出國報告(出國類別:研習)

因應氣候變遷之 國際農業科技交流合作 -耐逆境性狀之基因型篩檢平台建置與病害 育種技術交流

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出國期間:中華民國103年11月02-11月08日

報告日期:中華民國103年11月28日

一、摘要

經農委會支持與臺灣大學及農業試驗所之努力,於101年展開「因應氣候變遷之國際農業科技交流合作-抗、耐逆境水稻品種之開發」合作計畫,本計畫主要目的是為有效因應全球氣候變遷對我國糧食安全及相關產業所造成之衝擊,透過與國際稻米研究所 (IRRI) 及其相關機構密切合作,接軌新興之因應科技,選育適合於臺灣推廣之水稻品種,同時建置穩定抗、耐逆境水稻的外表型評估技術及擴大繁殖稻熱病/白葉枯病菌生理小種 (病原型) 之判別品種及接種評估技術,並擴大雙方水稻抗病育種及調查資料之交流,希望得以提升我國在氣候變遷下之水稻農糧產業之適應力及競爭力。本篇論述針對病害逆境學術交流與研習提出第三階段報告,亦加強交流稻熱病及白葉枯病研發近況。在103年11月02日至08日間,由國立臺灣大學、國立中興大學及農試所、臺中區農業改良場等12位水稻病害及育種專家前往IRRI 實務訓練心得及建議,以供日後臺灣水稻抗病育種之參用。

本次研習內容不僅包含耐逆境性狀之基因型篩檢平台建置與技術交流,另在擴大遺傳資源應用上,也學習利用種間雜交導入野生稻耐性基因,打破種間生殖障礙,以提升栽培品種耐受性的遺傳歧異度,另借助次世代定序技術已大規模解序各樣類水稻遺傳種原,如何利用線上生物資料庫進行目標性狀尋找優良基因或憑藉特定基因型挑選優良親本,均是與國際水稻研究趨勢緊密接軌的重要議題,藉由面對面討論上述議題與實務操作,累積我方稻作研發能力,期望降低氣候變遷對稻作生產的危害與衝擊。

關鍵字:白葉枯病、稻熱病、基因型分析技術、種原利用。

目次 一、摘要..... 2 4 二、目的..... 三、參訪行程..... 5 四、參訪內容 1. 水稻白葉枯病接種試驗田及溫室導覽 7 7 2. 分子輔助育種理論與實務簡介 9 3. 簡介稻熱病流行病學研發現況 4. 基因型分析應用實驗室觀摩 10 5. 参訪亞洲最大水稻種原中心 10 6. 水稻種原基因型生物資料庫之操作簡介與實務交流 11 7. 野生稻稻屬與滲入系族群選育 11 8. 流行病害監控與預警模式評估 12 9. 稻熱病判別品種選育概況與展望 12 10. 病蟲害篩檢中心與病圃導覽 13 11. 抗病種原開發與應用研究 14 12. 參訪米質中心 15 五、參訪心得...... 15 六、參訪建議..... 16 七、參訪記錄照片..... 17 八、抗病研習小組英文回饋報告.....

20

二、目的

本計畫依據農委會農業試驗所與國際稻米研究所 (International Rice Research Institute, IRRI) 共同協議合作架構下,制定目標並推動各項合作研究計畫,以有效導入抗、耐逆境基因,並改良臺灣水稻推廣品種。

隨著臺灣水稻栽培品種與氣候在時間性與地區性上的變遷,病原菌流行之生理小種(病原型)亦隨之消長,我們需要持續加強抗病育種與流行病學的研究;很明顯現今栽培狀況不僅是栽培品種的分布改變,臺灣的氣候環境也改變了,每日最高溫和每日最低溫上升,增溫以夜晚比白天明顯,冬季較其他季節明顯,高溫日數增加,百年來總降雨量變化不大,但總降雨日數(尤夏季)呈減少趨勢,強降雨機率明顯增加,乾濕季差異更加顯著,且2000年之後侵臺颱風由每年3.3個增加為5.7個,以上變化大幅影響水稻病害發生的機率及嚴重程度,菌群勢必也隨著逐漸改變。病害最主要的防治方法之一為種植抗病品種,然回顧目前臺灣育成品系的抗病性普遍不足,顯示可能在選育過程中,沒有抗性種原、選拔效率不彰或是檢定系統不穩定,希望能藉由本次研習與IRRI學術交流提升我方的能力建構。

