GAP-B2 and GAP-B3—Grimar, A. Perez[1] and Huang, Tzu-Pi[2] ([1] International Master Program of Agriculture, National Chung Hsing University, Taichung; [2] Depart-ment of Plant Pathology, National Chung Hsing University, Taichung)

Plant growth-promoting rhizo- bacteria (PGPR) are microorganisms with traits of being able to colonize plant roots and to promote plant growth. Ap-plication of certain PGPR may suppress plant diseases. The goal of our study was to isolate strains of *Bacillus subtilis* from natural environments in Taiwan that show significant antagonistic activity against various plant pathogens in-cluding *Ralstonia solanacearum* and to evaluate the potential of isolated strains as biocontrol agents of tomato bacterial wilt. We screened a total of 78 isolates which were obtained from various loca-tions in Taichung City, Taiwan. Two strains, GAP-B2 and GAP-B3 isolated from rhizosphere soils at Taiping Dis-trict, Taichung City showed significant antagonistic activity against *R. sola-nacearum*. These wild strains tested pos-itive for 2,3-butanediol, a known volatile for growth promotion, biofilm formation in defined medium as well as on tomato roots and leaves, and showed strong an-tagonistic activity against other plant pathogens including *Xanthomonas euvesicatoria*, *Xanthomonas oryzae*, *Pectobacterium chrysanthemi*, and *Fusarium oxysporum* f.sp. *niveum*. Under greenhouse conditions these strains reduced disease incidence by 60-70% and 50-60% in infested soil containing 1 x10^6 cfu per g and 1x 10^7 cfu per g of *R. solanacearum*, respec-tively. The strains were also able to promote plant growth as observed in a significant higher shoot fresh mass, shoot dry mass, root fresh mass, root dry mass, number of nodes, plant height and stem diameter of tomato when compared to the control treatments. Results indi-cate that *B. subtilis* strains GAP-B2 and GAP-B3 provide promising biocontrol efficacy of tomato bacterial wilt and plant growth promoting activity.

SB07 從土壤中篩選出可抑制茄科細菌性斑點病菌*Xanthomonas perforans*之細菌並鑑定其具抗菌活性之外泌物質－林立心[1]、Ronnie Gicana[2]、鄧文玲[1,2]、曾國欽[1] ([1]國立中興大學植物病理學系、[2]中興大學生物科技學研究所)

Characterization of the secreted sub-stances from soil bacteria which can in-hibit the growth of *Xanthomonas perforans*, the causal agent of tomato bacterial spot disease － Lin, L. S.[1], Gicana, R.[2], Deng, W. L.[1,2], and Tzeng, K. C.[1] ([1]Department of Plant Pathology and [2] Graduate Institute of Biotechnology, National Chung Hsing University, Tai-chung)

茄科細菌性斑點病廣泛分布於世界各地，並造成番茄產量嚴重損失。在台灣，*Xanthomonas perforans*為造成茄科細菌性斑點病之主要病原。為了尋找可抑制*X. perforans*之拮抗細菌，本研究從農用土壤中分離出240個菌株，以平板對峙試驗分析其抑制病原細菌生長情形，其中菌株SAn-03對*X. perforans*具有最佳之抑制能力。利用*X. perforans*脂肪酸圖譜、16S *rRNA*及*rpoB*基因序列比對等技術，將SAn-03鑑定為*Paenibacillus* sp.。以Imaging Mass Spectrometry (IMS)之技術分析拮抗菌分泌之化合物，結果顯示SAn-03分泌之化合物中可能具有拮抗特性的化合物質荷比 (*m/z*, mass to charge ratio) 分布在500-600 *m/z*、800-950 *m/z*及1,000-1,100 *m/z*之間。收取培養1-5天之SAn-03細菌培養濾液經20倍濃縮後，滴在已塗佈*X. perforans*的平板上，可觀察到濃縮濾液周圍有透化圈形成，證明SAn-03確實可分泌具有抑制*X. perforans*生長之抑菌物質。將SAn-03培養濾液通過C18管柱，並以10%-100%之甲醇洗脫，再以100%異丙醇進行最後洗滌。將所得之洗脫液濃縮10倍後進行薄層色層 (TLC, thin

