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中華民國植物病理學會一百零玖年度年會論文摘要

Abstract of the 2021 Annual Meeting of Taiwan Phytopathological Society

專題演講 Keynote and young scientist speech

KS01 利用傳統與分子流行病學策略剖析植物流行病害—洪爭坊 (國立中興大學植物病理學系)

Dissecting Plant Disease Epidemics by Conventional and Molecular Epidemiological Approaches—Hong, C. F. (Department of Plant Pathology, National Chung Hsing University, Taichung)

在傳統植物病理研究中，常利用選擇性培養基、誘釣植物 (baiting plants)、孢子採集器…等工具，檢測或偵測植物病原真菌侵入感染作物的情形，或是監測病原菌或病害在不同時間或空間維度上的傳播擴散情形，以利發展病害管理策略。舉例而言，筆者曾利用半選擇性培養基，研究番石榴立枯病菌 (*Nalanthamala psidii* (Sawada & Kuros.) Schroers & M.J. Wingf.) 的田間侵入感染點，結果發現番石榴根系為N. psidii重要的侵入感染位置，並提出移除罹病植株殘根的建議與做法。隨著分子流行病學研究技術的發展，利用不同的時間與空間上的採樣策略，配合多基因座序列分析 (multi-locus sequence analysis; MLSA)、簡單序列重複標誌 (simple sequence repeat marker; SSR markers) 或單核苷酸多型性 (single nucleotide polymorphism; SNP)…等不同分子標誌以及生物資訊分析平台，能更深入探討不同世代(有性或無性世代)的病原真菌對於植物流行病的影響、研究病原菌族群遺傳結構與親緣關係、以及病原菌在不同時空環境下的殘存、傳播與侵染生態…等課題。筆者曾利用MLSA策略與SSR標誌探討葡萄露菌病菌 *Plasmopara viticola* 的族群，在美國喬治亞州與佛羅里達州的時空變化，發現兩個州的露菌病族群可分為 *P. viticola* clade *aestivalis* (Pva)、*P. viticola* clade *vinifera* (Pvv) 與 *P. viticola* clade *vulpine* (Pvu) 三個隱匿種 (cryptic species)。其中，Pvv 與 Pvu 在地理分布上僅侷限於喬治亞州南部至佛羅里達州北部。進一步以 SSR 標誌分析地理分布較廣的 Pva 族群的遺傳結構，結果喬治亞州北部的 Pva 族群具有高度遺傳多型性，但越往南部，該族群的遺傳多型性有降低的趨勢。而在不同時間與空間可以偵測到相同基因型的 Pva 菌株，顯示該病原菌在喬治亞州北部與南部的殘存方式，以及造成病害的主要侵染源可能略有差異。由前述案例可知，傳統植物病理與分子生物研究工具不但可以相輔相成，配合生物資訊平台進行植物流行病研究分析，更能深化我們對於植物病原菌的殘存、侵入感染、以及不同時空中的病原菌族群結構…等病原及病害生態的瞭解，未來期能更準確的評估與管理植物流行病害。

KS02 大豆猝死病—張皓巽 (國立臺灣大學植物病理與微生物學系)

Sudden Death Syndrome of Soybean—Chang, Hao-Xun (Department of Plant Pathology and Microbiology, Nation Taiwan University, Taipei)

Fusarium virguliforme is the major soilborne fungal pathogen which causes sudden death syndrome (SDS) of soybean (*Glycine max*) in the United States. This fungus inhabits soils and it produces multiple phytotoxins, which are translocated from infected roots to leaves. Typical SDS foliar symptoms include interveinal chlorosis and necrosis, and because SDS foliar symptoms are solely induced by phytotoxins, it becomes a unique pathosystem to study plant-phytotoxin interactions. The genetic architecture of SDS resistance is quantitative, and among hundreds of quantitative trait loci (QTL) reported for SDS, only a few QTL were reproducible due to the complexity of SDS etiology. In screening 340 soybean germplasms for foliar resistance to phytotoxins, a differential system was discovered to separate foliar chlorosis and foliar necrosis. Using a combination of linkage mapping, genome-wide association, and genomic synteny to dissect soybean resistance to foliar chlorosis, two soybean *STAY-GREEN* genes were associated with severity of foliar chlorosis, and the resistance to foliar chlorosis was attributed to the double mutation of soybean *STAY-GREEN* genes. Moreover, genotyping-by-sequencing was applied on a F2 population crossed by a resistance germplasm and a susceptible variety for obtaining single nucleotide polymorphisms, and QTL mapping identified a locus for foliar necrosis. These studies together highlighted the importance of precise phenotyping, and symptom-specific rating may simplify the complexity of quantitative resistance. In addition to soybean resistance to SDS foliar symptoms, a putative effector FvNIS1 was discovered using RNA-Seq transcriptomics and FvNIS1 was shown

to induce SDS foliar symptoms via the *Soybean mosaic virus*-mediated overexpression. Since SDS-liked foliar symptoms could be observed in other soybean diseases such as red crown rot (RCR) caused by *Calonectria ilicicola*, the availability of *C. ilicicola* genome sequence will empower comparative genomics approach to discover the essential phytotoxin(s). In summary, research accomplishment on soybean SDS in aspects of host resistance and pathogen virulence have advanced the genetic insights of this pathosystem, and the experience would provide future direction to study not only RCR but also other soilborne diseases of soybean.

KS03 效應蛋白於真菌入侵過程中干擾植物細胞壁之功能性分析—陳禮弘[1,2] , Kračun, Stjepan K.[3,4], Nissen, Karen S.[3], Mravec, Jozef[3], Jørgensen, Bodil[3], Labavitch, John[5], and Stergiopoulos, Ioannis[2*]([1]國立中興大學植物病理系、[2]Department of Plant Pathology, University of California Davis, USA、[3]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark、[4]GlycoSpot ApS, Søborg, Denmark、[5]Department of Plant Sciences, University of California Davis, USA)
Functional elucidation of a novel effector that perturbs plant cell walls during fungal infections—Li-Hung Chen[1,2] , Kračun, Stjepan K.[3,4], Nissen, Karen S.[3], Mravec, Jozef[3], Jørgensen, Bodil[3], Labavitch, John[5], and Stergiopoulos, Ioannis[2*]([1]Department of Plant Pathology, National Chung Hsing University, Taipei. [2]Department of Plant Pathology, University of California Davis, USA. [3]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark. [4]GlycoSpot ApS, Søborg, Denmark. [5]Department of Plant Sciences, University of California Davis)

微生物入侵寄主時分泌效應蛋白去抑制寄主的免疫反應與幫助病原菌的感染，番茄黑黴病 (Black leaf mold) 其病原菌 *Pseudocercospora fuligena* 在入侵過程中分泌了一系列的效應蛋白至植物細胞間，其中Avr4效應蛋白是一個可以跟真菌細胞壁幾丁質 (chitin) 結合的蛋白質，Avr4 可以保護真菌細胞壁抵抗寄主的幾丁質酵素 (chintinase)。我們的研究發現一些真菌基因體中具有第二個同源的 Avr4，命名為 Avr4-2。Avr4-2 與 Avr4 一樣是一個具有 CBM14 domain 的分泌蛋白，為真菌的毒力因子，可以幫助 *P. fuligena* 入侵番茄葉片。但 Avr4-2 的生化功能與 Avr4 截然不同，Avr4-2 不能結合幾丁質，也不能保護真菌的細胞壁，同時番茄的 Cf-4 receptor 也不能藉由辨識 Avr4-2 產生免疫反應。我們的研究發現，Avr4-2 跟植物細胞壁中的 de-esterified pectin 結合，阻擾了植物細胞壁的功能，並且增加了真菌 endopolygalacturonases (endo-PGs) 分解 pectin 的活性。此研究指出新的效應蛋白的出現有可能是源自於基因重複 (gene duplication) 後產生了新的功能 (neofunctionalisation)，並且我們也發現了新的效應蛋白功能，Avr4-2 可以藉由阻擾植物的細胞壁，增加真菌 endo-PGs 的活性，進而幫助真菌的入侵。

KS04 農桿菌 VirB2 蛋白質之功能鑑定與高效率表現系統之建立—吳竑毅[1,2]、陳昭瑩[1]、賴爾珉[1,2] ([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 characterizations of the Agrobacterium VirB2 and development of an efficient transient expression system—Hung-Yi Wu[1,2], Chao-Yin Chen[1], and Erh-Min Lai1[2] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Academia Sinica, Taipei)

農桿菌為一種革蘭氏陰性的植物病原細菌，其能藉由演化上具保守性的細菌第四型蛋白質分泌系統 (type IV secretion system, T4SS) 將 T-DNA 傳送並嵌入植物基因體中。T4SS 主要由 VirD4 與 11 個 VirB 蛋白質 (VirB1 至 VirB11) 所組成。T 線毛 (T-pilus) 主要由 VirB2 與次要之 VirB5 蛋白質所組成，所有的 VirB 蛋白質都為產生 T 線毛所必需。VirB2 為一具保守性之 T4SS 必需組成元件，但是 VirB2 及其所組成的 T 線毛於農桿菌轉型過程中所扮演的角色仍然未知。本研究利用不同位置的 VirB2 單點胺基酸突變株，分析其細胞外 VirB2 的產生與否 (ExB2+ 或 ExB2-) 與在不同植物上產生腫瘤的能力 (Vir+ 或 Vir-)。其中五種突變株不會產生 T-pilus 但在番茄莖上或馬鈴薯塊莖上仍保有野生株程度的毒力，但是這種突變株在阿拉伯芥上的短暫表現效率卻大幅下降。因此，本研究之結果提供了 T 線毛於農桿菌轉型過程中所扮演的一個角色。有鑑於在阿拉伯芥上使用短暫基因表現的方法來進行植物功能性研究的潛力，我們更進一步在阿拉伯芥的小苗上分析不同生物因子與感染條件，並在使用 β-glucuronidase (GUS) 作為報導基因下，我們發現使用一種特定的農桿菌菌株並且配合毒性基因的預先誘導表現，並於感染的培養液中添加 AB 鹽類與 pH 5.5 的緩衝液能在阿拉伯芥免疫受器突變株 efr-1 及野生株 Col-0 均明顯提高短暫表現效率。此一簡單、快速又可靠的高效率表現系統 AGROBEST (*Agrobacterium*-mediated enhanced seedling transformation) 也證實得以應用在不同的研究當中，如檢視轉錄因子的動態表現情形、觀察生物時鐘基因的調控情形、蛋白質於細胞內的分佈位置與蛋白質間的交互作用等，提供阿拉伯芥上功能性分析的新平台，並可用於剖析農桿菌傳送DNA的分子機制。

KS05 Exploring rice resistance against bakanae disease —Chen, Szu-Yu[1], Lai, Ming-Hsin[2], Tung, Chih-Wei[3], Wu, Dong-Hong[2], Chang, Fang-Yu[4], Lin, Tsung-Chun[5] and Chung, Chia-Lin[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Crop Science Division, Taiwan Agricultural Research Institute, Taichung; [3]Department of Agronomy, National

Taiwan University, Taipei; [4]Kaohsiung District Agricultural Research and Extension Station, Pingtung; [5]Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung)

Bakanae disease caused by ***Fusarium fujikuroi*** is a rising threat to rice production in the past decades. Increasing occurrence and severity have been reported in several Asian countries. In Taiwan, an outbreak occurred during 2009-2012 and the disease incidence in severely infected fields were found up to > 30%. For a long time, rice seeds treated with fungicides was an effective method for bakanae control. However, fungicide-resistant isolates have been reported in Asian countries since 2010. Therefore, developing alternative control approach is necessary. In this study, loci conferring bakanae resistance were identified and utilized for future disease management. First, to identify resistance resources, resistant loci were investigated using Taikeng16 x Budda population and rice diversity panel 1 by linkage mapping and genome-wide association mapping, respectively. Total 16 quantitative trait loci (QTLs) were identified. Two resistant QTLs were mapped using linkage mapping from 166 F_9 recombinant inbred lines (RILs) derived from Taikeng16 x Budda. ***qBK1.8*** was co-localized with the previous reported ***qBK1.3*** and ***qBK2.1*** is a novel QTL. Among 14 QTLs identified from association mapping, ***qBK1.7*** was overlapped with the previously reported ***qFfR1*** and ***qBK1*** and also verified by RIL population derived from IR64 x Nipponbare. On the other hand, artificial inoculation was conducted on three populations (Tainung 71 x Budda, Taikeng16 x IR78581-12-3-2-2, and Tainung 71 x IR78581-12-3-2-2). After continuous resistance selection from F4 to F7 generations, a total of 17 progenies with great resistance to four representative ***F. fujikuroi*** isolates were selected. Moreover, pyramiding breeding for resistance to bakanae disease, bacterial blight, and rice blast is underway. We hope that this study can facilitate resistance breeding for bakanae disease and growing resistant cultivars can be an effective and eco-friendly alternative approach for bakanae control in the future.

KS06 Comparative pangenomics, phylogeny, and functional genomics of a novel rhizoid colony-forming biocontrol bacterium ***Bacillus nitratireducens*** BM02 — Je-Jia Wu[1,2], Jenn-Wen Huang[1,2] and Wen-Ling Deng[1,2] ([1]Ph.D. Program in Microbial Genomics, National Chung Hsing University and Academia Sinica, Taiwan; [2]Department of Plant Pathology, National Chung Hsing University, Taichung)

Bacillus nitratireducens BM02, a rhizoid colony-forming G+ bacterium, was isolated from the tomato rhizosphere and shown to promote plant growth and suppress tomato Fusarium wilt by producing plant hormones and antifungal metabolites. The 5,511,760 bp complete genome of BM02 contains a circular chromosome (GenBank accession no. CP047366) and three plasmids (CP047367 - CP047369). Whole-genome phylogeny revealed ***B. nitratireducens*** is closely related to ***B. mycoides***. Cytological experiments demonstrated BM02 reduced ***Fusarium oxysporum*** f. sp. ***lycopersici*** (Fol) invasion by reducing spore attachment and increasing hyphal deformation in hydroponics-grown tomato root tissues. Three antifungal volatiles, phenylacetic acid (PAA), methylphenyl acetate, and dimethyl disulfide (DMDS), were identified from BM02 culture supernatants by GC-MS analysis and verified to inhibit Fol spore germination in bioassays. Pangenome comparison revealed two chitinase-coding genes, ***chiA1*** and ***chiD*** that were absent in all ***B. nitratireducens***, were candidate species-specific biocontrol genes of ***B. mycoides***, whereas the enzymes for producing PAA and DMDS were conserved in the ***Bacillus*** core-genes. CRISPR/Cas9-mediated disruption of ***mgl*** coding for L-methionine gamma-lyase impaired DMDS production in BM02. The ***mgl***-defective strain exhibited reduced inhibitory activity against Fol spore germination, which was restored by ***in-trans***, native promoter-driven expression of ***mgl***. Functional genomics of the field-isolated ***B. nitratireducens*** demonstrated that BM02 produces various volatiles and antifungal metabolites to prevent tomato Fusarium wilt disease and promote plant health.

論文宣讀摘要 Abstracts for Oral Presentation

A.真菌及卵菌組

A01 褐根病菌與榕樹根部微生物相關聯性分析－劉則言[1,2]、陳昭翰[2]、楊玉良[3]、何攖寧4[†]、鍾嘉綾1[†] ([1]國立臺灣大學植物病理與微生物學系、[2]行政院農業委員會林業試驗所、[3]中央研究院農業生物科技研究中心、[4]國立臺灣海洋大學海洋生物研究所)

The correlation between ***Phellinus noxius*** and root-associated microbiota of ***Ficus*** trees — Liu, T. Y.[1,2], Chen, C. H.[2], Yang, Y. L.[3], Ho, Y. N.[4], and Chung, C. L.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Division of Forest Protection, Taiwan Forestry Research Institute, Taipei; [3]Agricultural Biotechnology Research Center, Academia Sinica, Taipei; [4]Institute of Marine Biology, National Taiwan Ocean University, Keelung)

植物微生物相 (plant microbiota) 涵蓋所有與植物相關的微生物，與植物的生長、耐抗病性、抗環境逆境等密切相關。褐根病為熱帶與亞熱帶地區的重要樹木病害，其病原菌 ***Phellinus***

noxius 可危害多種樹木根部，造成樹木枯萎死亡，然而目前對褐根病菌危害過程中，樹木根部微生物相的變化所知有限。本研究以臺灣都市常見行道樹－榕樹為研究材料，採集健康及罹染褐根病榕樹之立地環境土壤、根圈土及根部組織樣本，以次世代定序搭配微生物之分離培養，分析在健康與罹病樣本中，真菌與細菌族群的差異。經由微生物相分析發現，在罹染褐根病的根圈土及根部組織中，真菌的物種多樣性顯著降低，特別是在木質化的根部組織中，褐根病菌成為最顯著的真菌族群。此外，褐根病菌的侵染，雖然對細菌的多樣性沒有明顯影響，但仍會造成樹木根部細菌族群結構上的差異。進一步在屬的階層探討 *P. noxius* 與樹木根部微生物相的關聯，發現多數真菌與 *P. noxius* 呈現負相關，僅 *Cosmospora* 為正相關，顯示 *P. noxius* 與根部真菌族群在生態棲位上可能存在競爭關係，特別是其中的 *Aspergillus* 和 *Penicillum*，這裡並透過所分離到的菌株與 *P. noxius* 的對峙培養，驗證該兩屬真菌對 *P. noxius* 具有生長抑制的能力。有趣的是，本研究發現部分有益細菌 *Bacillus* 和 *Streptomyces* 與 *P. noxius* 呈現正相關，此現象是否與樹木在遭受 *P. noxius* 威脅時，會經由根部菌相的改變來抵抗病原菌，仍有待更進一步的驗證。透過樹木地下部微生物族群的分析，將有助於我們更全面了解褐根病菌在自然生態中所扮演的角色。

A02　首次報導由 *Phytopythium helicoides* 造成的芋軟腐病－<u>傅于珊</u>[1]、丁昭伶[2]、孫韻晴[1]、黃健瑞[1] ([1]國立嘉義大學植物醫學系、[2]行政院農委會苗栗改良場作物改良課)

First report of *Phytopythium helicoides* causing soft rot of taro corm in Taiwan－<u>Fu, Y. S.</u>[1], Ting, C. L.[2], Sun, Y. C.[1] and Huang, C. J.[1] ([1]Department of Plant Medicine, National Chiayi University, Chiayi; [2]Crop Improvement Division, Miaoli District Agricultural Research and Extension Station, Taichung)

芋 (*Colocasia esculenta*) 為天南星科 (Araceae) 芋屬植物，為國內重要雜糧作物之一，其地下球莖為主要的食用部位。芋球莖軟腐病常導致芋生產上的嚴重經濟損失，芋軟腐病研究團隊先前已確認芋球莖軟腐病包含 *Fusarium solani* 造成的芋球莖真菌性軟腐及軟腐細菌造成的細菌性軟腐病。此外，在 2018 至 2020 年間於苗栗及高雄地區採集具有褐色凹陷且邊緣呈現水浸狀病徵的芋頭球莖樣本，並取其病健部組織進行分離，純化後得到數個分離株，除 *F. solani* 外，亦陸續分離到菌落形態與卵菌相似的菌株，其在馬鈴薯葡萄瓊脂培養基 (PDA) 上培養，菌株生長快速且為白色菌落，富有氣生菌絲，菌絲透明；將菌絲塊接種在芋切片上，可造成水浸狀、軟腐病徵，初步確認其病原性。挑選 2 菌株利用 ITS (Internal Transcribed Spacer)、COX I (Cytochrome c oxidase subunit I) 基因片段序列進行分子鑑定，所增幅出之序列以 NCBI blastn 進行比對，與 GenBank 中 *Phytopythium helicoides* FL231 菌株 (GenBank accession no. KY084741.1) ITS 序列有 98.27－99.38% 以上的相似度，與 *P. helicoides* GDGJ6 菌株 (GenBank accession no. KT750956.1) COX I 序列有 98.22－99.12% 的相同度，確認所得分離株為 P. helicoides。另在 25 ˚C 及 37 ˚C 下以 Corn Meal Agar (CMA) 培養，觀察兩分離株之生長情況，兩者皆可在此兩溫度下生長，且在 37 ˚C 下生長速率高於 25 ˚C，並具有顯著差異，顯現出 *P. helicoides* 其耐高溫之特性。為確認其致病性，後進行芋球莖接種，將菌絲塊接種於人工傷口處，7 天後切開芋球莖，可於處理組之接種處觀察到水浸狀、褐色病徵，與田間所觀察到的病徵相符，並可再次分離到 *P. helicoides*；對照組則無病徵出現，也未分離到 *P. helicoides*，完成柯霍式法則，此為在臺灣證實 *P. helicoides* 可造成芋軟腐病的首次報導。

A03　由 *Sclerotinia sclerotiorum* 引起之麥桿菊莖腐病－<u>陳錦桐</u>[1]、褚哲維[1]、林玫珠[2]、吳容儀[1]、戴廷恩[1] ([1]行政院農業委員會農業試驗所花卉中心、[2]行政院農業委員會農業試驗所植物病理組)

Sclerotinia stem rot of *Helichrysum bracteatum* occurred in Taiwan－<u>Chen, J. T.</u>[1], Chro, J. W.[1], Lin, M. J.[2], Wu, R. Y.[1], and Tai, T. N.[1] ([1]Floriculture Research Center, Taiwan Agricultural Research Institute, Yunlin; [2]Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung)

麥桿菊 (*Helichrysum bracteatum*) 又稱為蠟菊、不老菊，為菊科臘菊屬植物，花瓣蠟質具光澤，除植株具觀賞性之外，也是新興理想的乾燥花材料。最近在南投縣信義鄉田區發現麥桿菊植株出現枯萎症狀，且零星分布在園區，植株由莖基部開始變褐色，下位葉逐漸萎凋枯黃變色，由下逐漸往上蔓延，迅速擴展，最終導致植物枯萎，腐爛和死亡。同時在莖桿基部葉柄伴隨著白色棉質菌絲體在莖桿表面與維管束組織內生長。在莖桿上的白色的菌絲體聚集體，其發展成深色的圓形至細長的菌核。由罹病組織分離出此真菌，於馬鈴薯葡萄糖瓊脂 7 天後，為白色圓形菌落，邊緣有白熱棉絮狀發展成深褐色的圓形至細長型的菌核。將所分離的菌株，切取 7 天左右的菌絲塊，以馬鈴薯葡萄糖培養基液態培養菌絲 5 天後，澆灌接種在 7-8 片本葉的麥桿菊植株，2 周後出現與田間相同的病癥，並能再分離得原接種的同種菌株，完成柯霍式法則，證實其病原性。評估此病原菌的型態特徵與 ITS (Internally Transcribed Spacer) 核酸序列鑑定結果，將此菌鑑定為 *Sclerotinia sclerotiorum*(Lib.) de Bary。Sawada (1919) 曾紀錄臺灣有此病菌，但至今相關研究闕如。麥桿菊莖腐病之 221 與 210 菌株菌絲最適合生長溫度為 12-28℃，高 於32℃ 即停止生長，菌核形成溫度在 16-24℃，溫度與病害發生有密切關係，田間發病多在於秋冬冷涼季節，尤其發生在高濕與低於 20℃ 以下環境。*S. sclerotiorum* 寄主範圍相當廣泛，多種蔬菜、花卉均可被害，目前麥桿菊莖腐病無推薦用藥，參考植保手冊相

關菌核病防治藥劑，在平板上試驗發現化學藥劑50% 撲滅寧可濕性粉劑、50%貝芬同可濕性粉劑、50% 快得依普同可濕性粉劑與62.5% 賽普護汰寧可濕性粉劑，這四種藥劑在抑制麥稈菊莖腐病菌菌絲生長，抑制率可達 100%。

A04 臺灣茶赤葉枯病之病原菌菌相調查－林秀榮[1,2]、林盈宏[3]、Ariyawansa, Hiran A.[2]、鍾嘉綾[2]、洪挺軒[2] ([1]行政院農業委員會茶業改良場、[2]國立臺灣大學植物病理與微生物學系、[3]國立屏東科技大學植醫學系)

Investigation of causal agents responsible for brown blight of tea in Taiwan－Lin, S. R.[1,2], Lin, Y. H.[3], Ariyawansa, H. A.[2], Chung, C. L.[2], and Hung, T. H.[2] ([1]Tea Cultivation Dept., Tea Research and Extension Station, Taoyuan; [2]Deptartment of Plant Pathology and Microbiology, National Taiwan University, Taipei; [3]Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung)

茶赤葉枯病為茶樹的一種重要病害，不僅會藉由傷口感染葉片，更會直接侵染幼嫩組織，若感染了嫩芽、嫩莖等部位，會使該嫩枝條容易折斷，繼而直接造成產量損失。然而需要正確選擇防治策略之前提下，首先需釐清造成本病害之病原菌種類，又因目前國內未有針對造成本病害之調查文獻，故本研究針對造成茶赤葉枯病之病原菌種類進行調查。本研究在臺灣重要產茶地區採集似茶赤葉枯病之罹病葉片，進行病原分離、純化及利用分子生物學方法進行分類鑑定，結果顯示分別來自 8 縣市 86 處茶園樣品中，共獲得 162 株病原性真菌，其中 141 株為 *Colletotrichum* 屬真菌，包括 *C. camelliae*、*C. fructicola* 及 *C. aenigma*，其他病原菌則為 *Ascomycota* sp.、*Fusarium oxysporum*、*Ceriporia lacerate*、*Pseudopestalotiopsis theae*、*Pestalotiopsis theae*、*Diaporthe* sp.、*Phomopsis* sp. 及 *Lasiodiplodia theobromae*，其中以 *C. camelliae* 為最主要之病原菌 (135株)。本研究確定了臺灣主要造成茶赤葉枯病之病原菌為 *C. camelliae*，且 *C. fructicola* 及 *C. aenigma* 為首次在臺灣茶樹上發表之病原菌。

A05 Phylogenetic diversity, morphological characterization and pathogenicity of fungi associated with leaf spot of tea in Taiwan－Tsai, I-C.[1], Lin, S.-R.[2], Hung, T.-H.[1], Chung, C.-L.[1] and Ariyawansa, H. A.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Department of Tea Agronomy, Tea Research and Extension Station, Taoyuan)

Camellia sinensis (L.) O. Kuntze, commonly known as tea, is widely cultivated around the world in tropical and subtropical areas. Several fungal pathogens and endophytes are associated with the tea plant, and fungal pathogens cause a significant threat to tea leaves. Tea is mainly manufactured using young shoots of tea plants. Therefore, it is essential to control foliar diseases. Although several studies have provided the base for the fungal diseases associated with *C. sinensis* in Taiwan, but up to now, no inclusive study has been carried out on fungal linages allied with leaf spots of C. sinensis. The main aim of the present survey was to fill this gap in the information of populations of fungal taxa on *C. sinensis* in the major tea producing areas in Taiwan and confirm their natural classification via morphology coupled with a phylogenetic analysis of single- and multi-locus sequence data. In total, 153 fungal strains were isolated in different regions of Taiwan between 2017 and 2019 from the leaves of *C. sinensis* plants with symptoms of leaf spot disease. These strains were evaluated morphologically and genotypically using multi-locus sequence analyses of the ITS, LSU, SSU, *rpb*2, *tef*1, *his*3, *cal* and *tub*2 genes. The study revealed a total of 31 Pleosporales strains, 98 pestalotiopsis-like strains and 24 *Diaporthe* strains associated with leaf spots of *C. sinensis*. Among the pestalotiopsis-like strains, seven well-classified taxa and seven tentative clades were included in three genera, i.e., *Pestalotiopsis*, *Pseudopestalotiopsis*, and *Neopestalotiopsis*. One novel species, *Ps. annellata*, was introduced, and *Ps. chinensis* was the taxon most frequently isolated from *C. sinensis* in this study. Our results demonstrated the affiliation of Pleosporales strains with the various families in Pleosporales and revealed the presence of one new genus (*Neoshiraia*) and eight new species. Furthermore, two novel *Diaporthe* species were proposed for leaf spotting fungi isolated from symptomatic leaves of *C. sinensis*. The results of pathogenicity assessments exhibited that, with wound inoculation, all assayed pestalotiopsis-like isolates and *Diaporthe* strain NTUCC 18-155-1 isolated in this study were pathogenic on tea leaves. Furthermore, to the best of our understanding over ten species were reported for the first time from *C. sinensis* in Taiwan. The present study improves our understanding of species associated with leaf spot symptoms on tea plants and provides useful information for effective disease management.

