J. Plant Med.

Vol. 65 No. 4, 2023 199

DOI:10.6716/JPM.202312_65(4).0005

中華民國植物病理學會一百一十二年度年會論文摘要

Abstract of 2023 Annual Meeting of Taiwan Phytopathological Society

專題演講 Keynote and young scientist speech

KS01 Plant-pathogen interactions of anthracnose disease and rice disease control－Lee, Miin-Huey, Hsieh, Dai-Keng, Kao, Chao-Yang, Tu, Chi-Kuan, Yen, Yu-Nung, Chiu, Yu-Hsuan, Syu, Zun-Jie, Wan, Ping, Kuo, Chia-Chi, Lin, Meng-Yi, Lin, Yung-Chu, Lin, Hsien-Che, Wang, Pei-Han, and Jang, Kai-Jie (Department of Plant Pathology, National Chung Hsing University, Taichung)

Anthracnose caused by *Colletotrichum* species is a destructive disease affecting many plants, especially vegetables and fruit producing plants such as chili pepper and mango. In Taiwan, anthracnose disease of chili pepper and mango is majorly caused by *C. scovillei* and *C. asianum*, respectively. Understanding the plant-pathogen interaction involved in the establishment of pathogen infection and pant defense can provide useful information for disease management. By comparing three *C. scovillei* strains with different virulences on chili pepper, we have characterized the infection process and potential virulence factors, and identified a special highly branched penetration structure formed in the cuticle layer of pepper fruit. Further analysis through fungal genome sequencing, transcriptomic analyses of different infection stages, and the establishment of *Agrobacterium tumefaciens* mediated T-DNA insertional fungal mutagenesis library, several candidate effector proteins and potential virulence factors were functionally characterized. Fungal infection regulated by host factors was studied in the *C. asianum*-mango pathosystem with a focus on host factor sensing and signalling in the G-protein signalling pathway. In rice disease control, we focus on rice seedling disease caused by *Pythium arrhenomanes*. By screening the TRIM library, we identified a TRIM line that displays resistance to *P. arrhenomanes*. Interestingly, the overexpression of one of the affected genes by T-DNA insertion made the overexpression lines more susceptible to sheath rot disease in the rice field. We also developed plant-associated microbes for use in controlling rice seedling diseases in nursery industry. Three bacterial endophytes, *Lysobacter firmicutimachus*, *Kitasatospora* sp., and *Bacillus* sp., displayed strong protection of rice seedlings against *P. arrhenomanes* in a small-scale assay. In the field trials, the three endophytes exhibited significant disease control efficacy on rice brown spot disease that naturally occurs in a commercial nursery field. Induced resistance was not found in rice seedlings colonized by the three strains.

KS02 Research and application of plant salicylic acid-mediated antiviral immunity－Yeh, Hsin-Hung (Agricultural Biotechnology Research Center, Academia Sinica, Taipei)

Viruses can cause substantial economic losses in crops, and as intracellular infections, cost-effective pesticides for controlling viral diseases are currently unavailable. To tackle this issue, we conducted research on how plants combat viruses and aimed to explore ways to improve plant immunity against viral infections. Salicylic acid (SA)-mediated plant immunity is known to play a central role in plant resistance to biotrophic and semi-biotrophic pathogens, including viruses. Initially, our research focused on investigating how SA-mediated plant immunity protects orchids against viruses. We later extended our research to study how bananas resist the semi-biotrophic fungus *Fusarium oxysporum* f. sp. *cubense*. Our findings reveale that *STRESS ASSOCIATED PROTEIN GENE*s act as central hubs in SA-mediated plant immunity in orchids, bananas, and Arabidopsis, providing valuable insights for the development of effective crop protection strategies. As well as conducting basic research, we also applied current knowledge on plant immunity to crop protection, and developed F8-culture, which primes SA-mediated plant immunity. Our study showed that F8-culture is highly effective in inducing resistance to viruses in tomatoes grown in fields.

KS03 植物線蟲研究之“慢漫”長路－陳珮臻 (國立中興大學植物病理學系)

The trail less traveled- my reflection on the study of plant nematology－Chen, Pei-Che (National Chung Hsing University, Taichung)

研究植物線蟲這幾年來的心路歷程，第一個想到的形容詞就是”慢”。多數線蟲是行雙性生殖的，但少數的重要線蟲例如*C. elegans*, 或是根瘤線蟲，它們不是雌雄同體，就是行孤雌生殖，飼養增量的過程相對簡單，造成多數人覺得植物寄生線蟲是好飼養的錯覺。雙性生殖的族群代表代表一直重複實驗室中少數個體的挑選與培養族群會面臨基因的漂移(genetic drift)或更甚者有了近親雜交衰弱(inbreeding depression)的現象。所以在飼養供試線蟲上，釐清其生殖模式，對後續如何保存或增量是極重要的一項資訊。當然植物寄生性線蟲相比於多數其他植物病原菌，其飼養的生活史也相對較長，所以要在這些物種上回答相似的問題，本就需要更多的時間。植物寄生性線蟲雖然很早就被發現，但它的研究進展卻是四大病原中進度較慢的，這跟它相對複雜的基因體也有很大的關係。本研究室有一個非常有趣的研究進程，剛好說明了這個現象；在2003年時，學生使用當時實驗室所採集保存的9個不同寄主來源的水稻葉芽線蟲 *Aphelenchoides besseyi* 進行雜交試驗。結果發現，9個品系中，具有兩種生殖模式，且跨寄主來源的品系雜交多數是不成功的，而幾個最初有產生子代的雜交事件，經過了幾次繼代後，有些族群就消失了。這樣的現象在近20年後跟中研院的合作文章中，終於有了答案，這些當初用型態鑑定所謂同種的線蟲，很可能就是內裡有差別的近似種，他們或許是正在”分手”的演化路上前進著而產生了生殖隔離。雖然演化是一個迷人的議題，但是身為植病人的正職是解決病害問題，於是成就了另一個”漫”。實驗室長期協助監測田間植物寄生線蟲種類，許多田間有趣的發現成了學生研究論文的題目，無論是新線蟲的鑑定與發表或是常見線蟲種類如何防治等等，陸續培植了許多植物寄生性線蟲研究的人才，期間還有不務正業的水域線蟲相調查，更是找到可幫助監測河川汙染狀態的線蟲科別。植物寄生性線蟲的研究具有其獨特的迷人之處，從形態觀察到分生試驗，從田間到實驗室，從基因研究到藥劑防治，只要你踏進來，很少找不到你喜歡的研究對象，期許未來，台灣的植物寄生性線蟲研究有更多不同專長的領域一起合作，回答更多有趣的研究議題。

Plant Nematology is a slow developed research subject compared to Mycology, Bacteriology, and Virology. The famous *Caenorhabditis elegans* and important *Meloidogyne* species are either hermaphrodites or reproduce parthenogenetically, giving the public the impression that raring nematodes is easy. In the lab, raring pathogens usually start by selecting a few individuals, and transferring them to a new host (media), this is will cause genetic drift in the amphimixis species. In some cases, the long-term inbreeding might also cause inbreeding depression and the population eventually die. It is important to clarify the reproduction mode of a certain plant parasitic nematode before we use the “pure culture” for further studies. With a longer life cycle, scientists asking the same questions would wait longer to get the answers on nematodes. Even though the first plant parasitic nematode ***Anguina tritici*** was seen in the 1740s, the progress of plant parasitic nematode study is slower compared to the other plant pathogens. Part of the reason might due to the much more complicated genomics than the other pathogens. The study in my lab offered an interesting example. In the early 2000s, my first graduate student used 9 isolates of ***Aphelenchoides besseyi*** that originated from 2 different hosts to perform the cross experiment. We found 2 reproduction modes in these 9 isolates, and most of the crosses between 2 host-originated isolates did not result in any offspring. Among the successful crosses, many populations die out after several generations. We did not have a good explanation of this phenomenon till the recent publication collaborated with SINICA. These morphologically inseparable isolates have very different genomic constituents, and they are on their way to speciation. Even though Evolution is a fascinating research topic, controlling diseases are considered a plant pathologist’s full-time job. Our lab has been conducting plant parasitic nematode monitoring projects for a long time, and many students built their thesis topics upon the interesting findings from the field survey. We even have an interesting de-tour publication on identifying nematode Families that could be good bio-indicators for river pollution. The trail of Plant Parasitic Nematodes study is unique, the research topics can cover classical morphological to molecular identification, from the field samples to bench works, from gene expression to chemical treatments. Use plant parasitic nematodes as your research model, you will certainly find the topic most suits your lifestyle. We are looking forward to more cross-disciplines collaboration and answering more interesting questions in Plant Nematology.

KS04 Dynamics of actin cytoskeleton in response to phytopathogenic bacteria—Lu, Yi-Ju (Department ouf Plant Pathology and Microbiology, National Taiwan University)

Actin is the most abundant protein among all organisms. While encountering fungal pathogens, plant cells tend to form callose-like depositions against all penetrations, and actin is regarded as one of the essential components in building up such physical barriers. However, not all pathogens invade plants via penetration, for example, phytobacteria enter hosts through natural openings such as stomata. To investigate the role of actin in plant-bacteria interactions, we monitored changes in actin architectures after bacterial infection and proved type III secretion system (T3SS) effectors are orchestrating the dynamics. From the plant side, molecular dissections of different components essential for actin cycling

demonstrate these molecules are also critical for plant immunity. The actin depolymerization factor 4 (ADF4) is one of the molecules that contribute to actin turnover and effector-triggered immunity (ETI). Our research found that the Arabidopsis Calcium protein kinase 3 (CPK3) is an upstream protein of ADF4 signaling and regulates the activity of ADF4 through phosphorylation. Beyond changing actin organization, CPK3 also controls the closure of apertures against the phytobacterium and further influences stomatal immunity.

KS05 臺灣草莓炭疽病菌與灰黴病菌對常用殺菌劑之感受性與抗性機制及生物防治－朱盛祺[1]、鍾文鑫[2] ([1]行政院農業委員會苗栗區農業改良場；[2]國立中興大學植物病理系)
Sensitivity and resistance mechanism of causal agent of strawberry anthracnose and gray mold to common fungicides and their biological controls in Taiwan－Chu, S.-C.[1] and Chung, W.-H.[2] ([1]Miaoli District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan, ROC; [2]Department of Plant Pathology, National Chung Hsing University, Taichung)

Colletotrichum gloeosporioides species complex (CGSC) 與*Botrytis cinerea*是引起臺灣草莓炭疽病 (anthracnose) 與灰黴病 (gray mold) 的主要病原菌，炭疽病菌會造成草莓種苗/本田植株冠部感染導致缺水枯萎死亡的情形，而灰黴病菌則會造成本田/儲藏期果實腐爛。苯丙咪唑類 (benzimidazoles)、史托比類 (strobilurins) 及二甲醯亞胺類 (dicarboximides) 殺菌劑是常推薦用來防治炭疽病與灰黴病的藥劑，但近十年來已觀察到田間防治效果不彰。本研究分別自臺灣主要草莓產區蒐集炭疽病菌株與灰黴病菌株，透過菌絲生長與孢子發芽測試菌株對三類殺菌劑的感受性，進一步探討對藥劑低感受性菌株產生耐藥性的可能機制，最後篩選具防治兩病害之潛力微生物進行病害防治評估。結果顯示，所分離108株炭疽病菌均屬*Colletotrichum gloeosporioides* species complex (CGSC)，其中三株屬於*C. fructicola*，其餘皆被鑑定為*C. siamense*。對苯丙咪唑類殺菌劑測試抑制菌絲生長之結果指出，該類藥劑對多數CGSC菌株之半數有效濃度(EC_{50})均大於500 μg a.i./mL；史托比類殺菌劑中，除百克敏外，對多數CGSC菌株之EC_{50}值亦大於500 μg a.i./mL。另一方面，在對所蒐集102株灰黴病株之菌絲生長測試，結果得知苯丙咪唑類殺菌劑中的貝芬替與甲基多保淨亦分別對41株與38株菌株之EC50值大於500 μg a.i./mL。於二甲醯亞胺類殺菌劑測試結果顯示，對灰黴病菌EC_{50}值大於500 μg a.i./mL的有依普同 (2株) 與撲滅寧 (9株)。此外，綜合上述測試結果證實，田間的CGSC與*B. cinerea*菌株已分別對相同作用機制的藥劑產生了交互抗性 (cross-resistance) 與不同作用機制的藥劑產生交叉抗性 (cross-resistance)。另，於史托比類藥劑測試結果，顯示供試CGSC菌株之菌絲生長對藥劑感受性高於孢子發芽。進一步分析菌株分離地區與藥劑敏感的相關性，不同地區所分離之CGSC菌株對苯丙咪唑類與史托比類殺菌劑的感受性會有差異；同樣情形亦發生在灰黴病菌。分析對史托比類藥劑低感受性CGSC菌株得知，cytochrome b基因第143密碼子無點突變且Alternative oxidase (AOX) 活性亦無參與耐藥劑性的產生；唯ABC transporter中的*cdr4*基因可能與菌株耐藥性相關。另分析對苯丙咪唑類藥劑表現低感受性炭疽病菌與灰黴病菌菌株，證實兩種病原皆於β-tubulin基因第198密碼子產生點突變，CGSC菌株與灰黴菌株由GAG (Glu)突變成GCG (Ala)；另表現中等低感受性的灰黴病菌株，於第198密碼子處由GAG (Glu)突變成GTG (Val)。對二甲醯亞胺類藥劑表現低感受性的灰黴病菌株，與*BcOS-1*基因Q369P和N373S點突變有關，中度敏感的灰黴病菌株，於I365S、I365N產生變異。於測試微生物防治資材結果指出，液化澱粉芽孢桿菌MLBA15-4所開發成的水懸劑 (SC) 與可濕性粉劑 (WP)，經溫室條件下對高架栽培草莓具有降低發生果實灰黴病的效果 (可降低12.8%)；而不同場域試測結果亦可有效防治草莓灰黴病 (平均防治率可達54~58.7%)。此外，結合微生物製劑與藥劑或不同作用機制殺菌劑交替使用可有效降低草莓炭疽病的發生。

論文宣讀摘要 Abstracts for Oral Presentation

A.真菌/卵菌/線蟲組

A01 Functional characterization of *Lilium* defense protein LsGRP1 in mediating plant induced systemic resistance－Lin, C.-H., Chiang, M.-J., Shih, Y.-T., Huang, P.-Y., and Chen, C.-Y. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

Induced resistance of plants is a fitness-optimized strategy that balances the energy consumption between growth and defense. Accordingly, induced resistance mediators have great application potential in plant protection. *LsGRP1* is a defense protein mediating monocot *Lilium* immune activation, such as callose deposition and reactive oxygen species production, crucial for resistance to gray mold caused by necrotrophic fungus *Botrytis elliptica* and also required for plant growth. Agroinfiltration with *LsGRP1* enhanced disease resistance, callose deposition and *LsGRP1* accumulation in systemic leaves of *Lilium*, indicating the systemic resistance-eliciting function of *LsGRP1*. Likewise, root drench with *LsGRP1* fusion protein crude extract protected *Lilium* from *B. elliptica* as well as dicot *Arabidopsis* from necrotrophic gray mold fungus *Botrytis cinerea* and hemibiotrophic bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*), in comparison to the control of fusion partner crude extract. Additionally, either constitutively expression with *LsGRP1* or root drench with *LsGRP1* crude extract was found to slightly improve *Arabidopsis* growth. These traits highly recommend the

application of *LsGRP1* in plant disease control. Both salicylic acid and pipecolic acid-associated long-distance signaling pathways were demonstrated to involve in *LsGRP1*-elicited systemic resistance since *LsGRP1* failed to enhance flg22-triggered callose deposition in systemic leaves of *Arabidopsis* mutants of related signaling molecule biosynthesis. Through comparing *Pst* proliferation among *Arabidopsis* transformants of wild-type and partial region-deleted *LsGRP1*. the key region of *LsGRP1* essential for disease resistance was identified. The presence of peptide representing this region enhanced the callose deposition against *B. elliptica* secretion challenge in *Lilium* leaves whereas mutated peptide did not. Besides, this peptide alone did not trigger callose deposition. These findings indicated its action mode of defense booster rather than inducer. In summary, *LsGRP1* is a systemic induced resistance-mediator with great potential forplant health management.