layer chromatography)分析，在波長254 nm 的紫外光下可觀察到三條帶，Rf值分別為0.2、0.51及0.69；而在波長366 nm的紫外光下則可觀察到兩條帶，R_f值分別為0.7及0.9。將IMS及TLC之結果與前人文獻比對，推測由Paenibacillus sp. SAn-03所分泌、可抑制*X. perforans*的物質可能為fusaricidins。在溫室試驗中，施用SAn-03於番茄植株根圈，再於葉面接種*X. perforans*，測試SAn-03對植物的保護作用。結果顯示，將番茄種子直接播種於混拌有SAn-03 (終濃度為10^7 CFU/g)之栽培介質中，不會影響種子之發芽率與番茄植株之生長情形。若以每週澆灌一次濃度為10^7 CFU/ml (50 ml/plant)之拮抗菌懸浮液、連續澆灌三週，對植株之生長亦不會影響。連續澆灌 SAn-03三週後，以葉部噴施接種濃度為10^8 CFU/ml之*X. perforans*， SAn-03處理之植株平均重量大於未施用之對照植株，顯示SAn-03菌株在溫室條件下，確實具有保護番茄植株，可降低*X. perforans*之危害效果。

SB08 以表面增效拉曼散射光譜指紋技術開發香蕉黃葉病菌之光譜檢測技術 — 林依佳、黃湘珊、楊峻毓、林盈宏 (國立屏東科技大學 植物醫學系)

De-velopment of surface-enhanced Raman spectroscopy (SERS)-based diagnostic method for spectral detection of *Fusarium oxysporum* f. sp. *cubense* — Lin, Y. J., Huang, S. S., Yang, J. Y., and Lin, Y. H. (Dept. of Plant Medicine, Na-tional Pingtung University of Science and Technology, Pingtung)

Fusarium oxysporum f. sp. *cubense* (Foc)會造成香蕉發生香蕉黃葉病，使香蕉產生系統性萎凋之病徵，是目前香蕉栽培時的限制因子之一。此病原菌為土傳性病原，且能夠以厚膜孢子殘存於土壤中超過十年，開發一個快速且準確的技術來檢測此病原菌，被認為是避免此病害大發生及傳播的可行策略之一。拉曼散射光譜分析法(Raman spectrum)，此技術主要依據拉曼散射(Raman scattering)原理，藉由不同樣品間之拉曼特異性圖譜(specific patterns)來區分出不同樣品，可做為一個光學檢測診斷工具，此分析法目前已被初步應用於生物醫藥、環境及工業等領域中之檢測工作。本研究擬利用拉曼光譜指紋技術開發一個快速檢測香蕉黃葉病(菌)之光學檢測平台，為增強拉曼散射訊號，本研究將表面增效拉曼散射技術法(Surface Enhanced Raman Scattering，SERS)導入本研究工作中，期望開發出此「表面增效拉曼散射光譜指紋技術平台」，藉此盡早檢測出患病香蕉，以降低此病害之傳播所造成香蕉產業上的危害。由目前的結果顯示，特定之拉曼圖譜對Foc的分生孢子(conidia)、菌絲(hyphae)與帶菌香蕉檢體具特異性，此顯示本表面增效拉曼散射光譜指紋技術對香蕉黃葉病(菌)具檢測可行性，未來將利用此表面增效拉曼散射光譜指紋技術檢測平台，進一步開發出田間帶菌土壤之快篩系統，建立一套香蕉黃葉病(菌)之田間現地檢測平台，以避免病原之傳播而造成香蕉產業上的危害。

SB09 Application of mild strain for cross-protection and culture filtrate de-rived from soil microorganisms on management of disease cause by *Cucumber mosaic virus*－Chen, Ying-Ju[1], Hung, Ting-Hsuan[1], Yeh, Hsin-Hung[1, 2] ([1]Master Program for Plant Medicine, National Taiwan University, Taipei; [2]Agricultural Biotechnology Research Center, Academia Sinica, Taipei)

Cucumber mosaic virus (CMV) is an economically important plant virus with a worldwide distribution and a very wide *host range*. We aim to use two strategies to manage CMV disease on crops. One is to develop mild strain virus for cross protection and the other is to induce plant resistance by spreading metabolites derived from soil microor-ganism. We first tested our strategies on *Nicontiana benthamiana*. The result showed that 2b gene-deleted CMV in-duced only mild symptom on *N. benthamiana* and protect plant from infec-tion by different severe strain of CMV. The application of culture filtrate de-rived from Fungi NTU1F8 isolated from soil, can decrease the infection rate and also delay the disease symptom on plants inoculated with CMV and *Turnip mosaic virus*.