A06 臺灣地區引起百香果果腐病與頸腐病之 *Fusarium solani* 菌株鑑定與特性分析－羅佩昕[1,2]、昌佳致[1]、張哲維[2]、鍾文鑫[2,3] ([1]行政院農業委員會臺中區農業改良場、[2]國立中興大學植物病理學系、[3]國立中興大學永續農業創新發展中心)

Identification and characteristics of *Fusarium solani* causing fruit rot and collar rot of passion fruits in Taiwan－Lo, P. H.[1,2], Chang C. C.1, Chang C. W.[2], and Chung, W. H.[2,3] ([1]Taichung District Agricultural Research and Extension Station, Changhua; [2]Department of Plant Pathology, National Chung Hsing University, Taichung; Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung)

百香果 (*Passiflora* spp.) 為西番蓮屬的蔓性果樹，原產於南美巴西，目前全球主要生產區包含澳洲、巴西、南非、哥倫比亞、越南、中國及臺灣等地區，商業栽培品種以紫百香果 (*P. edulis*) 及黃百香果 (*P. edulis* f. sp. *flavicarpa*) 為主。*Fusarium solani* 引起之百香果頸腐病 (collar rot) 為百香果栽培之重要病害，可感染植株造成莖基部褐化腐爛與植株萎凋病徵，於巴西、美國及中國等地區皆有被報導，臺灣則因濕度高常於病斑表面產生紅色子囊殼，導致產量下降與栽培區的嚴重損失。近來於百香果金昕、滿天星及台農一號果實觀察到褐色水浸狀病徵， 並於病斑表面產生大量紅色子囊殼，經分離純化得 11 株 *F. solani* 菌株; 另亦自發生百香果頸腐病之種苗分離到 1 株 *F. solani* 菌株。雖巴西已將引起百香果頸腐病之病原命名為 *F. solani* f. sp. *passiflorae*，然比較臺灣菌株與巴西菌株之特性，證實臺灣引起百香果頸腐病與果腐病之 *F. solani* 與巴西所發表之菌株不同。本研究目的為釐清引起臺灣百香果頸腐病與果腐病之 *F. solani* 菌株與其特性。依形態學特徵顯示，所蒐集菌株在 PDA 上之菌落形態分別呈現米黃色菌落，部分菌株菌落帶有橘色、淡紫色或藍色色素，其中 PaFS1-1 與 PaFS9 菌株，於 PDA 上不產生子囊殼與大孢子，可產生厚膜孢子，小孢子呈橢圓形; 其餘菌株皆可於 PDA 上產生紅色子囊殼，大孢子呈現彎曲鐮刀狀，具 3-7 個隔，小孢子為橢圓形，具有 0-2 個隔，可於菌絲上產生厚膜孢子。於接種金昕與台農一號果實試驗結果指出，PaFS1-1 與 PaFS9 菌株只能感染金昕果實，其餘菌株皆可感染上述兩品系果實，造成果實產生褐色水浸狀病斑。另於台農一號與滿天星品種種苗接種試驗證實，PaFS1-1 與 PaFS9 菌株於接種點周圍產生褐色乾燥侷限斑，其他菌株皆可感染種苗並造成百香果莖部褐化水浸狀病徵，最終造成植株萎凋。進一步透過增幅 ITS 與 TEF 序列並解序，將目前所蒐集之菌株與國外引起百香果病害之 *F. solani* 菌株進行分子親緣分析，結果得知所蒐集之菌株與國外菌株分屬不同分子群，而國內造成百香果病害之 *F. solani* 菌株亦可區分成不同分子群，證實臺灣地區引起百香果果腐病與百香果頸腐病之 *F. solani* 具多樣性，且與國外菌株不同。

A07 在臺灣發現由 *Leveillula taurica*引起之金蓮花白粉病及 *Podosphera fusca* 引起之豇豆白粉病－蕭伊婷[1]、歐海仁[2]、王照仁[1]、黃冬青[1]、沈原民[1] ([1]行政院農業委員會臺中區農業改良場、[2]國立臺灣大學植物病理與微生物學系)

The powdery mildews *Leveillula taurica* on *Tropaeolum majus* and *Podosphera fusca* on *Vigna unguiculata* subsp. *sesquipedalis* in Taiwan－Wei, S. H., Lin, M. Y. (Department of Plant Medicine, National Chiayi University, Chiayi 600, Taiwan)

於 2020 年 4 月在彰化縣大村鄉，發現金蓮花葉片出現過去未曾發現的不規則黃褐色壞疽斑，且大多出現在下位葉，園區發生的情形為 50-60% 左右，起初病斑為黃色暈圈，而到後期，葉片逐漸形成褐色壞疽斑，嚴重者病斑會癒合，並在葉背觀察到白色粉末狀的白粉病菌構造，依據光學顯微鏡下的形態及 ITS 序列特徵，將感染金蓮花的白粉病菌鑑定為 *Leveillula taurica s. str.*。此外，在發現金蓮花白粉病的臨近地點亦發現甜椒葉片遭 *L. taurica* 感染，結果顯示此白粉病菌在本地具有一種以上的寄主植物，金蓮花與甜椒上的白粉病菌有潛力在不同寄主植物間交互感染；在同一時間點於彰化縣大村鄉田間，另觀察到豇豆葉表與葉背覆蓋白色粉狀物，到後期葉片覆滿白色粉末，使豇豆逐漸萎凋，田間的發生率為 15%-30% 左右，依白粉病菌之形態及 ITS 序列特徵將其鑑定為 *Podosphera fusca*，此白粉病菌在臺灣具有多種寄主植物，寄主紀錄除甜瓜、木瓜、苦瓜、紅鳳菜等作物外，本研究確認 *P. fusca* 能夠感染豇豆造成豇豆白粉病。

A08 臺灣草莓葉枯病之發生與鑑定－吳竑毅[1]、蔡季芸[1]、吳意眉[2]、鍾嘉綾[1]、鐘珮哲[1,3] ([1]國立臺灣大學植物病理與微生物學系、[2]苗栗縣政府、[3]行政院農業委員會苗栗區農業改良場)

Wu, H. Y.[1], Tsai, C. Y.[1], Wu, Y. M.[2], Chung, C. L.[1] and Chung, P. C.[1,3] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei、[2]Miaoli County Government, Miaoli、[3]Miaoli District Agricultural Research and Extension Station, Miaoli)

草莓為苗栗地區重要觀光休閒產業，每年草莓季觀光採果遊客達百萬人次，為週邊產業帶來無限商機。目前我國草莓栽培現況，全國種植面積約 500 公頃，近 2~3 年草莓產業從原本豐香為主流品種，至 109 年 10 月於苗栗縣大湖鄉、獅潭鄉等草莓主要產區，香水品種栽植面積已高達 80% 以上，主因為豐香對炭疽病相當感病，育苗不易且產量不及香水。然而在品種汰換的同時，產業也面臨新病害的挑戰。香水品種於育苗期及產果期出現有別於炭疽病的病徵，該病害可感染葉片、葉柄、走蔓、冠部、根系及果實，在葉片上的病徵，初期為圓狀病斑，後期隨水蔓延並擴大病斑造成葉枯病徵，於潮濕環境下產生黑色環狀孢子堆。當此病害感染植株冠部造成壞疽褐化後，地上部逐漸弱化，新葉變小，葉肉顏色轉紫至紫紅色終至萎凋死亡。初步以孢子型態鑑定病原菌為 *Pestalotiopsis* sp.，進一步以 ITS、β-tubulin (*TUB*)、translation elongation factor 1-alpha (*TEF*) 三個基因序列進行多基因序列分析，序列以 Multiple Alignment using Fast Fourier Transform (MAFFT) 進行比對，再以貝葉斯推斷 (Bayesian inference analyses, BI) 及最大似然 (Maximum likelihood analyses, ML) 分析，結果顯示造成臺灣草莓葉枯病之病原菌與 *Neopestalotiopsis rosae* 歸於同一種類。目前此病害除了在苗栗地區發現外，台北內湖、桃園新屋、新竹關西、南投清境及嘉義等區域皆有發現，田間調查發現尤以香水品種特別感病。依發生情形推測，此菌可能具潛伏感染特性，經由雨水或噴灌水彈濺傳播，並可藉由種苗帶菌至

本田。因此，在防治策略上仍以健康種苗為重點，須澈底清除病葉及植株，並施用防治資材。初步從已推薦在草莓病害之藥劑於實驗室內進行感受性測試，結果顯示包含腐絕快得寧、待克利、賽普護汰寧、得克利 (於草莓炭疽病中推薦為三氟得克利)、百克敏與普克利等具有較佳抑制效果。本研究確認草莓產業面對之新病害種類，未來將篩選有效防治藥劑、非化學農藥資材及了解其流行病學，以建立綜合管理技術。

A09 宜花地區農友稻熱病防治用藥種類調查及病原農藥感受性測試－蔡依真[1]、謝文棟[1]、陳繹年[2] ([1]行政院農業委員會花蓮區農業改良場、[2]行政院農業委員會農業試驗所)

Survey on the types of fungicides used by farmers to control the rice blast disease and the fungicides sensitivity assay of *Pyricularia oryzae* in Hualien district－Tsai, Y.C.[1], Hsieh, W. T.[1], and Chen, Y. N.[2] ([1]Hualien District Agricultural Research and Extension Station, Hualien; [2]Taiwan Agricultural Research Institute, Taichung)

水稻為臺灣重要糧食作物，栽培期間發生的病蟲害種類多樣，其中以稻熱病菌 *Pyricularia oryzae* Cavara. 所引起的稻熱病 (rice blast) 為普遍發生且對產量影響甚鉅，慣行農友對此病之防治方法主要仰賴化學農藥施用。然而，近年宜花地區稻農曾反映部分稻熱病推薦用藥防治效果不甚理想；因此，本研究以問卷方式調查了解宜花稻農防治用藥情形，並進行室內測試初步評估本地區稻熱病菌對農友常用藥劑之感受性。蒐集 127 位慣行稻農訪談結果，受訪者年齡比例以 61 歲以上者為最高 (佔47.9%)，41~60 歲次之 (43.6%)，20~40 歲 (8.6%) 最低。從農年資 15 年以上比例最高 (50.3%)，10~15 年最低 (13%)。用藥選擇判斷方面，多數農友會自己判斷作物狀況選擇購置藥劑種類，不確定時優先詢問農藥行 (45.7%)，其次為依賴農藥行推薦藥劑者佔 31%，無法判斷用藥時會優先詢問試驗改良場所、相關院校或植物醫生者比例最低，佔 23.4%。用藥紀錄習慣部分，會記錄何時施藥者佔 42%，記錄用藥種類者佔 34.6%，兩者均無記錄者佔 23.4%。在稻熱病推薦藥劑種類選擇部分，以三賽唑使用最多 (74.8% 受訪農戶曾使用)、嘉賜黴素次之 (68.5%) 及亞賜圃再次之 (54.3%)。有關殺菌劑對稻熱病菌菌絲生長抑制試驗，本試驗將免賴得、亞賜圃、三賽唑、嘉賜黴素及撲克拉等五種藥劑分別以推薦倍數添加於 PDA 平板中培養稻熱病菌，結果以免賴得、亞賜圃及撲克拉抑制率最高 (100%)，其次為三賽唑 (96.7%)，嘉賜黴素最低 (17.7%)；另以菌絲塊浸藥法進行評估，則以撲克拉最高 (98.2%)、免賴得次之 (80.4%)，三賽唑為最低 (0.08%)。孢子發芽抑制結果，以撲克拉抑制率最高 (99.3%)、免賴得 (98.5%)次之，嘉賜黴素最低 (4.6%)。綜合以上調查試驗結果，宜花地區大部分稻農用藥遇到問題時多優先諮詢農藥行推薦藥劑，42% 受訪者有自行記錄何時施藥，三賽唑為最多農戶使用之藥劑，但在菌絲生長抑制測試結果顯示，以藥劑平板及菌絲塊浸藥方式測得之抑菌率差異甚大，顯示菌絲對此藥劑接觸時間較短時藥效較不理想，而撲克拉對稻熱病菌絲生長及孢子發芽抑制均有最佳表現，推論以此藥劑進行浸種消毒對降低稻熱病菌族群密度仍具一定功效。

A10 Identification of *AVR-Pib* genotypic polymorphism and population study of rice blast fungus in Taiwan－Syauqi, J.[1,2], Sulistyowati, L.[2], and Abadi, A. L.[2], and Shen, W.-C.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan; [2]University of Brawijaya, Malang, Indonesia)

Rice is one of the most commonly consumed staple food in the world. As the highest demanded food supply, rice productivity must be maintained at the high and stable level. Rice blast disease caused by *Magnaporthe oryzae* is one of the serious rice diseases faced by farmers around globe rice production areas. Frequent occurrence and highly adaptive nature of blast disease were caused by complicated mechanisms of pathogen evolution to escape host defense and resistance. Interaction between rice and rice blast fungus generally follows gene for gene relationship in which the host plant carrying a specific resistance (R) gene confers resistance against the pathogen with the corresponding avirulence (*AVR*) gene. Therefore, dissection and monitor of the *AVR* genes genotypes among field isolates are crucial to sustain field resistance. Taking advantage of 31 IRRI IRBL lines which carry single blast resistance gene, effectiveness of rice blast R genes in Taiwan was monitored and the genotypes of rice blast fungus population were studied. Surveillance data from five blast disease monitoring plots reveals that *Pii* and *Pia* genes were ineffective in Taiwan, while *Ptr* (formerly *Pita2*) gene was the most effective R gene against rice blast fungus population in Taiwan. Interestingly, IRBLb-B line carrying *Pib* gene which showed resistance in the past was observed to have various type of lesions in recent years, suggesting rice blast fungus population may have evolved and escaped from *Pib* resistance. Thus, we further characterized the *AVR-Pib* genotype of virulence isolates collected from IRBLb-B line of different monitoring plots. PCR screening and sequencing results confirmed 9 different *AVR-Pib* genotypes in Taiwan and the *AVR-Pib* locus was a hot spot of Pot3 insertion. Furthermore, compiling the genotypic screening results of three *AVR* genes, 22 groups which stand for 9 physiological races based on their abilities to infect rice lines or varieties containing *Pizt*, *Pik* and *Pib* genes were distinguished among Taiwan rice blast fungus population. We hope these findings will provide the pathogen surveillance data for breeding and deployment of rice varieties in Taiwan.

A11 臺灣引起洋桔梗根腐與萎凋病之*Fusarium*屬病原鑑

定－吳承峻[1]、鍾文鑫[1,2]、沈原民[3] ([1]國立中興大學植物病理學系、[2]國立中興大學永續農業創新發展中心、[3]行政院農委會台中改良場植物保護研究室)

Identification of *Fusarium* spp. causing stem rot and wilting in lisianthus in Taiwan－Wu, C. C.[1], Chung, W. C.[1,2], and Shen, Y. M.[3] ([1]Dept. of Plant Pathology, National Chung Hsing University, Taichung; [2]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung; [3]Crop Protection Research Office, Taichung District Agricultural Research and Extension Station, Chunghua)

洋桔梗 (*Eustoma grandiflorm* (Raf.) Shnn.) 為臺灣重要外銷切花。在溫室洋桔梗栽培期間常發生根腐與萎凋病徵，依台中改良場之初步鑑定可能屬於 *Fusarium oxysporum*，為確定引起洋桔梗根腐與萎凋之病原種類，自 2020 年 2 月開始蒐集發生根腐與萎凋病徵之洋桔梗罹病株，採集地點為彰化縣北斗鎮溫室內栽培之洋桔梗園。病害調查顯示，洋桔梗園區發生根腐與萎凋之比例約 3-5%。罹病植株發病初期僅維管束褐化，中後期受感染之莖部內呈中空或乾腐，褐化病徵延植株莖部擴展，並常於罹病組織表面出現粉狀與橘黃色分生孢子堆。有時褐化病徵亦可蔓延至葉片，造成葉部呈乾癟狀。將罹病組織攜回研究室進行病原分離與純化，結果指出所分離之菌株菌落形態相似，依 Leslie 與 Summerell 兩氏 (2006) 對 *Fusarium* 屬形態描述，初步鑑定所得菌株主要屬於 *F. oxysporum* species complex (FOSC)。進一步將單孢純化後之 FOSC 菌株以 PDA 培養，並置於 20℃光照 12 小時環境使其產生大孢子，另於 28℃ 避光環境下觀察 PDA 上之菌落特徵。培養 7 天後結果指出，菌落正面顏色為淡粉紅至紫色，氣生菌絲呈棉絮狀；背面顏色為白色淡橘色。大孢子形狀細長且略微彎曲、壁薄，長寬為 27.7-42.8 x 3.6-4.8 μm，具 3-4 個隔膜 (少數為 2 或 5 個隔膜)；小孢子為橢圓至棒狀，長寬為 6.5-11.8 x 2.79-4.36 μm，具 0-1 個隔膜，呈假頭狀聚集於產孢細胞尖端；厚膜孢子為球狀、壁厚且平滑，單生或雙生並間生於菌絲。於病原性測試方面，以混合 0.3% 水瓊脂含 10^5~10^6 spores/ml 孢子懸浮液 15 μl，接種在拉長 2~5 個節間之洋桔梗幼苗的第一與第二節間葉腋部，結果顯示，接種 2~3 週後，出現與田間相似之病徵。另以根部浸泡方式接種 10^5~10^6 spores/ml 孢子懸浮液 10 分鐘，結果得知，在接種 2~3 週後植株出現矮化與根系腐爛的病徵，且亦可自罹病組織分離到相同形態的菌株。進一步將可引起洋桔梗病徵之 *Fusarium* 屬菌株，萃取總DNA並增幅 TEF-1α 及 IGS 序列，經與 NCBI 資料比對與分子親緣分析，證實引起洋桔梗根腐與萎凋病之病原為 FOSC 中的 *F. nirenbergiae*。

B.細菌及病毒組

B01 利用人工接種評估梨葉緣焦枯病菌具替代性寄主潛力的雜草－馮擷安[1]、張哲銘[2]、蘇秋竹[1] ([1]行政院農業委員會農業藥物毒物試驗所農藥應用組、[2]行政院農業委員會農糧署中區分署)

Potential alternative weed hosts in association with *Xylella taiwanensis* assessed by artificial inoculation method－Fung, J. A.[1], Chang, C. M.[2] and Su, C. C.[1] ([1]Division of Pesticide Application, Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Taichung; [2]Central Region Branch, Agriculture and Food Agency, Changhua)

梨葉緣焦枯病由侷限導管細菌 *Xylella taiwanensis* 所引起，本病於全台橫山梨產區零星發生，感染後罹病梨樹於每年生育中期後才會顯現系統性葉緣焦枯病徵並於後期提早落葉，橫山梨母樹若高接過多梨穗進行梨果生產，則逐年顯現樹勢衰落現象。*Xylella* 屬之病原菌寄主範圍廣並藉由蟲媒傳播，全世界目前共紀錄到595 種寄主植物。本病為臺灣梨樹獨有之病害，為了解田間是否有替代性寄主植物，104 至 106 年主動於罹病梨園周圍採集不同植物，共調查 32 科、58 種、1,021 個植物樣本，利用 PCR 檢測及組織分離兩種技術同步進行檢測，均未在田間偵測到替代性寄主植物。為了解田間是否具有替代性寄主潛力的植物，107 年起利用人工接種方式對雜草進行替代性寄主之潛能評估，目前共測試 15 科 27 種雜草，人工接種一個月後陸續進行檢測，結果顯示 27 種雜草中菊科的艾草 (*Artemisia indica*)、小花蔓澤蘭 (*Mikania micrantha*)、唇形科的薄荷 (*Mentha canadensis*)、禾本科的升馬唐 (*Digitaria ciliaris*)、稗草 (*Echinochloa crus-galli*)、早熟禾 (*Poa annua*)、茄科的玉珊瑚 (*Solanum pseudocapsicum*)、葡萄科的漢氏山葡萄 (*Ampelopsis brevipedunculata* var. *hancei*) 可被 *X. taiwanensis* 纏據。綜合田間主動偵測及室內人工接種結果，推測 *X. taiwanensis* 之媒介昆蟲在田間可能傳播效率低？故人工接種後雖發現有雜草可被病菌纏據著，但過去在田間並未測到任何替代性寄主植物。本研究上述具有替代性寄主潛力的雜草，未來或許可作為田間管理防治策略擬定。

B02 Rhizosphere bacterial community differences among organic and conventional farms in Taiwan－Fan, J. Y. and Yang, J.-I. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

Organic farming is one of the popular farming practice strategies of the sustainable agriculture concept. It is believed that organic farming potentially supports the soil bacterial communities that provide important ecological services. However, how organic farming influence the assembly of rhizosphere bacterial communities in fields remains unclear. In this study, we investigated the diversity and composition of rhizosphere bacterial communities under organic and conventional farming. Rhizosphere soils of *Fragaria* ×

ananassa (strawberry) and *Camellia sinensis* (tea) were sampled from 17 organic farms and 16 conventional farms in Taiwan between July and December in 2016. Bacterial communities were profiled with amplicon metagenomic sequencing of the hypervariable region 3 and 4 of 16S rRNA genes using the illumina Miseq platform by the 2 x 300 bp paired-end runs. The DADA2 pipeline was adopted to infer amplicon sequence variants. The bacterial communities were summarized with Bray-Curtis dissimilarity and principal coordinate analysis, and the composition of communities were compared with permutational multivariate analysis of variance. The contribution of each taxon to the inter-community difference was assessed with similarity percentage analysis. The results showed that the richness and diversity of rhizosphere bacterial classes were not significantly different between organic farms and conventional farms. However, some rhizosphere bacterial classes were more abundant in the conventional farms. In strawberry rhizospheres, Alphaproteobacteria, *Betaproteobacteria*, *Opitutae*, and *Gammaproteobacteria* contributed over 40% of overall Bray-Curtis dissimilarity between samples from conventional and organic farms. Among those classes, *Betaproteobacteria* was more abundant in the conventional farms. In tea rhizospheres, *Acidobacteria Gp1*, *Acidobacteria Gp2*, Betaproteobacteria, and *Acidobacteria Gp3* contributed over 40% of overall Bray-Curtis dissimilarity between samples from conventional and organic farms. Among those classes, *Acidobacteria Gp3* was more abundant in the conventional farms.

