國立臺東大學 理工學院
NATIONAL TAITUNG UNIVERSITY COLLEGE of SCIENCE and ENGINEERING

教師指導學生專題製作與論文競賽補助 成果報告

一、 申請補助計畫基本資料

申請教師	黃祥恩	核定經費	8,000
單位系所	生命科學系	經費執行情況	□已請購核銷完畢 ■尚未請購核銷 □經費餘款_____
計畫執行 年度/學期	114 年度上學期	參賽期程	112 年 11 月 7 日 ~ 112 年 11 月 8 日
參加競賽/學術活動名稱	2025 植物學年會- 海報競賽	作品名稱	Enhancing Broad-Spectrum Basal Resistance of Tomato Against *Colletotrichum* spp. Fungi Using Inactivated *Bacillus thuringiensis* BtHS1 Bacterial Remnants
指導參賽學生姓名	林嘉盈	班級	生命科學系四年級
競賽性質	■國際性 □校際 □校內(院級以上)	參賽地點	國立中興大學
系所主管簽章		日期	
學院院長簽章		日期	

二、 參賽作品：(論文摘要或作品說明)

Enhancing Broad-Spectrum Basal Resistance of Tomato Against *Colletotrichum* spp. Fungi Using Inactivated *Bacillus thuringiensis* BtHS1 Bacterial Remnants

Jia-Ying Lin, Ching-An Chang, Hsiang-En Huang
Department of Life Sciences, National Taitung University, Taiwan R.O.C

Abstract

Bacillus thuringiensis (Bt) is a commonly used biocontrol bacterium in agriculture and has long been applied for pest management. Recent studies have shown that Bt not only suppresses insects but also has the potential to enhance plant resistance against pathogens and abiotic stresses. However, field applications have revealed that large amounts of viable Bt cells may pose potential risks to humans and animals as opportunistic pathogens, increasing the possibility of biological contamination. In previous studies, our laboratory isolated a Bt strain, HS1, which was shown to enhance tomato tolerance to drought, heat stress, and anthracnose disease caused by *Colletotrichum karsti* by upregulating ferredoxin (Fd) expression and activating salicylic acid (SA) and jasmonic acid (JA)-related defense pathways. To reduce the biosafety risk associated with live HS1 cells, a high–low temperature deactivation method, designated HS1-5s-10 (-20 °C for 4 h followed by 50 °C for 15 min), was developed. This treatment reduced the viable cell count from 10^8 CFU/mL to <100 CFU/mL, and the inactivated preparation was subsequently used for tomato biocontrol experiments. The results showed that HS1-5s-10 treatment alleviated disease severity in tomatoes infected with *C. karsti, C. gloeosporioides, and C. camelliae-japonicae*. HS1-5s-10 treatment had no significant effect on the levels of H_2O_2 and total peroxide in infected plants but enhanced the accumulation of total phenolic compounds without affecting soluble peroxidase activity. These findings suggest that HS1-5s-10 may strengthen tomato defense against pathogens by promoting the accumulation of secondary metabolites rather than inducing oxidative stress responses. Moreover, the metabolic fragments of HS1-5s-10 also exhibited direct inhibitory effects on *Colletotrichum* species.

Keyword

Bacillus thuringiensis 、*Colletotrichum*、tomato、ROS、HS1-5s-10

三、 參加之競賽活動：(請依據參加活動次數，依序附上相關活動簡章或海報、議程與參加證明等佐證資料)