A02 新擬盤多毛孢屬真菌引起臺灣草莓葉枯病之探討－趙巧娟、吳竑毅、鐘珮哲、陳儀嘉、李吉峰、涂鳳清 (行政院農業委員會苗栗區農業改良場)

Neopestalotiopsis species associated with strawberry leaf blight in Taiwan－Lai, Q.-J., Wu, H.-Y., Chung, P.-C., Chen, Y.-C., Li, J.-F., and Tu, F.-C. (Miaoli District Agricultural Research and Extension Station, COA, Miaoli)

草莓為一連續採收之高經濟作物，近年因品種汰換，以*Neopestalotiopsis rosae*所引起之葉枯病嚴重危害草莓香水品種，造成葉片產生褐色圓形斑，後期病斑蔓延擴大成葉枯病徵，病原感染冠部及根系後造成壞疽、新葉小葉化及地上部萎凋等病徵。本場於台北市、桃園市、新竹縣、苗栗縣、台中市、南投縣及嘉義縣草莓產區採樣草莓植株並經組織分離共得71株*Neopestalotiopsis*屬之菌株，經親緣分析有65株分離株 (92%) 隸屬於*N. rosae*，6株分離株 (8%) 為非N. rosae之種類。而後經形態分析，*N. rosae*菌株之分生孢子長度較非*N. rosae*菌株稍長，寬度無差異，軸部附絲 (apical appendage) 及基部附絲 (basal appendage) 長度亦較長。*N. rosae*以PDA培養於15-35℃環境下，經回歸分析後得出最適生長溫度約為22-27℃，與非*N. rosae*菌株相似。由於非*N. rosae*菌株皆由草莓植株所分離，為瞭解非*N. rosae*菌株是否對草莓具有致病力，後續以離葉接種之方式進行致病性測試，將菌株培養於1/4 PDA約1-2週，收取分生孢子及菌絲塊後，以針頭穿刺草莓葉片製造傷口，將孢子懸浮液 ($1x10^6$ conidia/mL) 或菌絲塊接種於傷口上，觀察接種後7天與14天的病徵表現。結果顯示不論以孢子懸浮液或菌絲塊方式接種，*N. rosae*菌株皆可造成葉片出現褐色壞死病徵，而非*N. rosae*菌株則無病徵表現。本研究顯示臺灣草莓葉枯病以*N. rosae*為主要病原菌，且其最適生長溫度涵蓋臺灣草莓育苗期與產果期，因此將可做為未來防治策略擬定之參考。

A03 Identification of *Pythium* species and screening for resistance cultivar to *Pythium* in Taiwan and Indonesia－Handoko, R.-N.-S.[1,2] and Lee, M.-H.[1,2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Advanced Plant Biotechnology Center, National Chung Hsing University, Taichung)

Pythium spp. are parasitic oomycetes and considered as soil-borne plant pathogens that can infect plants, causing root rot and seedling stunting, which can reduce plant yield. Other plant symptoms can also be observed after *Pythium* infection, including seedling damping-off and yellowing, and necrotic lesion on root. Investigation of *Pythium* for ecology and attack the host is important for disease control and relatively unexplored habitats. *Pythium* can be found in both cultivated and uncultivated fields, as well as in other environments, such as algae, human, animals. There are many different *Pythium* species associated with rice and some of them can cause seedling disease. Various rice varieties are grown in Taiwan and Indonesia, but their resistance/susceptibility to *Pythium* pathogens has not been investigated. Screening for resistance cultivar is indeed a very efficient and environmentally friendly method for disease management. By selecting resistant cultivars, growers can have harmful effects on the environment, non-target organisms (such as beneficial insects), and human health. By selecting resistant cultivars, growers can promote a more sustainable and eco-friendly approach to agriculture. Developing and using resistant cultivars is considered one of the most effective and sustainable strategies for disease management in agriculture. The aims of my research include the collection and identification of *Pythium* from rice seedling and the screening of rice resistant variety to *Pythium*. *Pythium* isolates were collected starting in 2019 in Taiwan and 2022 in Indonesia by surveying rice field for diseased seedlings with stunting, yellowing, and/or root rot. *Pythium* isolates were used for virulence assay on variety Tainan 11 (TN11) and the isolate that caused disease symptoms on TN11 was reisolated for identification by morphology and molecular diagnosis. The morphology of hyphae, oospore, antheridium, oogonium and sporangia was examined and measured. Molecular diagnosis was conducted using DNA sequencing from internal transcribed spacer (ITS), β-tubulin (tub), and cytochrome oxidase subunit II (COX II) for phylogenetic analysis. Two isolates, PyDU from Taiwan and SUB from Indonesia, were identified as *Pythium aristosporum* and *Pythium arrhenomanes*, respectively, based on both morphological and molecular identification. It is noteworthy that *Pythium aristosporum* had not been reported in Taiwan before, indicating that this study has contributed to the identification of a new pathogen in rice production in Taiwan. This

study need to highlights the importance of using molecular tools for accurate identification of plant pathogens, especially when morphological characteristics are not sufficient for identification. The resistance/susceptibility of rice varieties of Taiwan and Indonesia to *Pythium* species, particularly *Pythium aristosporum* and *Pythium arrhenomanes*, is being analyzed, and the results will be presented and discussed. This analysis could help in the development of effective management strategies for *Pythium* species in rice production in these regions, including the development of resistant rice varieties or the use of biological control agents.

A04 篩選抗褐根病之原生樹種－黃冠瑛、陳啟予 ([1]國立中興大學植物病理學系)

Screening for native plants resistant to brown root rot pathogen *Phellinus noxius*－Huang, G.-Y. and Chen, C.-Y. (Department of Plant Pathology, National Chung Hsing University, Taichung)

褐根病 (Brown root rot disease) 是病原菌*Phellinus noxius*感染樹木根系所導致之病害，罹病樹木出現葉部黃化、變小，後期造成植株萎凋、甚至死亡而倒伏，為危害熱帶及亞熱帶地區樹木的主要根部病害，在臺灣是最重要之公園、行道樹病害。臺中都會公園位於臺中市西屯區大肚山臺地，占地約88公頃，近年來園區內發生嚴重之褐根病，導致園區內多處樹木枯死，影響提供遊客遊憩遮蔭之功用，目前已於園區內之18個感染區域分離出20株褐根病菌株，其寄主涵蓋有美人樹、雀榕、銀葉樹、樟樹等樹種，由於發病範圍廣泛，因此若欲採用化學藥劑進行土壤澆灌後再補植樹種將所費不貲，因而以抗病樹種來補植於發病區域為最佳之選項。本研究即以大肚山臺地之原生樹種作為篩選對象，期待能篩選出對褐根病菌具抗病性之原生樹種，用以推薦補植於臺中都會公園內褐根病發病嚴重之病區。前期之溫室盆栽實驗中，以分離自樟樹 (Cin-1) 與雀榕 (Ficus-1) 之褐根病菌製備麥粒接種源，接種於32種原生樹種地基部，藉由褐根病菌選擇性培養基自植株地基部再次分離以確定成功接種後，共發現21種未曾有感病紀錄之原生樹種可被褐根病菌感染，如三斗石櫟、九節木、菲律賓饅頭果等，為本研究新發現之潛在褐根病寄主，顯示此類樹種不宜種植於高風險病區；有10種測試樹種有抗病潛力，然而需更進一步確認；較明確的是，魚木為具有抗病性之樹種，可建議種植於褐根病之發病區。

A05 *Lasiodiplodia* spp.引起之落花生基腐病－吳雅芳[1]、張智凱[1]、林語貞[1]、黃培真[2] ([1]行政院農委會臺南區農業改良場、[2]雲林縣元長鄉公所)

Peanuts collar rot caused by *Lasiodiplodia* spp.－Wu, Y.-F.[1], Chang, C.-K.[1], Lin, Y.-C.[1], and Huang, P.-C.[2] ([1]Tainan District Agricultural Research and Extension Station, COA, Tainan; [2]Yuanchang Township Office, Yunlin)

近年來，雲林元長地區落花生於採收期經常出現萎凋現象，罹病植株呈現乾腐症狀，且有一年比一年嚴重的趨勢。莖部病徵處可見密集凸起之黑色柄子殼，鏡檢可見兩種型態之孢子，分別為卵圓形、透明、單室之未成熟孢子與卵圓形、褐色、中央有一隔膜且具明顯縱紋之成熟孢子。病原菌經分離純化後，於PDA上菌落初呈灰白色，之後轉為灰黑色絨毛狀菌落，並有柄子殼形成，經型態鑑定，輔以ITS比對病原菌為*Lasiodiplodia theobromae*與*Lasiodiplodia pseudotheobromae*。將此病原菌接種於落花生植株上，可產生與田間相同病徵，並回分得相同病原菌。為釐清病原菌生長特性並擬定防治措施，進行溫度生長及藥劑篩選試驗，該病原菌於28-30℃生長快速。腐絕、依普同與脫克松等藥劑能有效抑制菌絲生長。觀察罹病植株發現，子房柄與豆莢上有產生病原菌之柄子殼，且種子上能分離出相同的病原菌。播種罹病植株之種子，發芽之植株會產生與田間相同之病徵，因此推測感染源可能來自於種子。後續將以藥劑拌種後播種，觀察發芽率與罹病率是否有改善。結果將提供農民於播種期進行防治，以減少感染源進入田間。

A06 開發大豆紅冠腐病帶菌植株與土壤之田間分子檢測－汪倖如[1]、施秉澤[1]、王郁雰[1]、陳泰元[2]、林盈宏[1]、劉軒豪[1] ([1]國立屏東科技大學植物醫學系、[2]行政院農委會高雄區農業改良場)

Construction of molecular detection of *Calonectria ilicicola* in soybean plant and soil－Wang, X.-R.[1], Shih, P.-T.[1], Wang, Y.-F.[1], Chen, T.-Y.[2], Lin, Y.-H.[1], and Liu, H.-H.[1] ([1]Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan; [2]Kaohsiung District Agricultural Research and Extension Station, COA, Kaohsiung)

大豆紅冠腐病 (Red crown rot of soybean, RCR) 於 2017 年在臺灣首次被記錄並報導。此病害是由土壤傳播性的病原真菌 *Calonectria ilicicola* 引起的，好發於大豆結莢期，並會影響豆莢及種子的產量與品質。及早檢測此病原對於防治大豆紅冠腐病的傳播具有重要意義。本研究希望能建立一套能夠應用於檢測臺灣大豆紅冠腐病的分子技術。根據試驗結果顯示。使用 TaqMan probe-based real-time PCR 技術，可檢測低至100 fg 紅冠腐病病原的基因組DNA。在檢測人工接種和田間隨機採樣的帶菌植株時，使用 TaqMan probe-based real-time PCR 技術可成功進行篩檢帶菌樣本。本研究後續擬利用此大豆紅冠腐病菌的分子檢測技術，分析不同時間種植大豆的田間土壤與罹病植株中的實時菌量，同時調查植株發病率，以探究大豆紅冠腐病感染植株的時機，以期未來能夠進行早期預防管理。

A07 中性化亞磷酸與柑橘精油防治胡蘿蔔白粉病之模式－蔡小涵 (行政院農業委員會臺南區農業改良場)

The model of neutralized phosphorous acid solution and citrus essential oil applied on carrot powdery mildew disease control－Tsai, H.-H. (Tainan District Agricultural Research and Extension Station, COA, Tainan)

胡蘿蔔 (*Daucus carota* subsp. *sativus*) 為繖形花科，一、二年生的草本植物，為臺灣主要食用根莖菜類之一，主要栽培地區為彰化、雲林，臺南。胡蘿蔔為冬季作物，秋冬為白粉病 (powdery mildew disease) 好發季節，雖然白粉病登記用藥不少，以農藥實施慣行防治並無困難，但農藥殘留問題仍屢見不顯，因此在農藥減量的政策推行及有機有善農業的擴展下，許多作物之病害仍有以安全資材防治之需求。本研究於溫室及田間，以800倍中性化亞磷酸及200倍柑橘精油進行胡蘿蔔 (彩譽品種) 白粉病防治試驗，結果顯示，於發病前使用3次800倍中性化亞磷酸及發病後繼續使用200倍柑橘精油2次，對胡蘿蔔白粉病的防治效果及持續力最佳，白粉病罹病度僅6.3%；單獨使用800倍中性化亞磷酸也能有預防效果，罹病度為42.3%；而發病後才使用柑橘精油控制，罹病度可維持在21.3%；而無任何處理之對照組，罹病度最終上升到72%。綜上所述，經由田間試驗結果顯示，防治胡蘿蔔白粉病最有效果之安全性植保資材搭配方式為於發病前使用中性化亞磷酸，並於發病後繼續使用柑橘精油；而單獨使用中性化亞磷酸或柑橘精油，相較於對照組，也可達具統計顯著差異之防治成效，均為值得推廣之安全資材應用方式，有助於農藥減量及環境友善之目標。

A08 胡瓜根腐病菌發病因子之探討－黃晉興、袁琴雅 (行政院農業委員會農業試驗所植物病理組)

Factors affect disease progression of *Pythium* root rot of cucumber－Huang, J.-H. and Yuan, C.-Y. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA, Taichung)