B03 Evaluation of an antibacterial weapon on *Agrobacterium tumorigenesis* and crown gall microbiota－Wang, S.-C.[1,2], Kuo, C.-H.[1], and Lai, E.-M.[1] ([1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei; [2]Department of Life Science, National Central University, Taoyuan)

The type VI secretion system (T6SS) is deployed by many proteobacteria to secrete effector proteins into eukaryotic cells for pathogenesis or prokaryotic cells for interbacterial competition. Previous studies demonstrated that *Agrobacterium tumefacien*, a soil-borne phytopathogen causing crown gall disease on various plant species, deploys T6SS to attack closely- and distantly-related bacterial species *in vitro* and *in planta*. Intriguingly, tumorigenesis was not affected by the loss of T6SS when *A. tumefaciens* was inoculated on host plants under sterile condition or directly on wounded site. Thus, it remains unknown whether T6SS influences tumorigenesis in natural infection process, or affects the composition of bacterial community inside crown galls. Here, we established a soil inoculation method on wounded tomato seedlings and performed 16S rRNA gene amplicon sequencing to address these questions through the comparison of *A. tumefaciens* C58 wild-type strain and two T6SS deficient mutants (i.e., Δ*tssL* and Δ*tssB*). Based on inoculation trials, all three strains could induce tumors through this method, however, the mutants have significantly lower disease incidences. With our newly developed PCR blockers, the optimized protocol greatly reduced host contamination in 16S amplicon sequencing. Composition comparisons indicated that the T6SS may not be important in shaping the crown gall microbiota. Future works are necessary to investigate if the role of T6SS in influencing disease incidence is through enhancement of *Agrobacterium* colonization in rhizosphere or on the wounding site. Furthermore, factors that may shape the crown gall microbiota development and composition are important for future studies to better understand plant-microbe interactions.

B04 毛西番蓮罹染 *East Asian passiflora virus*-AO之鑑定及不同百香果 potyvirus 病毒對其之感染力測試－陳金枝、江芬蘭、鄭櫻慧 (行政院農業委員會農業試驗所植物病理組)
Identification of *East Asian passiflora virus*-AO infecting Passiflora foetida and the infectivity of different passiflora potyviruses on seedlings of *P. foetida*.－Chen, C. C., Chiang, F. L., and Cheng, Y. H. (Taiwan Agriculture Research Institute, Taichung)

毛西番蓮 (*Passiflora foetida*) 為西番蓮科 (*Passifloraceae*) 西番蓮屬 (*Passiflora*) 之二年生蔓性草本植物，又稱為小時計果或野百香果，全球熱帶地區均可見其蹤跡，常見於臺灣野外田間；文獻紀錄上為百香果木質化病毒之野生寄主。本研究於 2020 年由埔里採集到葉片出現嵌紋徵狀且果實木質化之毛西番蓮，經由間接式-酵素連結免疫吸附反應 (indirect enzyme-linked immunosorbent assay, indirect ELISA)、反轉錄-聚合酶鏈鎖反應 (RT-PCR) 檢測和病毒鞘蛋白核酸定序結果，鑑定其乃屬 *East Asian passiflora virus*-AO 系統之病毒分離株。進一步於溫室接種試驗中，毛西番蓮之罹病組織液可成功接種於奎黎產生單斑，單斑組織回接健康的毛西番蓮以及黃百香果苗株，均能引起葉片嵌紋徵狀，確認由毛西番蓮上所分離而得之 EAPV-AO分離株 (代號 PL)，具有對原寄主以及百香果之感染力。本研究另取田間罹染 EAPV-AO 之毛西番蓮木質化果實之種子，播種後之實生苗均未檢出病毒，顯示 EAPV-AO 並未有種子傳播之現象；本研究另由員林地區採集所得之毛西番蓮罹染病毒病材料，經檢測鑑定均屬 EAPV-AO 所感染引起，進一步分析不同地理位置來源之百香果或毛西番蓮 EAPV-AO 分離株之鞘蛋白胺基酸序列之類緣關係，發現由員林所採集之毛西番蓮分離株自成一分群，而由埔里所分離之毛西番蓮分離株則與臺灣大坪頂的百香果分離株有較近的類緣關係，經田間觀察 PL 分離株來源之毛西番蓮周遭為百香果田區，有其百香果病毒來源之地緣關係。本研究另將不同百香果 potyviruses (包

括 EAPV-IB 和 *Telosma mosaic virus* (TeMV)) 接種於健康毛西番蓮苗株，均能引起黃化嵌紋病徵，除印證毛西番蓮為百香果 EAPV 病毒之寄主外，並首次證明其為 TeMV 之潛力寄主，可作為田間防治百香果病毒野生寄主之參考用。

B05 Development of transgenic plants expressing a ToLCNDV movement protein for studying mechanical transmission of begomovirus－Uslu, Y. E., Lee, C.-H., and Jan, F.- J. (Department of Plant Pathology, National Chung Hsing University, Taichung)

Begomoviruses belong to the family of Geminiviridae. Begomoviruses are usually transmitted by whitefly. Mechanical transmission of begomo-viruses is rare. Mechanical transmissibility has been shown to be associated with the movement protein (MP) of begomovirus DNA-B in the tomato leaf curl New Delhi virus (ToLCNDV). A ToLCNDV-OM strain isolated from diseased melon can be mechanically transmitted, however, a ToLCNDV-CB strain isolated from diseased cucumber cannot. Transgenic plants expressing the movement protein of ToLCNDV-OM or ToLCNDV-CB under the control of an estrogen inducible promoter were developed. Plasmid constructs built in a binary vector were independently transformed into *Nicotiana benthamiana* plants by agrobacterium-mediated trans-formation. MP was expressed either in cytoplasm or nucleus to study MP to determine how MP affects mechanical transmission. Transgenic plants were confirmed by PCR, and movement protein expression was confirmed by ELISA. Transgenic plants will be inoculated with the ToLCNDV-CB isolate by mechanical transmission to determine whether or not MP will affect mechanical transmissibility of ToLCNDV.

B06 Development of a bead-based assay for detection of three Tospoviruses－Kuan, C. P.[1], Hsiao, C. J.[1], Cheng, Y. H.[2], and Yang, T. C.[1] ([1]Division of Biotechnology and [2]Division of Plant Pathology, Taiwan Agricultural Research Institute, Taichung)

Simultaneous detection of three tomato viruses, Capsicum chlorosis virus, Tomato spotted wilt virus and Pepper chlorotic spot virus, were carried out using a multiplex bead-based assay, a novel detection technique that combines RT-PCR with the florescent detection. On the basis of the establishment of the optimal PCR and reverse transcription (RT)-PCR for the detection of a single virus, the RT-PCR method that employed virus-specific primers was developed for the detection and differentiation of all the three viruses in tomato or pepper plants. The multiplex RT-PCR based method has superior specificity, sensitivity, and high-throughput capacity compared to conventional RT-PCR and is an attractive alternative for the identification of different Tospovirus species. The virus specific probes were detected without electrophoresis analysis and effective removal of RT-PCR inhibitors. The assay was then validated using tomato/ pepper samples infected with one or more viruses collected from fields. The system offers a sensitive, high throughput and rapid detection method for tomato viruses.

B07 Identification of Tomato yellow leaf curl disease following genomic amplification－Kuan, C.-P.[1], Liu, Y.-T.[1], Hsiao, C. J.[1], Cheng, Y.-H.[2], and Yang, T. C.[1] ([1]Division of Biotechnology; [2]Division of Plant Pathology, Taiwan Agricultural Research Institute, Taichung)

Tomato yellow leaf curl disease caused by Begomoviruses have become one of the major significant crop losses in tomato production worldwide. The management of the virus in tomato is difficult and expensive in cultivation under a structure and open field production. In the field, tomatos are infected frequently with several viruses during a growing season, which leads to reduced yield and seedlings quality. A real-time PCR based assay combined with TaqMan chemistry was developed for detection of the TYLCTHV in tomato seedlings. The designed probe for specific to TYLVTHV was detected without electrophoresis analysis and effective removal of PCR inhibitors. The assay showed a relative higher sensitivity comparable to the PCR reaction. The assays presented here could assist in the implementation of quarantine measures for TYLCTHV on site identi cation and in routine indexing of TYLCTHV for the production of virus-free tomato.

B08 臺灣感染馬鈴薯之馬鈴薯Y病毒的分子特性－林玫珠、鄧汀欽、蔡錦慧、趙君皓、陳金枝、鄭櫻慧 (行政院農業委員會農業試驗所植物病理組)

Molecular characterization of Potato virus Y isolates infecting potato in Taiwan－Lin, M. J., Deng, T. C., Tsai, C. H., Chao, C. H., Chen, C. C., and Cheng, Y. H. (Taiwan Agricultural Research Institute, Taichung)

馬鈴薯 (*Solanum tuberosum* L.) 係採無性繁殖生產，病毒病易透過種薯進行傳播，導致產量減少 30~50%，因此無論是國內外對此等病毒之檢測與控管均有相當重要的需求。而臺灣發生之馬鈴薯病毒有馬鈴薯 Y 病毒 (Potato virus Y, PVY)、馬鈴薯 X 病毒 (Potato virus X, PVX)、馬鈴薯 S 病毒 (Potato virus S, PVS)及馬鈴薯紡錘塊莖類病毒 (*Potato spindle tuber viroid*, PSTVd)，田間感染以 PVY 及 PVS 為主。國際間 PVY 的病毒系 (strain) 以寄主的反應及與植物抗型基因交互作用進行分群，因此早期的病毒株分類系統較為混亂，2008年 Singh 等歐

美數位學者針對 PVY 的命名提出具體的建議，應結合生物學及分子生物學的特性進行命名。以本研究室蒐集的二株病毒株 PVY-M-1510 及 PVY-N-SY4 進行研究，PVY-M-1510 在馬鈴薯上呈現為嵌紋病徵，PVY-N-SY4 在馬鈴薯上呈現葉脈壞疽病癥。將病毒株 PVY-M-1510 及 PVY-N-SY4 之全基因進行定序，分別獲得 9737 bp 及 9467 bp 之序列，經全長度及鞘蛋白基因之比對，結果顯示 PVY-M-1510 屬於國際定名之 PVY strain O (PVY^O)，其鞘蛋白基因與英國病毒株 PVY-SCRI-O (AJ585196) 及加拿大病毒株 PVY-N:O-L56 (AY745492) 最為相近，相似度為 99.5%；PVY-N-SY4 屬於國際定名之 PVY strain NTN (PVY^{NTN})，其鞘蛋白基因與英國病毒株 PVY- SASA-61 (AJ585198) 及美國病毒株 PVY- RRA-1 (AY884984) 最為相近，相似度為 99.6及 99.4%。由於近年來歐美國家發現一 PVY 病毒株在馬鈴薯葉片上並無病徵顯現，但卻造成薯塊上有輪狀壞疽的病斑 (Potato tuber necrotic rings, PTNRD)，嚴重影響產量，損失達 50-70%。因此，將過去至今在臺灣所收集的 28 個 PVY 病毒株，以國際上 PVY^O、PVY^C、PVY^N、PVY^{NTN}、PVY^{NA} 之專一性引子對進行反轉錄聚合酵素連鎖反應 (RT-PCR) 分析，以確實掌握臺灣 PVY 病毒的現況。

B09 玫瑰感染李屬壞疽輪斑病毒(Prunus necrotic ringspot virus, PNRSV)的發生－林易賢[1]、陳宗祺[2]、陳煜焜[1] ([1]國立中興大學植物病理學系；[2]亞洲大學醫學檢驗暨生物技術系)

Occurrence of the Prunus necrotic ringspot virus (PNRSV) on rose (*Rosa rugosa*)－Lin, Y.S.[1], Chen, T.C.[2], and Chen, Y.K.[1] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Department of Medical Laboratory Science and Biotechnology, Asia University, Taichung)

玫瑰為薔薇科 (Rosaceae) 的作物，為世界上重要之園藝作物。有多種植物病毒，例如 Prunus necrotic ringspot virus (PNRSV)、apple mosaic virus (ApMV)、arabis mosaic virus (ArMV)、strawberry latent ringspot virus (SLRSV)、tobacco streak virus (TSV) 等，會在葉部引起多樣的病徵，包括嵌紋 (mosaic)、輪斑 (ringspot)、條斑 (line pattern)、斑駁 (mottle)、矮化 (stunt)、叢狀 (rossete)、變形 (distortion)。2020 年於嘉義番路鄉之有機玫瑰農園上發現在葉片帶有嵌紋及輪斑病徵的玫瑰，疑似由 PNRSV 所感染。將罹病葉片組織磨碎，機械接種於指示植物奎藜 (*Chenopodium quinoa*) 進行單斑分離，並未獲的單斑病毒株。由原始病葉進行總量RNA之萃取，使用 Malinowski and Komorowska (1998) 開發之 PNRSV 外鞘蛋白 (CP) 基因之專一性引子對 (PNRSV-CPF1: 5'-atggtttgc-cgaatttgcaatcat 和 PNRSV-CPR1: 5'-gagtgtgcttatctcactctag)，進行 RT-PCR 檢測。預期之 cDNA 片段大小約為大小為 700 bp。由三次採集的總數 43 份標本中，可自 16 個樣本中增幅出預期大小的 cDNA 片段，檢出率 43%。選殖 RT-PCR 所增幅的 cDNA 片段於 pCR Ⅱ Topo 載體，並送中興大學生科中心進行序列解析。解序結果顯示所選殖的片段全長 699 bp，涵蓋 PNRSV CP ORF。經 NCBI 比對解序比對結果，顯示與 NCBI 登錄的 PNRSV 病毒株的 CP 核苷酸和胺基酸序列相似度分別為98-99.23% 和 96.77-98.39%，最近似於 PNRSV isolate 143 coat protein (accession number DQ983498)，核苷酸和胺基酸序列相似度分別達 99.23% 和 97.49%，罹病率達 43%。在解序的同時也將罹病葉片組織磨碎，罹病組織粗汁液機械接種於奎藜、胡瓜及日日春，均未產生病徵，RT-PCR 檢測亦未自接種的供試植株檢出預期的 PNRSV 感染。以細菌表現載體 (pET-28 b) 表現 PNRSV CP，將表現蛋白免疫注射於紐西蘭白兔以製備 PNRSV 專一性抗血清，目前進行中。PNRSV 為 *Bromviridae* 科 *Ilavirus* 屬的病毒，於本國尚無發現之紀錄，但在國外的薔薇科重要果樹和花卉如蘋果、梨、桃、李、杏、櫻桃、玫瑰等是常見且重要的病毒。本次報告為 PNRSV 在臺灣的首次記錄。

B10 花蓮地區龍鬚菜病毒病田間發生與種類調查－劉亭君[1]、蔡依真[1]、謝佳珉[2]、洪挺軒[2] ([1]行政院農委會花蓮區農業改良場、[2]國立臺灣大學植物病理與微生物系)

Identification and occurrence of virus infecting chayote in Hualien－Liu, T. C.[1], Tsai, Y. C.[1], Hsieh, C. M.[1], and Hung, T. H.[2] ([1]Hualien District Agricultural Research and Extension Station, Hualien; [2]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

龍鬚菜 (*Sechium edule* (Jacq.) Swartz.) 為花蓮吉安鄉特色作物，為全台主要產區，近年來病毒病之發生日益嚴重，逐漸受到農民重視。由於龍鬚菜之採收大多為連續摘取嫩芽，考量此採收方式有利病毒之機械傳播，本研究於吉安鄉採集帶有病毒病徵之葉片，針對瓜類常見之機械傳播病毒：胡瓜綠斑嵌紋病毒 (*Cucumber green mottle mosaic virus*, CGMMV)，矮南瓜黃化嵌紋病毒 (*Zucchini yellow mosaic virus*, ZYMV)，胡瓜嵌紋病毒 (*Cucumber mosaic virus*, CMV) 進行檢測，目前尚未發現上述病毒感染情況。經分子檢測田間多為南瓜捲葉菲律賓病毒 (*Squash leaf curl Philippines virus*, SLCPHV)，文獻上 SLCPHV 為粉蝨傳播，另偵測田間粉蝨也可發現帶毒情形。於吉安鄉龍鬚菜田區設置黃色黏板偵測粉蝨族群，109 年 8 月族群數達到高峰，同年 11 月族群數開始明顯降低，至 110 年 1 月數量最低。調查田間病毒罹病率，於 10 處龍鬚菜田區，每處隨機選取 200 棵植株觀察病徵有無，可見 109 年 5 月田間罹病率達到最高 (24.9%)，而後開始下降，至 110 年 2 月罹病率最低 (6.8%)，109 年 8 月田間罹病之龍鬚菜可見全株葉片均出現斑駁病徵，同年 10 月調查則部分新葉轉為正常無病徵，12 月罹病株多數轉為正常，僅剩少數下位葉有病徵，此現象造成農民認為罹病株痊癒，但經 PCR 檢測，89%新葉仍能偵測到病

毒，且一年生新植田之罹病率遠低於二年以上舊田，如何在考量成本效益下，有效防治 SLCPHV 於田間傳播將是未來重要課題。

B11 番茄重要檢疫病毒核酸檢測系統之開發與應用－鄭櫻慧、蔡筱婷、林玫珠、陳金枝 (行政院農業委員會農業試驗所植物病理組)

Development and application of detection systems of tomato important quarantine viruses－Cheng, Y. H., Tsai, S. T., Lin, M. J., and Chen, C. C. (Plant Pathology Division, Agricultural Research Institute, Taichung)

煙草嵌紋病毒屬病毒是危害茄科作物重要的病毒，2013 年發現的番茄斑駁嵌紋病毒 (tomato mottle mosaic virus, ToMMV) 與2015年發現的番茄褐斑皺果病毒 (tomato brown rugose fruit virus, ToBRFV) 因為可以克服 Tm-22 抗病基因，感染具有此抗病基因的番茄，使國際間聞之色變，紛紛設立檢疫關卡欲拒此類病毒於國門之外，目前臺灣尚未發現此 2 種病毒。根據 NCBI 網站上登錄全長度完整基因體，ToBRFV 與最相近的菸草嵌纹病毒 (tobacco mosaic virus, TMV) 的相同度為 81.8%，ToMMV 與最相近的番茄嵌纹病毒 (tomato mosaic virus, ToMV) 的相同度為 84.3%，臺灣有紀錄的另外 2 病毒番椒微斑駁病毒 (pepper mild mottle virus, PMMoV) 與菸草微綠嵌纹病毒 (tobacco mild green mosaic virus, TMGMV) 與前述 2 病毒在親源距離更遠，基因體相同度更低。ToBRFV、ToMMV、TMV 與 ToMV 的鞘蛋白胺基酸序列相同度約高於 80%，無法以抗鞘蛋白之多元抗血清區分，需建立核酸檢測系統才能避免病毒間相互干擾。根據病毒的移動蛋白 (ORF3) 核苷酸相同度低的區域設計具有專一性的引子， 進行反轉錄聚合酶連鎖反 應，可以專一性檢出之標的病毒。從輸入番茄種子增幅得到預期 ToBRFV 產物大小約 680 bp，ToMMV 產物大小約 693 bp 的 DNA 產物後，經過定序分析確認種子的確攜帶標的病毒。動植物防疫檢疫局已於 110 年 1 月 21 日頒布將 ToBRFV 列為輸入植物檢疫規定項目，目前正進行 ToMMV 的風險評估等前置步驟。本試驗建立的方法可以有效地從輸入番茄種子樣本檢出 ToBRFV 或 ToMMV，防止此 2 種病毒入侵，確保我國非疫區地位。

B12 Begomovirus對抗病基因*Ty-1/3*及*Ty-2*堆疊之影響－賴玄春、梁專譯、簡宏益、蔡文錫(國立嘉義大學植物醫學系)

Influence of new emerging tomato begomovirus on pyramiding resistant genes *Ty-1/3* and *Ty-2* in Taiwan－Lai, H. C., Neoh, Z. Y., Jian, H. Y. and Tsai, W. S. (Department of Plant Medicine, National Chiayi University, Chiayi City, Taiwan)

於全球廣泛性危害番茄生產的捲葉病，可造成嚴重經濟損失，此病害病原為菸草粉蝨 (*Bemisia tabaci*) 傳播的雙生病毒科豆金黃嵌紋病毒屬 (Begomovirus)病毒。上世紀臺灣主要病毒病原為番茄捲葉臺灣病毒 (*Tomato leaf curl Taiwan virus*, ToLCTV)，番茄黃化捲葉泰國病毒 (*Tomato yellow leaf curl Thailand virus*, TYLCTHV)於2005年前入侵，並逐漸改變臺灣番茄病毒相為TYLCTHV及其與ToLCTV混合感染為主，此外尚有本世紀新興的洋桔梗贅脈捲葉病毒 (*Lisianthus enation leaf curl virus*, LELCV)。至2019年，臺灣本島番茄Begomovirus病毒共有6個病毒種，除已於臺灣發現的病毒種ToLCTV、TYLCTHV、LELCV、ToLCHsV (*Tomato leaf curl Hsinchu virus*) 外，新病毒種番茄捲葉嘉義病毒 (Tomato leaf curl Chiayi virus, ToLCCYV)，及番茄捲葉南投病毒 (Tomato leaf curl Nantou virus, ToLCNTV)。TYLCTHV可再分為TYLCTHV-B及TYLCTHV-D株系，LELCV可再分為4個株系 (A至D)。本研究針對ToLCTV及TYLCTHV-B有抗病效能之Ty-1/3與Ty-2抗病基因堆疊的番茄品系，以粉蝨傳毒測試對目前臺灣番茄Begomovirus病毒抗病之效能，結果顯示此番茄品系接種目前臺灣番茄Begomovirus病毒後，均無明顯病徵之顯現，而以PCR檢測病毒感染後之番茄植株，在ToLCTV、TYLCTHV-B與TYLCTHV-D測試組，病毒檢出率分別為83%、30%與10%，LELCV的四個株系中除LELCV-C檢出率為0%，其餘皆為百分之百。此外，新病毒種ToLCCYV檢出率亦達百分之百。此結果顯示Ty-1/3與Ty-2抗病基因堆疊的番茄品系對目前臺灣番茄Begomovirus病毒仍有耐病性，惟接種新興病毒後，病毒檢出率可達100%，未來需評估不同抗病基因對新興臺灣番茄Begomovirus之抗病效能，以作為番茄捲葉病抗病選育之基礎。

C.病害防治組

C01 溶裂型噬菌體之鑑定及其對甘藍黑腐病的防治效果評估－鄧舜誠[1]、陳煜焜[2]、林志鴻[1] ([1]國立嘉義大學植物醫學系、[2]國立中興大學植物病理學系)

Identification of lytic bacteriophage and evaluation of its control effect on cabbage black rot－Deng, S. C.[1], Chen, Y. K.[2], and Lin, C. H.[1] ([1]Department of Plant Medicine, National Chiayi University, Chiayi; [2]Department of Plant Pathology, National Chung Hsing University, Taichung)

由 *Xanthomonas campestris* pv. *campestris* (*Xcc*) 所引起的十字花科黑腐病，是蕓苔屬植物的重要細菌性病害之一，其危害遍及全球十字花科作物產區，極具經濟重要性。目前在臺灣防治甘藍黑腐病的推薦藥劑有嘉賜銅及維利黴素。已有報告指出 *Xanthomonas* 屬細菌對銅劑的耐受性較高，且抗生素施用不慎易對甘藍幼苗造成傷害及產生抗藥性菌株，使藥劑防治效果受限，甚至造成環境衝擊。故噬菌體作為植物細菌性病害的防治資材再次受到關注。前人研究顯示噬菌體可以有效降低植物細菌性病害造成的危害，且已有噬菌體相關的市售商品，如 Agriphage、Erwiphage 等。故本研究目的在於鑑定分離自甘藍