SB10 台灣芥菜黃葉病菌之病原特性及其生物防治試驗—陳薏安、李思儀、許雅婷、黃振文 (國立中興大學植物病理學系)

Pathogenic Characteristics of the Causal Agent of Mustard Yellows and its Biocon-trol Experiments—Chen, Y. A., Li, S. Y., Hsu, Y. T. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing Univer-sity, Taichung 40227)

芥菜黃葉病係由*Fusarium oxysporum* Schlecht. f. sp. *conglutinans* (Wollenw.) Snyder & H. N. Hansen所引起，可使植株矮化，葉片出現黃化、偏上生長及維管束褐變，嚴重時呈現萎凋，進而死亡。將由芥菜病株分離之菌株分別接種至芥菜、甘藍、芥藍、白菜及蘿蔔等數種十字花科蔬菜，結果發現由芥菜分離之菌株可感染芥菜、白菜及蘿蔔，但卻無法感染甘藍及芥藍的所有品種，顯示來自芥菜之菌株病原性異於由甘藍和芥藍所分離的菌株。利用營養體親合群(Vegetative Compatibility Group, VCG)測試分析，結果可將甘藍、芥藍的菌株與芥菜、白菜的菌株區分成兩群；進一步利用CNL12/CNS1引子對增幅不同菌株之內遺傳區間(intergenic spacer, IGS)後，分析比較各菌株間的親緣性，顯示甘藍與芥藍菌株屬於同一個演化支系，而芥菜、白菜及蘿蔔的菌株則歸屬於另一演化支系，另外採用各菌株的轉譯延長因子片段(translation elongation factor 1-α, EF1-α)進行親源性的比對，亦獲得相仿的結果。近年來社會大眾關心食安的議題，我們也嘗試進行芥菜黃葉病的生物防治試驗，首先自台灣中南部芥菜田土分離芥菜黃葉病菌的拮抗微生物，並在Nutrient agar平板進行對峙測試，發現其中有10菌株拮抗病原菌的能力最佳，進一步針對它們抑制病原菌分生孢子發芽百分率及植株生長進行測試，發現GL9-1菌株在Nutrient broth的發酵液可抑制87% Focn-05與81% Focn-38之分生孢子的發芽；至於TKM03菌株可促進芥菜植株根系生長率達31.27%，

而YBJ13菌株可提高20.3%的植株鮮重。將芥菜種子分別浸泡於YBJ13、TKM03及GL9-1三株拮抗細菌懸浮液後，播種於人造病菌土(10^4 spores /g soil)中，經過兩週，TKM03、YBJ13及GL9-1菌株可分別降低芥菜黃葉病罹病度達10、7.8-12.2及11.1-18.9%。評估土壤添加物對於植株生育之影響及抑制病害的試驗，發現蝦蟹殼粉可以顯著增加芥菜的株高及鮮重。將不同土壤添加物、拮抗菌懸浮液(10^7CFU/ml)及人造病菌土均勻混拌後，置於28℃生長箱保濕7天，結果顯示施用蝦蟹殼粉的不同處理皆可顯著降低病原菌的存活，其中蝦蟹殼粉搭配拮抗菌GL9-1菌株可使病原菌的存活率降至最低，是一種具有防治芥菜黃葉病潛力的最佳組合配方。

SB11　菸渣結合微生物防治白菜炭疽病之效果評估—楊謹瑜、黃振文 (國立中興大學植物病理學系)

Efficacy Evaluation for Tobacco Debris Combined with Microorganisms on Control of Cruciferous Vegetable An-thracnose — Yang, J. Y. and Huang, J. W. (Dept. of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan)