近年來在台灣溫室栽培的胡瓜經常發生嚴重的根腐病，植株出現矮化、猝倒、萎凋死亡等病徵，病原菌主要為腐霉菌*P. aphanidermatum* (Pap)、*P. irregulare* (Pir)、*P. myriotylum* (Pmy)、*P. spinosum* (Pspi)、*P. splendens* (Pspl)和 *P. sylvaticum* (Psy)。由上述6種腐霉菌的菌絲在5%CVA培養基於不同培養溫度的菌絲生長結果，可將病原菌分為高溫菌及中溫菌兩大類，高溫菌(Pap及Pmy)菌絲在8－44℃均可生長，最適生長溫度為32－36℃；中溫菌 (Pir、Pspi、Pspl和Psy) 菌絲於4－36℃均可生長，最適生長溫度為24－32℃。盆栽接種胡瓜苗結果顯示，高溫菌發病適溫為24－36℃，而低於24℃則不發病，其中以Pap致病性較強，28℃以上罹病度77.1－100%，造成植株萎凋，倒伏且死亡；中溫菌以Pspl發病溫度較廣，從12－32℃皆可造成病害，而其他3支菌株 (Pir、Pspi和 Psy) 發病溫度為12－28℃，最適溫為12－16℃。接種上述6種胡瓜根腐病菌於不同苗齡 (1、3及5週苗) 之胡瓜植株，多數菌株可感染苗齡1、3與5週的植株，造成植株根腐、萎凋與倒伏等病徵，只有Pmy會造成1週苗出現倒伏死亡，而3及5週苗僅出現植株輕微矮化，葉片較小且褪綠等病徵，不會造成植株死亡。以不同數量的Pap游走子接種於莖基部在28℃14天之發病度迴歸曲線方程式為$y=93.968(1-e^{-0.625x})$ (y=發病度, x=游走子量)，造成發病度50%之接種源濃度大約為1.2×10^3 zoospore/pot。將此Pap游走子量接種測試淹水對胡瓜根腐病的影響，試驗結果顯示在淹水 (partial submergence) 環境下植株發病嚴重，罹病度達100%，其次為浸水 (waterlogging)，罹病度為79.2%，而無浸水 (non-waterlogging) 的環境下，植株僅出現矮化病徵，罹病度為25%。上述Pap游走子量接種其他臺灣常見溫室作物，結果顯示胡瓜 (彩綠2號) 最為感病，其次為洋香瓜 (美華)，而接種美濃瓜 (嘉玉)沒有出現病徵，但每株植株的根部皆可分離到Pap；大果番茄 (農友301) 及小果番茄 (淑女) 僅1週苗出現矮化、倒伏等病徵，而3及5週苗皆無病徵，但亦可分離到Pap。

A09 應用非農藥資材防治設施香瓜與花胡瓜之根腐線蟲－林國詞、林語貞 (行政院農業委員會臺南區農業改良場作物環境課)

Application of non-pesticide materials to control root-lesion nematode of melon and cucumber in greenhouse－Lin, G.-C. and Lin, Y.-C. (Division of Crop Environment, Tainan District Agricultural Research and Extension Station, COA, Tainan)

根腐線蟲 (root-lesion nematode, *Pratylenchus* spp.) 為全球主要作物的重要病原線蟲種類之一，溫室栽培作物如瓜類等因無淋洗等因素容易受到寄生性線蟲危害，根腐線蟲近年檢出比例逐漸升高，已成為溫室栽培主流危害線蟲之一，於溫室密閉空間中作業，農友傾向施用非農藥方式進行防治，然現今非化學農藥防治方法多為防治根瘤線蟲之試驗結果，應用於防治根腐線蟲尚有調整空間。本試驗利用盆栽，進行防治根腐線蟲初步試驗，結果顯示固體資材蓖麻粕與苦茶粕添加增到5公克/2公斤土；澆灌液體資材皂素溶液2,000倍與肉桂油乳劑3,000倍，有明顯減低土壤線蟲密度效果。進一步利用固體與液體資材組合進行聯合防治試驗之防治效果均大於單一資材之使用，其中「蓖麻粕/皂素：5/3」(5公克/3000倍)組合防治效果最佳。分別於設施香瓜與花胡瓜溫室進行田間試驗，施用「蓖麻粕/皂素：5/3」(換算田間施用量蓖麻粕566公斤/0.1公頃+皂素3000倍)與「MA」(蓖麻粕446公斤+含放線菌有機質肥料300公斤/0.1公頃)處理，種植香瓜防治根腐線蟲結果較傳統上施用添加含放線菌有機質肥料，對根腐線蟲抑制率分別為50與23.2%，增加果實重量分別達24.9與7.4%。種植花胡瓜防治試驗結果，對線蟲抑制率為50與23.2%，增加收穫重量達40.3與13%。「蓖麻粕/皂素：5/3」共同防治處理組合有效降低根腐線蟲危害與避

免產量損失。可供作為友善耕作或農藥減量之防治策略參考。

A10 台灣中南部地區六種蔬果作物植物寄生性線蟲相調查－洪傳捷[1]、吳秋燕[2]、楊俊毅[3]、顏志恆[2] ([1]國立中興大學昆蟲學系、[2]國立中興大學農業推廣中心、[3]國立中興大學植物醫學暨安全農業碩士學位學程)

Investigation on plant-parasitic-nematodes of six different fruit and vegetable crops in central and southern area in Taiwan－Hong, C.-J.[1], Wu, C.-Y.[2], Yang, J.-Y.[3], and Yen, J.-H.[2] ([1]Department of Entomology, National Chung Hsing University, Taichung; [2]National Chung Hsing University Agricultural Extension Center, National Chung Hsing University, Taichung; [3]Master Program for Medicine and Good Agricultural Practice, National Chung Hsing University, Taichung)

包括葡萄 (*Vitis* spp.)、柑橘屬 (*Citrus* spp.)、番石榴 (*Psidium guajava*)、瓜類 (*Cucumis* spp.)、番茄 (*Solanum lycopersicum*)及紅龍果 (*Hylocereus* spp.) 等六種蔬果作物之土壤植物寄生性線蟲相被調查紀錄，植物病原線蟲產生危害的寄主植物可能造成葉片黃化、根部腐爛、根部產生根瘤、植株矮化或萎凋等現象，透過了解不同作物上的線蟲相，可以得知造成主要為害的線蟲種類，並做出合宜的防治措施。惟上述相關的報告較少，缺乏國內與較新的研究成果，比較田間的實際危害情形調查，與前人所留下的資訊不盡相同。結果發現，葡萄以根腐線蟲 (*Pratylenchus* spp.) 比例最高 (71.92%)，次高者 (22.93%) 為柑桔線蟲 (*Tylenchulus* spp.)，曾經常見的根瘤線蟲 (*Meloidogyne* spp.) 與鞘線蟲 (*Hemicriconemoides* spp.) 則比例僅1.56%與1.00%；柑橘屬則以腎形線蟲 (*Rotylenchulus* spp.) 為冠 (43.53%)，其次為根腐線蟲 (21.9%)，而以往最常見的柑桔線蟲剩19.09%；番石榴中最多者 (75.40%) 為釘線蟲 (*Paratylenchus* spp.)，其次為螺旋線蟲 (*Helicotylenchus* spp. & *Rotylenchus* spp.) 與根瘤線蟲，各占12.07%與8.32%；瓜類以腎形線蟲為大宗 (76.08%)，取代傳統上族群比例最多的根瘤線蟲 (19.76%)；番茄上以根瘤線蟲與腎形線蟲最多，各占50.00%與36.17%，而以前同樣常見的根腐線蟲與螺旋線蟲，如今分別剩0.03%及0.03%；紅龍果上則是以螺旋線蟲最常見 (51.79%)，仙人掌包囊線蟲 (*Cactodera cacti*)、矮化線蟲 (*Tylenchorhynchus* spp.)、腎形線蟲、根瘤線蟲各占13.24%、12.17%、11.11%、8.41%。綜合以上調查得知，主要出現的線蟲種類呈現多樣性，且因作物不同而有所差異。然而，目前國內針對線蟲的推薦防治藥劑，仍以防治根瘤線蟲為主，其次是根腐線蟲。尚缺乏多種作物與其他線蟲種類的推薦用藥。

A11 Transcriptome profiling of resistant and susceptible mungbean root in response to root-knot nematode infection－Lee, S.-K.[1], Liao, P.-Z[1], Chen, H.-W.[2], Lee, C.-R.[1,2], Yang, J.-I[3], and Ting, H.-M.[1] ([1]Institute of Plant Biology, National Taiwan University, Taipei; [2]Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei; [3]Department of Plant Pathology and Microbiology, National Taiwan University, Taipe)

RKNs (root-knot nematodes) are the most serious plant parasitic nematodes, causing billions of dollars in annual losses to crops. In particular, M. enterolobii is challenging to manage due to its high virulence, broad host range, and high reproduction rates. Mungbean is an economically and nutritionally valuable crop but is susceptible to many pests. Currently, little research on mungbean genotypes resistant to root-knot nematodes and defense mechanisms have been identified. Consequently, we aim to identify natural variants among wild populations from different countries that are able to resist M. enterolobii and propose the mungbean defense mechanism based on transcriptome profiling and gene expression analysis. A total of 1777 and 2730 differentially expressed genes (DEGs) have been identified in susceptible and resistant mungbeans after seven days of nematode inoculation (7 dpi), respectively. DEGs that were up-regulated in the resistant accession and down-regulated in the susceptible accession were similar, namely those related to plant-pathogen interaction, plant hormone signaling, oxidative stress, and immunity. Additionally, the transcriptomic results highlighted the induction of effector-triggered immunity (ETI) in the resistant accession and the inhibition of defense responses in the susceptible accession by nematode effectors. To verify the transcriptome results, qRT-PCR was used to validated the expression levels of selected significant plant defense related DEGs (WRKY, PAL, MAPK, PR protein) in mungbean and found that the response of those genes to resistant accessions was greater than the response to susceptible accessions. Furthermore, the phytohormonal analysis indicated that salicylic acid (SA) was involved in regulating the defense response of the resistant accession at 7 days post-infection. Summarized, the results of this study suggest that the induced immune response in plants might account for the differences in resistance between resistant and susceptible mungbean accessions. Additionally, we speculated that the resistant accession might protect against RKN by inducing ETI through SA regulation.

B.細菌/病毒組

B01 嘉義縣番路鄉罹病番茄田區兩種青枯病菌演化型菌株之特性分析－高之韋[1]、鄭慈靜[1]、劉偉誠[2]、林志鴻[1] ([1]國立嘉義大學植物醫學系、[2]嘉義縣中埔鄉農會)

Characteristics of tomato bacterial wilt caused by two phylotypes of *Ralstonia solanacearum* in a field of Fanlu, Chiayi－Kao, C.-W.[1], Zheng, C.-J.[1], Liu, W.-C.[2], and Lin, C.-H.[1] ([1]Department of Plant

Medicine, National Chiayi University, Chiayi; [2]Zhongpu Township Farmers Association, Chiayi)

2022年11月在嘉義縣番路鄉一處由果園轉種番茄的田區，植株出現不明的萎凋症狀，且持續蔓延擴展，經田間初步診斷為番茄青枯病。藉由採樣、分離及純化共獲得14株分離株，利用青枯病菌 (*Ralstonia solanacearum*) 種專一性引子對AU759f與AU760r進行PCR檢測，顯示供試的14株分離株皆可增幅出282 bp條帶。進一步利用演化型 (phylotype) 專一性多引子Nmult21:1F、Nmult21:2F、Nmult23:AF、Nmult22:InF、Nmult22:RR、AU759f及AU760r進行複合式PCR檢測，結果顯示11株分離株屬於phylotype I菌株，3株分離株屬於phylotype II菌株。故本研究欲探討兩種青枯病菌演化型菌株同時存在一處番茄田區，其菌株特性及病原性之差異。為進一步鑑定病原菌，分別自phylotype I及phylotype II菌株中各挑選2株進行16S rDNA序列分析，經序列同源性比對分析顯示供試菌株皆為 *R. solanacearum*。根據*egl*之基因序列分析與菌株對乳糖、麥芽糖及纖維雙糖等三種雙糖與甘露糖醇、山梨聚糖醇及甜己醇等三種糖醇之利用情形，分析14株 *R. solanacearum* 之序列型 (sequevar) 及生物型 (biovar)，結果有11株屬於 phylotype I、sequevar 13 及 biovar 3菌株，而3株屬於phylotype IIA、sequevar 7及biovar 1菌株。依菌株特性檢測結果顯示，該罹病番茄田區兩種演化型菌株具有不同的生理生化特性，但仍以phylotype I、sequevar 13及biovar 3的本土菌株為優勢族群。此外利用穿刺及澆灌接種檢測此兩種演化型菌株對番茄之病原性，結果兩種演化型菌株皆會引起番茄植株萎凋症狀。台灣過去已有研究報告指出，在桃園蘆竹與宜蘭壯圍的番茄田區存在*R. solanacearum* phylotype IIA、sequevar 7及biovar 1的外來菌株。本研究顯示此外來菌株已出現在嘉義番路的番茄田區，其如何在番茄產區擴散及對番茄生產的影響，值得未來持續追蹤探討。

B02　溶裂型噬菌體對番茄青枯病之防治效果評估－王申如、林志鴻 (國立嘉義大學植物醫學系)

Evaluation of the efficacy of lytic bacteriophage against tomato bacterial wilt－Wang, S.-R. and Lin, C.-H. (Department of Plant Medicine, National Chiayi University, Chiayi)

番茄青枯病 (bacterial wilt) 是由植物病原細菌 *Ralstonia solanacearum* 所引起的土壤傳播性病害，罹病的番茄植株呈現葉片萎凋及維管束褐化等徵狀，嚴重時導致植物死亡。目前有許多防治番茄青枯病之相關研究，如種植抗病品種或使用生物製劑等，其中利用噬菌體防治青枯病也是生物防治研究的一環。因噬菌體對細菌具有高度專一性，且對環境友善，利用於田間病害防治，除了不會影響人體健康外，亦不會危害田間其他非寄主生物。故本研究目的欲瞭解分離自番茄栽培田區土壤之噬菌體的寄主範圍、特性及其對番茄青枯病的防治效果。就寄主範圍測試結果，顯示實驗室純化保存之12株噬菌體可分為9群，第一群噬菌體 φRs34及 φRs13可溶裂供試的58株寄主菌株。在*In vivo*試驗中，將噬菌體 φRs34及 φRs13各別及共同與青枯病菌Rss4以MOI (multiplicity of infection) = 0.001混合震盪培養，每小時以分光光度計進行檢測，顯示 φRs34及 φRs13皆能有效降低混合液的OD600值，且以 φRs34效果較佳，利用十倍系列稀釋法以實際測試Rss4的菌量變化，顯示 φRs34能使5.7 × 108 cfu/mL的細菌數量降至1.3 × 10^5 cfu/mL。另外將 φRs34與Rss4分別以MOI = 10^{-7}、10^{-6}、10^{-5}、10^{-4}、10^{-3}、10^{-2}、10^{-1}、10^0及10^1混合，以瞭解不同MOI下噬菌體對青枯病菌的裂解情形及差異，結果發現MOI = 10^{-3}、10^{-2}、10^{-1}、10^0及10^1具有穩定的溶菌效果。在*In vivo*試驗中，將不同濃度的 φRs34與Rss4均勻混合後，以滲透注射番茄葉片進行接種，觀察 φRs34在感病番茄L390的植株體內是否具有溶菌作用，且可以有效抑制病害發生。當Rss4接種濃度為10^5 cfu/mL時，φRs34以10^8 pfu/mL混合滲透注射能將罹病度降低至16.7%，與單獨接種Rss4造成的100%罹病度具有顯著差異。此外亦利用幼苗試管接種試驗以瞭解噬菌體對番茄青枯病菌的防治效果，試驗結果顯示，當Rss4濃度為10^3 cfu/mL與濃度為10^9 pfu/mL之 φRs34均勻混合後，再接種至斷根處理的L390感病番茄幼苗，能有效降低萎凋率至40%，與單獨接種Rss4所引起的90%萎凋率相比較，具有顯著差異。考量處理噬菌體的時間差異對防治效果之影響，故先接種10^9 pfu/mL之 φRs34，間隔1及24小時再分別接種10^3 cfu/mL的Rss4，結果顯示兩種時間間隔之處理組與單獨接種Rss4之對照組皆有顯著差異，分別降低53.3%及36.7%之萎凋率。本試驗說明噬菌體能有效抑制青枯病菌的生長及降低病害之嚴重程度，未來將就盆缽試驗進行探討，以提供噬菌體作為番茄青枯病綜合管理因子之參考。