黑腐病罹病葉的噬菌體 φXcc2、φXcc14 及 φXcc25，探討環境對其存活的影響及在植體內對 Xcc 的溶裂作用，並評估其對甘藍黑腐病的防治效果。在噬菌體鑑定方面，利用 DNaseI 與限制性內切酶確認基因組的種類及長度，配合穿透式電子顯微鏡觀察噬菌體形態，結果顯示 φXcc2、φXcc14 及 φXcc25 皆為雙股 DNA，其大小約 34.5、44 及 46.5kb，均屬肌尾噬菌體科 (Myoviridae) 的噬菌體。以 φXcc25 探討不同溫度環境對其存活的影響，在 4、20、25℃ 環境下，經 14 天後的 φXcc25 仍維持原有數量，而 30 及 35℃ 的 φXcc25 數量在第 8 及 4 天開始下降，故高溫會影響 φXcc25 的存活時間；在日照環境下，上午 9 點至下午 6 點期間，φXcc25 數量從 ~10^7 降至 ~103 PFU，故日照會影響 φXcc25存活。利用滲透注射法以瞭解 φXcc25 在植體內對 Xcc 的溶裂作用，將~10^7 至 ~10^2 CFU/ml 的 Xcc53 懸浮液分別與無菌水及 10^9 PFU/ml 的 φXcc25 懸浮液，以 1：1 混合液注入甘藍葉肉組織，置於日溫 30℃、夜溫 25℃ 及光照黑暗各 12 小時的生長箱中，無菌水處理組顯示，Xcc53 濃度 ~10^7、~10^6、~10^5及~10^4 CFU/ml 的組別分別在第 2、2、4 及 5 天出現病徵，其餘組別至第 8 天仍無病徵；φXcc25 處理組顯示，Xcc53 濃度 ~10^7 及 ~10^6 CFU/ml 的組別分別在第 4 及 8 天出現病徵，其餘組別至第 8 天仍無病徵，故 φXcc25 與 Xcc53 的有效作用濃度比例為10000:1。在盆缽防治實驗中，利用小型噴瓶於甘藍四葉期進行噴霧接種，每株甘藍接種 8 ml~10^5 CFU/ml 的 Xcc53 懸浮液，經 1 小時再分別處理 8 ml 的無菌水、~10^9 PFU/ml 的 φXcc25 懸浮液、20% 歐索林酸可濕性粉劑 (1000×)、5% 維利黴素溶液 (300×) 及 81.3% 嘉賜銅可濕性粉劑 (1000×)，置於同前述之生長箱中，經 16 天，無菌水、φXcc25、歐索林酸、維利黴素及嘉賜銅等處理的發病率分別為 91%、10%、30%、29% 及 16%，統計分析顯示 φXcc25 處理具有顯著的防治效果，與藥劑處理無顯著差異。本研究結果顯示噬菌體 φXcc25 可以有效防治甘藍黑腐病，具有作為十字花科黑腐病防治資材的潛力。

C02 建立*Bacillus amyloliquefaciens* PMB04的發酵液配方於茄科細菌性斑點病之防治－<u>王菲</u>[1]、林宜賢[1] ([1]國立屏東科技大學植物醫學系)

Establishment of fermentation liquid formula of ***Bacillus amyloliquefaciens*** PMB04 to control bacterial leaf spot－W, F., and Lin, Y.-H.* ([1]Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung)

甜椒是世界上重要作物之一，在臺灣以中部地區為主要產區。甜椒栽培過程中，由 ***Xanthomonas perforans*** 所造成之細菌性斑點病會造成甜椒品質及產量嚴重降低。此病害通常以氫氧化銅等銅劑作為主要防治資材，長期施用銅劑容易導致抗病菌株產生，因此開發可防治病害之微生物製劑有其必要性。本研究擬應用已證明對十字花科黑腐病及草莓炭疽病具有良好拮抗效果之 *Bacillus amyloliquefaciens* PMB04 菌株，評估其在防治甜椒細菌性斑點病之潛力並建立其發酵液配方。首先，測試 PMB04 對不同病原菌之拮抗能力，結果顯示 PMB04 在平板上可抑制多種病原菌之生長，其中又以對 ***X. perforans*** 之拮抗效果最為顯著，且對 ***X. perforans*** 之不同菌株亦具有良好拮抗能力。進一步利用 PMB04 之細菌懸浮液處理，可在甜椒植株上降低細菌性斑點病之發生。為了提升 PMB04 之菌量及產胞率，以 PMBFL-2A 作為基礎發酵配方，分別利用 500mL 搖瓶及 30 公升發酵槽進行調整。結果顯示，於 500mL 搖瓶發酵中以碳氮比 0.5:0.5 及 1:1 之配方發酵後可達 100% 之產胞率。進一步利用 30 公升發酵槽測試不同配方對於 PMB04 拮抗能力之影響，結果顯示碳氮比 3:1、2:1 以及 0.5:0.5 之配方均可達 100% 之產胞率。隨後在接種測試的結果中顯示碳氮比 0.5:0.5 及 2:1 之配方可減少 35% 之罹病度。接著將 0.5:0.5 配方獲得之發酵液進行 200、500 倍稀釋處理，接種後之罹病度為 30% 及 40%，皆顯著低於對照組。為了瞭解 PMB04 與不同藥劑在田間共同使用之潛力，將 PMB04 與不同藥劑進行拮抗測試，結果顯示其他常見藥劑及銅劑均對 PMB04 沒有抑制效果，表示 PMB04 可能具有與藥劑共同使用之潛力。此外，為了瞭解細菌性病害常用之氫氧化銅與 PMB04 發酵液之相互影響，將氫氧化銅與前述 0.5:0.5 配方之發酵液共同處理於甜椒上，結果顯示單處理氫氧化銅之罹病度與單處理發酵液之罹病度均為 40%，共同處理之罹病度為 45%，三者間無顯著差異且皆顯著低於對照組，由此說明 PMB04 發酵液與氫氧化銅共同施用並無加成效果。最後，為了探討 PMB04 對病害防治之機制，利用 iturin、surfactin 及 fengycin 之引子對進行基因檢測，結果顯示 PMB04 具有合成 iturin 之基因。綜上所述，本研究所建立之 PMB04 發酵液配方可防治細菌性斑點病之發生，其確切機制仍需進一步探討。

C03 淹水與菌根菌接種對番茄根瘤線蟲感染力的影響－蘇俊峯[1]、林素禎[2]、簡蘭懿[1]、顏郁真[1]([1]農業試驗所植物病理組、[2]農業試驗所農業化學組)

Effects of flooding and inoculating mycorrhizal fungi on the infestation of tomato root-knot nematode.－Su, J. F.[1], Lin, S. J.[2], Chien, L. Y.[1], and Yen, Y. C.[1] ([1]Plant Pathology Division, and [2]Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung)

植物寄生性根瘤線蟲 (***Meloidogyne*** spp.,) 寄主範圍廣泛，可危害多種作物，包括番茄，嚴重時可造成 100% 的經濟損失。根瘤線蟲以可移動性的二齡幼蟲 (2nd stage juveniles, J2)，利用口針及分泌細胞壁分解酵素成功的侵入植株根部組織。幼蟲在根部組織內取食、成長後繁衍後代，並可導致該處組織細胞增多、增大，造成根瘤的病徵。近年來，因環保意識抬頭與施用後有藥害疑慮的影響，許多殺線蟲劑與土壤燻蒸劑

紛紛的從病害防治市場上消失。尋找可行防治根瘤線蟲的替代方案，成為線蟲研究工作者的首要目標。本研究將探討淹水與植前菌根菌處理對番茄根瘤線蟲感染力的影響。首先，將根瘤線蟲卵塊接種到組織培養的空心菜根系上，接種 60 天後，可於顯微鏡下觀察到有根瘤產生。將該些根片段放置到含清水玻璃皿中，第 1 天即有 J2 孵化出，平均每卵塊可孵化 8.3 隻 J2，卵塊在泡水狀態持續會有 J2 孵化出，直到第 70 天才停止。利用挑針將 J2 挑離含水地方，使蟲體乾燥處理一天，則該些線蟲即呈現不具活動力，活動力指數呈現 0%。將 J2 泡在水中 16 天，再以挑針刺激線蟲體表，則皆無反應，而泡水 14 天的 J2，其對感染番茄苗株根系的能力會有顯著的下降。據此，在植株種植前的整地，或可以淹水 14 天以上來降低田間根瘤線蟲的感染力。另一試驗取四株菌根菌菌株，包括*Claroideoglomus etunicatum* (LETC)、*Funneliformis mosseae* (LMOS)、*Rhizophagus clarus* (LCLM) 與*Rhizophagus intraradice*s (LITR)。供試菌根菌先行感染供試番茄植株農友 301 幼苗後，每種菌根菌處理再分接種南方根瘤線蟲 J2 與否的處理，每處理 15 盆，每盆接種 100 隻 J2。接種根瘤線蟲 5 週後，未接種菌根菌之對照組結瘤率為 18%，而接種菌根菌 LMOS 處理組的結瘤率為 0.7%、LETC 為 0.9%、LCLM 為 4.6%、與 LITR 為 9.2%。換算結瘤抑制率 LMOS 抑制率為 96%、LETC 為 95%、LCLM 為 73% 與 LITR 為 46%。然而，在比較各菌根菌處理有接種線蟲與否的植株株高、葉片數與地上部鮮重時，彼此之間並無明顯的差異。據此，推論接種 LMOS 與 LETC 可抑制根瘤線蟲感染的可能的機制，乃在於啟動植株的抗病性。

C04　不同土壤覆蓋栽培方式對杭菊萎凋病害之影響－劉東憲[1]、劉秋芳[2]、蔡正賢[1]、林鈺荏[1]、李吉峰[1] ([1]行政院農業委員會苗栗區農業改良場、[2]行政院農業委員會茶業改良場)

The influence of different soil covering cultivation methods on chrysanthemum wilt disease－Liu, T. H.[1], Liu, C. F.[2], Tsai, J. H.[1], Lin Y. R.[1], and Li, J. F.[1] ([1]Miaoli District Agricultural Research and Extension Station, Miaoli; [2]Tea Research and Extension Station, Taoyuan)

許多杭菊以覆蓋塑膠銀黑布的方式栽培解決雜草的問題，不然則是較費工完全除去雜草使表土裸露方式。近來常發生大規模杭菊萎凋枯死導致歉收，尤其是在梅雨季以及常降雨的夏季之後，導致萎凋的病害紀錄有許多種，從農藥殘留統計出最常被使用為疫病的防治藥劑。在不同土壤覆蓋栽培試驗採用 3 種處理：覆蓋塑膠銀黑布、除去雜草再以稻草覆蓋、添加苦土石灰改良土質，經過調查以覆蓋塑膠銀黑布能降低土溫，保持水分含量，在株高、株幅表現最好，優於其他 2 種處理，不過經稻草覆蓋後，杭菊生長情形明顯好轉，另外比較土壤檢測結果發現若以慣行肥量施肥，則有土壤有明顯酸、鹽化現象，尤其發現農友習慣於塑膠布的開口直接追肥、澆灌水分，容易造成肥傷、根系腐爛，被塑膠布覆蓋的表土累積的白色鹽分結晶，僅於塑膠布開口範圍沒有鹽化現象，經比較有逐步擴大塑膠布開口和沒有擴大的杭菊根系，塑膠布開口為直徑 25 公分時，根幅範圍僅直徑 27 公分，根發展明顯受到侷限，如農民又直接追肥的田區萎凋情形比例很高，杭菊存活率只剩 60~70%，若隨杭菊長大逐步擴大塑膠布開口或是改用稻草覆蓋方式，試驗區在沒有農藥使用下就能提升存活率至 95% 以上，故可推估大部分萎凋由土壤酸、鹽化造成。

C05　利用微波輔助萃取法改善硫鐵蛋白誘導型蘇雲金芽孢桿菌 *Bacillus thuringiensis* HS1 保護植物對生物性與非生物性逆境抵抗能力－蔡佳祐[1]、張育誠[1]、賴擴安[1]、邱詩茜[1]、黃祥恩[1] ([1]國立臺東大學生命科學系)

Using microwave assisted extraction (MAE) to improve the ferredoxin inducing *Bacillus thuringiensis*s HS1 to protect plant resistance to biotic and abiotic stress.－Tsai, J. Y.[1], Chang, Y. C.[1], Lai, K. A.[1], Chiu, S. C.[1], and Huang, H. E.[1] ([1]Department of Life Science, National Taitung University, Taitung)

硫鐵蛋白 (Ferredoxin, Fd) 是植物體內負責電子傳遞的蛋白，其藉由氧化還原反應控制眾多酵素活性，能幫助植物生長發育並幫助植物提升對逆境的抵抗力。過去研究中顯示藉由基因工程技術增量表現Fd異構蛋白表現量，能提升植物對於生物性與非生物性逆境的抗性。但直至今日基轉作物的安全性仍然受到外界質疑，為避免基因轉殖的安全疑慮，本研究從台東土壤中分離出 HS1 土壤分離株，發現HS1具抑制多種植物病原真菌生長與分解蛋白質及纖維素的能力。並具有誘導提升阿拉伯芥 *AtFd1*、*AtFd3* 及番茄 PT-Fd 基因表現量的能力，也會提升 ABA、乙烯、JA 及 SA 抗性路徑相關基因。在模式植物阿拉伯芥上，經過 HS1 處理後能提升對葉斑病菌 *Pseudomonas syringa*e DC3000的抵抗能力，同時增加45 ℃高溫抗性。而HS1的澆灌能幫助番茄抵抗青枯病菌 *Ralstonia solanacearum* Rd4的感染，以及提升番茄抵抗淹水逆境。在冬季 (11~2月2019) 溫室試驗中，HS1能提升番茄植株高度、青果對病原真菌抗性並降低果實大小，而在夏季 (5~7月2020) 會提升番茄植株高度、葉片數、繁殖組織數。為了避免使用高濃度菌體對於環境與人體可能造成的不確定風險，以及活菌因環境不穩定因素而影響HS1的保護效果，本研究發展出微波輔助萃取法 (Microwave Assisted Extraction，MAE) 來處理HS1，實驗結果發現經過MAE處理後的HS1死亡菌體依然可以保有誘導番茄葉部與根部型硫鐵蛋白及JA抗性路徑*LeCOI1*基因表現，但是卻無法有效提升*LePR1*的基因表現量。而如果使用翼豆培養基進行 HS1 的增量培養，則只能看到POD酵素活性的上升，其他效果均不明顯，推測此結果可能由於翼豆培養基可以直接在番茄誘導*LeCOI1*表現卻抑制了 SA 抗性路徑*LePR1*的表現。上述研究結果顯

示，通過 HS1 誘導確實能產生與基因轉殖增量表現Fd相似的保護結果，而且MAE的處裡也能在保留HS1效果的狀況下有效去除菌體的活性。但如果使用翼豆培養基來增量培養HS1 則只能有效增強JA及ABA路徑相關的抗性，而無法保護SA抗性相關路徑病原造成的感染。

C06 *Pseudomonas chlororaphis* as a potential biocontrol agent against Panama disease caused by ***Fusarium oxysporum*** f. sp. ***cubense*** — Sartika, L.[1,2], Yang, Y.-L.[3], Yeh, H.-H.[3], Sulistyowati, L.[2], Aini, L. Q.[2], and Shen, W.-Q.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]University of Brawijaya, Indonesia; [3]Agricultural Biotechnology Research Center, Academia Sinica)

Fusarium oxysporum f. sp. *cubense* (Foc) is the causal agent of banana soil-borne disease, known as Panama disease. The emergence of tropical race 4 (TR4) strain of Foc has posed serious threat to worldwide banana production. Management strategies such as the use of resistant varieties, fungicides, field sanitation, and other cultural practices have been suggested for Panama disease. Additionally, biocontrol agents have been considered to be environmentally friendly and can be implemented to sustain banana production. In our laboratory, one of the bacteria isolated from banana roots in an organic field was identified as *Pseudomonas* sp. AH1E1, which showed potent antagonistic effect and promising biocontrol ability against Foc TR4. In this study, using several housekeeping genes such as atpD, carA, recA, 16s rRNA, gyrB, rpoB, and rpoD, we further identified AH1E1 as *Pseudomonas chlororaphis* subsp. *aurantiaca*. Biochemical assays demonstrated that this strain exhibited various traits for plant growth promotion and ecological fitness, including the activities of siderophore, indole acetic acid, oxidase, and phosphate solubilization. LC-MS/MS analysis were performed with the dual culture samples and antifungal compounds such as pyrrolnitrin and pyochelin produced by P. chlororaphis AH1E1 were identified. Two operons, potentially responsible for the biosynthesis of pyrrolnitrin and pyochelin, were also identified from our sequenced genome. Colonization study with GFP-tagged strain was also conducted under laboratory condition. The presence of this bacterium in banana rhizosphere was also detected from the original organic field using the specific primers of 16s rRNA gene. Interestingly, this strain efficiently colonized the rhizosphere of banana roots and also grew limitedly inside root tissues. Pot biocontrol assay by soil drenching method showed *Pseudomonas chlororaphis* AH1E1 efficiently suppressed the disease. In the future, we hope to further develop a formulation and protocol of this candidate bacterium for field application.

C07 放線菌菌株W於田間防治香蕉黃葉病之效果評估－段國仁[1]、陳以錚[2]、郭洲獎[3]、楊晴晴[2] ([1]大同大學化學工程與生物科技學系、[2]財團法人臺灣香蕉研究所技術服務組、[3]大同股份有限公司)

Effectiveness evaluation of ***Streptomyces*** sp. W to bio-control on Banana Panama disease in field－Duan, k. J.[1], Chen, Y. J.[2], Kuo, C. C.[3], and Yang, C. C.[2] ([1]Department of Chemical Engineering and Biotechnology, Tatung University, Taipei; [2]Division of Technical Service, Taiwan Banana Research Institute, Pintung; [3]Tatung Company, Taipei)

香蕉 (banana) 為臺灣重要之果樹，主要栽培三倍體之北蕉 (Canvendish, AAA) 品系，近年栽培面積超過1萬6千公頃。由尖鐮胞菌古巴分化型熱帶生理小種第四型 (***Fusarium oxysporum*** f. sp. ***cubense*** tropical race 4, TR4) 引起之香蕉黃葉病 (Panama disease) 為香蕉最重要之病害，黃葉病目前無化學防治方法，且因病原菌為土壤傳播性 (soil-borne) 病原，一旦入侵田區及難以防除。因此，近年以有益微生物作為生物防治手段之方法漸被重視。本研究先前自臺灣北部地區農田土壤中篩選出來出一株在培養皿上對 TR4 具良好抑制效果之放線菌 (***Streptomyces*** sp.)，菌株代號W；經碳氮源需求測試發現其對葡萄糖、麥芽糖及大豆蛋白利用效率佳。進一步並完成大槽體液態發酵製程及粉劑開發。後續研究發現 ***Streptomyces*** sp. W 具溶磷能力，可分泌多種胞外酵素及產生吲哚乙酸 (indole3-acetic acid, IAA)，後續證實該菌株發酵液可促進纏據於香蕉根圈並促進香蕉幼苗生長。本研究進一步探討 W 菌株防治黃葉病之潛力。將北蕉組織培養苗於溫室馴化 1 個月後，定植於 5 吋塑膠軟盆，盆中含有 1.5 x 10^4 propagule/g soil TR4 isolte 272 之沙土，再澆灌經無菌水調整濃度為 10^8 cfu/ml W 菌株發酵液 30 ml 完成處理，並以澆灌無菌水為對照組，每株 1 盆，每處理 20 株。定植後於溫室 (25-35 ℃) 培養，每月觀察黃葉病罹病度。罹病度系將外部病徵訂為 0-4 個等級，0 為健康，1 為矮化或下位葉 1 葉黃化，2 為 2-3 葉黃化或假莖綜列，3 為 3 片以上到 1 半的葉片黃化，4 級 1 半以上葉片萎凋到植株死亡。結果第 3 個月時發現，W 菌株處理之北蕉，黃葉病平均罹病級數為 0.42 顯著低於對照組 0.85 ($p = 0.05$)；2020 年 12 月於南州黃葉病連作罹病田 (土壤病原菌濃度約 4 x 10^4 propagule/g soil) 進行田間效果評估。將馴化後之北蕉穴盤苗 (每盤24株) 浸泡於含有 10^8 cfu/ml W菌株發酵液 25L 之 100 L 方形水槽 1min 後完成接種，並以浸泡自來水之處理為對照組。浸泡後將北蕉苗以條區試驗設計 (split-block design) 方式定植於試驗田，浸根與非浸根處理各 4 畦，每畦 20 株；定植後，再各取 2 畦每月於根圈追加 10^8 cfu/ml W 菌株發酵液 500mL，共添加 4 次。結果發現，第三個月黃葉病罹病度在四種處理間分別為對照組 17.5 %、無植前處理但每月追加 10.0%、僅植前處理者 2.0 %、經

植前處理並每月追加 0；四處理之株高則分別為 49.3、64.9、63.0 和 70.4 cm；綜上，放線菌菌株 W 可在田間促進香蕉生長，並具有防治香蕉黃葉病之潛力，後續將評估其商品化之可行性。

C08 應用電漿活化水對草莓灰黴病之防治效果評估－許雅真[1]、林依佳[1]、蕭駿平[2]、黃濬佑[1]、吳宗信[2]、林盈宏[1,3] ([1]國立屏東科技大學植物醫學系、[2]國立交通大學機械工程學系、[3]國立屏東科技大學植物醫學教學醫院)

Evaluation of the effects of plasma activated water on control of strawberry gray mold－Xu, Y. Z.[1], Lin, Y. J.[1], Hsiao, C. P.[2], Huang, J. Y.[1], Wu, J. S.[2], and Lin, Y. H.[1,3] ([1]Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung; [2]Department of Mechanical Engineering, National Chiao Tung University, Hsinchu; [3]Plant Medicine Teaching Hospital, National Pingtung University of Science and Technology, Pingtung)

草莓 (*Fragaria* x *ananassa* Duch.) 具特殊風味及豐富營養價值，為全球重要園藝果菜類作物之一。草莓於栽種過程中，容易受到灰黴病菌 (*Botrytis cinerea*) 之潛伏感染，遇到合適之生長環境時極易發病，嚴重危害草莓果實，為儲藏過程中一項限制因子。目前對於草莓採收後貯藏期間之病害防治，多是以化學藥劑進行處理。近幾年因病原菌對藥劑產生抗藥性，對於環境、食品安全之意識逐漸抬頭，實現化學農藥施用減量為現今重視議題。本試驗擬利用電漿活化水 (Plasma activated water, PAW) 含有效抑菌物質的特性，希望藉由電漿活化水技術來防治灰黴病菌。由試驗結果證實，電漿活化水能抑制灰黴病菌 (*B. cinerea*) 之生長，並於電漿活化水的特定製備條件下，抑菌率可達 90% 以上。此外，我們也發現處理電漿活化水能降低草莓灰黴病菌人工接種的發病率。綜上所述，處理電漿活化水能對草莓灰黴病菌達到抑菌效果，且能延緩人工接種的果實上病斑之發展。電漿活化水技術，未來或許能成為替代化學藥劑施用減量之作物病害防治方法，進而降低生態汙染與延緩病害發生。

C09 Rhizosphere microbiota and its potential application in biocontrol of the roselle wilt disease－Yu, Y. H.[1], Wang, C. W.[2,3,4,5], Feng, R. Y.[6], Chen, Y. L.[1,6], and Tang, S. L.[3,4,5,7] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Taitung District Agricultural Research and Extension Station, Taitung; [3]Biodiversity Research Center, Academia Sinica, Taipei; [4]Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, National Chung Hsing University and Academia Sinica, Taipei; [5]Graduate Institute of Biotechnology, National Chung Hsing University, Taichung; [6]Master Program for Plant Medicine, National Taiwan University, Taipei; [7]Biotechnology Center, National Chung Hsing University, Taichung)

A roselle (*Hibiscus sabdariffa* L.) disease survey conducted recently in Taiwan reported that roselle wilt disease occurs widely; however, the causal agent was unknown. The stems of wilted roselle were browned, slightly constricted, and showed white aerial hyphae. Rotted pith was found in the vertically dissected stem base and macroconidia and microconidia typical of the *Fusarium* species were observed under a microscope, indicating that roselle wilt might be caused by *Fusarium* species. In this study, we isolated 119 strains from wilted plants grown in six roselle fields in Taitung county, Taiwan. Koch's postulates were used to evaluate the pathogenicity of these strains, among which we found that *Fusarium solani* K1 (FsK1) can cause wilting and rotted pith on roselles similar to those observed in the fields. This is the first demonstration that *F. solani* can cause roselle wilt in Taiwan. On the other hand, microbiome study was used to decipher the microbial composition in healthy and diseased roselle rhizosphere. By an overview of taxonomic composition plot, we found that family Nectriaceae showed higher abundance in diseased than healthy rhizosphere in all 3 fields in Taitung, Zhiben, and Taimali. The ITS sequences of isolated 119 putative pathogens were blasted to fungus OTU sequences. The otu_JX371352 hits most of the putative pathogens' sequences (with approximately 221 bp), and those strains were *F. solani*, which can serve as another evidence that *F. solani* was the main pathogen caused the roselle wilt disease. Furthermore, *Bacillus velezensis* SOI-3374, a potential biocontrol strain isolated from healthy roselle rhizosphere showed unsurpassed anti-FsK1 activity, which can use as potential biocontrol strategy against roselle wilt disease in the future.