IRRI 在水稻種原保存與利用之研究成果十分值得臺灣學習,由於作物改良的遺傳資源來自種原的多樣性,因此本次交流之目的將學習種子保存、野生稻之利用以及多親本互交高世代族群之建構。IRRI 有全世界最齊全的稻米種子保存庫,面對未來可能發生的逆境,遺傳資源是稻米糧食安全的最後一道防線,如何管理與利用這些遺傳資源是相當值得學習的方向。野生稻雖然與栽培稻的性狀差異較大,但野生稻的耐鹽性、抗病性及其他功能性基因對栽培稻而言是品種改良的關鍵來源,IRRI 的專家如何突破種間雜交的屏障,將功能性基因滲入栽培稻,值得臺灣借鏡。此外,IRRI 建構了數個「多親本互交高世代族群」,每一個族群有8個帶有可利用基因的親本,互交後在高世代產生各種排列組合的品系,此一方法可將功能性基因定位,並且可從中選拔適合用於育種的親本,值得臺灣學習與應用。水稻的性狀評估方法除了從外觀測量與病菌接種之外,如何分析米質與根系也是本次研習的重點之一,米粒的品質一向是消費者最關心的性狀,IRRI 的米質中心分析項目包含物理性質、化學成分以及飯粒質地,因此本次研習的目的之一為學習 IRRI 的米質中心如何分析米質性狀。水稻根部的採樣與測量皆具有困難度,但根部構造關係著耐旱性與抗倒伏性等重要性狀,因此 IRRI 在水稻根部的研究方法值得我們學習。

三、研習行程

日期	日數	時間	議題	地點
2-Nov	去程			
		07:30	桃園國際機場集合完畢	桃園機場
		09:30	登機	長榮 BR271
		12:10	抵達菲律賓(誤點)	馬尼拉機場
		13:30	抵達 IRRI	Swaminathan hall
11月3日				
3-Nov	Day 1	09:00	雙方計畫進度報告及問題討論	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
			Taiwan team introduction	PBGB Conf Rm 2, NCBL
			LUNCH	IRRI Dining Room
		13:30	Taiwan presentations	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
		15:00	Disease resistance Breeding-process and operations (field and greenhouse)	
11月4日				
4-Nov	Day 2	09:00	Marker-assisted selection biotic and abiotic stresses	PBGB Conf Rm 2, NCBL
			Coffee service	
			Visit Genotyping Service Laborary (GSL)	GSL
			LUNCH	IRRI Dining Room
		13:30	Disease situation in Asia	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
		15:00	Pathogen populations analysis	PBGB Conf Rm 2, NCBL
11月5日				
5-Nov	Day 3	08:30	Visit to Genebank	
		09:00	Exploring Genebank diversity (sequenced accessions)	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
		10:30	Molecular breeding for QTL: allele mining for disease resistance and other traits using <i>SNP SEEK</i> at IRIC portal	PBGB Conf Rm 2, NCBL

日期	日數	時間	議題	地點
		13:30	New sources of resistance from wild rices (Field and Greenhouse expt)	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
		15:00	Disease epidemiology—field survey and GIS zoning	PBGB Conf Rm 2, NCBL
11月6日		•		
6-Nov	Day 4	09:00	Specialized genetic stocks (NILs series)	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
		10:30	Biotic stress screening center, greenhouse and nursery	
		PM	LUNCH	IRRI Dining Room
			Discussion of breeding populations GWAS study for bacterial blight and other diseases	PBGB Conf Rm 2, NCBL
		15:00	Disease resistance germplasm for blast, blight, sheath blight and others	PBGB Conf Rm 2, NCBL
		16:00	Neck blast resistance, greenhouse expt	
11月7日				
7-Nov	Day 5	09:00	綜合討論與意見回饋	PBGB Conf Rm 2, NCBL
			Coffee service	PBGB Conf Rm 2, NCBL
		10:30	合作議題交流	PBGB Conf Rm 2, NCBL
			LUNCH out!	
		PM	Museum and store and others	
11月8日				
8-Nov	返程		Pick up at 0830h from MSSwamina 1250/1500h BR 0272)	than Hall (Mla/Taipei
		08:20-	搭車到機場	
		13:10-	登機	長榮 BR272
		16:10-	抵達台灣	桃園機場