B03　引起台灣薑細菌性萎凋病之病原特性探討－林志鴻[1]、陳亮嘉[1]、高之韋[1]、王誌偉[2]、吳雅芳[3] ([1]國立嘉義大學植物醫學系、[2]行政院農業委員會臺東區農業改良場、[3]行政院農業委員會臺南區農業改良場)

Characteristics of the pathogen causing bacterial wilt of ginger in Taiwa－Lin, C.-H.[1], Chen, L.-J.[1], Kao, C.-W.[1], Wang, C.-W.[2], and Wu, Y.-F.[3] ([1]Department of Plant Medicine, National Chiayi University, Chiayi; [2]Taitung District Agricultural Research and Extension Station, COA, Taitung; [3]Tainan District Agricultural Research and Extension Station, COA, Tainan)

近年來在台灣種植薑的田區陸續發生植株萎凋徵狀，且在罹病組織可見菌泥 (ooze) 產生，經分離純化後，利用青枯病菌種專一性引子對AU759f-AU760r進行PCR檢測，顯示分離自薑的菌株皆可增幅出282bp條帶。本研究探討在台灣引起薑細菌性萎凋病之病原菌菌系，為本土的race 1茄科菌系或是外

來的race 4薑菌系，並探討病原特性的差異，將有助於本病害的管理。22株供試菌株分離自嘉義番路 (2010)、台東 (2019) 及高雄六龜 (2022) 等地區之罹病薑田，經生物型 (biovar) 測試結果，10株嘉義菌株皆為biovar 3，4株台東菌株皆為biovar 4；而8株高雄菌株分別有4株biovar 3與4株biovar 4。進一步分析其演化型 (phylotype) 及序列型 (sequevar)，結果顯示供試的22株菌株皆屬於phylotype I，其中嘉義及台東共14株菌株為sequevar 30，而高雄菌株中4株biovar 4菌株為sequevar 13，而biovar 3菌株為sequevar 44，此三種序列型在台灣過去的研究資料中已經存在。根據egl基因序列分析顯示14株嘉義及台東菌株與日本發表之race 4的薑菌株同屬於sequevar 30。由於race 4菌株之生理生化特性與race 1菌系極為相似，同時存在biovar 3與biovar 4菌株，且皆為phylotype I，故不易區分，除了以寄主範圍作為分類依據外，目前尚可利用兩組race 4菌系之專一性引子對AKIF-AKIR或21F-21R進行PCR檢測，本研究以PCR檢測結果，僅sequevar 30的菌株可被引子對AKIF-AKIR增幅出165bp條帶，可視為race 4的薑菌系，而sequevar 13及sequevar 44的菌株皆無法增幅出專一性條帶，應屬於race 1的茄科菌系。此外亦測試供試菌株之寄主範圍，結果顯示穿刺接種或澆灌接種皆可引起薑、番茄、茄子、番椒及菸草等供試植物呈現萎凋徵狀，但菌株間存在差異性，此結果不符合race 4菌株只會感染薑之定義，故在台灣薑菌系之菌株間已存在致病性差異。本研究顯示台灣已存在青枯病菌第4生理小種，即薑菌系，且寄主範圍較廣，未來值得進一步探討。

B04 *Pseudomonas corrugata*感染番茄之研究－蔡佳欣、黃淑芩 (行政院農委會農業試驗所植物病理組)

The study of *Pseudomonas corrugata* infecting tomato－Tsai, C.-H. and Hwang, S.-L. (Plant Pathology Division, Taiwan Agricultural Research Institute, COA, Taichung)

2014年台中地區一處番茄園，部分植株出現植株衰弱及萎凋現象，莖表面可見暗褐色斑，切開莖部可見維管束褐化蔓延，切取莖部罹病組織於光學顯微鏡下可見細菌大量泳出，疑似細菌性病害。隨機採集5株植株，進行病菌分離，其中1株植株之罹病莖部組織可在營養培養基培養出1種淡黃色菌落，該細菌屬革蘭氏陰性菌，培養在King's B培養基無螢光色素產生，將其接種至萬國士煙草可誘導煙草葉片產生過敏性反應。以Biolog細菌鑑定系統分析該菌屬於*Pseudomonas corrugata*。將其16S rDNA序列於NCBI以blastn比對顯示與*P. corrugata*相似度最高，達99%以上。以*P. corrugata*專一性引子PC5/1與PC5/2對該菌進行聚合酶連鎖反應，可增幅出約1100 bp之特異性片段。將該菌接種至番茄植株，可產生與田間所見相同之病徵，且可再分離出相同細菌，顯示該菌為病原菌，此為*P. corrugata*感染番茄在臺灣的首次報告。該田區之後未再繼續種植番茄，未再發生該病害。

B05 探討光桿菌0805-P2R及2103-UV對偽二點葉蟎致死活性之最適化配方－楊寬敏[1]、謝建元[2] ([1]國立高雄師範大學生物科技系研究所碩士、[2]國立高雄師範大學生物科技學系)

Discussion on the optimum formula of *Photorhabdus luminescens* 0805-P2R and 2103-UV for lethal activity on *Tetranychus truncatus Ehara*－Yang, K.-M.[1], and Hsieh, C.-Y.[2] ([1]Postgraduate Master of Biotechnology, National Kaohsiung Normal University, Kaohsiung; [2]Department of Biotechnology, National Kaohsiung Normal University, Kaohsiung)

葉蟎繁殖力高、生長週期短且寄主植物種類繁多，造成多種經濟作物受損，影響農作物收成，大量使用化學農藥使葉蟎產生抗藥性並有環境汙染、農藥殘留等問題。本研究使用光桿菌 (*Photorhabdus luminescens* 0805-P2R) 以及本實驗室獲得另一株光桿菌 (*Photorhabdus luminescens* 2103-UV) 探討對偽二點葉蟎 (*Tetranychus truncatus Ehara*) 殺蟲活性，對照組為逆滲透純淨水，實驗結果顯示兩種菌株對偽二點葉蟎都具有殺蟲活性，以文獻為參考，將光桿菌0805-P2R及光桿菌2103-UV各以田口實驗設計直交表$L_9(3^4)$由四因子碳源、氮源 (水溶性澱粉、蔗糖、奶粉、YE/AA) 及三個比例不同之水準培養配方進行培養並進行實驗分析及蟲體切片觀察，以菌量濃度、蛋白酶、殺蟲活性因子反應圖得出光桿菌0805-P2R及光桿菌2103-UV最適化培養配方。光桿菌0805-P2R最適化配方為A2B2C3D3，實驗結果顯示添加0.4%水溶性澱粉、2.5%蔗糖、1.5%奶粉、0.6% YE/AA及0.1% Tween80下，菌株之菌量濃度最高11.87±0.03 Log (CFU/g) 及殺蟎活性最高為83.33%；光桿菌2103-UV最適化配方為A2B2C3D2實驗結果顯示添加0.4%水溶性澱粉、2.5%蔗糖、1.5%奶粉、0.4 % YE/AA及0.1% Tween80下，菌株之菌量濃度最高13.08±0.54 (CFU/g)、蛋白酶活性2.6±0.15 (U)及殺蟎活性最高為90%，以最適化配方培養光桿菌2103-UV殺蟎活性較高。

B06 大花曼陀羅黃斑病相關病毒之特性分析－楊佑勛、陳煜焜 (國立中興大學植物病理學系)

Characterization of a potyvirus associated to chlorotic spots of angel's trumpet (*Brugmansia suaveolens*)－Yang, Y.-S. and Chen, Y.-K. (Department of Plant Pathology, National Chung Hsing University, Taichung)

大花曼陀羅 [angel's trumpet, *Brugmansia suaveolens* (Willd.) Bercht. & Presl.)]為常見的茄科開花植栽，原產於南美洲。上世紀初由日本人引進台灣，常被當為庭院、公園中的景觀植物或路邊的行道樹。2022年12月於南投鹿谷溪頭 (XiTou) 發現大花曼陀羅葉面密布褪綠黃點或輪斑，整葉觀之如嵌紋狀等疑似病毒感染的病徵。病組織粗汁液中可觀察到大小約750-800 x 13 nm的長絲狀病毒顆粒，罹病細胞內有典型的卷

軸狀、層狀和風車狀的內含體。使用*Potyvirus*屬病毒簡併式引子對(PNIbF1: 5)-GGBAAYAATAGTGGNCAACC-3)、PCPR1: 5)-GGGGAGGT-GCCGTTCTCDATRCACC-3)) 於 RT-PCR可增幅出預期的1.0-1.2 kb、涵蓋potyvirus NIb 3)-後半和 CP 5)-前半的cDNA片段。該片段經選殖於pCR™Blunt II-TOPO™載體，並送中興大學生科中心進行序列解析後，實際長度為1022 nt，並即進行序列比對。比對結果顯示增幅的cDNA片段序列與哥倫比亞曼陀羅病毒 (Colombian datura virus, CDV) 有 98%的相似度，故單斑分離後之供試病毒暫以哥倫比亞曼陀羅病毒溪頭分離株 (CDV-XT) 稱之。以已知的 CDV 全長度基因體序列 [CDV (JQ801448)、CDV-AT-Kr (MW075268) 和CDV-TA-Br2 (OL999301)] 為模版，取外鞘蛋白基因 (CP) 序列設計專一性引子對進行CDV-XT CP基因序列解析。結果顯示CDV-XT之鞘蛋白基因具有822個核苷酸序列，與其它已發表之CDV鞘蛋白基因核苷酸序列相似度在98.3-99.1%之間；胺基酸序列相同度則在98.5-99.3%之間。 CDV首先於荷蘭的番茄上被發現，隨後陸續於澳洲、匈牙利和加拿大等國家被報導，紀錄上可自然感染番茄、香瓜茄與燈籠果等茄科作物。初步之CDV-XT寄主範圍測試顯示：奎藜 (*Chenopodium quinoa*) 為單斑寄主，番茄 (*Solanum lycopersicum*)、茄子 (*S. melongena*)、菸草 (*Nicotiana benthamiana*, *N. tabacum* cv. Xanthi, *N. rustica*) 等則為系統性寄主。

B07 Identification of Melon aphid-borne yellows virus by quantitative reverse transcription- polymerase chain reaction－Kuan, C.-P. and Liu, Y.-T. (Division of Biotechnology, Taiwan Agricultural Research Institute, COA, Taichung)

Cucurbits are important fruit crops across the world. Plant viruses that infect cucurbits cause significant economic losses and limit cucurbits production. Melon aphid-borne yellows virus (MABYV) is one of the important viruses infecting cucurbits, which belong to the Polerovirus genus, part of the Luteoviridae family. The symptoms exhibited on leaves include mild mottle, mosaic, vein banding, ringspots, various types of necrosis, dis- colouration and deformation. In the field, melon are infected frequently with several viruses during a growing season, which leads to reduced yield and fruit quality. To better detect and quantitate MABYV that may be present in field and greenhouse samples, we developed a rapid and sensitive real-time RT-PCR assay for the detection of MABYV RNA. Leaves of MABYV-infected melon or watermelon were collected from the field and tested by real-time, RT-PCR analyses to quantify the number of MABYV present in test samples. This TaqMan real-time PCR assay for detection and quantitation of MABYV would be a useful tool for application in quarantine and certification of MABYV in cucurbit seedlings as well as in the research of disease resistance and epidemiology. The assays presented here could assist in the implementation of quarantine measures for MABYV identi cation and in routine indexing of MABYV for the production of virus-free cucurbit seedlings.

B08 Identification of Cucumber green mottle mosaic virus by polymerase chain reaction-based methods－Kuan, C.-P., and Hsiao, C.-J. (Division of Biotechnology, Taiwan Agricultural Research Institute, COA, Taichung)

Cucumber green mottle mosaic virus (CGMMV) infects cucurbit crops all over the world and is a typical tobamovirus that can be transmitted by mechanical contact and by seeds. CGMMV causes severe mosaic symptoms on infected cucumber leaves and fruits. CGMMV induces green mottle on cucumber, melon, squash, or watermelon leaves at an early time and fruit decay in the harvest period. Due to threats on cucurbit production, CGMMV is regarded as a virus with significance for plant quarantine. Hence, it is necessary to develop a rapid, sensitive and specific method for CGMMV identification. Here, we describe the sensitivity and specificity of this real-time RT-PCR －based assay and RT-PCR was compared of CGMMV in melon plants extracts. Using primers specifically designed for CGMMV, the protocols for amplification allow to compare the amount of CGMMV present in different plant tissues. The CGMMV-infected or CGMMV-free status of these plants was verified based on PCR assay for 1 months post-inoculation. The method developed in this study can diagnosis CGMMV plants, even before the appearance of symptoms of mosaic disease. This method is sensitive, rapid, less prone to contamination, economical, and has potential for large-scale application in surveys, quarantine, and certification programmes.

B09 香莢蘭病毒Cymbidium mosaic virus發生調查及分子鑑定－陳金枝、江芬蘭 (行政院農業委員會農業試驗所植物病理組)

Investigation and molecular identification of Cymbidium mosaic virus (CymMV) infecting *Vanilla* spp.－Chen, C.-C. and Chiang, F.-L. (Department of Plant Pathology, Taiwan Agricultural Research Institute, COA, Taichung)

香莢蘭 (*Vanilla* spp.) 為蘭科 (Orchidaceae) 多年攀緣性的常綠植物；有「香料皇后」之稱的香草，乃香莢蘭發酵後的果莢。香莢蘭為淺根性之半日照植物，栽培時需在半遮蔽的環境中，種植後約需3-5年才會量產果莢。全球主要的香莢蘭產區在馬達加斯加、印尼、巴布亞新幾內亞和墨西哥等國家。台灣商業化培香莢蘭與自產香草莢已超過十年，國內栽培面

積超過20公頃。香莢蘭栽培期間會受到真菌病害、蟲害及病毒病影響，文獻上記載可感染香莢蘭的病毒至少十種。其中蕙蘭嵌紋病毒 (Cymbidium mosaic virus; CyMMV) 為蘭科植物常見的病毒，CyMMV可感染香莢蘭的紀錄最早於1987年於南太平洋島嶼被發現 (Wisler et al., *Plant Dis*. 71:1125–1129)，罹病香莢蘭葉片出現微斑駁或微黃化嵌紋，但大多數出現無徵狀。本研究以間接式-酵素連結免疫吸附反應 (Indirect enzyme-linked immunosorbent assay; indirect ELISA)及反轉錄-聚合酶鏈鎖反應 (Reverse transcription-polymerase chain reaction; RT-PCR) 進行檢測，調查國內香莢蘭罹染CyMMV概況。觀察香莢蘭單獨檢出有CymMV者之病徵，由埔里所採集之罹病樣品其葉片出現微黃化嵌紋；由桃園或屏東所採集之罹病樣品其葉片出現微斑駁或無徵狀。以罹染有CymMV之香莢蘭進行扦插苗繁殖，此病毒可隨扦插苗而傳播。利用NCBI GenBank上已知之蘭花CymMV、以及本研究之香莢蘭CymMV之鞘蛋白核苷酸序列所分別設計的引子對，顯示以香莢蘭CymMV核酸序列所設計之引子對，更能有效檢出香莢蘭的CymMV。針對不同地方採樣來源之香莢蘭CymMV之鞘蛋白基因進行選殖與定序，以鞘蛋白胺基酸序列分析香莢蘭與蘭花CymMV分離株之類緣關係，初步結果顯示分離自香莢蘭之CymMV分離株之間，有較相近之親緣關係，但受不同採集地來源影響：分別由桃園、埔里、后里和屏東來源之分離株，依地區各自形成較相近的親緣關係，此等親緣關係之確認，有待更多不同地區來源之香莢蘭CymMV分離株做進一步的比較。國際間已有CymMV感染香莢蘭之紀錄，本研究為國內首次調查香莢蘭罹染CymMV之報告，並開發可有效檢測香莢蘭CymMV之引子對，用以精準地檢測。而香莢蘭之繁殖量化以組織培養或枝條扦插為之，CymMV的傳播途徑可透過繁殖用母本罹染本病毒而傳播，因此無性繁殖之香莢蘭母本應強化對此病毒之追蹤檢測。

B10 邊境攔截之蝴蝶百合 (*Calochortus uniflorus*) 進口種球檢出一種*Potyvirus*屬新病毒之分子鑑定與其檢測試劑製備應用－<u>陳金枝</u>、江芬蘭、廖家翌 (行政院農業委員會農業試驗所植物病理組

Identification of a distinct potyvirus on bulbs *Calochortus uniflorus* collected from border interception and preparation of virus detection reagents－<u>Chen, C.-C</u>, Chiang, F.-L., and Liao, J.-Y. (Department of Plant Pathology, Taiwan Agricultural Research Institute, COA, Taichung.)