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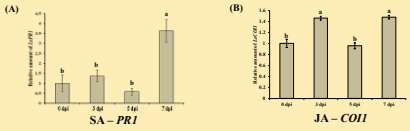
Bacillus thuringiensis (Bt) is a commonly used biocontrol bacterium in agriculture and has long been applied for pest management. Recent studies have shown that Bt not only suppresses insects but also has the potential to enhance plant resistance against pathogens and abiotic stresses. However, field applications have revealed that large amounts of viable Bt cells may pose potential risks to humans and animals as opportunistic pathogens, increasing the possibility of biological contamination. In previous studies, our laboratory isolated a Bt strain, HS1, which was shown to enhance tomato tolerance to drought, heat stress, and anthracnose disease caused by *Colletotrichum karsti* by upregulating ferredoxin (Fd) expression and activating salicylic acid (SA) and jasmonic acid (JA)-related defense pathways. To reduce the biosafety risk associated with live HS1 cells, a high–low temperature deactivation method, designated HS1-5s-10 (-20 ˚C for 4 h followed by 50 ˚C for 15 min), was developed. This treatment reduced the viable cell count from 10^8 CFU/mL to <100 CFU/mL, and the inactivated preparation was subsequently used for tomato biocontrol experiments. The results showed that HS1-5s-10 treatment alleviated disease severity in tomatoes infected with *C. karsti, C. gloeosporioides, and C. camelliae-japonicae*. HS1-5s-10 treatment had no significant effect on the levels of H_2O_2 and total peroxide in infected plants but enhanced the accumulation of total phenolic compounds without affecting soluble peroxidase activity. These findings suggest that HS1-5s-10 may strengthen tomato defense against pathogens by promoting the accumulation of secondary metabolites rather than inducing oxidative stress responses. Moreover, the metabolic fragments of HS1-5s-10 also exhibited direct inhibitory effects on *Colletotrichum* species.

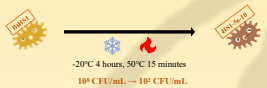
Result

BtHS1 Activates Both SA and JA Defense Pathways in Tomato



▲ Induction of tomato defense genes by BtHS1 irrigation
Tomato plants were grown in a controlled growth chamber and, after 45 days, irrigated with 10^8 CFU/mL HS1 bacterial suspension. Leaf tissues were harvested at 0, 3, 5, and 7 days post-irrigation, and total RNA (1 μg) was extracted for semiquantitative PCR analysis using the △△CT method. Transcript levels were normalized to *LeEF1α* as the internal control. (A) represents the *LePR1* defense gene, and (B) represents the *LeCOI1* defense gene. Statistical analysis was performed using SPSS software via ANOVA, followed by Fisher's protected least significant difference (LSD) test. Different letters (a, b, c) indicate significant differences ($n = 3$, $p < 0.05$). Data are presented as mean ± standard error. Growth conditions: 12 h light / 12 h dark photoperiod, light intensity 400 μmol m^{-2} s^{-1}, 25 °C.

BtHS1 Inactivation by -20˚C and 50˚C Temperature Treatment



▲ Inactivation of BtHS1 by high-low temperature treatment (HS1-5s-10)
Bacillus thuringiensis BtHS1 was cultured in Lysogeny Broth (LB) medium with shaking for 18 hours, reaching a cell density of 10^8 CFU/mL. The culture was subjected to a combined temperature treatment consisting of incubation at -20 ˚C for 4 hours followed by a 50 ˚C water bath for 15 minutes. After serial dilution and plating, the bacterial count was reduced to <10^2 CFU/mL, indicating effective inactivation.

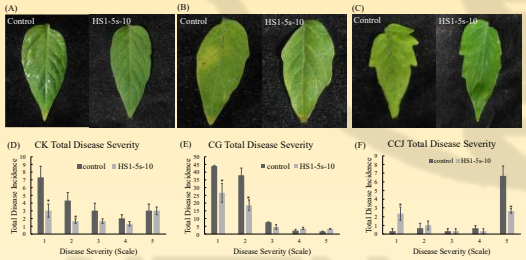
Colony Growth and Spore Morphology of *Colletotrichum* spp. on PDA

▲ Mycelial growth and spore morphology of *Colletotrichum karsti*, *C. gloeosporioides*, and *C. camelliae-japonicae* on PDA medium
Colletotrichum karsti, *C. gloeosporioides*, and *C. camelliae-japonicae* were cultured on PDA medium under dark conditions at 28 ˚C. After 7 days of incubation, colony morphology was photographed to observe mycelial growth. Each *Colletotrichum* species was also cultured in PDB liquid medium under shaking conditions (dark ; room temperature) for 14 days, and the spore morphology was examined under a microscope. (A) *C. karsti* colony and spore morphology ; (B) *C. gloeosporioides* colony and spore morphology ; (C) *C. camelliae-japonicae* colony and spore morphology.