四、參訪內容

(一)、水稻白葉枯病接種試驗田及溫室導覽

田間導覽係下午參觀IRRI的試驗田,其中田間試驗材料為IRRI和先正達的合作試驗。當地氣候 6~10 月為濕季,12~4 月為乾季。田中間為IRBB54、59、64 和 66 號跟先正達的品種進行雜交的後代,包括 F2、F4、F6 和對照品系。目的主要是為了瞭解各抗病品系和先正達的品種進行雜交後,哪個組合的抗病性最佳。田間接種 IRRI 當地的主流菌種 PXO61 和PXO88,每一單株接種兩支不同菌種。利用剪葉法接種後 14 天進行調查,應為氣候較炎熱穩定、水量充足,病斑在 14 天後即可進行調查。抗病性檢定有 3 個方法,(一)依照病斑面積比率判定,依無病斑到 100%得病共分為 9 個等級;(二)依病斑的長度,低於 3 cm 為抗病;(三)依病斑長度低於 5 cm 抗,5~10 cm 為中抗,10 cm 以上為中感。總共 3 重複隔周進行插秧,之後接菌也是隔周進行以方便接菌和調查。每一次的檢定,會種植感病對照品種 IR24,根據以往的經驗 IR24 的病斑長度會大於 20 公分。

溫室的部分,利用架高的鐵架用木板用成一小水槽,將水稻直播種於 25*12 的打動塑膠盆中,每一盆種植三株。整齊放置於水槽中,行為不同品系列則接種不同的白葉枯病菌株,菌株為菲律賓分離之菌株族群 Race1~10。種植後 45~50 天進行接種,單一植株可接種 1~3 個不同的菌株,不同菌株間用不同顏色的吊牌加以區隔。溫室中調查的近同源品系除了進行病斑調查的外表型檢定外,也利用分子標誌或微陣列序列確認基因型,以確保基因確實有表現。溫室中不失用任何的除草劑和殺菌劑,僅利用農藥進行褐飛蝨的防治,避免影響溫室內植株的生長。當溫度太高時則不進行任何的試驗,避免植株受到環境影響導致生長不佳及造成病菌的活性降低。

IRRI的菌株,有些年代十分久遠,最早有來自 1980的菌,為避免保存影響菌株活性,一般每兩個月會活化保存一次,在接種前,也會先過一次寄主。雖然菌株保存時間過長的確會讓病原性降低,但並不是每株菌都會發生一樣的情況,所以實驗一定要有 IR24 作為對照。IRRI 也將目前已知的 Xa gene 建構出 IRBB 的 NILs,品系的維持是藉由接種鑑別菌株,觀察抗感性表現,確認品系的純度。

(二)、分子輔助育種理論與實務簡介

在 IRRI 所進行的分子標幟輔助育種,所關心的性狀可以分成四個類型:分別是非生物性逆境的耐受性、生物性逆境的抗性、產量與外表型多型性以及品質。其育種目標分成可以依據目地分成兩類:一是新的品種發展或育成,這個目的需要遺傳背景分子標幟的促成;另外是特殊性狀提供親的發展,這個目的則不需要背景標誌。