蝴蝶百合 (*Calochortus uniflorus*) 為百合科的蝴蝶百合屬植物，分佈於北美西部、墨西哥和瓜地馬拉南部。Calohortus一詞源於希臘語，有“美麗的草”之意。本研究之受測樣品來源乃由行政院動植物防疫檢疫局新竹分局於2021年從海關邊境抽樣之荷蘭進口蝴蝶百合種球。本研究採取種球組織及種植後長出之葉片以Agdia公司出品之Potyvirus單元抗體進行間接式-酵素連結免疫吸附反應 (Indirect enzyme-linked immunosorbent assay, indirect ELISA)，受測之19個樣品中有1個 (樣品代號Calo6) 與Potyvirus單元抗體產生正反應；進一步純化Calo6之全量核酸進行反轉錄-聚合酶鏈鎖反應 (Reverse transcription-polymerase chain reaction, RT-PCR)，受測樣品與Potyvirus簡併式引子對 (HRP5/Oligo-dT$_{(14)}$) 有產生預估約1.3 kbp大小之核酸片段，經選殖與定序分析後，乃屬於Potyvirus病毒之核酸序列，將其帶有281個胺基酸之鞘蛋白 (coat protein, CP) 序列與美國國家生物科技資訊中心 (National Center for Biotechnology Information, NCBI) 基因資料庫 (GenBank) 已登錄的*Potyvirus*屬病毒之CP胺基酸序列比對後，與之相同度 (identity) 最相近者為67.6%的Tobacco vein banding mosaic virus (TVBMV)；初步由CP胺基酸比對結果鑑定本分離株為國際間尚未有登錄之一種potyvirus。本研究進一步以Potyvirus單元抗體針對其他642個蝴蝶百合進口種球進行檢測，篩檢出5個產生正反應之樣品，此5個樣品並與所設計的Calo6專一性引子對 (Calo-u/Calo-d) 於RT-PCR反應中，可被檢出有預估907 bp的病毒核酸片段；進一步經選殖病毒鞘蛋白核酸與定序後確認，此等分離朱與Calo6分離株之CP胺基酸相同度均高於97%，確認為相同病毒。檢出有此病毒者之植株出現的病徵為微斑駁或無徵狀。本研究以Calo6分離株之CP核酸序列構築於pET28a表現載體，再於*E. coli* BL21宿主中進行表現蛋白 (分子量預估約31 kDa) 之誘導與純化，以作為抗原製備出多元抗體，可成功用於蝴蝶百合上對此病毒之免疫檢測。本研究為首次自國外截獲蝴蝶百合的新種Potyvirus病毒紀錄；本研究成功研製之蝴蝶百合Potyvirus-Calo6核酸及免疫檢測用檢測試劑，可提升對蝴蝶百合病毒之邊境檢疫效能。

B11 利用催化髮夾組合系統 (catalytic hairpin assembly) 偵測柑橘磷砧類病毒 (*Citrus exocortis viroid*)－<u>簡浩原</u>、古大欣、沈湯龍 (國立台灣大學植物病理與微生物學系)

Catalytic hairpin assembly detection of *Citrus exocortis viroid*－<u>Chien, H.-Y.</u>, Ku, T.-H., Shen, T.-L. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

Viroids are small, circular, single-stranded RNAs. Due to its lack of an open reading frame (ORF), no protein can be transcribed. *Citrus exocortis viroid* (CEVd) is predominantly found infecting citrus varieties such as Trifoliate orange, Citrange, and Rangpur lime, and the development time of symptoms (bark shelling) takes 4-5 years to produce, so it is not easy to notice in the field. Currently, the detection methods for viroids are mainly bioassay and reverse transcription polymerase chain reaction (RT-PCR), but they take longer and require stricter experimental environments. To this end, we designed the Catalytic Hairpin Assembly (CHA) system, which uses two different hairpin DNAs to react with viroids: first, the viroid

fragments are identified by the hairpin DNA (I), and then the other hairpin DNA (II) activates the function of spontaneous assembly, continuing with the former and replacing the viroid fragment to keep it circulating. This study uses Python programming to design the hairpin DNA (I) and the hairpin DNA (II) and NUpack to assess the interaction between the hairpin DNAs and viroid and non-self-coupling between the two hairpin DNAs. The optimized reaction conditions for CHA chain replacement, including modification of reaction time, ratio of hairpins and type of working buffer, are investigated by gel electrophoresis. This enzyme-free, isothermal amplification strategy will be further combined with other optical detection methods in the future, allowing the technology to be read faster and more accurately.

B12　番茄黃化捲葉泰國病毒基因體DNA-B對臺灣新興番茄捲葉病毒之影響－賴玄春、梁專譯、蔡文錫 (國立嘉義大學植物醫學系)

The influence of tomato yellow leaf curl Thailand virus genomic DNA-B on new emerging tomato leaf curl begomoviruses in Taiwan－Lai, H.-C., Neoh, Z.-Y., and Tsai, W.-S. (Department of Plant Medicine, National Chiayi University, Chiayi)

番茄捲葉病於全球廣泛性的危害番茄生產，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，其可分單基因體與雙基因體 (DNA-A與DNA-B)。2019年在臺灣本島進行的番茄Begomovirus病毒調查，結果顯示共有6個病毒種，包含番茄捲葉臺灣病毒 (*Tomato leaf curl Taiwan virus*, ToLCTV)、番茄黃化捲葉泰國病毒 (*Tomato yellow leaf curl Thailand virus*, TYLCTHV) 與番茄捲葉新竹病毒 (*Tomato leaf curl Hsinchu virus*, ToLCHsV)，以及新興的洋桔梗贅脈捲葉病毒 (*Lisianthus enation leaf curl virus*, LELCV)、番茄捲葉嘉義病毒 (Tomato leaf curl Chiayi virus, ToLCCYV)與番茄捲葉南投病毒 (Tomato leaf curl Nantou virus, ToLCNTV)。TYLCTHV可分為TYLCTHV-B及TYLCTHV-D株系，LELCV則可分為4個株系 (A至D)，且臺灣番茄上以TYLCTHV-B與TYLCTHV-D、LELCV-A或LELCV-D混合感染為主。利用病毒基因體DNA-B廣效性引子對以PCR進行檢測，感病樣品DNA-B檢出率為30%，其基因體序列分析後與TYLCTHV-B DNA-B核酸序列相似度最高，達96%以上。感染臺灣番茄Begomovirus病毒中， TYLCTHV-B為雙基因體，ToLCTV為單基因體。在探討TYLCTHV-B DNA-B對臺灣其他番茄Begomovirus病毒之影響性上，藉由農桿菌分別將病毒DNA-A 與TYLCTHV-B DNA-B共同接種至圓葉菸草及番茄植株，結果顯示TYLCTHV-B DNA-B除與TYLCTHV-B DNA-A共感染外，僅能與LELCV-D共感染圓葉菸草及番茄，TYLCTHV-B DNA-B檢出率皆為百分之百，與無TYLCTHV-B DNA-B共感染者比較，除捲葉病徵外，尚有明顯的黃斑點。以粉蝨傳毒將LELCV-D與TYLCTHV-B DNA-B共感染番茄植株時，在帶有*Ty-1/Ty-3*，或帶有*Ty-1/Ty-3*與*Ty-2*抗病基因堆疊之番茄植株上，雖無明顯病徵，但皆能以PCR檢測到病毒與TYLCTHV-B DNA-B；在共感染帶*Ty-2*抗病基因番茄植株上，植株均有明顯病徵，且皆能測得病毒與TYLCTHV-B DNA-B，在與僅以LELCV-D進行感染者比較，共感染TYLCTHV-B DNA-B之植株，有縮短發病時程及出現明顯黃斑點病徵之情形。由此結果可以得知TYLCTHV-B DNA-B 除與TYLCTHV-B DNA-A共感染外，亦能與新興病毒LELCV-D共感染番茄，且能藉由粉蝨成功傳毒，此外TYLCTHV-B DNA-B之共感染，能縮短發病時程與改變病徵形態，在對臺灣防治番茄捲葉病上，是否產生一定的影響性，則需進一步探討。

學生論文宣讀比賽 Student oral presentation contest

SA01　印度梨形孢菌應用於褐根病防治之探討－劉沛軒[1]、蔡志濃[2]、彭婉兒[1]、鍾嘉綾[1, 3] ([1]國立臺灣大學植物醫學碩士學位學程、[2]行政院農委會農業試驗所植物病理組、3國立臺灣大學植物病理與微生物學系)

Investigate the potential of using *Serendipita indica* for the control of brown root rot disease－Liu, P.-H.[1], Tsai, J.-N.[2], Pang, Y.-Y.[1], and Chung, C.-L.[3] ([1]Master program for plant medicine, Nation Taiwan University, Taipei; [2]Plant Pathology Division, Agricultural Research Institute, COA, Taichung; [3]Department of Plant Pathology and Microbiology, Nation Taiwan University, Taipei)

Phellinus noxius 引起的褐根病 (Brown root rot disease) 導致樹木根基部腐朽，倒伏風險增加，影響公共安全。防治褐根病的眾多方法之中，生物防治法不會有抗藥性產生且對環境友善，值得進一步發展。印度梨形孢菌 *Serendipita indica* 為可人工培養的植物內共生真菌，可促進植物生長並協助其抵抗非生物及生物逆境。本研究首先針對印度梨形孢菌之培養及接種方式進行測試，發現將印度梨形孢菌以液態培養，並將孢子懸浮液濃度調整至10^6 spore/ml，以100 mL混拌至650 g土壤中進行接種 (約1.5×10^5 spores/g soil)，可有效促進茄苳苗的根系生長，也能透過台盼藍染色法在根部觀察到最多的厚膜胞子。為了解印度梨形孢菌能否運用於褐根病防治，使用茄苳苗進行接種試驗，發現相較於只接種褐根病菌的組別，接種印度梨形孢菌後兩週再接種褐根病菌的組別萎凋率較低，根系的褐根病菌分離率也較低，且接種較高濃度印度梨形孢菌防治效果較佳。為了進一步瞭解印度梨形孢菌與殺菌劑共同施用的可行性，也作為未來開發印度梨形孢菌選擇性培養基之參考，測試其在含有100 ppm (mg a.i./L)、10 ppm、1 ppm、0.1 ppm 的殺紋寧、百克敏、嘉賜黴素、得克利、鋅錳乃浦、克熱淨及腐絕快得寧共 7 種藥劑之馬鈴薯葡萄糖瓊脂培養基上菌絲生長情形，結果顯示印度梨形孢菌對殺紋寧呈現高度抗性，對百克敏為低敏感性，

對得克利則具有敏感性。期望未來將印度梨形孢菌運用於樹木健康管理，達到預防褐根病之效果。

SA02 鎳離子對象耳豆根瘤線蟲胚胎發育與卵孵化影響－郭浩宇、楊爵因 (國立臺灣大學植物病理與微生物學系)

The effect of nickel on ***Meloidogyne enterolobii*** embryogenesis and egg hatching－Kuo, H.-Y. and Yang, J.-I. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

根瘤線蟲 (*Meloidogyne* spp., RKN) 遍布全球，寄生於植株根部會使其產生結瘤並影響作物生長，由於其寄主廣泛，每年造成約1.7兆美元的全球經濟損失。二齡幼蟲 (J2) 為根瘤線蟲侵染植物的關鍵時期。然而，J2時期前的胚胎發育過程及卵孵化對於後來成功寄生宿主至關重要。根瘤線蟲蟲卵由多個膜層組成，可以保護胚胎免受外界環境的壓力影響。脂酶、幾丁質酶等酵素的分泌及口針戳刺在這些過程中扮演重要的角色。近年，本實驗室發現，環境中的鎳離子縮短了象耳豆根瘤線蟲 (*M. enterolobii*) 胚胎發育的時間但延遲了卵孵化的時間，暗示象耳豆根瘤線蟲可能具有未曾被報導過的重金屬壓力反應機制。因此，本研究透過一系列的生理實驗，探討不同濃度的鎳離子 (100、2000和3000 ppm) 對象耳豆根瘤線蟲的胚胎發育及卵孵化機制的影響。異常卵及未孵化卵比率於濃度2000 ppm時顯著增加，且孵化率在濃度3000 ppm時降低約63.3%。隨著鎳離子濃度增加，孵化率達50%所需的時間也有增加的趨勢。胚胎發育試驗中，在濃度100 ppm時從多細胞時期到一齡幼蟲時期發育有加速的趨勢。此外，在2000和3000 ppm濃度下，胚胎發育異常的比率分別高達71.1%和78.8%。未孵化的J2口針戳刺頻率在濃度2000 ppm時下降了14.4%，且在濃度3000 ppm時下降了46.5%。然而，鎳離子沒有影響未孵化J2的型態；口針的長度、角質層和肌肉纖維的厚度與對照組無差異。後續研究將探討鎳離子對卵殼通透性的影響，並利用轉錄體分析和qRT-PCR綜合評估鎳離子調控象耳豆根瘤線蟲胚胎發育與卵孵化可能機制。

SA03 蝴蝶蘭黃葉病菌之檢測技術開發－杜易禾[1]、曹維鈞[1]、王智立[1, 2, 3] ([1]國立中興大學植物病理學系、[2]國立中興大學植物醫學碩士學位學程、[3]國立中興大學植物保健學程)

Development of detection methods for ***Fusarium solani*** f. sp. ***phalaenopsis*** causing leaf yellows of phalaenopsis－Du, Y.-H., Tsao, W.-C., and Wang, C.-L. ([1]Department of plant Pathology, National Chung Hsing University, Taichung; [2]The Master Program for Plant Medicine and Good Agricultural Practice, National Chung Hsing University, Taichung; 3Plant Health Care Master Degree Program, National Chung Hsing University, Taichung)