Different Severity Levels of Anthracnose Disease in Tomato Caused by *Colletotrichum* spp.

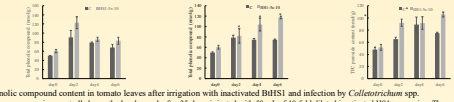
▲ Disease severity scale (Level 1 – 5) of tomato leaves infected with *Colletotrichum* spp.
Tomato plants were grown in a controlled growth chamber and, after 45 days, irrigated with 50 mL of 10-fold diluted inactivated HS1 suspension. The control group was irrigated with 50 mL of 10-fold diluted Luria-Bertani (LB) medium. Five days after irrigation, a Colletotrichum spp. spore suspension ($1x10^6$ spores/mL) mixed with 1/5 PDB and 10 mM $MgSO_4$ (20 mL per plant) was uniformly sprayed onto the abaxial surface of tomato leaves. Disease symptoms were photographed 7 days after inoculation. (A) Representative tomato leaves showing disease severity (Level 1–5). (B) Criteria for disease severity classification. Growth conditions : 12 h light / 12 h dark photoperiod, light intensity 400 μmol m^{-2} s^{-1}, 25 ˚C.

HS1-5s-10 Effectively Enhances Tomato Resistance Against *C. karsti*, *C. gloeosporioides*, and *C. camelliae-japonicae*



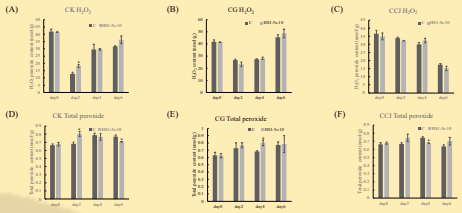
▲ Inactivated BtHS1 irrigation enhances tomato resistance against *Colletotrichum* spp. induced anthracnose
Tomato plants were grown in a controlled growth chamber and, after 45 days, irrigated with 50 mL of 10-fold diluted inactivated HS1 suspension. The control group was irrigated with 50 mL of 10-fold diluted Luria-Bertani (LB) medium. Five days after irrigation, a *Colletotrichum* spp. spore suspension ($1x10^6$ spores/mL) containing 1/5 PDB and 10 mM $MgSO_4$ (20 mL per plant) was uniformly sprayed onto the abaxial surface of tomato leaves. Disease symptoms were photographed 7 days after inoculation. (A ~ C) Representative leaf symptoms of *C. karsti* (CK), *C. gloeosporioides* (CG), and *C. camelliae-japonicae* (CCJ) in control and HS1-5s-10 treatments. (D ~ F) Total disease severity per plant. Growth conditions : 12 h light / 12 h dark photoperiod, light intensity 400 μmol m^{-2} s^{-1}, 25 ˚C. Statistical analysis was performed using SPSS software with ANOVA followed by an independent sample t-test. Asterisks (*) indicate significant differences ($n = 3$, $p < 0.05$). Data are presented as mean ± standard error.

HS1-5s-10 Increases Total Phenolic Compound Content in Tomato



▲ Total phenolic compound content in tomato leaves after irrigation with inactivated BtHS1 and infection by *Colletotrichum* spp.
Tomato plants were grown in a controlled growth chamber and, after 35 days, irrigated with 50 mL of 10-fold diluted inactivated HS1 suspension. The control group was irrigated with 50 mL of 10-fold diluted Luria-Bertani (LB) medium. Five days after irrigation, a spore suspension of *C. karsti*, *C. gloeosporioides*, or *C. camelliae-japonicae* ($1x10^6$ spores/mL) mixed with 1/5 PDB and 10 mM $MgSO_4$ (20 mL per plant) was uniformly sprayed onto the abaxial surface of tomato leaves. Total phenolic compound content was measured at 0, 2, 4, and 6 days post-inoculation. (A) Total phenolic compound content after inoculation with CK. (B) Total phenolic compound content after inoculation with CG. (C) Total phenolic compound content after inoculation with CCJ. Growth conditions : 12 h light / 12 h dark photoperiod, light intensity 400 μmol m^{-2} s^{-1}, 25 ˚C. Statistical analysis was performed using SPSS software with an independent sample t-test. Asterisks (*) indicate significant differences ($n = 3$, $p < 0.05$). Data are presented as mean ± standard error.