妥善利用農業廢棄物研製植物保護製劑，不但可以解決廢棄物汙染環境的問題，亦可促進農業經營的永續。過去本研究室已曾有利用農業廢棄物與肥料調製植物保護製劑的成功案例，例如S-H混合物、CH100植物健素及SSC-06栽培介質等，其中CH100植物健素防治韭菜銹病的原理已證實酵母菌*Rhodotorula* spp. 參與夏孢子發芽的抑制作用；同時也發現CH100的組成分之一菸渣具有防治紅蜘蛛與紋白蝶的功效。本研究的主要目的在於評估菸渣搭配微生物防治*Colletotrichum higginsianum* Sacc.引起白菜炭疽病的效果。首先以四種溶劑分別萃取菸渣的抑菌成分，結果發現菸渣水萃液可顯著降低73% *Puccinia polysora* 夏孢子與52% *C. higginsianum* 分生孢子的發芽率，然而該萃取液經過100˚C 間歇性蒸氣消毒或121˚C 高溫高壓滅菌處理後，卻隨即喪失抑菌功效。自菸渣水萃液中分離出96株細菌，經由抑菌測試與分析它們對白菜植株生長的影響後，獲得Tw04、Tw07、Tw12及Tw22菌株可顯著抑制*C. higginsianum* 的菌絲生長與孢子發芽百分率外，並可促進白菜植株的生育。進一步利用Biolog、16S rDNA及16S-23S rDNA ITS分析，將Tw04與Tw22菌株鑑定為*Bacillus pumilus*；而Tw07與Tw12則為*Bacillus licheniformis*。將Tw04與Tw22分別培養於菸渣基質加入菜籽粕 (代號TR315) 或加入花生粕 (代號TP315)中，結果兩種配方均可顯著提高兩菌株的抑制效果，且其400倍的發酵稀釋液抑制孢子發芽率仍可高於70%。利用TR315作為基礎配方分別添加不同植物油後，培養Tw22菌株，結果顯示添加未經乳化的辣木油、大豆油、葵花油和乳化過的玉米油、花生油等處理均可提高該配方的抑菌效果。此外，在TR315中添加不同的無機鹽類後，發現添加1%碳酸氫鉀或碳酸氫鈉亦可顯著提高Tw22的抑菌功效，其中添加兩種鹽類的600倍發酵稀釋液可使孢子發芽率分別降為8.60% 與10.03%。若進一步在發酵液中加入5%酒精或生石灰亦可些微提高其抑菌功效。在TR315發酵液中分別加入不同氮、磷、鉀、鈣及鎂等鹽類後，以400倍稀釋液進行試驗，結果發現硝酸鈣、硫酸鎂及硝酸鎂均可提高發酵液對病原菌孢子的發芽抑制效果。將前述證明具有提高抑菌功效的成分逐一與TR315發酵液進行調配混合後製成「TR315植物健素」，在溫室噴佈於白菜植株後，發現其具有顯著降低白菜炭疽病發生的功效。

SB12　利用 *Bacillus* spp. 防治草莓炭疽病及其可能機制探討—吳意眉[1]、鄭秋雄[1]、林宜賢[1*] ([1] 國立屏東科技大學植物醫學系；* 聯絡作者，E-mail: yhlin@mail.npust.edu.tw)

Studies of *Bacillus* spp. on control strawberry anthracnose and possible mechanisms involved—Wu, Y. M. [1], Cheng, C. C. [1], Lin, Y. H. [1] ([1] Depart-ment of Plant Medicine, National Ping-tung University of Science and Tech-nology)