蝴蝶蘭 (*Phalaenopsis* species) 為台灣重要出口花卉，占外銷花卉市場比例達70%，為我國不可或缺的重要農產品，然而有許多因素會導致蝴蝶蘭折損，增加外銷成本，其中黃葉病就是相當重要的因素之一；蝴蝶蘭黃葉病是由***Fusarium solani*** f. sp. ***phalaenopsis***所引起的病害，該病害造成葉片黃化、葉鞘壞疽及落葉，使蝴蝶蘭失去其商品價值或死亡，且因其潛伏期長和藥劑防治效益不佳，常造成蝴蝶蘭栽培生產以及出口產業嚴重的損失。本研究欲開發偵測蝴蝶蘭黃葉病菌的方法，以應用於探討病原菌生態，期可建立有效的防治策略。首先開發*F. solani*的半選擇性培養基，提升分離目標病原菌的效率，半選擇性培養基以Nash & Snyder medium進行改良，保留其基底成分，去除管制藥品pentachloronitrobenzene (PCNB)及Streptomycin，改為添加殺菌劑及Rose bengal等作為抑菌物質，並以蝴蝶蘭園常見之真菌為參照，提升蝴蝶蘭黃葉病菌的分離效率；並利用全基因比對的方式，設計針對蝴蝶蘭黃葉病的專一性引子對，將蝴蝶蘭黃葉病菌之全蛋白基因，與***Fusarium proliferatum***、***Fusarium ambrosium***、***Fusarium oxysporum*** f. sp. ***lycopersici***、***Fusarium solani***、***Fusarium vanettenii***等五菌株之全蛋白基因以orthovenn2比對，並進一步比對NCBI資料庫，篩選黃葉病菌獨有之專一基因，結果選出基因編號g1689以及g6345兩基因，根據兩基因序列設計之引子對，可分別針對蝴蝶蘭黃葉病菌增幅出大小662 bp以及557 bp之條帶，並以*F. solani* species complex的其他種菌株驗證其專一性。未來應用於調查田間接種源時，可先以培養基進行分離，再以專一性引子對確認獲得之菌株，來進一步增加對黃葉病菌生態的瞭解。

SA04 台灣與金門地區高粱穗腐病之鐮胞菌鑑定－蕭兆良[1]、陳以錚[2]、林韋汝[2]、蘇士閔[3]、王智立[1, 4, 5] ([1]國立中興大學植物病理學系、[2]國立嘉義大學植物醫學系、[3]行政院農業委員會種苗改良繁殖場、[4]國立中興大學植物醫學碩士學位學程、[5]國立中興大學植物保健學程)

Identification of ***Fusarium*** spp. causing sorghum grain mold in Taiwan and Kinmen－Hsiao, C.-L.[1], Chen, Y.-J.[2], Lin, W.-J.[2], Su, S.-M.[3], and Wang, C.-L.[1, 4, 5] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Department of Plant Medicine, National Chiayi University, Chiayi; [3]Taiwan Seed Improvement and Propagation Station, COA, Taichung; [4]The Master Program for Plant Medicine and Good Agricultural Practice, National Chung Hsing University, Taichung; [5]Plant Health Care Master Degree Program, National Chung Hsing University, Taichung)

在台灣政府近年來推廣旱作及雜糧作物的政策下，高粱 (*Sorghum bicolor*) 栽培面積逐漸的上升，重要性也逐漸提高。高粱受到許多的病原菌危害，例如紋枯病 (sheath blight)、葉斑病 (target leaf spot) 及穗腐病 (grain mold)，其中造成穗腐病的病原菌主要為鐮胞菌屬 (*Fusarium* spp.)，此病害會覆蓋白色、粉紅色的黴狀物於穗部的穀粒，造成穀粒品質及產量下降，甚

至影響種子的活性及發芽率。早期以形態特徵為主鑑定病原菌，但是容易受到培養環境的影響混淆相似的病原菌種類；在台灣植物病害名彙的紀錄中，造成高粱穗腐病的鐮胞病菌為 *Fusarium moniliforme*，目前該種名已不再使用，新的鑑定技術已將該種的菌株分類為不同的物種。本次研究以分子鑑定為主，形態鑑定為輔，釐清台灣高粱穗腐病菌的病原菌種類，並確認病原性。自桃園市新屋區、台中市新社區、雲林縣土庫鎮、台南市學甲區和金門烈嶼等地區蒐集高粱穗腐病樣本，進行病原菌分離，共收集到42株分離株，首先利用 translation elongation factor 1-alpha (*tef1α*) 分析菌株，初步鑑別菌株分別屬於 *Fusarium fujikuroi* species complex (FFSC) 與*Fusarium incarnatum-equiseti* species complex(FIESC)，從中挑選代表菌株進行多基因分析，FFSC利用the second largest subunit of RNA polymerase II (*rpb2*)及*tef1α* 二基因序列；而FIESC也是合併*rpb2* 及*tef1α* 二基因序列進行分析。鑑定結果顯示在42株分離株中FFSC的種類為*F. verticillioides*、*F. proliferatum*、*F. andyiazi*、*F. thapsinum*，FIESC的種類為*Fusarium pernambucanum*、*F. sulawense*及一株未知種。並分別記錄每物種之代表菌株在carnation leaf agar (CLA)上分生孢子和產孢構造及PDA培養基的菌落形態。以科霍式法則確定每物種之代表菌株在穗部的病原性，以接種高粱幼苗出現苗枯、根腐及根系褐化等病徵，檢測各分離株對高粱的病原性。本研究顯示台灣各高粱產區引起穗腐病的鐮胞菌種類具多樣性，可提供日後之學術研究及田間栽培管理參考。

SA05 Microbiome study of asymptomatic and symptomatic Welsh onion naturally infected by the leaf blight pathogens in Taiwan－Jayasinghe, H.[1], Yang, S.-H.[2], Liu P.-Y.[3], Ariyawansa, H.-A.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, [2]Institute of Fisheries Science, National Taiwan University, Taipei, [3]Biodiversity Research Center, Academia Sinica, Taipei)

Fungal pathogenic attacks are one of the major threats to the growth and productivity of Welsh onion (*Allium fistulosum* L.) in Taiwan. Our recent study identified that Welsh onion plants are severely threatened by fungal foliar diseases such as Stemphylium leaf blight and Anthracnose, greatly affecting their yield and quality. Plants are associated with enormous microbes and this complex plant-associated microbial community is critical for plant health. However, very little is known about the influence of plant pathogens on the configuration of microbial communities allied with Welsh onion during natural outbreaks in agricultural fields. Therefore, characterizing the microbial communities associated with leaf blight disease incidence may provide a better insight into the disease management of Welsh onion. Increasing evidence suggests that the rhizosphere may recruit beneficial microbes to suppress soil-borne pathogens, but the microbiome assembly due to foliar pathogen infection is not fully understood. Thus, to provide a comprehensive view of the Welsh onion-associated microbiome, we analyzed the microbiome associated with the phyllosphere and rhizosphere of healthy (without visual symptoms) and symptomatic Welsh onion naturally infected by leaf blight pathogens by amplicon sequencing targeting Internal Transcribed Spacer (ITS) region and 16S rRNA genes for fungi and bacteria respectively. Our results revealed a difference in the fungal and bacterial community's abundance, diversity, and similarity between diseased and healthy Welsh onions. With regard to fungal communities, fungal genera like *Malassezia*, *Phaeosphaeria*, and *Hannaella* were abundant in the phyllosphere of healthy plants. Being enriched in the symptomatic phyllosphere, the fungus *Stemphylium* was identified as the most probable causative agent for the development of leaf blight symptoms. Meanwhile, an increase of genera such as *Athelia* and *Colletotrichum* was observed in the diseased rhizosphere. Remarkably, an enriched *Fusarium* was observed in a healthy rhizosphere. With regard to bacterial communities, the dominance of Firmicutes was observed in the healthy phyllosphere while the dominance of Proteobacteria was observed in the healthy rhizosphere. In the healthy phyllosphere, bacterial genera like *Clostridium*, *Terrisporobacter* and *Turicibacter* were more abundant compared to the diseased phyllosphere. Shannon diversity indicated that, in both phyllosphere and rhizosphere, species diversity of bacteria and fungi was high in healthy plants compared to diseased plants. Higher microbial diversity found in healthy plants could be indicative of pathogen suppression events preventing or minimizing disease expression. Moreover, principal coordinate analysis separated both fungal and bacterial communities in the phyllosphere of symptomatic and asymptomatic plants. This comparative study of the microbiota in the two plant conditions might provide fundamental information on the microbial community structures between symptomatic and asymptomatic Welsh onion and will serve as a basis for further research investigating the development of improved disease management systems and preventive counteractions.

SA06 Development of molecular marker for detecting *Fusarium oxysporum* f. sp. *eustomae* causing lisianthus wilting and population dynamics in fields－Wu, C.-C.[1] and Chung, W.-H.[1, 2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Master Program for Plant Medicine and Good Agricultural Practice, National Chung Hsing University, Taichung)

Lisianthus is one of the most important cut-flowers in Taiwan

for export to Japan. According to field investigation, *Phytophthora* sp., *Fusarium oxysporum*, *F. solani* were frequently isolated from wilting/stem rot lisianthus, especially, *F. oxysporum*. Previous study indicated that *Fusarium oxysporum* f. sp. *eustomae* (*Foe*) was the causal agent to cause root/stem rot and wilting in Taiwan. However, two molecular groups (I and II) were separated by molecular phylogenetic analysis. Currently, *F. oxysporum* is considered as species complex (FOSC) and reidentified by multi-locus genes (*cmdA*, *rpb2*, *tef1*, and *tub2*) analysis. Here, the Foe isolates causing root/stem rot and wilting in lisianthus in Taiwan are clarified their species in FOSC and the molecular markers are developed to study their population dynamic in field. The multi-locus genes analysis showed that group I formed same group with *F. nirenbergiae* and group II is distinguished from other species in FOSC. Moreover, the pathogenicity test indicated that group I isolates have higher virulence in cultivar of Arena III pink flash and group II isolates have higher virulence in cultivar of Voyage II first love. For molecular marker developing, two primer pairs, *SIX6*-220628-F/R for group I and *SIX1*-220813-F/R for group II, were designed from *SIX* (*Secreted in Xylem*) genes, and the two primer pairs showed specificity to group I and group II based on multiplex and touchdown PCR. Furthermore, the sensitive test indicated that *SIX6*-220628-F/R and *SIX1*-220813-F/R could amplified 5 ng and 1 ng of purified fungal DNA, respectively. According to the PCR examination, group I isolates were dominant agent during lisianthus growth season; meanwhile, group II isolates were mostly detected in winter.

SA07 Fungal flora and survival of *Fusarium solani* in the sphagnum moss for phalaenopsis production—Lo, Y.-H. and Hong C.-F. (Department of Plant Pathology, National Chung Hsing University, Taichung)

Taiwan is a major exporter of phalaenopsis orchids with sphagnum moss to the United States, New Zealand, and Australia. During the extended shipping period, fungal disease is one of the limiting factors threatening the exportation of the commodity. To mitigate potential loss caused by fungal pathogens, the objectives of the study were: 1. to investigate the pathogenic fungal flora associated with sphagnum moss; 2. to compare the fitness traits of fungal pathogens in sphagnum moss, and 3. to investigate the effect of fungicide treatment on the fluctuation of fungal pathogen populations in sphagnum moss. Among 334 sphagnum samples collected from 12 phalaenopsis orchid nurseries, more than 7 fungal genera were found associated with sphagnum moss. However, *Fusarium solani* was the only species found in sphagnum moss that could cause necrosis and leaf yellowing on phalaenopsis. To investigate why *F. solani* was dominating in sphagnum moss comparing with other three previously documented phalaenopsis-pathogenic *Fusarium* spp. in sphagnum moss, spores of the four *Fusarium* spp. were treated with different sphagnum moss extracts. After 12 hours, spore germination rate and length of the germ tubes of *F. solani* were generally higher than other *Fusarium* spp., suggesting that *F. solani* might better fit in sphagnum moss than other *Fusarium* spp. To further investigate the survival and the effect of fungicide treatment on population fluctuation of *Fusarium* spp. in sphagnum moss, 10 fungicides were respectively amended with sphagnum mosses and inoculated with *F. solani*, *F. oxysporum*, and *F. proliferatum*. Prochloraz was found reducing the population of *Fusarium* spp. in the beginning of treatment whereas propamocarb and procymidone not only increased the *Fusarium* spp. populations but also worsened the root symptoms on phalaenopsis orchid. Our results suggested that some caveats should be taken into considerations when selecting fungicides for disease management.

SA08 Biocontrol potential of novel rhizobacterial strains against *Fusarium oxysporum* f. sp. *lycopersici* infecting Tomato (*Solanum lycopersicum*)—Khayamali, Sunil[1] and Chang, Pi-Fang Linda[2,3] ([1]International Master Program of Agriculture, National Chung Hsing University, Taichung; [2]Department of Plant Pathology, National Chung Hsing University, Taichung; [3]Program of Plant Health Care, Academy of Circular Economy, National Chung Hsing University, Nantou)

Plant stresses have urged farmers to heavily rely on chemical fertilizers, fungicides, and pesticides. This is also the case with managing devastating tomato Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Hence, it is important to foster an effective, economical, and eco-friendly solution. Silicon is the second most abundant element on the earth's crust; however, its potential in crop protection has not gained much attention due to its poor availability to plants. Beneficial microbes that facilitate the solubilization of inorganic nutrient pools in the soil to enhance nutrient availability for plants and possess antimicrobial properties can act as green alternatives to agrochemicals. Therefore, this study aims to obtain rhizobacteria with multiple beneficial attributes and determine key factors in stress mitigation. The bacterial strains were screened for their ability to solubilize inorganic silicate and phosphate, tolerate salt and drought conditions, and produce indole acetic acid and siderophore. Strains were further assessed for in vitro inhibition of five economically important phytopathogens, namely, *F. oxysporum* f. sp. *niveum*, *Magnaporthe oryzae*, *Colletotrichum gloeosporioides*, and two races of FOL isolates.

Thirteen potentially beneficial strains were obtained, and the two best-performing *Burkholderia* spp., which confirmed positive for all tested in vitro attributes and inhibited the growth of all tested phytopathogens, were chosen for pot experiments in a greenhouse along with silicon supplementation. Disease severity of tomato plants challenged with FOL was reduced by both stains regardless of silicon supplementation. The use of these biocontrol agents is expected to regulate plant growth and defense-related genes.