HS1-5s-10 Has No Effect on H_2O_2 and Total Peroxide Content in Tomato



▲ H_2O_2 and Total peroxide content in tomato leaves after irrigation with inactivated BtHS1 and infection by *Colletotrichum* spp.
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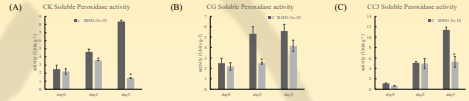
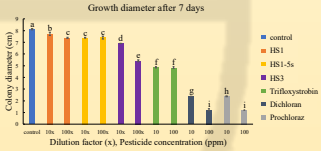
HS1-5s-10 Does Not Increase Soluble Peroxidase Activity in Tomato Infected with Anthracnose



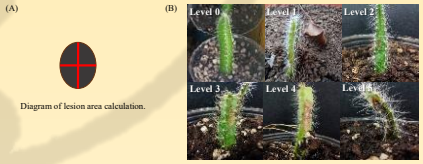
▲ Soluble peroxidase activity in tomato leaves after irrigation with inactivated BtHS1 and infection by *Colletotrichum* spp.
Tomato plants were grown in a controlled growth chamber and, after 35 days, irrigated with 50 mL of 10-fold diluted inactivated HS1 suspension. The control group was irrigated with 50 mL of 10-fold diluted Luria-Bertani (LB) medium. Five days after irrigation, a spore suspension of *C. karsti*, *C. gloeosporioides*, or *C. camelliae-japonicae* ($1x10^6$ spores/mL) mixed with 1/5 PDB and 10 mM $MgSO_4$ (20 mL per plant) was uniformly sprayed onto the abaxial surface of tomato leaves. Soluble peroxidase activity was measured at 0, 2, and 5 days post-inoculation. (A) Soluble peroxidase activity after inoculation with CK. (B) Soluble peroxidase activity after inoculation with CG. (C) Soluble peroxidase activity after inoculation with CCJ. Growth conditions : 12 h light / 12 h dark photoperiod, light intensity 400 μmol m^{-2} s^{-1}, 25 ˚C. Statistical analysis was performed using SPSS software with an independent sample t-test. Asterisks (*) indicate significant differences ($n = 3$, $p < 0.05$). Data are presented as mean ± standard error.

Effects of Trifloxystrobin, Dichloran, Prochloraz, and HS1, HS1-5s, HS3 EPS Media on the Growth of *C. gloeosporioides*



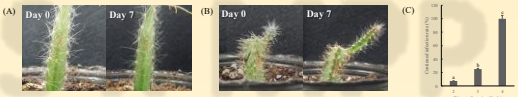
▲ Trifloxystrobin, Dichloran, Prochloraz, and HS1, HS1-5s, HS3 EPS media effectively inhibit the growth of *C. gloeosporioides*
C. gloeosporioides was cultured on Potato Dextrose Agar (PDA) containing 10 ppm or 100 ppm of trifloxystrobin, dichloran, or prochloraz, as well as 10-fold or 100-fold diluted HS1, HS1-5s, and HS3 EPS media. Seven days after inoculation, the colony diameter of *C. gloeosporioides* was measured on each medium.Statistical analysis was performed using SPSS software with ANOVA followed by Fisher's protected least significant difference (LSD) test. Different letters (a–i) indicate significant differences ($n = 3$, $p < 0.05$). Data are presented as mean ± standard error.