C10 非農藥防治資材對西洋南瓜貯藏性病害防治效果評估－羅佩昕[1]、林煜恒[1]、賴奕佐[1] ([1]行政院農業委員會臺中區農業改良場)

The efficacy of non-pesticide materials on controlling postharvest diseases of pumpkin－Lo, P. H., Lin, Y. H., and Lai, Y. T. (Taichung District Agricultural Research and Extension Station, Changhua)

西洋南瓜 (*Cucurbita maxima*) 又稱栗子南瓜，是臺灣常見的南瓜栽培種類，南瓜果實採收後，放置於溫度10-13℃環境下，可貯藏 2-5 個月。然於臺灣地區，南瓜果實貯藏期間常因 *Phomopsis* sp.、*Colletotrichum* sp.、*Fusarium solani* f. sp. *Cucurbitae* 及 *Fusarium* sp. 等病原菌引起果實腐爛，影響商品價值進而造成損失。因此，為減少西洋南瓜貯藏期間，因病害引起致果實腐爛，本研究以非農藥防治資材於南瓜貯藏前進行處理，並低溫模擬貯藏 3 個月，測試分別以不同濃度之次氯酸水與幾丁聚醣處理後，對南瓜貯藏性病害之防治效果，並進行

果實品質分析，包含總可溶性固形物、澱粉、粗纖維及粗蛋白含量，以瞭解非農藥防治資材是否影響南瓜果實品質。結果顯示，於南瓜貯藏第 3 個月，40 ppm 之次氯酸水經 100 倍與 500 倍稀釋處理後，可有效降低病害發生，而幾丁聚醣則 以0.1% 和 0.01% 幾丁聚醣溶液處理，可有效降低病害發生，其中又以幾丁聚醣較次氯酸水處理，可減少南瓜貯藏性病害之發生，於貯藏第 3 個月可降低貯藏性病害罹病率達 41.66%。另不同非農藥防治資材處理對西洋南瓜果實品質分析結果顯示，次氯酸水與幾丁聚醣於貯藏前處理南瓜果實，對貯藏之果實品質無影響。

C11 臺灣青蔥疫病防治之研究－黃晉興、袁琴雅 (行政院農業委員會農業試驗所植物病理組)

Study on disease management of green onion diseases caused by *Phytophthora nicotianae* in Taiwan－Huang, J.-H., and Yuan, C.-Y. (Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung)

臺灣青蔥主要產區在彰化縣與雲林縣，栽培面積佔全臺 70% 以上。近年來臺灣發生強降雨的頻率增加，造成上述地區由 *Phytophthora nicotianae* Breda de Haan (syn. *Phytophthora parasitica* Dastur) 引起之青蔥疫病非常嚴重，常導致作物根腐、莖腐、葉枯，甚至死亡。為篩選防治資材，先於培養基上測試化學藥劑對青蔥疫病菌菌絲生長之影響，藥劑對青蔥疫病菌絲生長抑制有效濃度 (EC_{50}) 小於10mg/L 者有凡殺克絕、氟比拔克、銅滅達樂、賽座滅、依得利、達滅芬、安美速、曼普胺、滅達樂、達滅脫定與鋅錳座賽胺。進一步以人工接種盆栽青蔥測試供試藥劑與資材對青蔥疫病之防治潛力，施用化學農藥中的氟比拔克、銅滅達樂、曼普胺、滅達樂、達滅芬、達滅脫定或鋅錳座賽胺等處理的發病度 6.1 15.6%，低風險資材中的亞磷酸新配方或中性亞磷酸等處理的發病度 24.4 35.6%，以及微生物製劑中的液化澱粉芽孢桿菌製劑或蕈狀芽孢桿菌製劑等處理的發病度 28.1 35.4%，與對照組的發病度 62.4% 皆有顯著差異，較具防治潛力。在 2019 年的田間試驗中，亞磷酸新配方或氟比拔克處理的發病度分別為 12.8% 或 16.5%，皆與對照組發病度 29.3% 有顯著差異，而液化澱粉芽孢桿菌製劑處理的發病度為 21.6% 則與對照組無顯著差異。以人工接種盆栽青蔥測試氟比拔克農藥、亞磷酸新配方、液化澱粉芽孢桿菌製劑之施用時機對疫病的防治效果，結果顯示氟比拔克農藥在接種病原菌前 14 天至接種後 1 天施用 1 次之發病度為 5.2 11.5%、亞磷酸新配方在接種前 14 21 天施用 1 次之發病度為 21.3 25.0%、液化澱粉芽孢桿菌製劑在接種前 1 4 天施用 1 次之發病度為 42.6 42.9%，與對照組 67.0% 有顯著差異。在 2020 年的田間試驗中，四至五月每 10 14 天施用一次亞磷酸新配方、五月下旬梅雨季前 7 天內施用 2 次及梅雨中後施用各 1 次氟比拔克農藥，以及綜合上述兩種措施皆能顯著減少發病度與產量損失，發病度分別為 25.9%、24.4%、19.3%，皆與對照組 53.1% 有顯著差異，而產量分別為 19.8 kg/plot、19.2 kg/plot、24.0 kg/plot，皆與對照組 9.7 kg/plot 有顯著差異，其中又以綜合施用的防治效果顯著優於與其他處理。本研究顯示平時施用亞磷酸新配方，加上雨季前中後施用化學農藥，可減少病害的發生與產損失，並可避免長期施用防治疫病的化學農藥。

C12 油茶葉枯病防治資材篩選－呂柏寬[1]、林瑞珍[1]、陳宜豐[2] ([1]行政院農業委員會花蓮區農業改良場、[2]國立嘉義大學植物醫學系)

The control materials screening of oil tea leaf blight disease－Lu, P. K.[1], Lin, R. C.[1], and Chen, Y. F.[2] ([1]Hualian District Agricultural Research and Extension Station, Hualien; [2]Department of Plant Medicine, National Chiayi University, Chiayi)

油茶 (*Camellia oleifera*) 上之新紀錄病害葉枯病係由 *Haradamyces foliicola* 造成之病害，葉片病徵型態分為兩型，一為白色至褐色圓形病斑，病斑呈乾枯狀，病斑外圍有褐色至深褐色暈環，黃暈不明顯，病斑成型後不容易向外擴展，葉片不會掉落；二為黃褐色圓形病斑，病斑呈軟腐狀，病勢發展迅速，產生此種病斑之葉片極易掉落，田間的病斑，會產生灰白色鈕扣型之繁殖體 (propagules)，嚴重發病植株幾乎全株落葉，造成樹勢衰弱死亡。室內藥劑篩選試驗以微量平板法做測試，以保存菌株 HL_CO-31、HL_CO-35、HL_CO-41 及 HL_CO-42 進行室內藥劑篩選，測試非農藥資材為 4-4 式波爾多液、亞磷酸中和液 1000 倍、80% 可濕性硫磺 1000 倍，以及化學農藥 25.9% 得克利、75% 嘉保信 1600倍、23% 亞托敏 2000 倍、84.2% 三得芬 1000 倍、33.5% 快得寧 1000 倍、70% 甲基多保淨、40% 克熱淨 1500 倍、5% 保粒黴素丁 2000 倍、39.5% 扶吉胺 2000 倍，進行三日菌絲生長抑制試驗。試驗結果顯示非農藥資材菌絲生長抑制率均低於 65% 以下，抑制效果不明顯，而一般化學合成農藥試驗結果顯示得克利、三得芬、快得寧、甲基多保淨、扶吉胺均可達 95% 以上之抑制率。自抑制率較佳藥劑中，選用油茶可使用之得克利進行盆栽防治試驗，分為接種前一日與接種後一日施藥全株，植株以快速接種法進行病原菌接種，無菌 PDA 塊接種作為對照組，每處理 4 重複，觀察 5 日後其落葉率。試驗結果顯示接種後施用得克利之落葉率為 23.5%，接種前施用得克利之落葉率為 20.5%，對照組落葉率為 100%，顯示於盆栽試驗中，得克利具防治油茶葉枯病之效果，而接種前後施藥處理未影響其防治成效。

學生競賽SA組

SA01 Evaluation of *Luffa* accessions for resistance to *Fusarium oxysporum* f. sp. *Luffa*－Namisy, A.[1], Chung, W.-H.[1, 2] and Rakha, M.[3] ([1]Department of Plant Pathology, National Chung Hsing

University, Taichung; [2]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung; [3]Horticulture Department, Faculty of Agriculture, University of Kafrelsheikh, Kafr El-Sheikh, Egypt)

Fusarium wilt caused by ***Fusarium oxysporum*** is a serious plant disease that causes great loss to cucurbits worldwide, such as watermelon, cucumber, luffa, etc. Among these cucurbit Fusarium wilt, the ***F. oxysporum*** f. sp. ***luffae*** is limitation factor to produce luffa. Grafting major cucurbit crops onto Luffa rootstock has been reported but resistance to soil-borne diseases in Luffa is largely unknown. The objective of this study was to screen 20 accessions belong to ***Luffa aegyptiaca*** and 8 accessions from ***Luffa aegyptiaca*** for resistance to ***Fusarium oxysporum*** f. sp. ***luffae*** isolate (Folust). Folust is a very high virulent Fusarium strain which isolated from Luffa rootstock in Nantou in center of Taiwan. Among 28 Luffa accessions evaluated, two accessions (L30 and L26) of ***Luffa aegyptiaca*** were completely resistant to (Folust) with no disease symptoms. In addition, three accessions of ***Luffa aegyptiaca*** (L7, L9, and L11) were highly resistant with disease index ranged from (4.4 to 6.7%) and six accessions were moderately susceptible with disease index (43.3 to 49.3%). Resistant accessions were selected and further evaluations for resistance to more isolates of ***Fusarium oxysporum*** f. sp. ***luffae***. These finding might useful for breeding resistant Luffa rootstocks and cultivars that can be used to manage this endemic disease.

SA02 Characterization, pathogenicity, and phylogenetic analyses of fungal species associated with Welsh onion foliar diseases in Sanxing, Taiwan—Wang, J.-Y.[1], Tsai, I.[1], Wang, C.-H.[2], Lin, Y.-C.[1], Hsu, C.-H.[1], Cho, Y.-T.[1], Hung, T.-H.[1], Tsai, Y.-C.[2], and Ariyawansa, H. A.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Hualien District Agricultural Research and Extension Station, Hualien)

Welsh onion (***Allium fistulosum*** L.) is one of the main vegetable crops in Taiwan. Comparing to other crop growers, Welsh onion planters are concern more on leaf diseases. Leaves of Welsh onion are subject to various fungal diseases such as anthracnose; purple blotch; rust and Stemphylium leaf blight (SLB). Purple blotch was considered the most prevalent foliar disease of Welsh onion in Taiwan. However, during 2018–2020, leaf blight symptoms somewhat similar to those described for purple blotch caused by ***A. porri*** were observed throughout Welsh onion fields in Sanxing, Taiwan. The severity of the disease varied among cultivars, causing up to 10% yield losses in most conventional and organic Welsh onion commercial fields. The initial symptoms consisted of either small, yellowish-brown to tan water-soaked lesions or whitish lesions with purple centres, which are very similar to purple blotch. When the disease was severe, the leaf spots merged and the leaves turned prematurely chlorotic and senescent, ultimately drying up and resulting in leaf dieback. For the past few decades, continuous application of fungicides recommended by Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI) such as chlorothalonil, difenoconazole and iprodione was carried out to manage the foliar disease of Welsh onion fields in Sanxing, Taiwan. Moreover, in our recent field practice, we observed that these recommended fungicides and their commercial doses do not control the disease. However, correct species identification, epidemiology and control of leaf blight of Welsh onion is not well established, therefore, the main objective of this study was to investigate the causal agents of Welsh onion leaf blight in Sanxing and determine their pathogenicity. In this study, we surveyed the 27 Welsh onion fields located in seven villages commercial in Sanxing, Yilan. In total 769 diseased leaves were collected and among them, 394 lesions caused by ***Stemphylium*** sp. (51%), and 359 lesions caused by ***Colletotrichum*** sp. (47%). In the present study, 82 fungal isolates were obtained and preserved. During the field observation, two major types of lesions were observed, of which ***S. vesicarium*** caused limited oval leaf spots in light to dark brown, with a darker spot core producing spores, and ***Colletotrichum*** species caused light to dark brown leaf spot without a certain size and usually covering the whole leaf. Remarkably, we did not obtain ***Alternaria porri*** which was used to be the major pathogen causing leaf blight in Taiwan onion felids in our survey. The morphological identification was based on features such as fungal colony, sexual or asexual spores and reproducing structures. Moreover, the molecular-based identifications were carried out via multi-locus sequence analysis, and the isolates were differentiated up to species level via polyphasic approaches. DNA alignments of multi-gene data set consists of ITS, ***gapdh***, ***cal***, and ITS, ***tub2***, ***gapdh***, were used to developed phylogenetic trees for ***Stemphylium*** and ***Colletotrichum***, respectively. The isolates frequently obtained from fields were identified as ***Stemphylium vesicarium***, ***Colletotrichum spaethianum*** (***C. spaethianum*** species complex), and ***C. circinans*** (***C. dematium*** species complex). To determine and compare the pathogenicity of each species, the inoculation of fungal isolates on the cultivar 'Siao-Lyu' was performed by spraying spore suspension onto the leaf surface. In this study, the Welsh onion plants were susceptible to all three species. Based on the results of disease incidence, we observed that Welsh onion plants were more susceptible to ***C. spaethianum*** compared to the other two species. Further, the mature leaves were more prone

for leaf blight compared to younger leaves. Fungal strains used in the pathogenicity assay were re-isolated from infected leaves to determine their identity in order to confirm Koch's postulates and pathogenicity of ***S. vesicarium***, ***C. spaethianum*** and ***C. circinans***. In the present study, we carried out a comprehensive investigation and revealed the pathogenic association towards the Welsh onion foliar disease at fields in Sanxing, Yilan, Taiwan. This progress builds the key reference to developing accurate strategies for disease management.

SA03 Characteristics of ***Bacillus amyloliquefaciens*** R8-43 on inhibition of ***Alternaria brassicicola*** and control of black spot on cabbage－Wang, S.-Y.[1], and Chung, W.-H.[1,2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Innovation and Development Center Sustainable Agriculture, National Chung Hsing University, Taichung)

Cabbage (***Brassica oleracea*** var. ***capitata***) is the most common leafy vegetable. In Taiwan, there are 8,000 to 9,000 ha for cabbage production and the major plating area locate in Yunlin, Changhua and Yilan. Many microorganisms can cause diseases during the growth period of cabbage, such as fungi, bacteria, virus and nematode. Among the fungal diseases, black spot caused by ***Alternaria brassicicola*** in Taiwan is important pathogen that could be transmitted by air or seed. Presently, fungicides, such as Polyoxins, are considered as strategy for controlling black spot. For safety of cabbage production, the biological control is the alternative method. Previous study indicated the endophytic ***Bacillus*** strains from banana have the efficacy on control of anthracnose in Chinese cabbage and may induce the disease resistance. For confirming the phenomenon, we tested these ***Bacillus*** strains whether have similar potential for control of black spot in cabbage. Results showed that ***B. amyloliquefaciens*** R8-43 strain showed the good ability to inhibit mycelia growth of ***A. brassicicola*** ABA-1 isolate. Further study indicated that the filtrate of R8-43 strain also have efficacy on growth inhibition of ABA-1 isolate. Although fermentation liquid of R8-43 strain could not reduce spore germination of ABA-1 isolate, it could induce the hyphae showed malformation, such as tip swelling or vesicle-like deformation. The phenomenon might result the cell wall thinning and leakage. Moreover, filtrate of R8-43 strain is heat tolerant to 100°C. In the greenhouse condition, the fermentation liquid of R8-43 strain could reduce the severity of black spot significantly in cabbage. Simultaneously, the R8-43 strain could be detected on surface of cabbage leaf for 21 days after sprayed. This result demonstrated that R8-43 strain has ability to survive on cabbage leaf for long time. These results indicated that R8-43 and its metabolites has biocontrol potential to against the black leaf spot.

SA04 Molecular detection of ***Fusarium oxysporum*** f.sp. ***rapae***.－Chu, H.-H.[1], and Wang, C.-L.[1] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung)

There are four formae speciales of ***Fusarium oxysporum*** that cause yellows of cruciferous plants. They are distinguished by VCG groups, pathogenicity, and phylogenetic analysis. However, formae speciales may overlap in host ranges due to cross breeding between cruciferous crops. It becomes a challenging task to determine formae speciales of cruciferous pathogens. Recently, molecular detection has been developed to quickly identify specific formae speciales. Specific primers for ***F. oxysporum*** f. sp. ***conglutinans*** (FoCN) and ***F. oxysporum*** f. sp. ***raphani*** (FoRF) have been developed, respectively. ***F. oxysporum*** f. sp. ***rapae*** (FoRP) caused severe yellows of leaf mustard, radish, and pakchoi in Taiwan, but there are only few studies on FoRP. Here, we plan to develop a molecular detection tool for FoRP. We compared the secreted in xylem (***SIX***) genes of FoRP with the other three formae speciales and found that ***SIX14*** gene is unique to FoRP among the four formae speciales. We designed specific primers FORP1-F/FORP1-R on ***SIX14*** gene which showed a high specificity for detecting FoRP but not for other 20 formae speciales. The sensitivity of the primers was at 0.01 ng of genomic DNA. In addition, we established a multiplex-PCR that combined the FORP1-F/FORP1-R and the abovementioned FoRF-specific primers (FOR2-F/FOR2-R), FoCN-specific primers (Focs-1/Focs-2) and universal primers for ***Fusarium*** species (CL1/CL2A) to detect formae speciales of ***Fusarium oxysporum*** that cause yellows of cruciferous plants. The results showed that the designed multiplex-PCR distinguished the four formae speciales in one reaction with high specificity.

SA05 整合轉錄體學與抑制活性氧以探討炭腐病菌微菌核之形成調控－劉軒豪[1]、曾敏南[2]、張皓巽[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作物境課)

Integration of RNA-Seq and reactive oxygen species inhibition assay to study microsclerotia development of ***Macrophomina phaseolina***－Liu, H. H.[1], Tseng, M. N.[2], and Chang, H. X.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan; [2]Kaohsiung District Agricultural Research and Extension Station council of Agriculture, Pingtung)

炭腐病菌 (***Macrophomina phaseolina***) 為廣宿主性的植物病原真菌，可感染多種重要經濟作物如大豆和紅豆。炭腐病

菌以微菌核 (microsclerotia) 於土壤中傳播及長期存活，並作為來年的初級感染源，其功能類似菌核 (sclerotia) 。前人研究指出活性氧 (Reactive Oxygen species, ROS) 誘導菌核病菌 (*Sclerotinia sclerotiorum*) 之菌核形成，能使初期菌絲細胞分化與纏繞，以及後期菌核成熟與黑色素生合成。然而目前菌核與微菌核研究多以子囊菌 (Ascomycetes) 中的菌核病菌與黃萎病菌 (*Verticillium dahliae*) 為主，關於活性氧是否在演化分歧後仍參與座囊菌綱 (Dothideomycetes) 炭腐病菌之微菌核形成，目前尚無實驗探究。本研究計劃進行核糖核酸測序 (RNA Sequencing)，分析炭腐病菌由菌絲至微菌核形成的四個時期中之基因表現差異，並在基因表現趨勢分析 (cluster analysis) 中發現諸多基因與氧化還原之功能性相關。相較於菌絲階段，菌核形成後的顯著差異表現基因 (differential expression genes, DEG) 與抗氧化物活性 (antioxidant activity)、二次代謝 (secondary metabolism)、過氧化體 (peroxisome)、電子傳遞鏈 (electron transport chain) 等生理代謝功能相關。為證實活性氧於微菌核形成中確實扮演重要角色，另於培養基中添加活性氧抑制劑，發現其微菌核形成受到影響；應用活性氧染色 (ROS staining) 亦發現不同的活性氧物質對於微菌核形成扮演不同的角色。整合轉錄體與活性氧染色結果，本研究證實活性氧參與炭腐病菌微菌核之形成調控。

SA06 *Trichoderma atroviride* Tri-104 抑菌代謝物質成份分析－侯曉瑩[1]、陳瑞祥[2] ([1]國立嘉義大學植物醫系、[2]國立嘉義大學生化科技系)

Analysis of antimicrobial metabolites produced by *Trichoderma atroviride* Tri-104－Hou S. Y.[1], and Chen R. S.[2] ([1]Department of Plant Medicine and [2]Department of Biochemical Science and Technology, National Chiayi University, Chiayi)

Trichoderma spp. 是土壤和植物根圈常見的微生物，具有病害防治和促進植物生長的活性，其主要的作用機制為超寄生 (mycoparasitism)、分泌水解酵素、產生具有抗生物質的二次代謝物、與病原菌競爭養分和空間以及誘導植物產生抗病性，已廣泛被發展為生物防治劑 (Biocontrol Agents, BCAs)。由於*Trichoderma* spp. 產生的二次代謝物在對抗植物病原菌具有重要的角色，本研究探討自台南柳營地區土壤中分離的中的*T. atroviride* Tri-104 菌株的抑菌活性及其抑菌代謝物質的成份。首先以Internal Transcribed Spacer (ITS)、translational elongation factor (TEF) 進行序列分析，在GenBank 進行 BLAST 結果顯示和*Trichoderma atroviride* 分別為 99% 和 97 % 的相似度，並以親緣關係樹分析，Tri-104和T. atroviride 歸屬於同一群。以玻璃紙抗生測試結果發現，其代謝物質對於 *Rhizoctonia solani*、*Sclerrotium rolfsii*、*Phellinus noxius*、*Fusarium oxysporum*、*Phytophthora capsici* 等病原真菌皆具有 100% 的生長抑制率。在胞外水解酵素活性測試方面，Tri-104 具有 Chitinase、Cellulase 和 Protease 的活性。另外發現 Tri-104 產生的揮發性氣體亦具有延緩病原菌生長的效果，其中對於 *R. solani* 和 *P. capsici* 有較佳的抑制效果。以 Solid-phase microextraction (SPME) 和 In-Tube Extraction (ITEX) 兩種方式收集揮發性有機化合物 (volatile organic compounds, VOCs)，並以 GC－MS 進行分析，所收集的 VOCs 含量最多的皆為 6-pentyl-2-pyrone (6-PP)，未來將進一步確認抑菌代謝物質之作用機制，並評估田間防治植物土壤傳播性病害的潛力。

SA07 Phylogeny, pathogenicity and morphology of Fusarium oxysporum causing basal rot in Cymbidium in Taiwan－Chang, A.[1], and Chung, W.-H.[1, 2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung)

Orchidaceae were important ornamental plants and wildly distributed over the warm and humidity area. Previous studies indicated that *Fusarium* spp. were important pathogens to be limitation factor for production. Among these orchids, the Cymbidium disease caused by *F. oxysporum* was serious problem in garden. Cymbidium, some of them called oriental cymbidium, was popular in East Asia region, usually included *Cymbidium ensifolium*, *C. sinense*, and *C. goeringii*. In Taiwan, basal rot disease caused by *F. oxysporum* was limitation factor for export Cymbidium in orchid industry. During infection process, the common symptoms are leaf yellowing in early stage and pseudobulb showing brown rot, sometimes the shoots are showing necrosis and rotting. Previous study indicated that two morphological type of *F. oxysporum* (type I and type II) were existed in field in Taiwan. However, more isolates collection from diseased *Cymbidium* demonstrated that one more morphological type of *F. oxysporum* was different from type I and type II. Thus, classifying the *F. oxysporum* from *Cymbidium* still needed to add more characters, especially, pathogenicity test and molecular analyses. According to the molecular analyses, the *F. oxysporum* isolates from diseased *C. ensifolium* could be divided into three molecular groups based on IGS and TEF-1 sequences. The results of molecular analyses were associated with morphological characteristics. For pathogenicity test, the certain *F. oxysporum* isolates form *Cymbidium* spp. could infect other orchids and showed symptom, such as *Phalaenopsis* and *Anoectochilus formosanus*. However, the pathogenicity did not correspond to morphological characteristics.