SA09 Volatile compounds of beneficial rhizobacterium *Bacillus cereus* C1L alter *Arabidopsis* root exudate composition for better root colonization—Ruan, T.-H., Lin, C.-H., and Chen, C.-Y. ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

Plant roots exude photosynthetically fixed carbon sources into rhizosphere, which attract the colonization of specific microbes. Colonization of rhizobacteria is critical for their beneficial effects on plants such as plant growth promotion and induced systemic resistance (ISR). Volatile compounds (VCs) emitted from beneficial rhizobacteria function as inter-kingdom signal molecules mediating physiological responses of plants. As known, rhizobacterial colonization changed root exudate composition and further recruited beneficial rhizobacteria. Presumably, VCs from beneficial rhizobacteria could act as signals to alter root exudates that affect rhizobacterial behaviors and survival. In this study, a gnotobiotic system was used to assay the effect of the VCs emitted from beneficial rhizobacterium *Bacillus cereus* C1L on *Arabidopsis* root exudates. *B. cereus* C1L VCs-treated *Arabidopsis* root exudates increased C1L colonization on *Arabidopsis* roots. Through the bioassay on a C1L mutant reduced in the production of VC constituents, a decrease in C1L colonization was observed on *Arabidopsis* roots pretreated with mutant VCs compared to that pretreated with wildtype C1L VCs. Meanwhile, pretreating the VC constituents individually showed some of them were able to increase colonization of *B. cereus* C1L on *Arabidopsis* roots. The involvement of secondary metabolite coumarins from *Arabidopsis* roots treated with C1L VCs will be investigated by qRT-PCR to explicit whether the recruitment of *B. cereus* by root exudates is related to coumarin production. Overall, this research increases our knowledge of molecular interactions between plant hosts and beneficial rhizobacteria, and facilitates agricultural production.

SA10 Interaction between *Bacillus subtilis* and *Ralstonia solanacearum* in tomato rhizosphere—Yen, X.-C.[1] and Huang, T.-P.[1,2] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Plant Health Care Program, Academy of Circular Economy, National Chung Hsing University, Nantou)

Tomato bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating soil-borne diseases in tomato, resulting in significant yield losses. Due to the soil-borne characteristics and long survival time in soils by *R. solanacearum*, chemical controls of tomato bacterial wilt are considered to be less effective. Beneficial microbes such as *Bacillus* species have been shown to compete the root niche with *R. solanacearum* and reduce the population density of *R. solanacearum*, and exhibit efficacy for the control of tomato bacterial wilt. Sugars are the major metabolites of plant root exudates, and can be transported by sugar transporting systems. Among the sugar transporting systems, *S*ugar *W*ill *E*ventually be *W*xported *T*ransporters (SWEETs) were demonstrated to participate in plant pathogen infection process. Our previous study showed that *B. subtilis* MCLB2 can suppress tomato bacterial wilt and colonize the roots of tomato. *AtSWEET2* was shown to be involved in Arabidopsis root colonization by *B. subtilis* MCLB2. The objectives of my research are to assess the root colonization by *B. subtilis* and *R. solanacearum* on tomato; to investigate the roles of root exudates and sugars in biofilm formation by *B. subtilis* and *R. solanacearum*; to find possible association of *Solanum lycopersicum* SWEET with *B. subtilis* and *R. solanacearum*. Our results indicated that *B. subtilis* MCLB2 was chemotactic to root exudates of tomato. Root exudates of tomato were found to induce the biofilm and pellicle formation by *B. subtilis* MCLB2. *B. subtilis* MCLB2 could interfere the colonization and prevent the infection on tomato root by *R. solanacearum*. Our data also showed that *B. subtilis* MCLB2 exhibited chemotaxis toward glucose and fructose which could be transported by SWEETs. Additionally, glucose, galactose, fructose and mannose enhanced biofilm and pellicle formation by *B. subtilis* MCLB2. *SlSWEET2b* and *SlSWEET16* were differentially expressed when the tomato plants were inoculated with *B. subtilis* MCLB2 and *R. solanacearum* suggesting *SlSWEET2b* and *SlSWEET16* may be involved in the root colonization by these beneficial and pathogenic bacteria.

SA11 Investigation on viral diseases of kenaw (*Allium macrostemon* Bunge) in Hualien area—Lin, C.-C.[1], Tasi, Y.-C.[2], and Chen, T.-C.[1] ([1]Department of Medical Laboratory Science and Biotechnology, Asia University, Taichung; [2]Hualien District Agricultural Research and Extension Station, Hualien)

Allium macrostemon Bunge is a wild onion widely distributed in East Asia and has become a featured crop of the Amis people in

Hualien, who named it kenaw. Since September 2021, kenaw plants showing virus-like symptoms, such as stunting and yellowing, were observed in Shoufeng Township, Fonglin Town, Guangfu Township and Ji-an Township. Filamentous virus particles of varying lengths were examined in symptomatic kenaw samples by transmission electron microscopy. A combination of total RNA extracted from collected kenaw samples was used as a template to construct a random primer-derived cDNA library. High-through sequencing (HTS) was performed using the Illumina NovaSeq 6000 sequencing system for virus diagnosis. A total of 47,357,824 reads were obtained. After filtering plant sequences, 46,787,466 reads were retained and used for *de novo* assembly, resulting in 398,764 contigs. Of these, 567 contigs were annotated as viral sequences, including four known *Allium* viruses, asparagus virus 3 (AV3, *Potexvirus*) and garlic virus C (GarV-C, *Allexivirus*) of the *Alphaflexiviridae* family, shallot latent virus (SLV, *Carlavirus*) of the *Betaflexiviridae* family, and scallion mosaic virus (ScaMV, *Potyvirus*) of the *Potyviridae* family. The presence of SLV was verified by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using the newly designed species-specific primers. The PCR products were cloned and sequenced to confirm the correctness of the RT-qPCR results. The 2022-2023 survey on the incidence of SLV in kenaw in Hualien showed that the detection rate was 98.3%.

SB01 百合灰黴病菌效應子BeSerp增加病原菌毒力及活化寄主防禦之現象探討－許家偉、陳昭瑩 (國立臺灣大學植物病理與微生物學系)

Phenomena of the increase of pathogen virulence and activation of host defense by *Botrytis elliptica* effector BeSerp－Hsu, C.-W. and Chen, C.-Y. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

百合灰黴病菌 (*Botrytis elliptica*) 為百合屬 (*Lilium*) 植物的專一性死體營養型病原真菌，在低溫高濕環境下感染百合組織，造成葉片焦枯、花苞畸形及花瓣枯萎等病徵，嚴重影響百合切花及種球的產量與品質。東方型葵百合 (*Lilium* ‘Star Gazer’) 的防禦蛋白LsGRP1之LsGRP1 N端區段 ($LsGRP1^{N}$)，在體外試驗標定於百合灰黴病菌之菌絲表面，推測$LsGRP1^{N}$與百合灰黴病菌胞外蛋白具有交互作用。續經免疫共沉澱法釣取百合灰黴病菌BeSerp為LsGRP1N假定交互作用蛋白，並推定其為一類枯草桿菌蛋白酶 (subtilisin-like proteases, subtilases)。以RT-qPCR分析百合灰黴病菌感染感病性亞洲型百合*Lilium* ‘Tresor’之*BeSerp*表現時間圖譜，發現感染初期的*BeSerp*表現量會提高五倍再微幅下調，並於接種後三天增加至20倍。以噴灑誘導基因靜默 (spray-induced gene silencing, SIGS) 的方式處理dsRNA，發現相較於對照組，處理*BeSerp* dsRNA會顯著降低*BeSerp*表現量以及百合灰黴病菌所造成的病斑面積，揭示BeSerp為一重要之毒力因子。以雙分子螢光互補技術 (bimolecular fluorescence complementation) 在菸草葉組織驗證LsGRP1與BeSerp的交互作用，可於細胞表面偵測到訊號。另一方面，以大腸桿菌系統表現BeSepΔSS融合蛋白並自包涵體 (inclusion body) 進行純化，所得BeSepΔSS融合蛋白在脫脂奶粉培養基及蛋白酶活性檢測螢光試劑中均未測得酵素活性。為了解BeSerp是否經由直接或間接殺滅百合細胞以促進感染，將BeSepΔSS融合蛋白單獨或輔以能夠毒殺百合細胞的百合灰黴病菌外泌液注入百合葉盤，經由偵測細胞質電解質滲漏以量化百合死亡程度，發現BeSerpΔSS融合蛋白非但不會促成百合細胞死亡，反而能抑制由百合灰黴病菌外泌液誘發的百合細胞死亡。據此，以BeSerpΔSS融合蛋白預處理*Lilium* ‘Tresor’，透過比較接種百合灰黴病菌所造成的病斑面積以及處理百合灰黴病菌外泌液所誘發的癒傷葡聚醣 (callose) 沉積量，得知BeSerpΔSS融合蛋白應具有激發子功能。初步推測大腸桿菌表現之BeSerpΔSS融合蛋白無法表現天然BeSerp具有的酵素活性，但不排除BeSerp效應子可能具有毒力因子及激發子之雙重角色。

SB02 A Protein-dsRNA complex to increase dsRNA stability for spray-induced gene silencing against *Botrytis cinerea*－Chang, L.-P. and Chen, L.-H. (Department of Plant Pathology, National Chung-Hsing University, Taichung)

Cross-kingdom RNA interaction between pathogen and host has been reported in several fungal species giving a new sight of the plant protection strategy called spray-induced gene silencing (SIGs). The dsRNA that targets fungal essential or virulence genes sprayed on leaves or fruits is absorbed by fungi and activates the RNA interference machinery in fungi. Silencing these target fungal genes influences fungal pathogenicity or virulence leading to mild symptoms and fungal growth inhibition. Different species of fungi exhibit different dsRNA uptake efficiency that is positively related to the success of SIGs. Moreover, SIGs application is restricted in lab conditions due to the instability of dsRNA caused by various environmental factors such as high UV, rainfall, and ribonuclease resulting in dsRNA degradation. We proposed a protein-based carrier composed of a dsRNA binding domain (DRBD) from *E. coli* RNase III (*rnc*) to increase the stability of dsRNA. We have already produced *rnc*-DRBD and confirmed that *rnc*-DRBD interacting with dsRNA is sequence and size-independent by electrophoretic mobility shift assay (EMSA). In the ribonuclease protection assay, *rnc*-DRBD protects dsRNA against RNase A digestion. Finally, using fluorescent-labeled dsRNA has shown that the rnc-DRBD complex with dsRNA can be absorbed by *Botrytis cinerea* the same as naked

dsRNA.

SB03 Elucidating the role of helper NLR proteins in the plant innate immunity mediated by receptor-like proteins－Huang, L.-T.[1], Wu, C.-H.[2] and Chen, L.-H.[1] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei)

Plant immune receptors can be divided into cell-surface pattern recognition receptors (PRRs) such as receptor-like proteins (RLPs) and receptor-like kinases (RLKs), and intracellular nucleotide-binding, leucine-rich repeat receptors (NLRs). Both PRRs and NLRs directly or indirectly recognize molecules from pathogens. The activation of these receptors after molecules perception triggers downstream signaling, resulting in immune responses against pathogen infection, including Ca^{2+} influx, reactive oxygen species (ROS) production, and cell death called hypersensitive response (HR). NLRs are mainly grouped into three classes according to their N-terminal domains, including CC-type (CNLs), TIR-type (TNLs), and RPW8-type (RNLs). In NLR-mediated immunity, sensor NLRs responsible for molecule recognition require helper NLRs for downstream signaling and triggering immune responses. In Arabidopsis, the RNLs ACTIVATED DISEASE RESISTANCE 1 (ADR1s), and N REQUIREMENT GENE 1 (NRG1s) act as helper NLRs for some CNL and most TNLs mediated immunity. In Solanaceous plants, the helper NLRs in the NLR-REQUIRED FOR CELL DEATH (NRC) family form signaling networks with several sensor NLRs. Recent studies showed that helper NLRs are also involved in RLP-mediated immunity, but the detailed mechanism remains to be further investigated. In this study, we found that RLP Cf-2, Cf-4, Cf-9, and RXEG1-mediated HR were reduced in the absence of helper NLRs NRC2, NRC3, and NRC4 in *Nicotiana benthamiana*. In contrast, the immune responses mediated by RLP protein ELICITIN RESPONSE (ELR) were unaffected, indicating that RLPs-mediated HR was not completely dependent on NRCs. According to our study, the RLPs-mediated HR may be regulated by other downstream signaling component in NRCs-independent manner in solanaceous plants. Whether different RLPs have different signaling pathway respectively and how those RLPs induce HR remains to be elucidated.

SB04 探討番茄萎凋病菌C2H2鋅指蛋白*FOXG_00031*的基因功能－范家安、陳穎練 (國立台灣大學植物病理與微生物學系)

Functional characterization of zinc finger protein *FOXG_00031* in tomato *Fusarium wilt fungus*－Fan, J.-A. and Chen, Y.-L. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei)

土傳性真菌番茄萎凋病菌(*Fusarium- oxysporum* f. sp. *lycopersici*; *Fol*) 所造成的番茄萎凋病是全球番茄重要的病害之一，受感染的植株初期會有明顯的半邊萎凋，下位葉逐漸向上黃化和萎凋，最終死亡。而番茄萎凋病菌所生成的厚膜孢子會長時間留存在土壤中，導致病害不易解決。在許多病原菌中，帶有C2H2鋅指結構的Crz1轉錄因子已被證實對生長、環境壓力、毒力以及細胞壁穩地性有著重要的調控。相對於*Fusarium graminearum*的Crz1，也帶有C2H2鋅指結構的Fg01350轉錄因子對於菌絲生長、鈣離子耐受性以及毒力有更明顯的影響。在番茄萎凋病菌中，本實驗室研究發現FolCrz1的影響並不明顯，然而*Fol*鈣調磷酸酶其它下游蛋白的調控機制尚不清楚。在本研究中，分析在*Fol*中和Fg01350胺基酸序列相似度最高，並且也帶有三個C2H2鋅指結構的FOXG_00031的基因功能。透過基因剃除，ΔFOXG_00031在滲透壓力及在鈣離子環境中的表現和野生株相似，這和之前研究其它病原菌的結果迥異。然而ΔFOXG_00031的菌絲生長有明顯的下降，且在番茄盆栽試驗中發現ΔFOXG_00031的毒力明顯降低，顯示FOXG_00031可能在菌絲生長及感染番茄的過程中扮演重要角色。

菌能固定大氣中的游離氮氣，將其轉化為氨 (NH_3) 等含氮化合物，可促進植物生長，減少氮肥用量。固氮根瘤菌亦具有競爭養分、產生抗生物質、分泌細胞壁分解酵素等多種抑菌機制，也可作為生物防治資材。台灣目前尚無豆科固氮根瘤菌之微生物肥料商品上市，僅透過各地區農業改良場推廣應用於大豆及紅豆的生產。故本研究欲瞭解台灣農田土壤中存在的固氮根瘤菌種類及特性，並初步測試其對大豆植物生長之影響，以供未來作為微生物肥料評估之參考。本研究之供試菌株分離自彰化縣竹塘鄉及嘉義大學蘭潭校區兩地種植之金珠大豆的根瘤，經柯霍氏法則 (Koch's postulates) 驗證後，共獲得 10 株分離株。根據分離株生長在 Yeast extract mannitol (YEM) agar 培養基的菌落形態及生長速度，可區分為快生表面具光澤、慢生表面具光澤、慢生表面不具光澤及慢生流質狀等 4 種類群。經由 16S rDNA、*nodC*、*nifH* 等基因序列對分離株進行親緣分析，結果顯示供試 10 株分離株中有 4 株為 *Bradyrhizobium yuanmingense*，1 株為 *B. elkanii*，5 株為 *Ensifer* (*Sinorhizobium*) spp.，其中 *B. yuanmingense* 在台灣首次被發現。將子葉出土兩天的金珠大豆幼苗接種 5 mL 的 10^8 CFU/mL 分離株 SB006-1-2，至第二週形成直徑約 1.6 mm 之根瘤，其內部呈現粉紅色，顯示已具有固氮活性，隨著培養週次增加，根瘤逐漸增大至直徑約 5 mm，至第八週根瘤仍具固氮活性。將上述同樣大小的金珠大豆幼苗分別接種 CyPM1 與 SB006-1-2 分離株，接種後第六週結果顯示此兩株分離株引起大豆之根瘤數及植株地上部乾重皆無顯著差異，經回歸分析得知兩者之間有正相關性 (R^2=0.4825)，顯示兩分離株形成的根瘤