其中在近來 IRRI 進行的非生物逆境耐受性的研究,包含淹水(submergence)、乾旱 (drought)、磷缺乏(P-deficiency)、鹽分(salinity)、低氧發芽(anaerobic germination)、熱(heat) 等等。抗病基因如水稻白葉枯病、水稻稻熱病、水稻飛蝨、病毒病;農藝性狀,如米質、產量與雜種優勢。QTLs 和基因的偵測和導入皆進行中。耐非生物逆境不見得有系統性的研究成果(如耐鹽性),代謝路徑不見得明朗,也會因為定位的族群不同而得到不一樣的QTL。但還是可以藉由比較多個族群,得到主效QTL,把可能得位置侷限在更小的片段。知道QTL在哪條染色體的哪個位置以後,就能得到與其連鎖的分子標誌(marker)—可能是上資料庫找的分子標誌,或是進行該區間的定序以後得到的SNP。

對於各種不同性狀的研究,Dr. Chin 指出在 MAS 技術依據兩種不同的目的需求會影響著研究的方向,一是用以針對新性狀進行育種或品系的育成,另一是作為初期基因貢獻親本(donor)的基因導入進行品種改良,前者除須目標基因或性狀的分子標幟外,仍需背景標誌進行篩選,但後者則僅需目標基因的分子標幟再配合接受親本的回交即可。在相同的耐性性狀 (tolerance)中,可能有各種不同的基因利用不同的機制控制著,所以多個基因的堆疊是有助性狀提升的。因此,可藉由不斷改良增加貢獻親本在目標性狀上的育種價值,增進育種的效率。

在每一次的雜交育種流程當中,所需要用的分子標誌,依據目的可以分成前景(foreground)、背景(background)以及重組分子標誌三類。對於 MAS 技術的增進除了對目標性狀的瞭解與分子標誌的掌握外,仍需有基因體學與資訊學的支援、快速的基因型判別、有效的分子標幟及背景篩選系統。例如在 IRRI 近期的研究當中,會將這上述三類標誌同時放在一晶片中,組成 integrated marker sets(整合分子標誌組),在同一塊晶片當中同時進行大量的基因型分析,減少所耗時間。目前在水稻中,有許多的資源可供使用,例如基因型的部份,有許多的 SNP 資料庫可供查詢,包含了世界各地的代表水稻品種的基因型,甚至有部份性狀的表現型資料,配合 44K、700K 的基因晶片或是各種高通量的基因型鑑定技術(Fluidigm, GBS...),可有效進行 MAS。

由於原本耐受性基因所在的種源可能有其他性狀不良,或是雜交的效率不佳,IRRI利用分子標誌回交育種育成帶有各個抗耐性基因的提供親。例如已 IR64 為主要遺傳背景的近似同源系。再進行各個組合的基因堆疊,就可以在短時間內得到具有各個抗性基因的 IR64 同源系。同理,運用在其他現行品種當中,就可以擴大方便育種的 donor parent。利用Muti-parents advance generation inter-cross(MAGIC)將帶有個特殊抗耐性的性狀直接堆疊,就可以育成新品種。

(三)、簡介稻熱病流行病學研發現況

稻熱病病原菌的 AVR 基因會被植物的 R 基因所辨認以至於讓病原菌無法侵入植物體內。早期研究認為 AVR 基因是病原菌的"叛徒",但是近期認為應該是演化上病原菌的 AVR 偶然被植物的 R 基因所辨認的結果。稻熱病菌的族群分析,主要是由周波博士介紹他在稻熱病菌上的研究。目前 IRRI 針對菲律賓的稻熱病菌族群做分析,(因為菲律賓並不允許輸入國外的病原菌),經過先前與今年的比較,有 100%的分離株都含有 AvrPi9 這個無毒基因,而其相對應的水稻 R 基因是 Pi9 基因,這是一個從野生稻 Oryzaminuta 發現的 R 基因。而 AvrPizt的檢測頻率原為 50%分離株且今年下降至 2.5%而已。AvrPik 的頻率原為 96%現已下降到 42%。 AvrPiks 及 AvrPii 原本各為 76%及 26%,今年卻反而為 18%及 80%。而所有的 isolate 中皆無值測到 AvrPia。而針對 AvrPik haplotypes 目前已知可分為 A, B, C, D, E types,但是在菲律賓的分離株中發現新的 F type,是在 423 的 T 置換為 A 的 SNP,而綜合來看,D type 的出現頻率高達 88%分離株,由此推論,應該找到是否有能有效辨識 AvrPik-D type 稻熱病菌之水稻 Pik 基因,以利有效進行抗病篩選工作。