C. Gloeosporioides Cause Varying Levels of Infection in Pitaya



▲ Varying degrees of rot in pitaya following injection inoculation with *C. gloeosporioides*
Pitaya (5–6 cm in diameter) were grown outdoors and injection-inoculated 35 days later. A spore suspension of *C. gloeosporioides* ($1x10^6$ spores/mL) mixed with 1/5 PDB and 10 mM $MgSO_4$ was injected at 0.5 mL per fruit. Seven days post-inoculation, fruit appearance was photographed, and disease severity was assessed. (A) Illustration of lesion area calculation. Lesion area was calculated as the product of the longest and shortest lesion diameter rs. (B) Appearance of fruits at different disease severity levels. Level 0 : Healthy. Level 1 : Lesion with yellow–brown discoloration. Level 2 : Black lesion < 30 mm^2. Level 3 : 30 mm^2 ≤ Black lesion < 90 mm^2. Level 4 : 90 mm^2 ≤ Black lesion < 150 mm^2. Level 5 : Black lesion > 150 mm^2.

HS1-5s-10 Suppresses Persistent Infection in Mildly Anthracnose-Infected Pitaya



▲ Injection of inactivated BtHS1 suspension halts persistent infection in level 2 and 3 Anthracnose-infected pitaya
Pitaya fruits with anthracnose symptoms were classified as Level 2, 3, or 4. Each lesion was treated by injecting 0.5 mL of inactivated HS1 suspension per fruit. (A) Appearance of Level 2 infected fruits before and 7 days after HS1 treatment. (B) Appearance of Level 3 infected fruits before and 7 days after HS1 treatment. (C) Rate of persistent infection. Statistical analysis was performed using SPSS software with ANOVA followed by Fisher's protected least significant difference (LSD) test. Different letters (a–c) indicate significant differences ($n = 3$, $p < 0.05$). Data are presented as mean ± standard error.

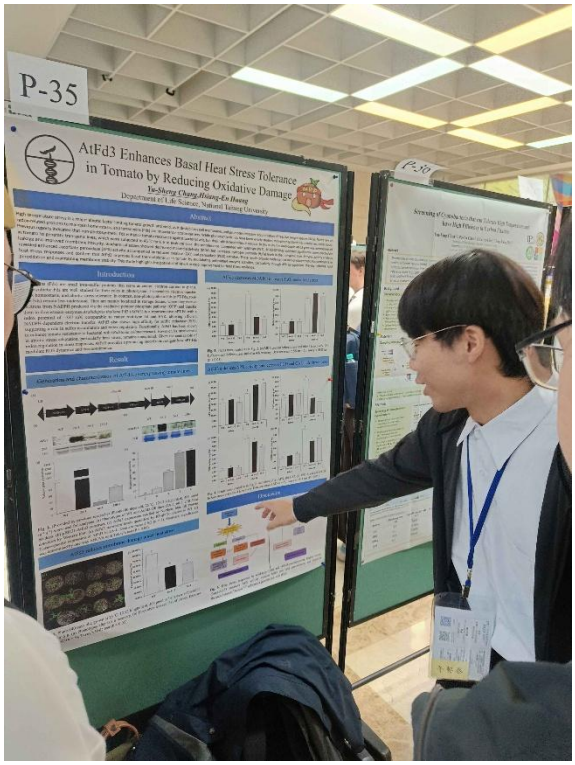
Conclusion

1. HS1-5s-10 reduced disease severity in tomato infected with *C. karsti*, *C. gloeosporioides*, and *C. camelliae-japonicae*.
2. HS1-5s-10 increased total phenolic compound content.
3. HS1-5s-10 did not increase soluble peroxidase activity.
4. HS1-5s-10 may enhance defense via secondary metabolite accumulation.
5. HS1-5s-10 metabolic fragments directly inhibited anthracnose pathogens.
6. Inactivated HS1-5s-10 induces resistance while avoiding biosafety risks of live bacteria.