草莓炭疽病菌會造成草莓萎凋，為嚴重影響草莓生產的真菌性病害。在安全有效防治此病的前提下，有益微生物的應用為可行的策略。本研究將以 *Bacillus* spp. 探討防治草莓炭疽病之能力與可能參與機制。首先，以對峙試驗分析 *Bacillus* spp. PMB03、PMB04 及 PMB05 對草莓炭疽病菌之菌絲生長情形，結果顯示三菌株對草莓炭疽病菌均有明顯之抑制。同時探討上述菌株之濾液對草莓炭疽病菌孢子發芽之影響，顯示 PMB03 及 PMB05 濾液處理後，孢子仍可正常發芽，但發芽管長度與對照組相比顯著降低 4.6 倍及 11.5 倍；而 PMB04 濾液處理後，孢子即喪失發芽能力。由上述結果說明三個菌株均可能具防治草莓炭疽病之潛力。在以草莓果實進行接種之結果顯示，PMB03、PMB04 及 PMB05 菌液處理後草莓炭疽病菌的罹病度可與對照組之 94.4%，分別降低至 61.1%、44.4% 及 50.0%。此結果說明此些菌株均可有效抑制草莓果實的炭疽病菌之發生。為進一步了解 PMB03、PMB04 及 PMB05 防治草莓炭疽病菌之可能原因，將此些菌株濾液處理於草莓炭疽病菌之孢子，發現 PMB03 與 PMB05 雖不抑制孢子發芽，但均可造成孢子發生細胞凋亡的現象，且 PMB03 可顯著進一步造成菌絲之膨大死亡。為進一步探討 PMB05 濾液處理後，雖不造成孢子及菌絲死亡卻具有極佳防治草莓炭疽病菌之效果是否與 PMB05 具強化草莓細胞免疫之能力有關，分別以激活化氧及癒傷葡聚醣之累積進行分析。結果顯示僅 PMB05 可增加草莓細胞辨識炭疽病菌後所誘發激活化氧及癒傷葡聚醣之累積。綜上所述，PMB03、PMB04 及 PMB05 可分別導致草莓炭疽病菌發芽管膨大死亡、抑制發芽長度、孢子細胞凋亡及強化草莓免疫反應等多種機制來防治草莓炭疽病。

SB13　紅龍果病毒病害與去病毒技術之初步研究—李勝軒[1]、郭庭禕[2]、張雅君[1] ([1] 國立臺灣大學植物病理與微生物學系、[2]國立臺灣大學植物醫學碩士學位學程)

Study of pitaya virus diseases and virus elimination techniques—

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紅龍果(pitaya, dragon fruit)屬於仙人掌科(*Cactaceae*)、三角柱屬(*Hylocereus* spp.)的熱帶果樹，由於其富含營養價值、栽培容易以及產期長，因此逐漸興起並受到重視。近幾年的研究發現田間的紅龍果受到三種*Potexvirus*屬病毒的感染，包括仙人掌病毒X (*Cactus virus X*, CVX)、紅龍果病毒X (*Pitaya virus X*, PiVX)以及蟹爪蘭病毒X (*Zygocactus virus X*, ZyVX)，同時我們也發現這些病毒存在於仙人掌科觀賞植物中。在去年的田間調查發現，幾乎所有的紅龍果植株皆受到這三種病毒感染，推斷是由於國內紅龍果多以扦插的方式進行大量繁殖，若母本植株帶有病毒，則容易使病毒在田間迅速擴展開來。為了保留母本植株的優良性狀，本研究將利用前人報導的植物脫毒技術，如莖頂分生組織培養以及冷凍處理技術，嘗試得到去除病毒的紅龍果健康植株。在組織培養的部分，首先將枝條進行表面消毒後置於MS培養基中，發現田間的紅龍果枝條內有內生菌，而枝條刺座的絨毛內亦含有許多真菌，雖嘗試利用殺真菌劑進行處理，仍沒有顯著的效果；但若將植株栽種於室內，切取新生的枝條，可以降低或避免此情況發生。當將肉質莖培養於含低濃度(1～3 mg/L)細胞分裂素(benzylaminopurine, BAP)之MS培養基時，能誘使較多的側芽長出新枝芽，所獲得的新生枝條可利用於紅龍果的大規模繁殖。而在莖頂分生組織的培養實驗，發現須在培養基中額外添加生長所需的維生素，否則培植體容易出現組織褐化、壞死，嚴重影響生長；而目前的研究結果顯示，同時含有植物生長素(1-naphthaleneacetic acid, NAA)與BAP之MS培養基，莖頂分生組織有較佳的生長效果。至於冷凍處理的部分，目前尚未克服莖頂組織經處理後，組織白化死亡的現象，需要進行調整，以得到最適合紅龍果的冷凍處理條件。本研究期望使用上述方式達到去除紅龍果病毒之目的，並同時保存母株優良的園藝性狀，以解決病毒病害廣泛存在於田間的現況。

SB14 簡易區分十字花科黑腐病原細菌及葉斑病原細菌的方法—<u>陳慧真</u>及李永安(輔仁大學生命科學系)

A simple method to differentiate *Xan-thomonas campestris* pv. *campestris* and X. campestris pv. raphani isolated from cruciferous seeds—<u>Chen, Hui-Chen</u>, and Lee, Yung-An (Dept. of Life Science, Fu Jen Catholic University, New Taipei City).