IRRI 目前利用幾個無毒基因型來對稻熱病菌分成幾個 race,若某些 race 的頻度越高則表示利用其相對應的 R gene 導入栽培品種能夠最有效率地減少病害發生。在這次的報告中,菲律賓當地最有潛力的候選 R gene 是 Pish, Pii, Pi3, Pi5, Piks, Pikp, Pi9, Piz-5, Piz-t, Pi12, Pita, Pi19, Pi20。目前許多植物的 R 基因已經被發現並定位。Dr. Bo Zhou 建議地區性新品種若發現稻熱病抗性,經初定位後,應先檢查其抗性是否由鄰近已知的 R 基因所造成的。特別要提醒的是,連鎖分子標誌(Linkage marker)不應視為等同抗性基因,因為不同水稻品種其基因體不會一樣,將兩者視為相同會有誤判的風險。但是若用在育種上,在兩親本的分子標誌的情況已知之下,連鎖的分子標誌就有其應用性。

目前有發展新的策略去尋找新的植物 R 基因。首先先收集大量稻熱病病原菌,分析其基因體尋找其共有的核心因子(Core effectors)。建立帶有或不帶有核心因子的病原菌,分別篩選種原庫水稻品種。從帶有核心因子病原菌篩選出的抗性水稻品種,與不帶有核心因子病原菌篩選出的感性水稻品種,其兩者交集品種即帶有廣福抗性(Broad spectrum) R 基因的水稻品種。此策略亦可反向搜尋病原菌的 AVR 基因。

在亞洲流行病害監控研究上,IRRI身為國際組織,對於全球的水稻病害狀況非常重視,帶領進行對東南亞、南亞各地區水稻病害的調查。在過去一年內,對於菲律賓、越南、泰國、馬來西亞、印度、以及非洲東岸的水稻栽培地區設置熱點,種植對稻熱病、白葉枯病感病之水稻品系實驗田,對這些檢測地點進行病害發生程度的長期檢測,調查亞洲地區水稻病害的狀況,藉此擬訂對當地農民有利的病害管理策略,並提供育種家重要且即時的參考資訊。

(四)、基因型分析應用實驗室觀摩

IRRI 過去使用 STS markers 與 SSR markers,現在使用 SNP(Single-nucleotide polymorphism) marker 輔助育種,此實驗室開發新的 marker 提供育種者進行篩選。實驗室內有許多最新技術的儀器可提供快速、大量的基因型鑑定工作,如 IlluminaBeadXpress platform 是用雷射可識別的玻璃微珠為載體分別與 DNA 互補序列結合,可用來做 gene mapping、SNP 鑑定等,GSL 提供 384-SNPs 的資料庫。而 IlluminaInfinium platform 則是利用晶片上的矽珠矩陣,可與 DNA 互補序列結合,一個晶片可設計 6000 個專一性互補序列,與樣品 DNA 結合後 extention 單一核酸辨識某一位點的 SNP,可運用於背景篩選、DNA 指紋分析、QTL mapping。而 Fluidigm system 是一種自動化 PCR plate,又稱育種家晶片,最多可同時對 96種 marker 進行分析,GSL 提供 24個 SNP*192個樣品或者是 96個 SNP*96個樣品,後於儀器上進行 controller code/mix 與 PCR 程序後以搭配之 reader 讀取,可直接觀看螢光染色,辨別 A(紅色)、B(綠色)、H(藍色)。可用來分析 SNP、QTL mapping、及回交背景篩選。

為了快速取得植物樣品,基因型分析應用實驗室使用了一種叫 PlantTrak 的工具,其功能類似打洞機,並有專用的收集盒,每個樣片在剪取時,會剪 2-12 次,採完一個樣品,再轉至下一個樣品槽(cup),待葉片取樣結束後,會利用機器將採集器的樣片裝至 96 孔盤,使得田間到實驗室之間的工作方便許多。