▲ 2025 台日聯合研討會植物學年會海報-海報競賽

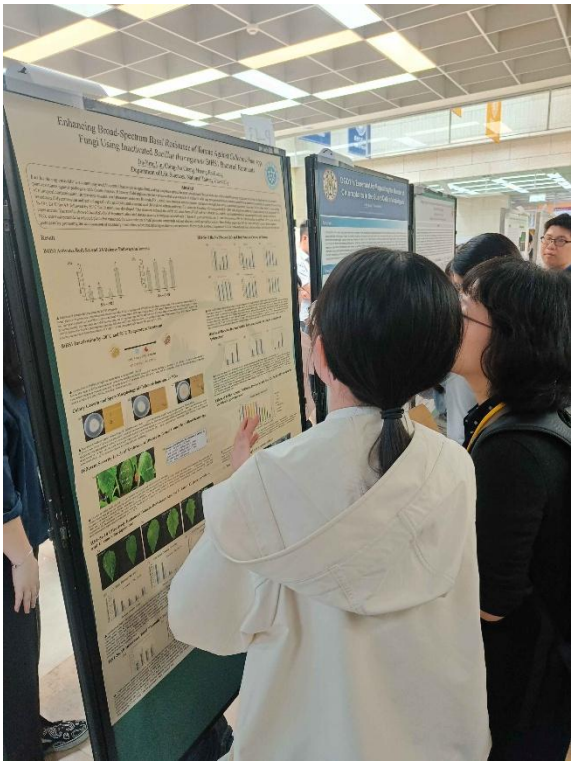
▲ 2023 台日聯合研討會植物學年會流程

四、參賽準備與活動記錄



圖說明：

遇到已畢業學長他為我們介紹海報



圖說明：

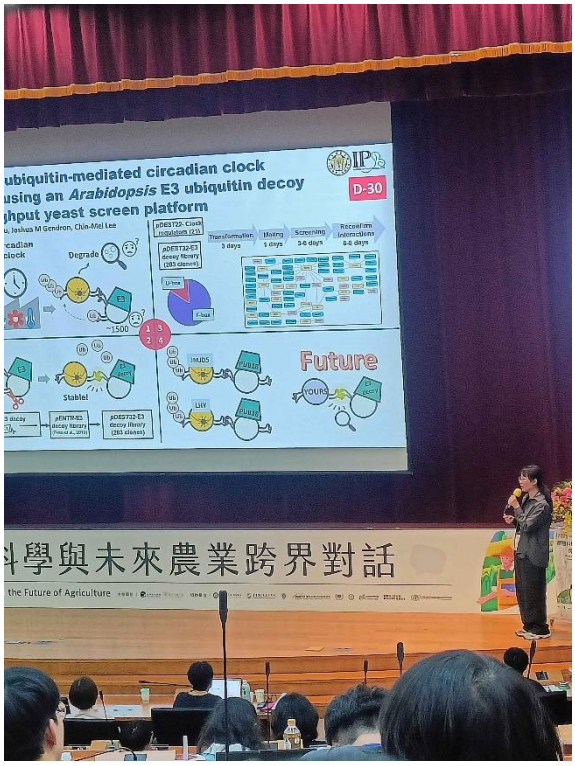
壁報參賽過程中與研究員討論議題

圖說明：

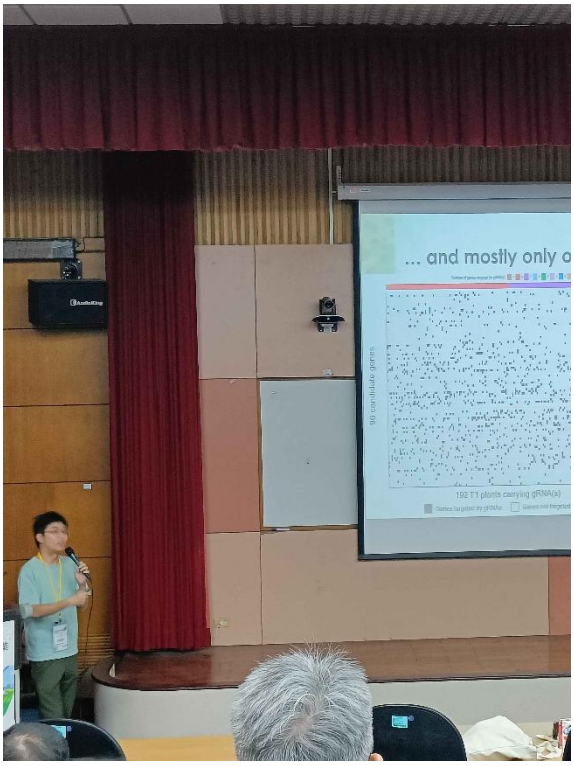
參與終身成就獎頒獎典禮

圖說明：

實驗室成員與中研院研究員在晚宴一同共餐



圖說明：
觀摩三分鐘論文短講競賽



圖說明：
聆聽馬家威博士關於多基因編輯的演講

五、 參加競賽成果 (參賽證明、得獎證明或學生心得)