以*Xanthomonas* differential (Xan-D) 培養基檢測十字花科種枴子，有些種子可分離出的*Xanthomonas*屬病原細菌，經由植物接種及PCR檢測，發現有些為十字花科黑腐病菌 (*X. campestris* pv. *campestris*; XCC)，有些則為葉斑病原細菌(*X. campestris* pv. *raphani*；XCR)，而XCC及XCR在Xan-D培養基上，無法以生長情形加以區別。實驗發現在含有X-gal的Luria-Bertani agar培養基(LA-X)，XCC菌株呈黃色，XCR菌株則呈黃綠，但加入葡萄糖至LA-X培養基(LAG-X)後，XCC在LAG-X呈黃色，而 XCR則呈藍綠，可明顯的將XCC及XCR區分出來。為探討XCC及XCR在LAG-X上呈色差異的原因，利用LA培養基含有的成份，配置TY-X (tryptone, yeast extract, X-gal)、含有葡萄糖(G)的TYG-X及TYGNa-X (TYG-X含NaCl) 三種培養基，在TY-X培養基中， XCC呈黃色，XCR呈藍綠色，但在TYG-X培養基上，XCC及XCR均呈藍綠色，而在TYGNa-X上，XCC又呈黃色，XCR則呈藍綠色，故判定XCC及XCR均有水解X-gal能力，且葡萄糖可增加XCC水解X-gal能力，但NaCl卻能抑制XCC水解X-gal的能力，而不會抑制XCR。另外進一步發現，不單是NaCl，其他離子如KCl、MgCl2也可抑制XCC水解X-gal的能力。以目前自十字花科種子分離出的10株XCC菌株及8株XCR菌株，XCC在TYGNa-X培養基上均呈黃色，而XCR菌株則均呈藍綠色，因此在十字花科種子檢測上，若以Xan-D培養基分離出Xanthomonas屬病原細菌後，可將菌株再培養於TYGNa-X培養基上，若呈黃色則為XCC，呈藍綠則為XCR，可簡易且明顯的區分XCC及XCR菌株。

SB15 Application of *Streptomyces* sp. strain S1 as a bio-agent for strengthening the effectiveness of fungicides for controlling *Peronophythora litchii* on Litchi —<u>Tseng, Hsiao-Tzu</u>, and Tzeng, Der-Syh (Department of Plant Pathol-ogy, National Chung Hsing University, Taichung, Taiwan, R.O.C.)

Downy blight disease caused by *Peronophythora litchii* has long been a major limiting factor severely impacting the yield and quality of litchi in Taiwan. The control measures currently practiced in litchi commercial production rely mainly on application of chemical pesti-cide mostly known with broad-spectrum activity rather than that specifically tar-geting only oomycetes. Development of effective disease control measure with improved environmental friendliness and reduced need of pesticide application is urgently needed for strengthening the international competitiveness of the litchi industry. The main objective of this study was to explore the feasibility of integration of an antagonistic *Streptomyces* sp. strain S1 as an additive to strengthen the effectiveness of chemical control thus to reduce the need of its ap-plication. The *Streptomyces* sp. strain S1 was shown of great potential as a microbial fungicide for the control of plant diseases-especially those caused by soil borne fungal or fungal-like pathogens. The effectiveness of dis-ease control was known due greatly to the mycoparasitic effect rendered by the superior chitinase and glucanase activi-ties of the bacteria. Screening of chemical resistance indicated that the growth of S1 was not affected by a total of 14 tested fungicides widely adapted for oomycetes at the concentration up to 1000 ppm. Among these 14 tested fungicides, the mixed application of metalaxyl at