(五)、參訪亞洲最大水稻種原中心

International Rice Genebank 由 IRRI 管理,主要工作為確保稻的多樣性並長期保存,收集了世界各地稻的種類達 117,000 種,包含現行品種與野生品種。每種類型的稻分兩個地存放,一為 base,長期保存於 -20℃冰箱;另一則為 active,短期存放於 2-4℃冰箱。在 International Rice Genebank 的稻種需要長期保存至幾十年,所有種子需要進行嚴格的檢測。在基因庫外的環境,因為菲律賓的高溫及高相對濕度,種子僅能保存六個月至一年,但在基因庫內則能長期保存。

從各地收集來的稻種,進行登記並編號。生長勢差與種子數少的品種,在旱季於田間 與溫室進行繁殖。收穫後的稻種以紙袋裝取後,放置於乾燥室中進行乾燥,乾燥室溫度約維 持至 15℃,相對濕度約為 15%,減少昆蟲及真菌對稻種的危害,乾燥後的稻種會經由人工去 除品質不良者,經篩選過後的稻種才會送入冷藏保存的環境,而在人工檢查稻種的過程中, 也會與原保存的稻種外觀比對,確認稻種品種正確,之後為確保乾燥後的稻種不會受潮,依 照目的分裝於鋁袋或鋁罐。中期保存 (20~40 年) 的冷藏庫溫度約 4℃,長期保存 (50~100 年)的冷藏庫溫度約-20℃,此外,也會定期檢測稻種稻種發芽率,狀態不佳者便會更新保存的稻種,大約每 5~10 年會重新繁殖,進行稻種更新一次,在每次到種保存前也會記錄其發芽率是否達 90%,低於 90%以生產新鮮種子進行保存,並進行昆蟲、線蟲、真菌等檢測作為稻種健康的評估,以確保品質。

(六)、水稻種原基因型生物資料庫之操作簡介與實務交流

主要是介紹 Rice SNP-Seek Database 之內容與操作方法,並以水稻胡麻葉枯病為例,講解此資料庫應用於關聯定位之使用, Rice SNP-Seek Database 整合水稻的基因型、表現型及品種資訊,其中 SNP 基因型資料的來源為 3,000 Rice Genomes Project,並與 Nipponbare 比較後所得之結果,此資料庫有助於研究者進行基因型及表現型關聯性的相關研究。

當有基因型及表現型整合而完成定位之後,從所有候選基因座中檢視實驗族群該區域的 haploid type,以縮小與性狀相關的區段範圍,進一步確認該區段與表型的相關性,之後便能 利用 Rice SNP-Seek Database 了解資料庫中的水稻品種是否有與目標區域相同型態者,也能 經由資料庫更清楚此區段中的組成,最後選擇適合的親本可作為後續驗證或育種之使用。

(七)、野生稻稻屬與滲入系族群選育

水稻在分類地位上屬於禾本科稻屬 Oryza,總共有 24 個種,這 24 個種可以分成三級 gene pool: primary, secondary 和 tertiary gene pool。其中有兩種栽培稻,分別是亞洲栽培種 (*Oryza sativa*)和非洲栽培種(*Oryza glaberrina*),亞洲栽培種又可再被細分為秈稻和稉稻,其餘的 22 種皆為野生稻。在野生稻中,有豐富而大量有用的基因,如:稻熱病抗性基因 Pi9 和水稻白葉枯病抗性基因 Xa27 皆來自於 *Oryza minuta*。但進行栽培稻和野生稻雜交時,常會遇到障礙。於是,依照遺傳基因轉移的難易度,可將野生稻分為三類:

Cross Incompatible:雜交不親合,為基因庫分級的基本原則。

Primary gene pool (基因體結構為 AA):包含眾所皆知的兩個栽培種 *O. Sativa* (含 japonica 和 indica 兩個亞種)及 *O. glaberrima*,再加上其它 6 種,共 8 種組成 Primary gene pool。可直接進行雜交育種。