SA08 辣椒炭疽病菌效應蛋白CaEC5833 與寄主免疫反應之功能性分析－游宗達[1]、羅方里[1, 2]、莊淑錚[3]、謝岱庚[1]、施明哲

[3]、李敏惠[1, 2]、陳禮弘[1, 2] ([1]國立中興大學植物病理學系、[2]中興大學前瞻植物生技研究中心、[3]中央研究院農業生物科技研究中心)

Functional analysis of *Colletotrichum acutatum* effector candidate CaEC5833 in plant immune response－Yu, T.-T.[1], Lo, F.-L.[1, 2*], Chuang, S.-C.[3], Hsieh, D.-K.[1], Shih, M.-C.[3], Lee, M.-H.[1], and Chen, L.-H.[1] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Advanced Plant Biotechnology Center, National Chung Hsing University, Taichung; [3]Agricultural Biotechnology Research Center, Academia Sinica, Taipei)

真菌病原體感染植物寄主時會分泌多種效應蛋白，以對抗植物的免疫反應或促進其感染。炭疽病菌 (*Colletotrichum* spp.) 被列為世界第八大最重要的植物病原菌屬，其中 *Colletotrichum acutatum* 是造成臺灣辣椒炭疽病的主要病原菌，但關於其分子致病機制目前仍尚未了解。本研究藉由分析辣椒炭疽菌感染寄主時所建構之 RNA-seq 資料庫，選定只在 *C. Acutatum* 感染辣椒的活體營養期 (<72 h) 表現之基因 caEC5833，CaEC5833 為一具有 EG45-like domain 的小分子外泌蛋白 (130 amino acids)，NCBI BLASTp 的比對結果顯示，在親緣相近的 *C. salicis* 中也具有一個高度相似的蛋白 (Identity: 90.35%)，而在其他 *Colletotrichum* spp. 或是 *Fusarium* spp. 中也有發現類似的蛋白 (Identity:55~65%)，除此之外，該序列相似的蛋白也存在於不同的植物中，像是咖啡、玫瑰、葡萄等。植物中具有 EG45-like domain 之蛋白可能與細胞壁鬆弛或植物賀爾蒙相關，但具有此 domain 之蛋白在病原真菌感染植物中的角色依然未知。本研究中我們利用 *Agrobacterium*-mediated transient gene expression 將 CaEC5833 表現在菸草上，二十四小時後，再表現可於細胞內誘導植物細胞死亡之 BAX 蛋白，5 天後觀察 CaEC5833 對 BAX 引發的細胞壞死有無抑制或加劇現象。結果顯示，在表現 CaEC5833 的 8 個葉片中有 7 個葉片顯示 CaEC5833 抑制了 BAX 誘導的細胞死亡，因此 CaEC5833 可能藉由抑制寄主植物之細胞自體死亡幫助病原真菌的感染。未來我們也會將於真菌中剔除此基因來深入的了解 CaEC5833 在病原菌感染寄主過程中扮演的角色。

SA09 Evaluation of the translocation of fungicides and their efficacy for control of brown root rot disease of trees－Liao, T.-Z.[1], Chen, Y.-H.[2, 3], Tsai, J.-N.[4], Chao, C.[2, 3], Huang, T.-P.[2, 3], Hong, C.-F.[2, 3], and Chung, C.-L.[1, 5] ([1]Master Program for Plant Medicine, National Taiwan University, Taipei; [2]Department of Plant Pathology and [3]Pesticide Residue Analysis Center, National Chung Hsing University, Taichung; [4]Plant Pathology Division, Agricultural Research Institute, Taichung; [5]Depart- ment of Plant Pathology and Micro-biology, National Taiwan University, Taipei)

Brown root rot (BRR), caused by a white rot fungus *Phellinus noxius*, is an important disease of over 200 species of woody plants in tropical and subtropical areas worldwide. Infected trees with root and basal stem rot are easier to fall over during strong winds, which poses a potential threat to public safety. Nowadays, the only registered fungicide for BRR in Taiwan is prochloraz. To prevent the emergence of fungicide resistance due to long-term use of a single fungicide, it is necessary to screen for alternatives from the fungicides of different modes of action. Besides, since P. noxius can colonize the underground root system, it is difficult to apply fungicides to the whole infected tissues. To achieve high efficacy of chemical control for BRR, whether a fungicide has a good systemic property should also be taken into consideration. In this study, 14 fungicides with various modes of action were tested for the inhibition effects on representative *P. noxius* isolates from Taiwan (4 isolates), Hong Kong (4 isolates), Malaysia (4 isolates), Australia (5 isolates), and other islands in Pacific Ocean (16 isolates), using potato dextrose agar containing 0.1, 1, and 10 ppm fungicide. The results showed that the fungicides belonging to FRAC G1 group, in particular cyproconazole, epoxiconazole, and tebuconazole, could inhibit 97.8-99.8% of the colony growth of *P. noxius* at 1 ppm. The other effective fungicides were cyprodinil (FRAC D1) + fludioxonil (FRAC E2) and mepronil (FRAC C2), which showed inhibition rates of 76.7-100% and 79.1-100% at 10 ppm, respectively. The upward translocation of different fungicides was evaluated in the seedlings of *Bischofia javanica*, by treating the root tips with 100 ppm of mepronil, cyproconazole, tebuconazole, epoxiconazole, prochloraz, and cyprodinil + fludioxonil, followed by liquid or gas chromatography tandem-mass spectrometry (LC- or GC-MS/MS) analysis of consecutive segments of root, stem, and leaf tissues at 7 and 21 days post-treatment (dpt). Cyproconazole was found evenly distributed in the whole plant with the highest concentrations (1.39-29.72 ppm at 7 dpt and 0.58-68.61 ppm at 21 dpt) among tested fungicides. At 21 dpt, tebuconazole and epoxiconazole showed even distribution pattern with lower concentrations (0.26-4.44 ppm and 0.04-1.59 ppm, respectively), whereas only trace amount (0.02-1.28 ppm) of mepronil, prochloraz, and cyprodinil + fludioxonil could be translocated to the basal part of the stem (< 15 cm from the crown). The six fungicide products and potassium phosphite were tested for the efficacy of BRR control by drenching before or after stem inoculation of *P. noxius* isolate 2248. The seedlings treated with cyproconazole had lower wilting rate, re-isolation rate, and discoloration area of the stem comparing with other treatments. Further research is needed to find out the appropriate dose and application method to achieve effective control of BRR in planta.

SA10 Efficacy and potential mechanism of *Streptomyces griseorubiginosus* LJS06 on controlling cucumber anthracnose caused by *Colletotrichum orbiculare*—Chai, C. H.[1], Hong, C.-F.[1, 2, 3], and Huang, J.-W.[1, 3] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Pesticide Residue Analysis Center, National Chung Hsing University, Taichung; [3]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung)

Effective biocontrol alternatives to fungicides for disease management is still limited in organic farming of cucumber. In this study, twenty-eight bacterial strains isolated from Chinese herb extract, beer fermentation wastes, the soil sample from a cucumber farm, and raw oyster shell were evaluated for their efficacy on controlling cucumber anthracnose. Among the 28 bacterial strains, the strains TG01, TG02, LJS06 and LJS08 were found effectively reduced the mycelial growth of *Colletotrichum orbiculare* COC3 on PDA media. The bacterial strain LJS06 had the broadest inhibition spectrum against phytopathogenic fungi. Spore suspension of the strain could also significantly (p=0.05) reduce the severity of cucumber anthracnose. Hence, LJS06 was chosen for further identification and biocontrol experiments. Based on the multi-locus sequence analysis of partial 16S rDNA, *atp*D, *arpo*B and *trp*B genes, the phylogenetic tree, and the morphology observed under a scanning electron microscopy, LJS06 was identified as *Streptomyces griseorubiginosus* (Ryabova and Preobrazhenskaya) Pridham *et al*. Biochemistry tests showed that *S. griseorubiginosus* LJS06 could produce hydrolytic enzymes (amylase, cellulase, chitinase, protease), siderophore, IAA and polyamines. Therefore, 5-day cultural filtrate of LJS06 was diluted 100-fold, amended with 0.5%(w/v) of K_2HPO_4 (namely SL06P05 solution), and the effect of SL06P05 solution on conidial germination and appressorial formation of *C. orbiculare* COC3 was tested. Results showed that SL06P05 solution significantly reduced conidial germination and appressorial formation by 2.4-times and 8.6-times, respectively, comparing with water control. The mechanism of reduced conidial germination and appressorial formation may be associated with damaged membrane integrity and induced ROS accumulation in *C. orbiculare* COC3 conidia. Further *in-planta* experiments were conducted to test the efficacy of SL06P05 solution against cucumber anthracnose. The results showed that pre-inoculation spraying and drenching of SL06P05 solution significantly reduced anthracnose severity by 6.9-times and 5.9-times, respectively, comparing with water control. However, SL06P05 solution lost its biological activity after being heated in 40 ℃ water bath for 10 minutes. We hypothesize that SL06P05 solution may have similar effect with pyraclostrobin, which impairs the ATP metabolism of the pathogen thereby reducing the disease severity. In conclusion, our results suggest that *S. griseorubiginosus* LJS06 is a potential biocontrol agent against cucumber anthracnose. ◦

SA11 Investigation and identification of the fungal pathogens causing tea dieback/canker in Taiwan—Hsieh, Y.-C.[1], and Chung, W.-H.[1, 2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung)

Twig dieback or branch canker of tea is a serious problem in tea gardens in Taiwan. Previous studies indicated that the twig dieback or branch canker are major caused by *Macrophoma theicola* Petch. However, other fungi, such as *Fusarium* or *Phomopsis*, are also reported to be pathogens that could cause dieback or canker in tea tree. According to the List of Plant Diseases in Taiwan, the tea dieback or canker could be caused by *Cryptomyces theae*, *Macrophoma theicola*, *Nectria diversispora* and *Phomopsis* sp. However, all of information might not enough to support the new situation in tea garden in Taiwan. Thus, reinvestigation and reidentification is necessary to carry out the major fungal pathogens causing tea dieback and their diversities in Taiwan. The samples were collected from January to November in 2020, including TTES No.12, TTES No.13, TTES No.18, TTES No.20, Sijichun, Chin-Shin-Oolong and Chin-Shin-Dapan locate in Nantou, Chiayi, Pingtung, Miaoli and Taoyuan. A total of 234 fungal isolates were obtained from the tea tissues showing dieback or canker symptom. The investigation showed that the percentage of tea dieback and canker is 0.67% ~30%. Based on morphology and molecular analyses, these fungal isolates could be identified as 10 genera in 7 familiae and the major genera are *Diaporthe* (36.8%), *Cophinforma* (22.2%) and *Colletotrichum* (11.5%). For pathogenicity test, the genera of *Diaporthe*, *Cophinforma*, *Lasiodiplodia*, *Neofusicoccum*, *Botryosphaeria*, *Pestalotiopsis*, *Colletotrichum* and *Fusarium* could infect and cause twig blight symptom on one-year-old tea tree seedling (cv. Chin-Shin-Oolong) based on wound inoculation at 25℃ under 12 hr of light after 3 weeks of inoculation. Among these fungal genera, the *Diaporthe* spp. isolates are the main pathogens to cause dieback symptom (44%) on tea seedlings. For clarifying relationship of the *Diaporthe* isolates in our study, phylogenetic trees were constructed using sequences of internal transcribed spacers (ITS), translation elongation factor 1-alpha (EF1) and beta-tubulin (TUB). Results showed that Diaporthe isolates could be divided into 4 major clades. The non-pathogenic isolates were grouped as single clade with high bootstrap value (100%) and formed same clade with *D. ueckera* and *D. miriciae*. On the other hand, among pathogenic

isolates clades, one of clade is closely related with *D. tulliensis* supported by a high bootstrap value (100%). The primary results indicated that the fungal isolates causing tea dieback or canker are high diversity in Taiwan.

SA12 奈米氧化鋅與三氟敏藥劑複合使用對草莓炭疽病菌抗藥性之影響－陳宜琪[1]、張道禾[2, 3]、薛涵宇[4]、黃振文[2, 3]、張碧芳[2, 3] ([1]國立中興大學植物醫學暨安全農業碩士學位學程、[2]國立中興大學植物病理學系、[3]國立中興大學永續農業創新發展中心、[4]國立中興大學材料科學與工程學系)

The impacts of combined application of nano-ZnO and trifloxystrobin in antifungal resistance of *Colletotrichum* spp. causing strawberry anthracnose－Chen, Y.-C.[1], Chang, T.-H.[2, 3], Hsueh, H.-Y.[4], Huang, J.-W.[2, 3], and Chang, P.-F. L.[2, 3] ([1]Master Program for Plant Medicine and Good Agricultural Practice, National Chung Hsing University, Taichung; [2]Department of Plant Pathology, National Chung Hsing University, Taichung; [3]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung; [4]Department of Materials Science and Engineering, National Chung Hsing University, Taichung)

由炭疽病菌 (*Colletotrichum* spp.) 引起的草莓炭疽病 (anthracnose) 為草莓生育期主要病害，可危害全株，其中以子苗的冠部感染造成移植後倒伏之影響最為嚴重，目前田間病害管理仍以化學防治為主，然而相同作用機制的藥劑頻繁噴施，容易促使草莓炭疽病菌產生抗藥性。三氟敏 (trifloxystrobin) 屬於史托比類 (strobilurin) 藥劑，為常用於防治草莓炭疽病之慣行藥劑，大湖草莓產區曾有紀錄該藥劑針對草莓炭疽病的防治效果不彰，且苗栗區農業改良場於 105 年年報曾報導分離自該區菌株超過半數對史托比類藥劑具有抗性。為創造永續與環境友善之農業栽培技術，開發替代性非農藥資材為目前農業科技發展之方向，奈米材料為一新穎資材，具有穩定、無毒及抑制病原菌生長之特性，已有研究指出奈米材料可以降低植物病原菌抗藥性並延長藥劑效力。本研究目標為評估多枝花狀奈米氧化鋅 (multibranched flower-liked nano zinc oxide, 3D nZnO) 與農用藥劑複合使用對草莓炭疽病菌抗藥性的影響。已自台中與苗栗草莓產區蒐集 20 株炭疽病菌株，利用比濁法與盤式分光光度儀分析不同劑量之三氟敏與炭疽病菌共培養的生長曲線，計算最低抑菌濃度 (minimum inhibitory concentration, MIC) 加以評估病原菌對農藥的感受性。依據分析結果挑選抗藥性及敏感性菌株，將 3D nZnO、三氟敏及草莓炭疽病菌複合培養，結果顯示 20 ppm 3D nZnO 與三氟敏複合施用，可提升抗藥性菌株對三氟敏之感受性，同時也提升低劑量三氟敏之抑菌效果，預期未來可應用於永續農業栽培環境中，達到降低抗藥性產生之風險、減少農藥殘留及延長老舊農藥在田間應用之商品壽命。

SA13 大豆紅冠腐病之抗病品種及防治藥劑篩選－吳秉祐[1]、曾敏南[2]、張皓巽[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作物環境課)

Assessments of fungicide sensitivity and soybean resistance to red crown rot fungus *Calonectria ilicicola*－Wu, P. H.[1], Tseng. M. N.[2], an d Chang, H. X[1]. ([1]Department of Plant Pathology and Microbiology. National Taiwan University. Taipei; [2]Kaohsiung District Agricultural Research and Extension Station, Kaohsiung)

紅冠腐病 (Red crown rot) 是由病原真菌 *Calonectria ilicicola* 造成的重要大豆 (*Glycine max*) 病害，近年來於部分高屏毛豆產區發生嚴重。地上部病徵通常於播種後 50 至 60 天顯現，包括莖基部褐化、葉片壞疽、葉柄枯萎、充實不完全等，降低豆莢品質及產量。然紅冠腐病菌主要危害植株根系造成根腐，除了造成產量降低外，病害嚴重時形成的「鉛筆根」病徵，使得機器採收時容易將植株連根拔起，造成豆莢品管與加工成本提高。為提供病害防治之策略參考，本研究針對 14 個臺灣大豆及毛豆品種進行抗病測試，亦針對紅冠腐病菌進行藥劑篩選。品種抗感性分為種腐及根腐，種腐抗性以消毒後的種子放置在載有病原菌的培養皿上進行測試，結果顯示台南選 1 號最為感病，恆春黑豆則抗性較強。根腐抗性以麥粒接種原混拌土壤，種植後 14 天評估植株根腐程度，結果顯示 14 個品種之間皆無顯著差異。藥劑篩選共測試 25 株分離株、6 種農藥，其中以得克利、鋅錳乃浦、賽普護汰寧的抑制菌絲生長效果最佳。總結本研究成果，目前並未發現對紅冠腐病抗病性較高的品種，藥劑防治可能為此病害的主要防治方法，未來將需測試以藥劑披衣防治紅冠腐病菌的可能性。

學生競賽SB組

SB01 聖誕紅分枝誘導性植物菌質體的遺傳多樣性以及其族群量與植物性狀和基因表現量之關聯性－李昕[1]、朱建鏞[2]、朱家慶[1] ([1]國立中興大學植物病理系、[2]國立中興大學園藝系)

Investigation on the genetic diversity and infection patterns of poinsettia branch-inducing phytoplasma－Lee, S.[1], Chu, C.-Y.[2], and Chu, C.-C.[1] ([1]Dept. of Plant Pathology, National Chung Hsing University, Taichung; [2]Dept. of Horticulture, National Chung Hsing University, Taichung)

聖誕紅分枝誘導性植物菌質體 (poinsettia branch-inducing phytoplasma; PoiBI) 在 1997 年即被證實會造成聖誕紅分枝性的增加，對於聖誕紅園藝性狀的影響非常重要。然而，目前有關 PoiBI 的生態以及其與寄主的交互影響仍然有許多未知之處。本研究利用專一性引子對與聚合酶連鎖反應對不同聖誕紅品種中所感染的 PoiBI 進行偵測以及序列分析，發現在不同聖誕紅品種中的 PoiBI 存在序列差異並可被區分為不同的基因型

(genotypes)。此外，本研究亦應用即時定量聚合酶連鎖反應測定 PoiBI 在植物寄主中的感染密度，並探討其族群量大小與植物性狀與基因表現量之關聯性。試驗結果顯示 PoiBI 的感染密度會因植株個體與組織種類的不同而有所差異，而 PoiBI 的族群量變化對植株性狀以及特定植物基因之表現量可能皆會造成影響。這些研究內容除了能增進各界對 PoiBI 生態之了解，未來也可能有助於聖誕紅育種產業的發展。

SB02 Functional characterizations of membrane-associated diguanylate cyclases Atu1207 and Atu5372 in *Agrobacterium tumefaciens* C58－Lai, X.[1, 2], Yu, M.[2], Wang, Y.-C.[2], and Lai, E.-M.[1, 2] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei)

Agrobacterium tumefaciens is the causative agent of crown gall disease on wide range of plants. *A. tumefaciens* can sense the plant signals for induction of several virulence (*vir*) genes encoding proteins for T-DNA processing and assembly of a type IV secretion system (T4SS) to transfer T-DNA from bacterial cells into the plant cells. Cyclic-di-GMP, a universal eubacterial second messenger, has been shown to regulate multiple biological processes, such as biofilm formation, exopolysaccharide production, and motility in *A. tumefaciens*. However, the function in regulating virulence associated with T4SS remains unclear. Our previous study showed that overexpression of two putative diguanylate cyclases (DGC) which synthesize cyclic-di-GMP, Atu1207 and Atu5372, caused the reduced mRNA levels of selected *vir* genes and attenuation in *Agrobacterium*-mediated transient transformation efficiency. Herein, the biological functions of Atu1207 and Atu5372 and their role on virulence were characterized with gene-deletion mutants and overexpression strains. The results showed that overexpression of Atu1207 or Atu5372 reduced the weight of tumors induced on tomato stems, whereas Δ*atu1207*/ *atu5372* double but not single Δ*dgc* mutant caused an increased virulence. In addition, overexpression of Atu1207 or Atu5372 decreased while the Δ*atu1207*/ *atu5372* double mutant increased the promoter activity of *virB* and *virE*. Accordingly, mRNA and proteins levels of selected *virB* genes were reduced in strains with Atu1207 or Atu5372 overexpression but slightly increased in the Δ*atu1207*/ *atu5372* double mutant. These results suggested that DGCs Atu1207 and Atu5372 may regulate the virulence of *A. tumefaciens* C58 through negative regulation of vir promoters. We also investigated the physiological phenotypes and showed that over-expression of Atu1207 or Atu5372 reduced planktonic growth and swimming motility while increased biofilm formation; however, no significant change of these phenotypes was observed in single or double Δ*dgc* mutants. In summary, Atu5372 and Atu1207 negatively regulates the virulence and swimming motility of *A. tumefaciens* C58 while positively regulate biofilm formation. The present study lays a foundation for future investigation on the underlaying molecular mechanisms of how DGCs regulate *vir* gene expression, which may provide new insights for the improvement of *Agrobacterium*-mediated plant transformation and crown gall disease control.

SB03 臺灣蝴蝶蘭上細菌性軟腐病菌之遺傳多樣性與表型分析－魏憲雍、鄧文玲、朱家慶 (國立中興大學植物病理學系)
Genetic and phenotypic analysis on soft rot bacteria isolated from *Phalaenopsis* orchid in Taiwan－Wei, X.-Y, Deng, W.-L. and Chu, C.-C. (Department of Plant Pathology, National Chung Hsing University, Taichung)

蝴蝶蘭 (*Phalaenopsis* sp.) 為臺灣重要之觀賞作物，而細菌性軟腐病是影響蝴蝶蘭栽培的重要病害。過去研究顯示引起蝴蝶蘭細菌性軟腐病之病原菌大多被鑑定為 *Dickeya* spp. (*Erwinia chrysanthemi*)。近年來依據多位點序列分析 (MLSA) 已將該屬病原菌區分為不同的種 (species)，而同種之菌株在遺傳與表型上也常具有差異。本研究在臺灣蘭花產區與實驗室菌種庫取得了 29 個由蝴蝶蘭分離出的 *Dickeya* spp. 菌株，與其他寄主分離得到的菌株一同進行了系統發生學與表型的分析。試驗結果發現由蝴蝶蘭上分離出之軟腐細菌皆屬於 *D. fangzhongdai*。在表型測試上，比較各菌株在營養瓊脂培養基上的生長情形，可發現多數由蝴蝶蘭分離出的軟腐細菌較其他寄主的 *Dickeya* 菌株有更不規則的菌落型態。此外，常用於鑑別 *Dickeya* 屬軟腐細菌之藍色素 (indigoidine) 在產量上多呈現菌株差異性，且此差異與寄主植物種類無關。將供試菌株接種至不同植物寄主後，可發現由蝴蝶蘭上取得之菌株相較於其他寄主分離到的軟腐細菌在馬鈴薯與蝴蝶蘭上具有較強的致腐能力。另外，本研究亦利用 Biolog GENIII 系統對蝴蝶蘭與其他植物分離得到的軟腐細菌進行了適合之營養源與生長條件的測試。本研究之內容除了有助於增進對臺灣蝴蝶蘭細菌性軟腐病菌之生態與多樣性的了解，相關之試驗結果亦可應用於病原菌的診斷與防治。

SB04 鏈黴菌對番茄疫病之防治潛力及其在番茄根系之纏據能力－吳宗育、黃振文、黃姿碧 (國立中興大學植物病理學系)
Biocontrol of *Streptomyces* species on phytophthora blight of tomato and the root colonization on tomato plants by *Streptomyces* species－Wu, T.-Y., Huang, J.-W., and Huang, T.-P. (Department of Plant Pathology, National Chung Hsing University, Taichung)

番茄 (*Solanum lycopersicum*) 為全球栽培面積位居第二大

之重要蔬菜作物，而由 *Phytophthora capsici* Leonian 所引起的幼苗疫病，為危害番茄生產造成經濟損失之重要病害，然而疫病菌多以雙價菌體存在於自然環境中，若不當施用化學藥劑易有抗藥性菌株之產生，因此應用兼具多重作用機制之微生物製劑為具防治疫病潛力且避免抗藥性發生之可行策略。本研究室前期從臺灣本土植物根圈 植物病原菌具優異拮抗活性之鏈黴菌，分別為 *Streptomyces cavourensis* PES4、*Streptomyces fimicarius* PT-3、*Streptomyces* sp. N3 及 *Streptomyces* sp. 3A-3。其中在趙 (2020) 研究中已證實 *S. cavourensis* PES4 具番茄疫病防治潛力，且可以受阿拉伯芥根系分泌物誘引而纏據於根系。本研究旨在篩選及探討本土所分離鏈黴菌 PES4、PT-3、N3 及 3A-3 對番茄疫病之防治潛力，並瞭解鏈黴菌於番茄根系纏據能力與番茄植株之交互作用對疫病防治效用之影響。為初步確認本研究中使用之四株鏈黴菌株不具植物毒性，將此四株本土鏈黴菌進行 16S rRNA基因序列解序分析，並與美國國家生物資訊中心 (National Center for Biotechnology Information) 資料庫中鏈黴菌 16S rRNA 序列進行比對與親緣關係分析，結果顯示此四株本土鏈黴菌與可造成馬鈴薯瘡痂病的鏈黴菌分屬不同分支群。另利用聚合酶連鎖反應分別以可增幅馬鈴薯瘡痂病病原致病相關基因 *txtA* (thaxtomin synthetaseA) 、*necl* (necrotic protein) 以及*tomA* (tomatinase) 之專一性引子對 TxtAl/TxtA2、Nf/Nr 及 Tom3/Tom4 進行 PES4、PT-3、N3 及 3A-3 和馬鈴薯瘡痂病原菌 *Streptomyces scabies* T6 偵測分析，僅 T6 菌株可增幅出相對應條帶。由上述結果初步判定 PES4、PT-3、N3 及 3A-3 菌株不具植物致病相關基因，且非屬馬鈴薯病原菌。由測試本土分離四株鏈黴菌對 *P. capsica* 菌絲生長抑制活性之結果顯示 PES4、PT-3、N3 及 3A-3 菌株對 *P. capsica* 生長抑制率分別為 50 ± 8.3%、38 ± 3.1%、13 ±5.7% 及 49 ±1.6%，其中以 *S. cavourensis* PES4 具最強之番茄疫病菌拮抗活性，因此以此鏈黴菌菌株進行後續試驗。將 *S. cavourensis* PES4 於 SMG-M 之培養濾液添加入置有 *P. capsici* 菌絲圓盤之 V8 固態培養基及 *P. capsica* 游走孢子懸浮液中，可見 PES4 菌株培養濾液對 *P. capsica* 可達 60 ± 0% 菌絲生長抑制率及 98 ± 2% 游走孢子發芽抑制作用。此外於番茄植株每週一次共三次連續澆灌 100 倍稀釋之 PES4 菌株培養液，可見 PES4 菌株處理組相較於水及 SMG-M 養液之對照組可顯著提升番茄植株鮮重，推測與此四株鏈黴菌具有產生吲哚乙酸、蛋白質分解酵素 (protease)、澱粉分解酵素 (amylase)、纖維素分解酵素 (cellulose)、幾丁質分解酵素 (chitinase) 等植株生長激素及所需養分分解之能力相關。另外，為瞭解鏈黴菌與疫病菌及番茄植株間之交互作用，透過將可表現綠色螢光蛋白載體轉殖嵌入鏈黴菌株之基因體中，並測試其在番茄根系的纏據能力，結果顯示 *S. cavourensis* PES4 可纏據於番茄根部之成熟部。本研究後續將以對番茄疫病最具拮抗能力且可促進番茄生長之 *S. cavourensis* PES4 進一步評估其對番茄疫病之防治效用及施用時機，並探討此鏈黴菌、疫病菌及番茄植株在根系之交互作用關係。