林嘉盈同學

今年的植物學年研討會對我而言是一段特別的經驗。過去的幾年我都以參加者的身分前往，單純聽演講、看別人的成果展示。今年則第一次以發表者的身分站在壁報前，向不同領域的研究者說明自己的研究內容，這讓我對學術交流更有深刻印象，也更有參與其中的感覺。

我發表的題目是：

「利用去活化的蘇雲金芽孢桿菌 Bacillus thuringiensis BtHS1 細菌分離株殘骸提高番茄對於炭疽病菌屬真菌的廣泛性基礎抗性」。

這次壁報競賽讓我真正練習到如何把複雜的研究背景、實驗設計、結果，用簡短的時間清楚地說給不同背景的聽眾與評審理解。每位停下腳步觀看壁報的人都會提出不同角度的問題，有的人關心實驗設計的細節，有的人提出可能的機制，也有人分享他們遇過類似問題的經驗。這些討論讓我覺得很新鮮，也讓我重新思考研究中一些容易忽略的環節。透過這樣的互動，我也感受到學術交流真正的意義，不是單方面說明結果，而是透過溝通讓研究變得更完整、更有方向。

除了自己的壁報之外，我也參加了多場不同領域的研究演講。講者們分享的最新發現與研究方法讓我看到植物科學的多樣面貌，不論是病害機制、生理反應、基因調控還是應用策略，都讓我獲得新的靈感。

這次研討會最大的收穫，是讓我從聽眾變成分享者。站在壁報前一次次的講述與回答問題的過程讓我更有信心，也讓我意識到自己還有很多可以進步的地方，例如資料在壁報的呈現方式、敘述的流暢度，以及對研究整體脈絡的掌握。這次植物學年研討會的經驗對我而言不只是一次活動，而是一個讓我成長的機會。它提醒我，做研究不是關在實驗室裡，而是要不斷與他人交流、反思與調整。未來我也希望能持續參與類似的學術場合，累積更多分享與學習的經驗，讓自己的研究之路越走越穩。

邱炳榮同學

我很榮幸能參加 11 月 7 日於國立中興大學舉辦的「2025 植物科學與未來農業跨界對話研討會」年會。對我而言，這次活動具有特別的意義。回想三年前剛加入實驗室時，因為得知資訊太晚而無法報名，錯失了向外界學者學習與交流的機會。經過這三年的努力，我參與了中研院與國科會的大專生研究計畫，也逐漸具備自行設計實驗的能力，並修習更多植物科學相關課程，使我對這個領域有了更深的認識。

這次能與祥恩實驗室的夥伴一起參加年會，讓我感到非常開心。會場中各式海報展示豐富而多元的研究主題，每一張海報都凝聚了研究者的心血，讓我深刻體會到科學研究的廣度與挑戰性。由於海報數量很多，我挑選了幾張特別感興趣的主題進行詢問，其中有部分是馬家威實驗室夥伴的研究，聚焦於阿拉伯芥根聯細菌對植物的影響。這類研究不僅有助於理解植物與微生物的互動，也可能對農業生產帶來實際應用。

在與研究者討論的過程中，我更清楚地理解了他們的研究方法與結果，也激發了我對未來研究方向的思考。雖然目前我在生物資訊方面的技術仍不夠熟練，但透過他們的講解，我對一些基礎概念與應用有了初步的掌握，這也是我未來想加強的能力之一。這次經驗讓我更確信，若能掌握更多工具與分析方法，將能大幅提升研究的深度與廣度。