具有促進植物生長的能力。比較不同栽培介質對SB006-1-2在大豆結瘤能力的試驗，結果顯示栽培介質為泥炭土與蛭石等比例混合時，具有最多的根瘤數。根據上述的初步試驗結果，可作為未來本土固氮根瘤菌在促進植物生長及生物防治等相關研究之參考

。

SB05 Functional analysis of cyclophilin A of ***Fusarium solani*** f. sp. ***Phalaenopsis*** －Zhou, Z.-Y.[1] and Wang, C.-L.[1, 2, 3] ([1]Department of Plant Pathology, National Chung Hsing University, Taichung; [2]The Master Program for Plant Medicine and Good Agricultural Practice, National Chung Hsing University, Taichung; [3]Plant Health Care Master Degree Program, National Chung Hsing University, Taichung)

Phalaenopsis is the important economic flower crop for Taiwan that brings over 100 million export income per year. Leaf yellows of phalaenopsis caused by ***Fusarium solani*** f. sp. ***phalaenopsis*** (Fsp) is one of the important diseases, causing an high economic loss every year. The typical symptoms include basal rot, leaf necrosis, leaf yellowing, and defoliation. Although the disease has caused significant impact on the industry, its pathogenesis is largely unknown. Here, we explored the potential functions of cyclophilin A (***CYPA***) gene of Fsp in this pathosystem. CypA is a ubiquitous peptidyl prolyl cis-trans isomerase in most organisms, participating in protein folding and trafficking. The protein may form a conjugated complex with cyclosporin A, which is an immunosuppressive drug often used in organ transplant. In addition, ***CYPA*** of ***Pyricularia oryzae*** and ***Botrytis cinerea*** is reported to play a role in virulence, fungal development, and fungal sensitivities to cyclosporine A and abiotic stresses. Thus, we hypothesized that ***CYPA*** of Fsp plays a role in the interaction between phalaenopsis and Fsp, and tried to focus on ***CYPA*** function. From whole genome sequencing data, we found 11 cyclophilin genes in Fsp FUZ10S. Among these genes, gene g3763 clustered in the same clade of ***CYPA*** of other fungi was considered a homolog of ***CYPA*** of Fsp. To reveal the functions of Fsp ***CYPA***, we generated gene knock-out mutants of ***CYPA*** in Fsp to examine the potential mutant phenotypes. Current results showed that the mutants of Fsp ***CYPA*** (△***cypA***) grew slower than WT strain with much irregular colony edges. △***cypA*** produced less conidia, and slightly reduced lesions. △***cypA*** showed no differences in germination, penetration ability, cyclosporin A sensitivity, and sensitivities to abiotic stresses including congo red and H_2O_2. Notably, phenotypes of △***cypA*** on cyclosporin A sensitivity and abiotic stress sensitivities were different from those ***CypA*** mutants of other fungi, suggesting that Fsp ***CYPA*** may play atypical roles in fungal cells.

SB06 Functional analysis of G-protein-coupled receptor proteins in mango pathogen ***Colletotrichum asianum*** －Chiu, Y.-H. and Lee, M.-H. (Department of Plant Pathology, National Chung Hsing University, Taichung)

Mango (***Mangifera indica*** L.) is an important tropical fruit in the world and a major economic crop in Taiwan, providing for both domestic consumption and export markets. However, the hot and humid climate in Taiwan is conducive to the growth of pathogens, including ***Colletotrichum asianum*** which causes anthracnose disease on mango. This pathogen can infect inflorescences, branches and leaves of mango, leading to reduced yields. The pathogen can also cause latent infections on mango leaf and fruit where no obvious symptoms were observed on the surface. However, these infections may develop into anthracnose lesions during fruit ripening or due to changes in environmental factors. G-protein-coupled receptors (GPCRs) are transmembrane receptor proteins located on cell-membrane that receive external environmental signals and regulate gene expression through intracellular signal transduction. Previous studies have shown that ethylene produced during mango ripening can promote spore germination, appressorium formation, and disease development. The G-protein α1 subunit mutants of ***C. asianum*** lost the ability to promote appressorium formation by ethylene and decreased virulence to mango leaves. To understand Gα1 mediated ethylene sensing in ***C. asianum***, we analyzed the function of three GPCR genes in TYC-2 and explored possible environmental signals that could be received by the three GPCRs. The three GPCR genes were found to be involved in sensing ethylene, sugars, organic acids and/or other environmental factors in ***C. asianum***. The related data will be presented and discussed.

SB07 以組織病理學與轉錄體分析探討褐根病菌侵染機制－巫宗錡[1]、柯怡君[1]、蔡怡陞[2]、林盈仲[3]、蔡志濃[4]、鍾嘉綾[1] ([1]國立臺灣大學植物病理與微生物學系、[2]中央研究院生物多樣性研究中心、[3]國立臺灣大學植物科學研究所、[4]農業試驗所植物病理組)

Unveiling the infection mechanisms of ***Phellinus noxius*** by histopathology and transcriptomic analysis－Wu, Z.-C.[1], KO, Y.-C.[1], Tsai, I.-J.[2], Lin Y.-J.[3], Tsai, J.-N.[4], and Chung, C.-L.[1] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Biodiversity Research Center, Academia Sinica, Taipei; [3]Institute of Plant Biology, National Taiwan University, Taipei; [4]Plant Pathology Division, Taiwan Agricultural Research Institute, COA, Taichung)

褐根病菌 (*Phellinus noxius*) 有別於其他木材腐朽菌，具有侵染活體植物並導致植物病害的能力，可感染200種以上的樹種，造成樹木褐根病 (brown root rot disease)，為熱帶與亞熱帶地區林木、果樹與行道樹的重要病害。然而對於褐根病菌之致病機制研究匱乏，本研究以組織病理學與轉錄體分析為主軸，透過接種模式木本植物毛果楊 (*Populus trichocarpa*) 組培苗莖基部，配合石蠟切片法觀察發現，褐根病菌在接種後第三天從表皮傷口或自然開口入侵，且表皮與木質部之間的薄壁組織呈現部分空洞化，在接種後第四天植株萎凋時，皮層組織空洞化加劇且大量褐根病菌菌絲殖據其中，並在接種後第五天分別藉由射線薄壁細胞與導管進行水平與垂直向擴張。分析高毒力與低毒力擔孢子菌株接種毛果楊後第二天、萎凋病徵尚未出現時的轉錄體，並與培養於麥粒接種源上的褐根病菌樣本相互比較，發現325個差異表現基因，其中19個基因在高毒力菌株之表現量顯著高於低毒力菌株；而高達123個碳水化合物活性酶基因 (carbohydrate-active enzymes, CAZymes) 中，以61個*glycoside hydrolase family*蛋白基因為大宗，其中有許多已知的病原菌毒力因子，此外植物毒素*cerato-platanin* (*CP*) 與一些功能未知的蛋白也被預測為可能的效應子。毛果楊轉錄體則顯示，多個*pathogenesis-related* (*PR*) 蛋白基因以及細胞壁強化相關基因可能參與毛果楊在褐根病菌感染初期的防禦反應。田間罹病組織常可見大量褐根病菌菌絲與菌絲索纏據於表皮與木質部之間，此現象與本研究觀察到薄壁組織空洞化的現象相互呼應，從轉錄體分析可推測，褐根病菌侵染初期可能分泌CP蛋白或其他效應子造成細胞凋亡，並藉由大量CAZymes導致產生防禦反應的活細胞在短時間內死亡崩解，本研究將進一步透過功能性分析，釐清褐根病菌候選毒力基因與植物組織壞死及空洞化之關聯。

SB08　抗露菌病非洲鳳仙花之抗病基因分子鑑定－<u>李冠緯</u>[1]、蔡文錫[2]、沈榮壽[3] ([1]嘉義大學園藝系、[2]嘉義大學植物醫學系、[3]嘉義大學園藝系)

Molecular identification of resistence gene on resistence to downy mildew in *Impatiens walleriana*－<u>Li, G.-W.</u>[1], Tasi, W.-S.[2], and Shen, R.-S.3 ([1]Departmet of Horticulture, National Chiayi University, Chiayi; [2]Department of Plant Medicine, National Chiayi University, Chiayi; [3]Departmet of Horticulture, National Chiayi University, Chiayi)

鳳仙花露菌病(*Plasmopara obducens*)於2009年開始出現並蔓延至全球，造成非洲鳳仙花幼苗生長不良及成株葉片乾枯掉落並死亡，使栽培業者經濟損失嚴重，本實驗室歷經抗病選拔出WALIPi、WFYPe及SPi等耐病品種，其中WALIPi及WFYPe在露菌病選拔中有100%的存活率，葉片亦極少出現露菌病之病徵或病兆，極有潛力成為抗病之品種，根據2021年所發表之研究報導指出，有四種R-gene有可能為鳳仙花中參與抗露菌病之基因，本實驗將四種R-gene之引子對做PCR，確認抗病篩選出的耐病品種是否具有該四條基因存在，結果顯示極耐病之WALIPi及WFYPe並無存在該四條基因，表示此兩個品種的抗病基因與研究報導中所述不同，推判為全新的鳳仙花抗露菌病基因。

SB09　Genome analysis of '*Candidatus* Phytoplasma pruni' strain PR2021 associated with poinsettia－<u>Pei, S.-C.</u>[1, 2], Hung, T.-H.1, and Kuo, C.-H.[2] ([1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei; [2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei)

The branching performance of ornamental plants is a crucial aspect in cutting production and marketing value. Previous study demonstrated that Poinsettia Branch-Inducing (POIBI) phytoplasmas are a key factor that promotes branching on poinsettia. Therefore, infecting poinsettia plants with POIBI phytoplasmas has become a common practice in commercial production. However, the mechanisms that POIBI phytoplasmas use to induce branching remains unknown. In this study, we performed whole genome sequencing of a POIBI phytoplasma from the poinsettia cultivar 'Princettia ROSA' to investigate the candidate genes. Based on sequence analysis, this strain PR2021 was classified as '*Candidatus* Phytoplasma pruni', and provides the first complete genome sequence for this species-level taxon. Examination of gene content revealed that this strain has two homologous genes that encode SAP11, which have been experimentally demonstrated as effectors that induce branch proliferation in other phytoplasmas, thus likely explains the phenotype of POIBI phytoplasmas. To further investigate the diversity of POIBI phytoplasmas, we evaluated the branching phenotype among 10 poinsettia cultivars. Based on the phenotyping results, 6 cultivars that likely harbor POIBI phytoplasmas with strong or weak abilities for inducing branching were selected for whole genome shotgun sequencing. Upon the completion of genome assembly of these additional POIBI phytoplasma strains, we plan to conduct comparative analysis among different strains to identify the genetic variations that may explain the phenotypic variations. In addition to providing a basic understanding of POIBI phytoplasma biology, the knowledge produced in this work may help future poinsettia breeding programs for selecting POIBI phytoplasma strains with desired branch inducing ability.

SB10　夏南瓜虎斑嵌紋病毒弱症病毒株之構築與應用於交互保護可行性之探討－<u>莊立晟</u>、陳煜焜 (國立中興大學植物病理學系)

Construction of mild strains and the feasibility of their application in cross protection against zucchini tigre mosaic virus (ZTMV)－Chuang, L.-C. and Chen, Y.-K. (Department of Plant Pathology, National Chung Hsing University, Taichung)

夏南瓜虎斑嵌紋病毒 (zucchini tigre mosaic virus, ZTMV) 為一種馬鈴薯Y群病毒 (potyvirus)，1982年在法屬西印度群島瓜地洛普的夏南瓜上首次報導，最初被認為是木瓜輪點病毒 (papaya ringspot virus, PRSV) 的一個系統 (PRSV-T strain)。但隨後之生物學、血清學和分子生物學研究表明其為馬鈴薯病毒屬中的一獨立物種。2017年，於彰化北斗田間發現ZTMV (和squash leaf curl Philippines virus, SqLCPV複合) 感染冬瓜 (wax gourd, ***Benincasa hispida***) 的案例。本研究目的為探討利用輕症病毒株經由交互保護機制防治瓜類作物感染ZTMV的可能性。將感染冬瓜之ZTMV-TW (Acce. No. LC371337) 完整的病毒cDNA構築於含有CaMV 35S啟動子及NOS (nopaline synthase) 終止子的載體 (pCAMBIA 1304) 中，以生成ZTMV-TW具感染力選殖株 (infectious clones)。以Agro-infiltraion方式接種ZTMV-TW具感染力選殖株於冬瓜 (***B. hispida***)、南瓜 (pumpkin, ***Cucurbita pepo*** var. ***pepo***) 及夏南瓜 (zucchini, ***C. pepo*** var. ***cylindrica***) 上可引起與野生型ZTMV類似的病徵，並於罹病細胞內形成典型的potyvirus病毒顆粒及內含體，顯示所構築之ZTMV-TW具感染力選殖株具有活性與病原性。參照前人製備馬鈴薯Y病毒屬病毒輕症病毒的研究，在ZTMV-TW的HC-Pro蛋白中突變四個與致病性相關的高度保守鹽基序列，即Phe_{7}→Ile_{7} (F7I)、Arg_{181}→Ile_{181} (R181I)、Phe_{206}→Leu_{206} (F206L) 及Glu_{397}→Asn_{397} (D397N)，分別形成7個在其HC-Pro上具有單點或雙點組合突變的突變株。這7個突變株同法接種至夏南瓜及冬瓜皆具有感染力。於夏南瓜，突變株F206L造成嚴重的虎斑狀嵌紋病徵，突變株F7I、D397N、F7I+F206L和F206L+D397N造成略輕微的嵌紋病徵，而突變株R181I和R181I+D397N則不會造成明顯的病徵；於冬瓜上，突變株F206L和F7I+F206L造成捲曲、畸形及嵌紋病徵，突變株F7I、D397N、R181I、R181I+D397和F206L+D397N則不會引起明顯的病徵，顯示R181I和R181I+D397N等突變株具有輕症病毒之潛質 (potential)。觀察突變株病毒在夏南瓜和冬瓜等供試植物中經時累積的變化，可發現突變病毒株R181I和R181I+D397N在感染夏南瓜的初期，病毒累積量先緩慢增加，再隨著時間增加而病毒濃度緩慢下降，之後在寄主植物體內維持在低濃度水平動態平衡的狀態，表明其具有交互保護的潛力。目前，弱症病毒株 (即突變株R181I和R181I+D397N) 已接種於供試之冬瓜 (農友・綠虎) 和夏南瓜 (農友・阿滿)，ZTMV-TW挑戰接種進行中，以測試弱症病毒株交互保護。