SB05 臺灣本土馬鈴薯瘡痂病病菌遺傳及表型多樣性之研究－吳家蓉[1]、王至正[1]、邱燕欣[2]、吳雅芳[3]、蘇士閔[2]、朱家慶[1] ([1]國立中興大學植物病理學系、[2]行政院農業委員會種苗改良繁殖場、[3]行政院農業委員會臺南區農業改良場)

Investigation on the genetic and phenotypic diversity of phytopathogenic Streptomycetes in Taiwan－Wu, J. R.[1], Wang, J. J.[2], Chiu, Y. H.[2], Wu, Y. F.[3], Su, S. M.[2], and Chu, C. C.[1] ([1]Dept. of Plant Pathology, National Chung Hsing University, Taichung; [2]Taiwan Seed Improvement and Propagation Station, Taichung; [3]Tainan District Agricultural Research and Extension Station, Tainan)

馬鈴薯瘡痂病 (Potato common scab) 是由 *Streptomyces* 屬細菌所引起的重要土壤傳播性病害；其病原細菌一般主要危害馬鈴薯地下部，可在塊莖上引起大小不一的瘡痂斑塊，大量發生時可導致嚴重的經濟損失。過去研究顯示 *Streptomyces* 屬內有多個能夠引起類似病徵的細菌種類 (species)，而瘡痂病菌亦可能藉由水平基因轉移的方式將其致病基因送至腐生菌中；因此不同馬鈴薯栽培地區可能在瘡痂病菌的遺傳多樣性與其他特性方面會有所差異。為了對臺灣本土馬鈴薯瘡痂病病菌的遺傳與表型多樣性能有進一步的認識，本研究於 2019 年於苗栗、臺中、雲林、嘉義、臺南、南投及臺東共 7 個縣市進行了大規模的採樣與菌株分離作業。獲得大量菌株後，本研究測試了各菌株是否能夠抑制白蘿蔔苗生長，並對具有抑制能力的菌株之 *rpoB* 基因完成了定序與序列分析。此項試驗一共將分離的菌株區分為 8 個不同的基因型 (genotypes a-h)。針對這 8 個基因型的代表性菌株完成多位點序列分析與系統發生樹的繪製後，可發現其與常見的瘡痂病菌種類一同被歸類在一個較大的分群；其中 genotype a 與 b 在親緣關係上與 *S. scabiei* 較為接近，而在其他基因型方面則較難確認其所歸屬之物種。進一步以細菌懸浮液對馬鈴薯植株進行接種，並以專一性引子對測試 8 個基因型的代表性菌株是否具有與致病能力相關的 *txtAB*、*nec*1 與 *tomA* 基因後，發現雖然並非所有篩選出的 8 個基因型皆可造成病徵，但無論是偵測到致病基因的菌株或是未偵測到這些基因的菌株皆有可能成功地感染植株。整合而言，本研究之內容有助於增進對臺灣馬鈴薯瘡痂病病菌多樣性的了解，也顯示這些病原菌的生態特性仍有許多值得被探討的空間。

SB06 應用 PMA- qPCR 及 PMA-PCR 對臺灣本土之茄科細菌性斑點病菌進行偵測與定量－盧志弘[1]、王怡馨[1]、蘇士閔[2]、朱家慶[1] ([1]國立中興大學植物病理學系、[2]行政院農業委員會種苗改良繁殖場)

Application of multiplex PMA-qPCR and PMA-PCR assays in detecting and quantifying Xanthomonads causing bacterial leaf spot of tomatoes in Taiwan－Lu, C. H.[1], Wang, Y. H.[1], Su, S. M.[2], and Chu, C. C.[1] ([1]Dept. of Plant Pathology, National Chung Hsing

University, Taichung; [2]Taiwan Seed Improvement and Propagation Station, Taichung)

番茄細菌性斑點病是世界上重要的細菌性病害，目前在臺灣主要由 *Xanthomonas euvesicatoria* 與 *X. perforans* 引起。這些病原細菌可以藉由種子傳播，而歐洲和地中海植物保護組織 (EPPO, European and Mediterranean Plant Protection Organization) 也將其視為重要的檢疫有害生物，將它們列在其 Alert List 2 (A2 list) 中。發展具有高度專一性與靈敏度的檢測技術對於茄科細菌性斑點病的防治至關重要，本研究之目的為優化及發展能夠偵測臺灣本土茄科細菌性斑點病菌之 quantitative polymerase chain reaction (qPCR) 及 polymerase chain reaction (PCR) 技術，並將其搭配 propidium monoazide (PMA) 處理，使增幅出的訊號能更有效地反映出樣本中的活菌量。試驗結果顯示，若以本研究篩選出的 *X. perforans* 專一性引子對，搭配前人用於檢測 *X. euvesicatoria* 的引子，可有效地對臺灣本土之茄科細菌性斑點病菌菌株進行 PCR 多重檢測 (multiplex assay)。而在 qPCR 方面，使用前人開發的 qPCR 引子與探針 (probe) 對臺灣分離到之菌株進行測試亦發現有良好的靈敏度與專一性。將上述檢測技術與 PMA 處理進行整合與測試後發現，無論在 PCR 與 qPCR 試驗中，病原細菌死細胞 DNA 產生的訊號皆有被 PMA 抑制的情形。進一步以本研究使用的 PMA-qPCR 技術偵測人工帶菌種子以及人工接種的番茄葉片中的病原細菌後，亦發現此技術可有效地對種子洗出物與葉片組織樣本中的病原菌進行偵測與定量；此外，而在這些試驗中，病原菌死細胞 DNA 被偵測到的訊號量也有被抑制的現象。整合而言，本研究使用之技術在未來或許能幫助提升病害診斷的精準度，或是被應用於病原菌生態之研究。

SB07 Phc quorum signal-mediated regulation of *Ralstonia solanacearum* virulence on tomato－Lin, C.-Y., and Deng, W.-L. (Department of Plant Pathology, National Chung Hsing University, Taichung)

R. solanacearum, a plant pathogenic bacterium causing "bacterial wilt" on diverse crops. The extensive genetic diversity of *R. solanacearum* strains has led to the concept of an *R. solanacearum* species complex (RSSC) in recent years. RSSC invades xylem vessels of roots and disseminates into the stem and rapidly spread to the whole plants. The typical symptom of *R. solanacearum* is wilting which was because the excessive exopolysaccharide production causes the dysfunction of the vascular system. Besides EPS production, RSSC also has other virulence factors, e.g., cellulase, type III secretion system. RSSC employs the quorum sensing (QS) system composed of the phcBSR operon to regulate their virulence on plants. Previous studies have revealed that according to their phc QS signals, PhcB QS signal synthase will produce neither methyl 3-hydroxymyristate (3-OH MAME) nor methyl 3-hydroxypalmitate (3-OH PAME). The phylogenetic analyses of signal synthase PhcB from 10 RSSC strains showed that these proteins and nucleotides have two clades dependent on their QS signal types. Additionally, there is a specific restriction enzyme cutting site located in the variable region of phcB gene, which could recognize different QS signal types by restriction fragment length polymorphism (RFLP) analysis. Base on this founding we analyze 96 RSSC strains collected from 1974 to 2020 in Taiwan. The results demonstrated there are two different phc QS systems of RSSC in Taiwan. To identify the correlation between phc QS and virulence, we quantify RSSC virulence via plant assay, EPS production, and cellulase activity. The results indicated the RSSC strains employ 3-OH PAME as signal molecules perform higher virulence than 3-OH MAME strains. These results point out the uniqueness of QS systems evolution in *R. solanacearum*.

SB08 Genomics and phenotypic (virulence) characteristics of *Xanthomonas oryzae* pv. *oryzicola* in Taiwan－Chang, C.-P., and Deng, W.-L. ([1]Department of Plant Pathology, National Chung Hsing University, Taichung)

Rice is one of the most important staple crops in the world, especially for Asian country. According to FAO statistics, the rice production in Asia accounts for 90.6% of the world. Bacterial blight and bacterial leaf streak are two major bacterial disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv. *oryzicola* (Xoc), respectively. Chemical treatment and resistant cultivars are commonly used disease management for controlling these two diseases. Gene-for-gene theory is the mainly concept for breeding resistant cultivars to against bacterial blight. To date, more than 40 resistance (*R*) gene have been identified against Xoo, but no similar avirulence (*avr*) -*R* gene interaction between Rice and Xoc has been found. In previous virulence assay, we discover a Xoc strain X019 showed low virulence on IRBB5 and IRBB7, which contain *R* gene *xa5* and *Xa7*, respectively. The result indicated that there might be an *avr*-*R* gene interaction between X019 and rice. After analyzing the whole genome sequence of X019, an *avrXa7* homologous gene *Tal9a* was found. To further determine the virulence difference of Xoc strains, the target gene of *Tal9a* were predicted using PrediTALE. After analyzing the expression of several target genes, the expression pattern cannot reflect the virulence. Surprisingly, the patho-genesis-related gene *PR1a* were upregulated in rice cultivar TK9 inoculated with X019. *PR1a* were reported involving in pathogen-associated molecular pattern (PAMP) triggered defense pathway, including salicylic acid (SA) pathway.

The upregulation of *PR1a* might confers the tolerance of TK9.

SB09 臺灣青枯病菌菌株之重新分類－曾世良[1]、吳雅芳[2]、林志鴻[1] ([1]國立嘉義大學植物醫學系、[2]行政院農業委員會臺南區農業改良場)

Reclassification of *Ralstonia solanacearum* strains from Taiwan－Tseng, S.-L.[1], Wu, Y.-F.[2], and Lin, C.-H.[1] ([1]Department of Plant Medicine, National Chiayi University, Chiayi; [2]Tainan District Agricultural Research and Extension Station, Tainan)

由 *Ralstonia solanacearum* (*Rs*) 所引起的植物細菌性萎凋病 (bacterial wilt)，又稱青枯病，是熱帶、亞熱帶及溫暖的溫帶等地區多種經濟作物的重要細菌性病害之一。*Rs* 是由不同菌系所組成的複合種 (species complex)。鑑定 *Rs* 菌系的方法有生理小種 (race)、生物型 (biovar) 及演化型 (phylotype) 等 3 種。近年來學者利用Rs的基因體學與蛋白質體學等分析，輔以硝酸還原反應 (nitrate reduction)，根據 4 種演化型的架構，進一步將 *Rs* 重新分類為 3 種菌種，分別為 *Ralstonia pseudosolanacearum* (phylotype I and III strains)、*R. solanacearum* (phylotype II strains) 與 *Ralstonia syzygii*，其中 *R. syzygii* 包含 3 種亞種，分別為 *R. syzygii* subsp. *syzygii*(*R. syzygii* strains)、*R. syzygii* subsp. *indonesiensis* (phylotype IV strains) 與 *R. syzygii* subsp. *celebesensis* (BDB strains)。故本研究目的欲瞭解臺灣青枯病菌菌株在重新分類中的歸屬。供試的 *Rs* 菌株共 125 株，分離自番茄、茄子、番椒、馬鈴薯、絲瓜、印加果、薄荷及白鶴靈芝等作物，利用演化型專一性多引子 PCR 檢測、*egl* 基因序列分析、*mutS* 基因序列分析及硝酸還原反應測試，進行*Rs*重新分類，結果顯示供試的 125 株 *Rs* 菌株中有 90 株屬於第一演化型，其餘 35 株屬於第二演化型；在 90 株第一演化型菌株中有 55 株為第三生物型、35 株為第四生物型；在 35 株第二演化型菌株中有 32 株為第二生物型、3 株為第一生物型。經 *egl* 與 *mutS* 之部分基因序列分析，再利用 neighbor-joining 建構親緣樹，結果顯示 90 株第一演化型菌株皆歸類至親緣樹的第一演化群 (phylotype I)，而 32 株第二演化型菌株被歸類至親緣樹中寄主範圍較窄的第二演化群 (phylotype IIB)，另外3株第一生物型則被歸類至寄主範圍較廣的第二演化群 (phylotype IIA)。利用硝酸還原反應測試結果，第一演化型有 89 株菌株可還原硝酸，比例為 98.9%，其中又有 85 株菌株會產生氮氣，產氣比例 95.5%，故供試的臺灣 Rs 第一演化型菌株屬於重新分類的 *R. pseudosolanacearum*。第二演化型的 35 株菌株可還原硝酸，比例為 100%，其中僅 1 株菌株會產生氮氣，產氣比例為 0.03%，屬於重新分類的 *R. solanacearum*。本研究結果顯示，臺灣 *Rs* 菌株經重新分類後，分別歸屬於*R. pseudosolanacearum* 與*R. solanacearum* 兩種。而供試的臺灣 *Rs* 菌株中，第一生物型、phylotype IIA 的菌株如何出現在番茄田區及對產業的衝擊，未來值得進一步探討。

SB10 Antagonistic potentials of phyllosphere *Pseudomonas* sp. against *Xanthomonas citri*－Huang, R.-H., and Deng, W.-L. (Department of Plant Pathology, National Chung Hsing University, Taichung)

Citrus production is greatly affected by plant pathogens, and the primary practice for pest management is the application of agrochemicals. Among the infectious agents, phytopathogenic bacteria are usually controlled by copper-based bactericides and pruning to reduce bacterial population in the phyllosphere. Alternatively, biological control using natural enemies and their derived compounds against pests is employed in the integrated pest management system. Pseudomonad bacteria are common phyllosphere-inhabiting microorganisms for their capability of catabolizing various organic compounds and high tolerance to abiotic stresses on plant surfaces. Other features, including competition for niches and nutrients, production of antibiotics, and induction of induced systemic resistance (ISR), make *Pseudomonas* spp. as ideal candidates for promoting plant healthcare. In this study, five leaf-isolated bacterial strains were identified as *Pseudomonas* spp. and exhibited inhibitory activities against *Xanthomonas citri*, the causal agent of citrus bacterial canker, in confrontation assays. Physiological and biochemical analyses showed the five pseudomonad strains produce non-fluorescent siderophores, and three of them hydrolyze esculin and casein. One of the antibacterial compounds in the pseudomonad culture supernatant was extracted by ethyl acetate, partially purified by reverse-phase HPLC, and identified to be lactic acid by nuclear magnetic resonance spectroscopy. Further studies on the genetics of lactic acid biosynthesis and its involvement in bacterial survival in the phyllosphere will be conducted to reveal its biological functions and potential applications in pest management.

SB11 多黏類芽孢桿菌TP3應用於番茄與草莓病害防治之評估－馮全[1]、陳昭瑩[1, 2] ([1]國立臺灣大學植物醫學碩士學位學程、[2]國立臺灣大學植物病理與微生物學系)

Evaluation of *Paenibacillus polymyxa* TP3 application in the control of tomato and strawberry diseases－Fong, C.[1] and Chen, C. Y.[1, 2] ([1]Plant Medicine Master Program, National Taiwan University, Taipei; [2]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

為了減少化學農藥的使用與風險，需要開發化學農藥的替代品，微生物農藥即為其中之一。多黏類芽孢桿菌菌株 TP3 分離自田間草莓植株的地上部組織，先前研究顯示其施用於草莓

植株上具有對灰黴病與炭疽病之防治功效。多黏類芽孢桿菌會產生內生孢子，相較於營養細胞有較佳的環境耐受性與倉儲壽命，因此較適合開發為微生物農藥。初步研究得知，菌株 TP3 可採用黃豆粉取代一般實驗室使用的培養基進行發酵培養，為較經濟的內生孢子生產方法；其次，多項在番茄與草莓推薦使用的殺蟲劑與殺菌劑對菌株 TP3 不具抑菌活性，這些條件有利於菌株 TP3 在田間實務操作應用。本研究亦將菌株 TP3 延伸應用至番茄上，探討菌株 TP3 在不同作物的病害防治是否有效，於盆栽試驗得知施用菌株 TP3 可抑制番茄灰黴病之病徵發展，並且於接種前 7 天施用菌株 TP3 亦具有防治效果。另一方面，對於草莓新興病害葉枯病，菌株 TP3 對於病原菌之菌絲生長及孢子發芽具有抑制作用，且於田間試驗之果實病徵發展具有抑制作用，施用菌株 TP3 並能增加花與幼果的數量，指出其具使草莓增產的潛力。綜合上述，多黏類芽孢桿菌 TP3 應得以延伸應用於番茄灰黴病及草莓葉枯病之防治。

SB12 LsGRP1誘導系統抗病性與其長距離傳訊之相關路徑－黃博洋、陳昭瑩 (國立臺灣大學植物病理與微生物學系)
LsGRP1-induced systemic resistance and the related long distance signaling pathway－Huang, P. Y., and Chen, C. Y. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

植物受病原菌侵擾時會啟動植物防禦反應- PTI (PAMP-triggered immunity) 以抵抗病原的入侵，而病原菌會產生效應子抑制 PTI 防禦反應，植物則可藉由辨識效應子啟動更強烈的防禦反應—ETI (effector-triggered immunity)；此外，受到感染的部位會累積水楊酸並進行長距離傳訊以啟動系統性抗病反應。百合防禦蛋白 LsGRP1 在抵抗百合灰黴病上扮演相當重要的角色，將大腸桿菌蛋白表現系統生產之 LsGRP1 衍生性重組蛋白對百合進行葉片浸潤處理，可系統性抑制百合灰黴病之病徵發展。另利用農桿菌浸潤法於百合葉片表現 ***LsGRP1***，亦可明顯抑制系統葉的灰黴病病徵發展。這些結果指出，LsGRP1 具有誘導植物系統性抗病的能力，然其機制仍有待釐清。本研究以農桿菌浸潤法於阿拉伯芥葉片表現 ***LsGRP1*** 或浸潤 LsGRP1 衍生性重組蛋白，分析系統葉接種番茄細菌性斑點病菌後的病原菌增殖情形，及在處理病原相關分子模式後的癒傷葡聚醣累積動態。由實驗結果得知以葉片表現 ***LsGRP1*** 可以抑制系統葉的病原細菌增殖菌量，並使系統葉處理 flg22 後有較多癒傷葡聚醣累積。使用大腸桿菌生產之重組蛋白 SUMO-LsGRP1△SS 浸潤阿拉伯芥葉片，亦可有效降低系統葉受病原細菌增殖，於系統葉處理 flg22 也有較多的癒傷葡聚醣累積。另一方面，植物系統性抗病的發生與受到刺激的葉片所產生的長距離傳訊有關，已知阿拉伯芥誘導系統性抗病的長距離傳訊路徑有水楊酸 (salicylic acid, SA) 與 2-哌啶甲酸 (pipecolic acid, Pip) 相關路徑。於阻斷 SA 及 Pip 相關路徑之阿拉伯芥 *bsmt1* 與 *sard4* 突變株浸潤融合蛋白 SUMO-LsGRP1△SS 及於系統葉接種病原菌，皆使 LsGRP1 誘導系統性抗病的作用降低；浸潤 SUMO-LsGRP1△SS 則可以提升SA相關路徑之 ***AtSID2*** 及Pip相關路徑之 ***AtALD1*** 及 ***AtSARD4*** 的表現量。綜合以上研究，驗證 LsGRP1 誘導系統性抗病的作用及其長距離傳訊的參與路徑。

SB13 Diversity and biological characterization of ***Cephaleuros*** in Taiwan－Huang, Y.-C., and Wang, C.-L. (Department of Plant Pathology, National Chung Hsing University, Taichung)

The genus ***Cephaleuros*** was named by Kunze in 1827 and accommodated in Trentepohliaceae. Species of ***Cephaleuros*** commonly occurs on leaves, stems and fruits of diverse trees and ornamental plants. It was considered as a plant disease pathogen causing red rust disease. ***Cephaleuros*** significantly reduces yields of tea and fruit trees, but there are few researches on these diseases. ***Cephaleuros virescens***, the type species, has been recorded on at least 100 plant families in Taiwan. However, recent studies indicated that classification of ***Cephaleuros*** needs to be reassessed and found that morphological species of ***Cephaleuros*** are a polyphyletic species based on molecular phylogenetic analysis. A previous research found that Japanese ***C. virescens*** could be divided into 5 species, suggesting that ***C. virescens*** is a species complex. Thus, we planned to re-examine ***Cephaleuros*** spp. collected from Taiwan by molecular analysis, and to compare characteristics between clades. With phylogenetic analysis of sequences of SSU, ITS and LSU, we revealed that ***Cephaleuros*** in Taiwan can be separated into 11 clades. Besides, 13 new hosts were recorded with algal spots. Isolates phylogenetically closed to reference strains of ***C. parasiticus***, ***C. karstenii*** and ***C. expansus*** were also found. Furthermore, since subcuticular and intercellular colonization of hosts are important characters to identify morphological ***Cephaleuros*** species, we will assess the correlation of the two colonization types with phylogenetic clades. ***Cephaleuros*** isolates with distinct colonization types were selected to compare biological characteristics related to pathogenicity and to elucidate the interactions of ***Cephaleuros***s and plants.

學生競賽SC組

SC01 Investigating the role of glycoside hydrolase family 16 and 17 in ***Phytophthora parasitica***-plant interaction－Chiang, B.-J., Ke, T.-Y., and Liou, R.-F. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

To facilitate successful invasion, plant pathogens secret a range of glycoside hydrolase (GH) to degrade plant cell wall components. Interestingly, in response to pathogen infection, plants

have evolved the ability to recognize certain of these enzymes as microbe-associated molecular patterns (MAMPs) or in some cases their degradation products as damage-associated molecular patterns (DAMPs), thereby to elicit basal defense response. Previously, we found OPEL secreted by *Phytophthora parasitica*, a notorious oomycete pathogen with a wide host range, may elicit basal defense response on *Nicotiana tabacum* cv. Samsun (NN). Moreover, mutation in the conserved beta-1,3-glucanase active site present in the GH16 domain of OPEL significantly comprised the elicitor activity. To know whether other genes containing putative beta-1,3-glucanase consensus also induce plant defense response, we cloned and analyzed candidate genes obtained from the *P. parasitica* genome database, focusing on members of the GH16 and GH17 families. Among those belonging to GH16, we found four genes homologous to OPEL. Recombinant proteins prepared from *E. coli* for three of these genes induced ROS production and callose deposition on *N. tabacum* cv. Samsun (NN), which demonstrate their elicitor activity. The remaining one was neglected due to the presence of a GPI anchor in its C-terminus. Of the genes containing a putative GH17 domain, we found one causing severe symptoms of yellowing, mosaic, and crinkling on the systemic leaves of *Nicotiana benthamiana* at 10 days post agro-infection by using pGR106 as a vector. We are in the process to explore how this gene involved in plant-*P. parasitica* interaction.