除了學術面向，我也把握機會與來自不同機構的研究者建立聯繫。這讓我感受到科研社群中的互助與支持，也從彼此的交流中獲得許多靈感，對於我未來的研究方向帶來新的啟發。我開始思考如何把目前習得的實驗技術運用到更廣泛的植物研究之中，這讓我對未來充滿期待。

這次年會不僅讓我看見最新的研究成果，也提供了一個與各界學者交流的寶貴平台。透過這次參與，我對植物科學的熱情更加提升，也深刻體會到持續學習與交流的重要性。我希望未來的學弟妹也能擁有這樣的機會，從中獲得啟發並開拓視野。

林崇育同學

這次植物學年會，比起去年，多去了第一天的壁報比賽，聆聽到了各學校，中研院各實驗的內容，有些內容畢竟還實驗室的實驗方向大同小異，這次前去年會在聽講解時，也和同學聊了一下，在對於幾個在壁報上的問題，並討論和自己實驗相關的改進或是能夠更注重的地方，這在第一天的活動有了一些的心得，而第二天則是聽各學校、實驗室當下做的實驗或者實驗室研究的方向，讓參加年會的學者、同學看看實驗的結果，但因為這天有三個不同研究

方向的大講廳在同時分享，所以就也只能從中選擇幾場題目有興趣的場次去聽。

吳東儒同學

身為初入實驗室的我很自然而然的參與了這次有關本實驗室研究主題的研討會活動，在這裡讓我因為經驗的不足而看不懂參賽海報的內容，在這裡讓我因為語言能力欠佳導致許多場演講都無法理解，但在這裡我可以明白自己的能力有何需改進，有什麼樣有興趣的內容可以再回來深入思考並鑽研，對於我一個領域新人來說實際體驗差距是難得也是很美好的體驗。

陳怡綸同學

作為剛加入實驗室的人，能夠參加與實驗室研究方向相關的研討會對我來說是一個很寶貴的經驗。雖然因為經驗尚淺，海報的內容常常難以抓住重點，演講時也因語言障礙而無法完全理解，但正是這些挑戰讓我更清楚自己還有哪些能力需要加強。

在研討會中，我也注意到一些令我感興趣的研究題目，雖然目前還無法完全理解，但我希望未來能回過頭去深入學習。

此外，研討會中我了解到，SLs 最初只是植物與菌根真菌之間的訊號分子，隨著演化中的基因變化，它們才逐漸擁有了調控植物生長的功能。這讓我意識到，許多植物機制其實都是從基本需求逐步演化而來的，背後的脈絡比我想像中更有連貫性，也讓我對相關知識的理解更為清晰。

吳承諺同學

這次去植物學年會聽到許多很有趣很厲害的演說，其中我印象深刻的是，利用特定病毒將建構好的 DNA 片段送入植物中，而此方式的轉入效率比一般在用的轉入方法還要高，這需要對該病毒有了解才做得到，而我這學期的病毒學也剛好有學到病毒的七種類型、複製方式，能夠在聽演講的時候把剛學到的知識套用上去，我覺得很開心，同時也讓我知道了，我還要學習更多相關領域的知識，才有機會像這些講者一樣厲害。

王鈞綸同學

這次在中興大學舉辦的植物學年會，雖然我沒有發表海報，但我真的學到了很多。

除了聆聽我們實驗室學長姊的報告外，我特別感謝學長和我去看其他學校的海報，並且一起討論。這讓我對實驗設計的思路與方向有了更深層次的了解，對我之後讀研究所有非常實質的幫助。

看別人的海報也讓我對自己現在的實驗產生了更多想法。特別有一篇關於熱逆境的海報令我印象深刻，可以透過參考其他人的方式來精進自己的實驗方式。

除了海報，也有許多教授分享他們的最新研究成果與突破，他們分享了自身經歷，告訴我們在研究路上感到迷惘時要如何調適與面對，讓我感到非常受用。

除了學術之外，這次研討會的伙食也很奢，非常期待明年在日本舉辦的研討會，希望屆時能換我貼海報，分享我的成果。