80ppm, fluopicolide at 40 ppm, mandipropamide at 40ppm or di-methomorph at 10ppm, each respectively together with S1 showed additive in-hibitory effect of the bacteria on the mycelial growth of *P. litchii*. As for inhibitory effect on sporangial germina-tion, the mixed application of metalaxyl at 10 ppm, trifloxystrobin at 1 ppm, kresoxim-methyl at 1ppm or amisulbrom at 1ppm, each respectively together with S1 also showed remarkable additive effectiveness. An additive control effectiveness against *P. litchii* infection on litchi fruits was also demonstrated where that metalaxyl, fluopicolide, azoxystrobin, and cyazofamid each at 10ppm respectively were sprayed with the addition of S1 broth culture. In ad-dition, on a detached leaf system, spray treatment of S1 at 100X-dilution was shown to deter the disease development for 12hr. And a pretreatment of met-alaxyl, fluopicolide, cyazofamid, and dimethomorph each respectively togeth-er with strain S1 24 hours before inocu-lation reduced the infection to nearly nil. Although continued effort is needed for the optimization of the combined use of strain S1 together with tested chemicals, the accumulated evidence indicated clearly the value of S1 as an aid for the integrated management of the targeted disease.

SB16 Development of a wettable pow-der formulation of *Streptomyces griseobrunneus* SGS3 for the control of pepper blight caused by *Phytophthora capsici*—Liu, Su-Yu, Huang, Wen Di, and Tzeng, Der-Syh (Department of Plant Pathology, National Chung Hsing Uni-versity, Taichung, Taiwan R.O.C.)

Phytophthora blight of sweet pep-per caused by *Phytophthora capsici* is one of the most important diseases in pepper cultivation. Severe damage was usually observed during typhoon or raining seasons in Taiwan. The antag-onistic *Streptomyces griseobrunneus* SGS3 isolated from Taichung was shown of great potential as a microbial bio-agent for the control of plant diseas-es caused by Oomycetes. And a stand-ard operation protocol for mass produc-tion of arthrospore formulation of the bacteria was established by a 750L fer-menter. The spore yield reached ap-proximately $2x10^{11}$ CFU/ml. This re-search was aimed to develope a wettable powder (WP) formulation for the control of pepper Phytophthora blight. Using bentonite and micro-cellulose as the car-riers, and kaolin as additives to enhance the suspensibility, a wettable powder with $5.2x10^{10}$ CFU /g free of contamina-tion was prepared by a Fluid Bed Dryer. Examination of the shelf life of the at-tempted formulation after one year stor-age indicated a better performance of WP comparing to that of liquid culture broth. For broth culture kept at 6℃, the survival count was $3.6x10^{6}$ CFU /ml. Whereas for those kept at room temper-ature, a rapid decline of the bacterial propagule was detected. As for the compared WP formulation, the survival count detected after stored for one year at 6℃ and at room temperature respec-tively was $1.1x10^{9}$ CFU /g and $7.7x10^{8}$ CFU /g. The WP formulation also gave a better colonization competence of the applied bacteria on pepper leaves. On a detached pepper leaves and fruits system artificially inoculated with *P. capsici*, the application of broth culture and WP formulation each at 100-fold dilution both showed significant effectiveness in reducing the fungal infection. In a Petri plate system, the application of SGS3 broth culture at 100-fold dilution showed similar effectiveness on inhibit-ing hyphal growth of *P. capsici* as com-pared to that by the commercially avail-able fungicides including metalaxyl, fluopicolide, dimethomorph, mandipro-pamid and Orvego® (mixture of dime-thomorph and ametoctradin) each at recommended concentration. As for inhibition of sporangium and zoospore germination, a better performance of SGS3 was detected. In greenhouse tri-al, application of 100-fold diluted SGS3 broth culture in simultaneous with inoc-ulation effectively controlled the infec-tion of *P. capsici* on pepper seedlings; the application of 100-fold diluted WP formulation 2 days after inoculation showed an even better effectiveness. And for the pathogen-infested soil sys-tem, amendment of 10% (w/w) WP formulation was also effective in con-trolling the fungal infection. The evi-dence accumulated indicated clearly the usefulness of both liquid broth culture and the attempted WP formulation for controlling Phytophthora blight on sweet pepper.