SC02 Development of rice endophytes for controlling rice damping off disease caused by *Pythium* species—Tu, C.-K., and Lee, M.-H. (Department of Plant Pathology, National Chung Hsing University, Taichung)

Rice (*Oryza sativa*) is the most important crop in Asia, providing as staple food for over four billion people. Modern rice practices usually raise rice in nursery boxes before transplanting to paddy. High planting density, warm temperature, and high humidity provide a favorable environment for microbes to cause diseases on rice. Among the rice diseases, damping off disease caused by *Pythium* species. sometimes results in severe economic losses to nursery growers. Chemical pesticides are commonly applied to nursery boxes for disease prevention, which often cause the emergence of pesticide resistant pathogens. In addition, paddy fields with organic farming are largely increasing in Taiwan, but rice nursery is still difficult to be practiced with organic farming. Therefore, eco-friendly products for disease prevention in rice nursery, such as plant beneficial microbes, are required to be developed. In this study, two bacterial strains, 5-7 and 6-4, were isolated from rice cultivar TN11 which showed strong antagonistic ability to different *Pythium* species and other rice pathogens. *In planta* assay also revealed a significant reduction in Pythium damping off disease by applying to nursery boxes with 50 to 100 times dilution of the two bacterial fermentation broths. For identification, 16S rRNA sequences identified strain 5-7 and 6-4 as *Lysobacter* and *Kitasatospor*a spp., respectively. By observing GFP-labeled strain under a microscope, 5-7 can colonize well on root tips, root hairs, crown roots, and lateral root surface. Dual culture of the pathogen and 5-7 on water agar and examined under a microscope revealed remarkable abnormal hyphae and sporangia production inhibition of the tested pathogen. The two strains also exhibited multiple enzymes activities. To sum up, two strains, 5-7 and 6-4, isolated from TN11 showed multiple biocontrol mechanisms for Pythium disease control. In the future, more experiments are needed to evaluate the application for Pythium disease control on nursery.

SC03 Functional characterization of a rice endoglucanase gene on rice growth and disease resistance to *Pythium*—Wan, P.[1,4], Syu, Z.-J.[1,2], Chang, K.-J.[1], Yu, S.-M.[3,4] and Lee, M.-H.[1,4] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Tainan District Agricultural Research and Extension Station, Tainan; [3]Institute of Molecular Biology, Academia Sinica, Taipei; [4]Advanced Plant Biotechnology Center, National Chung Hsing University, Taichung)

Rice is a very important staple food crop that can feed more than half of the world's population. There are many diseases occurred in the rice field in Taiwan, such as seedling blight, rice blast, Bakanae disease, sheath blight, brown spot, and bacterial leaf blight, which significantly decrease grain yield. Among these diseases, seedling blight of rice is caused by several different pathogens, and *Pythium arrhenomanes* is one of the major pathogens in Taiwan. *Pythium* is known as a water-mold pathogen and causes seedling root rot and damping off by zoospores or oospores spreading in the paddy field and seedling tray. *P. arrhenomanes* is one of the highly virulent pathogens in the *Pythium* species to rice. By screening 500 lines of Taiwan Insertional Rice Mutant (TRIM) library for resistance to *P. arrhenomanes*, a resistance line was identified and further characterized previously in Dr. Lee's Lab. The gene potentially involved in this resistance has been identified as an endoglucanase gene. In order to confirm the endoglucanase gene function in rice resistance to *P. arrhenomanes* and to understand its role on rice development, this gene was constructed by fusing to a constitutive promoter, transformed into TNG67, and then screened the overexpression (OE) lines for functional characterizations. The

transgenic lines were screened for T-DNA single insertion and the endoglucanase gene overexpression by hygromycin selection, Southern blotting, and semi qRT-PCR. In the *Pythium* inoculation tests in a growth chamber, however, the results showed that the endoglucanase OE lines were not more resistant to *Pythium* than the wild-type (WT) lines. In addition, the phenotypes related to plant height, grain yield and diseases caused by various pathogens were evaluated in paddy fields for three growing seasons. The rice height in tillering stage of the endoglucanase OE lines showed significantly shorter than the WT lines, and the grain weight per panicle of the OE lines showed significantly lighter than the WT lines. In addition, OE lines were more sensitive to sheath blight disease in the field for three seasons. The reasons that the OE lines were not resistant to *Pythium* infection and more susceptible to the sheath blight disease than WT lines need to be discovered in the future.

SC04 Transcriptome analysis unravels the *Nicotiana tabacum* response to OPEL, an apoplastic effector from *Phytophthora parasitica*—Lai, P.-Y., Ke, T.-Y., and Liou, R.-F. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

To combat the infection by pathogens, plants employ a multilayer surveillance system, with pattern-triggered immunity (PTI) at the first level. It involves recognition of microbe- and damage-associated molecular patterns (MAMPs and DAMPs) by pattern recognition receptors, followed by the activation of signaling pathways and a series of plant defense responses. Previously, we demonstrated that OPEL from *Phytophthora parasitica* induces reactive oxygen species (ROS) accumulation, callose deposition, defense gene expression, and cell death in *Nicotiana tabacum* cv. Samsun-NN. In addition, it confers systemic acquired resistance against variable pathogens. Therefore, OPEL very likely functions as an apoplastic effector. In this study, to know how OPEL elicits defense responses in *N. tabacum*, we treated the plants with OPEL recombinant protein (or MES buffer as a control) and performed RNA-seq analysis. In total, we identified 6,460 genes (hereafter called DEGs) which were differentially expressed in response to OPEL treatment, with a fold change $\geqq 2$ and p value < 0.01. KEGG pathway enrichment analysis indicates that OPEL treatment induced the expression of many genes involved in the plant defense pathways, especially *N. tabacum* homologs of BAK1, SOBIR1, and RBOHD. In contrast, OPEL suppressed the expression of genes involved in photosynthesis. Further analysis of DEGs employing Plant Resistance Gene database (PRGdb) identified 339 genes which encode receptor-like kinases (RLKs) or receptor-like proteins (RLPs). Of special interest are those encoding CERK1-like and several LysM domain-containing genes. Among them, the expression of *NtCERK1-like* and *NtLYM2* was shown by qRT-PCR to be up-regulated not only by OPEL treatment but also by *P. parasitica* infection. In addition, overexpressing NtLYM2 on *Nicotiana benthamiana* enhanced *P. parasitica* infection. These results not only verify the role of OPEL as an apoplastic effector, but facilitate the identification of potential key players involved in OPEL-elicited plant immunity as well as in *P. parasitica*-plant interaction.

SC05 Complementary studies on mechanical transmissibility of begomo-viruses—Gustian, D.[1], Chang, H.-H.[1], and Jan, F.-J.[1,2] ([1]Departement of Plant Pathology, National Chung Hsing University, Taichung; [2]Advanced Plant Biotechno-logy Center, National Chung Hsing University, Taichung)

Most of begomoviruses are transmitted by whiteflies, whereas only a few begomoviruses can be transmitted mechanically. The mechanical transmissibility of the begomovirus affects its distribution in the field. This study is aimed to analyze whether mechanically transmissible begomo-viruses [tomato leaf curl New Delhi virus-oriental melon isolated (ToLCNDV-OM) and tomato yellow leaf curl Thailand virus (TYLCTHV)] enable non-mechanically transmissible begomo-viruses [tomato leaf curl New Delhi virus-cucumber isolated (ToLCNDV-CB) and tomato leaf curl Taiwan virus (ToLCTWV)] to become mechanically transmissible. *Nicotiana benthamiana* inoculum were prepared by agro-infiltration as either single virus infection (SI) or two viruses co-infection (CI) (ToLCNDV-OM with ToLCNDV-CB or TYLCTHV with ToLCTWV). After 14 days post inoculation, the crude sap derived from SI and CI plants were used for mechanical inoculation. Mechanical inoculation with mixed sap (MS) from SI ToLCNDV-OM and SI ToLCNDV-CB or SI TYLCTHV and SI ToLCTWV symptomatic leaves was also performed as third treatment in this study. After symptom development, virus detection was conducted with PCR analysis. PCR results showed that nonmechanically transmissible begomoviruses (ToLCNDV-CB and ToLCTWV) can be detected in CI and MS mechanical inoculated-plants. In contrast, ToLCNDV-CB and ToLCTWV on SI plants were not detected by PCR analysis. These results indicated that a mechanically transmissible begomovirus can complement the mechanical transmissibility of other non-mechanically transmissible begomo-viruses that belong to the same or different species. This finding provides information about begomovirus distri-bution which in turn is essential for developing disease management in the field.

SC06 Selection of nitrous acid-induced mild strains of ***Pepper veinal mottle virus*** for cross protection in solanaceous crops—Wang, G.-D., Kang, Y.-C., and Chen, T.-C. (Department of Medical Laboratory Science and Biotechnology, Asia University, Taichung)

Pepper veinal mottle virus (PVMV) is a member of the genus Potyvirus of the family ***Potyviridae*** and can be transmitted by aphids in a non-persistent manner. As all potyviruses, PVMV genome possesses a single linear positive sense ssRNA molecule in the size of 9.7 kb, with a poly A tail at the 3' terminus and a viral genome-linked protein (VPg) at its 5' end, for encoding a polyprotein that is subsequently processed into 10 functional proteins in order of P1, helper component-protease (HC-Pro), P3, a first 6K peptide (6K1), CI, second 6K peptide (6K2), nuclear inclusion-VPg, nuclear inclusion a (NIa), nuclear inclusion b (NIb) and coat protein (CP). Tomatoes (***Solanum lycopersicum*** L.) and peppers (***Capsicum annuun*** L.), including bell peppers and chili peppers, are the most important vegetable and fruit solanaceous crops in the world, with a global planting area of about 38.4 million ha and a total planting area of about 7,000 ha in Taiwan. Virus diseases are the most important threat to the production of economic solanaceous crops. PVMV is one of the prevalent solanaceous viruses in Taiwan. In this study, nitrous acid-induced mutagenesis was conducted to generate PVMV mild strains for cross protection. The attenuated PVMV strains were screened based on the biological characteristics that the wild-type (WT) PVMV induces conspicuous necrotic lesions on the inoculated leaves of ***Chenopodium quinoa*** but its attenuated mutants do not. A stable attenuated PVMV mutant, denoted m4-8-4, was obtained from 1288 local lesions in seven nitrous acid treatments. The accumulation of CP of m4-8-4 was significantly slower than that of WT PVMV in ***Nicotiana benthamiana*** plants. In addition, m4-8-4 effectively protected ***N. benthamiana*** from WT PVMV challenge. Genome sequence analysis revealed that 14 nucleotides leading to 13 amino acid (aa) changes can be found in the m4-8-4genome by comparison with the parental WT PVMV strain. The aa residues involved in the virulence of PVMV will be investigated. Our results showed that m4-8-4 has potential to be a protector against severe PVMV strains, and its cross-protective effect on solanaceous crops, such as tomatoes and peppers, will be evaluated in the future.

SC07 Functional analysis of the helper-component proteases (HC-Pros) of nitrous acid- induced attenuated mutants of Zucchini yellow mosaic virus—Goh, R.-P.[1], Xie, X.-Y.[1], Lin, Y.-C.[1], and Yeh, S.-D.1,2 ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Advanced Plant and Biotechnology Center, National Chung Hsing University, Taichung)

Aphid-borne potyviruses encode helper-component protease (HC-Pro) as viral suppressors to antagonize host defense of RNA silencing for infection. Through modification of the reported conserved motifs of HC-Pros, highly attenuated potyviral mutants suitable for cross protection can be generated. In this study, we attempted to find other conserved motifs of HC-Pro responsible for RNA silencing suppression by analysis of mutations on zucchini yellow mosaic virus (ZYMV) induced by nitrous acid treatment. Eleven attenuated mutants of ZYMV were previously obtained, all mutants did not form local lesions on ***Chenopodium quinoa*** leaves and induced attenuated symptoms on systemic hosts. Results of whole genome sequencing of six mutants showed that most of the amino acid changes for attenuation were located at HC-Pro gene. Four mutants (ZG-2-4, ZG-3-10, ZG-4-1 and ZG-4-10) with different degrees of attenuated symptoms were selected for further analysis on the roles of their mutated HC-Pros. Replacement of wild type HC-Pro with each mutated HC-Pro was conducted, pathogenicity analysis of the resulted recombinants indicated that the mutations on their HC-Pros are correlated to symptom attenuation. RNA silencing suppression (RSS) assay by agroinfiltration of these mutated HC-Pros indicated that silencing suppression ability of each mutated HC-Pro was reduced. Our results indicated that nitrous acid mutagenesis is an efficient way to obtain mutants with random mutations on HC-Pro, resulting in highly attenuated symptoms with a great potential to be used as protective viruses for control of ZYMV by cross protection.

SC08 Identification and isolation of nitrous acid-induced attenuated mutants of turnip mosaic virus from ***Chenopodium quinoa*** leaves for cross-protection—Wu, H.-J.[1], and Yeh, S.-D.[1,2] ([1]Department of plant pathology and [2]Advanced Plant Biotechnology Center, National Chung Hsing University, Taichung)

Turnip mosaic virus (TuMV) belongs to the genus ***Potyvirus*** of the family ***Potyviridae***. This virus has a wide host range and causes severe damage to crops in the family ***Brassicaceae***. Cross protection is a natural phenomenon of induced host resistance against a plant virus and has been widely used as an effective strategy to control plant viruses. Previous studies have revealed that single local infection induced by a mild strain of a potyvirus in ***C. quinoa*** leaves is invisible under white light. Thus, TuMV genome was modified to carry a reporter gene green fluorescent protein (GFP) and was used as the virus source for nitrous acid induction to facilitate the selection of attenuated mutants. After nitrous acid treatment, the infection sites without lesion formation in ***C. quinoa*** leaves were identified by

fluorescent signals with UV light, and the virus within was isolated. From five independent experiments, five mutants were obtained from *C. quinoa* leaves and transferred to *Nicotiana benthamiana* plants, on which all mutants induced attenuated symptoms. In order to evaluate suitable candidates for cross protection, virus titer was monitored at three to 21 days post inoculation in *N. benthamiana* plants by indirect ELISA. The viral accumulation dynamic of a good attenuated candidate should show a zigzag-pattern, in which the virus titer gradually increased after initiation of infection, but decreased sharply and then maintained at low levels up and down in the host plant. After time-course accumulation analysis and pathogenicity assay in *N. benthamiana* plants, the highly attenuated mutant TuMV 4B-1 with a typical zigzag accumulation pattern was selected and considered as a feasible protectant for control of TuMV by cross protection.

SC09 建立柑橘系統性病害之同時偵測方法與應用於柑橘健康種苗調查之研究－姚舜閔[1]、洪挺軒[1] ([1]國立臺灣大學植物病理與微生物學系)

Development of multiplex RT-PCR for simultaneous fiagnosis of vitrus systemic diseases and survey of citrus disease-free seedlings－Yao, S. M., and Hung, T. H. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipe)

柑橘為臺灣重要的果樹之一，種植面積廣且栽種品種多樣。栽種柑橘時所伴隨的病害也是相當複雜，其中以系統性流行病害之危害最為嚴重，包括柑橘黃龍病、柑橘萎縮病、柑橘破葉病及柑橘鱗砧病。植株受感染後會影響其樹勢、生育，導致植株壽命減短、產量下降。目前此四大系統性病害並無有效的防治方法，僅能透過健康種苗制度、拔除田間病株及防治媒介昆蟲來防止病害的擴散。過去本實驗室針對此四種系統性病害已分別開發出分子檢測技術，但因為需進行多次檢測而使得完整檢測過程相當耗時費力。本研究擬開發可同時偵測四大柑橘系統性病害之多重反轉錄聚合酶連鎖反應方法 (multiplex RT-PCR)，藉此降低檢測過程所消耗之時間與成本。本研究首先測試不同引子對間的搭配，從中挑選出最佳組合並進行後續優化，測試結果顯示所開發出之 multiplex RT-PCR 方法具有良好的專一性與靈敏性。本研究亦利用此檢測方法對 2017 年至 2019 年送檢之柑橘健康種苗進行柑橘系統性病害之調查，調查結果顯示送檢柑橘種苗中均無柑橘黃龍病與柑橘鱗砧病之發生，僅有極少數樣本有偵測到柑橘萎縮病或柑橘破葉病的存在。

SC10 探討仙人掌X病毒與紅龍果X病毒之基因功能與協力作用－陳柏因、吳悅民、張雅君 (國立臺灣大學植物病理與微生物學系)

Investigation of the gene functions and the synergistic interaction of *Cactus virus X* and *Pitaya virus X*－Chen, P. Y., Wu, Y. M., and Chang, Y. C. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

紅龍果為原生於中南美洲的仙人掌科植物，因其果實營養價值高，近年來頗受市場歡迎。根據本實驗室多年調查，臺灣田間紅龍果受到 Potexvirus 屬病毒感染的情形相當普遍，且仙人掌X病毒 (*Cactus virus* X, CVX) 與紅龍果X病毒 (*Pitaya virus* X, PiVX) 複合感染的情形十分常見，由此推測兩種病毒間具有交互作用。實驗室前人將 CVX 與 PiVX 轉錄體同時接種於紅龍果原生質體或圓葉菸草 (*Nicotiana benthamiana*) 原生質體時，兩病毒的累積量相較於單獨接種時皆有所提升，顯示 CVX 與 PiVX於單細胞中應具有協力作用。同屬病毒之間發生協力作用的相關報導較為罕見，而我們的研究結果是 *Potexvirus* 屬首例，故欲對 CVX 與 PiVX之協力作用機制進行探討。因許多與協力作用機制的相關研究中皆顯示，病毒之 RNA 靜默抑制子在其中扮演了重要的角色，故首先針對 CVX 與 PiVX 具有抑制 RNA 靜默能力的蛋白進行探討，透過農桿菌注射法共同表現 GFP 基因與單一病毒蛋白於 GFP 轉基因之圓葉菸草 line 16c，證實 CVX 與 PiVX的鞘蛋白與 triple gene block 1 (TGB1) 蛋白皆具有 RNA 靜默抑制子之功能。為探討 TGB1 與鞘蛋白如何影響協力作用，我們於圓葉菸草原生質體進行CVX 與 PiVX轉錄體之接種測試，發現在額外添加 TGB1 或是鞘蛋白轉錄體時，皆有助於雙方病毒的累積。此外我們也製備出 CVX 與 PiVX 的病毒突變株，經北方雜合分析法發現當一病毒喪失產生 TGB1 或是鞘蛋白之能力，同時也會失去其幫助對方病毒於單細胞中累積的能力，以上結果皆指出此二種蛋白在 CVX-PiVX 協力作用中具有一定的重要性。由於 TGB1 抑制植物RNA 靜默作用的能力較鞘蛋白高，因此推論 TGB1可能在協力作用中扮演著關鍵的角色。為探討TGB1抑制 RNA 靜默的能力與協力作用的發生是否具有關聯性，我們針對 CVX 與 PiVX之 TGB1 蛋白進一步分析，參考 potexviruses 之 TGB1 相關研究並進行胺基酸序列比對及結構預測，建構出兩病毒 TGB1 蛋白上 T115A、T190A 與 T211A 之三種胺基酸單點突變株，並經由農桿菌短暫表現法篩選出喪失抑制 RNA 靜默能力之 TGB1 突變株 CVX-TGB1-T190A、CVX-TGB1-T211A 與 PiVX-TGB1-T211A。目前於原生質體和植株測試 TGB1 的單點突變是否會影響到病毒的複製、移動以及協力作用的發生，而其中又是否牽涉到其抑制 RNA 靜默的能力。期望這些研究結果對於 Potexvirus 屬病毒之間協力作用的發生機制有進一步的了解。

SC11 Diagnosis of a new emerging cucurbit virus using a Nanopore sequencing platform－Dong, Z.-X.[1], Chen, Y.-K.[2], Chou, C.-C.[3], and Chen, T.-C.[1] ([1]Department of Medical Laboratory Science

and Biotechnology, Asia University, Taichung; [2]Department of Plant Pathology, National Chung Hsing University, Taichung; [3]National Center for High-Performance Computing, National Applied Research Laboratories, Hsinchu)

Cucumber is a valuable economic crop in Taiwan. The planting area of cucumber in Taiwan was about 1893 ha with an annual yield of 48968 tons in 2019. In June 2020, an unknown virus, designated CX-2, was obtained from a diseased sample of greenhouse grown-cucumber in Xizhou Township, Changhua County by standard single lesion isolation on ***Chenopodium quinoa*** leaves. Double-stranded (ds) RNA isolated from CX-2-infected ***C. quinoa*** leaves was used as the template to create a random primer-primed complementary DNA (cDNA) library, the Invitrogen SuperScript IV reverse transcriptase was use for cDNA synthesis and the Klenow fragment was used to synthesize the complementary strand of cDNAs. The synthesized dsDNAs were purified and ligated with sequencing adaptor using the Nanopore SQK-LSK109 ligation sequencing kit. The Nanopore MinIon device was used for sequencing, running for 8 hr. The sequenced reads were analyzed by the EPI2ME WIMP workflow. A total of 7408 reads were obtained from the sequencing, of which 13 reads were classified as cucumber Bulgarian latent virus (CBLV), a ***Tombusvirus*** species. The full-length genome sequence of the original CBLV isolate (GenBank accession number: AY163842) was used as a reference to align with the classified reads by the basic local alignment search tool (BLAST). The BLAST results showed that the reads shared a high nucleotide identity of 81.89-93.5% with the reference sequence, with a genome coverage of 68.4%. Our result suggests that CX-2 should be CBLV. The full-length genome sequence of CX-2 will be completed in the future.

SC12 Characterization and detection of Passiflora mottle virus and other two potyviruses causing passionfruit woodiness disease in Vietnam—Do, D.-H.[1], Chong, Y.-H.[1] and Yeh, S.-D.[1, 2] ([1]Department of Plant Pathology, , National Chung Hsing University, Taichung; [2]Advanced Plant Biotechno-logy Center, National Chung Hsing University, Taichung)

Passionfruit plantation in Vietnam increased to 10,000 ha in 2019. However, the outbreaks of passionfruit woodiness disease (PWD) have become a serious threat for the production. In this study, five virus isolates DN1, DN4, NA1, GL1 and GL2 were collected from different areas of Vietnam. Their causal roles for PWD were verified by back inoculation to passionfruit. Analyses of coat protein (CP) and genomic sequences revealed that GL1 isolate is closely related to East Asia Passiflora virus (EAPV) AO strain of Japan (polyprotein nt/aa identities of 98.1% / 98.2%), while GL2 isolate is related to Telosma mosaic virus (TelMV) isolate PasFru, China (polyprotein nt/aa identities of 87.1% / 90.9%). CP comparison, host range and cytological characterization indicated that DN1, DN4 and NA1 are potyviruses, but different from EAPV and TelMV. Phylogenic analyses of their CP and genome sequences indicated that these three isolates and passionfruit severe mottle-associated virus Fujian isolate of China belong to a distinct clade, which does not satisfy the threshold (76% nt identity of polyprotein) to be regarded as any of potyviral species. Thus, a new species name of "***Passiflora mottle virus***" has been proposed by ICTV. A rabbit antiserum was produced against the CP of DN1 and it can discriminate Passiflora mottle virus (PaMoV) from TelMV and EAPV in western blotting and ELISA without cross reactions. Field surveys of 240 samples by ELISA and RT-PCR disclosed that PWD in Vietnam is mainly caused by PaMoV; followed by EAPV, mixed-infection of PaMoV/EAPV, and rare cases of TelMV.

SC13 Concurrent control of two most important aphid-borne potyviruses in cucurbits by two-in-one live vaccine—Tran, T.-N.-B.[1], Xie, X.-Y.[1], Chen, K.-C.[1] and Yeh, S.-D.[1, 2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Advanced Plant Biotechnology Center, National Chung-Hsing University, Taichung)

Papaya ringspot virus (PRSV) and ***Zucchini yellow mosaic virus*** (ZYMV) cause severe damage to cucurbits. The strategy to control a potyvirus by cross protection is the use of a mild strain of the virus as a plant vaccine. Because a plant is inoculated by an attenuated strain, this plant cannot be superinfected by the same strains of the same viral species. In this study, the cross-protection effectiveness of combining the protective mild strain PRSV HA5-1 (Yeh et al. 1984) and ZYMV AC (Lin et al. 2007) against both PRSV P type and ZYMV on plants of ***C. metuliferus*** was first attempted. Consequently, the mild strain of PRSV W, PRSV W-AC was created by mutating two conserved amino acids, $Arg_{181} \rightarrow$ Ile and $Asp_{397} \rightarrow$ Asn in the HC-Pro gene of the infectious cDNA clone of a severe strain of PRSV W from Taiwan (Chen et al. 2008). The highly attenuated symptoms, virus accumula-tion, the stability, and the cross-protection effectiveness of the generated mild mutant PRSV-WAC against PRSV W were investigated. PRSV-WAC and ZYMV-ZAC were combined together and applied as 2 in 1 protective viruses, our results indicated that the co-infection two mild strains applied on horn melon, melon, and watermelon, provide complete or high degrees of cross protection against the challenge with severe strains PRSV W and ZYMV-GFP, alone or together. The absence

of the challenge severe viruses in cross-protection tests was verified by local lesion assays or RT-PCR with specific primers. Our results indicated that the mutant WAC is an excellent protective virus for the control of PRSV W by cross protection. The co-infection WAC and ZAC propagated equally well in the inoculated horn melon, no obvious interference on viral accumulation, and no synergistic effects of symptoms were observed. Intriguingly, co-infection with WAC and ZAC in horn melon, muskmelon, and watermelon plants provides concurrent complete protection against both severe PRSV W and ZYMV infection in these cucurbits. This two-in-one attenuated vaccine has a great potential to be used for the control of the two most important unrelated aphid-borne virus species threatening cucurbits by cross protection.