剩下六種是:1. O. rufipogon,為在亞洲熱帶及大洋洲,被認為是 O. Sativa的祖先;2. O. nivara,亞洲大陸,與 O. Sativa 親緣較近;3.O. barthii,與 O.glaberrima 親緣較近。被認為是 O.glaberrima 的祖先);4. O. longistaminata,多年生具地下莖。為非洲特有種。具有廣福抗性的 Xa21 就是此種而來;5. O. meridionalis 和 O. glumipatula (O. glumaepatula)主要分布都在澳洲及中南美洲。

這個 gene pool 可以用一般的雜交方式,將目標基因(如抗病基因等)導入栽培種水稻當中。 為了尋找並利用野生稻豐富的資源,IRRI 也建立了不同的 MAGIC (Multi-parent advanced generation inter-cross) population,選用的親本皆為 improved varieties,帶有優良的農藝性狀或 是對生物/非生物逆境具有抗性。建構出這樣的族群,希望能利用 QTL mapping 以及 GWAS 的方法,定位出調控多重性狀 (multiple traits)的區域。在 IRRI 的溫室中,種植了各種野生稻, 其植株高度、葉片質地、分蘖情況等皆有很大的差異,有些較高的水稻甚至能長到 3 公尺。

一般胚拯救流程:以 20%漂白水消毒種子 10min,以無菌水潤洗三次,在解剖顯微鏡下挑出胚,移到 1/4 MS medium。生長一個月後,移到水耕液進行馴化,再移植到土中。IRRI 有專門進行此工作的員工,一天最多能做 500-800 管。

(八)、流行病害監控與預警模式評估

Philippines Rice Information System (PRISM) 計畫中想要獲得的是關於水稻的資訊,關於菲律賓有種植水稻的區域分布以及水稻的種植狀態,此演講主要介紹即時偵測病蟲害的發生的部分,因為實地監測需要有多各地人員的參與,因此,,首先訓練各地方部分人員,有關辨認病蟲害以及設備的使用,當他們具有進行調查的能力後,回到當地區域去教導更多的人此技術;田間資料的收集是利用智慧型手機操作,使用智慧型手機可以直接輸入資料、獲得GPS 資訊,可以簡化以前用紙本記錄再以電子郵件傳輸資料所需的時間,使得資料獲取可以更為即時,將所有獲得資訊包括電腦、手機、紙本等作物資訊傳回雲端,進行更進一步的資料分析,如:作物生產資訊、病蟲害及雜草發生情況、殺蟲劑使用、產量等,將所有資訊整合至地圖上,可以觀察各地病蟲害發生情形,藉由各年度資料整合,以可以了解在時間軸上的作物種植情況的變化,經由手機獲得當地資訊,可以在經由手機將分析後的資料傳輸回去,使得整個系統能更即時及有效率。

此外,所得的資料也可以應用於病害流行病學上的研究,獲得環境因子:降雨量、溫度、相對濕度等資訊後,結合當地作物資訊,如:種植時間、水分應用、肥料管理等,希望能預測病害發生情形及產量損失,目前是以白葉枯病及葉稻熱病為初步研究目標。

(九)、稻熱病判別品種選育概況與展望

水稻稻熱病的判別系統是利用判別菌株與判別品種間不同的交互作用進而對稻熱病相關基因研究的工具,可用以鑑別或監測不同的病原病理小種以及進行基因型的預測。國際判別品種由8個品種所組成,普遍用於病理小種的判別與研究,但其中的抗病基因未知,且各品種間並非同一遺傳背景;日本判別品種有兩套,一於1976年開始使用共有9個品種,另

一套報導於 1981 年共由 12 個品種組成,日本判別品系可有效判別日本的病原生理小種,且知道其中相對應之抗病基因,但對於熱帶地區的病原菌,可能有其它的抗病基因在其中作用, 且該系統中的品種並不適應熱帶氣候。

對於理想的判別品種有兩大重要條件,一是應具備單一的抗病基因,二則是需具感病的遺傳背景,因此,IRRI 遂開始進行 Monogenic line(ML) 與 Near-isogenic line (NIL)的研究與育成,目前育成了以 CO39 與 LTH 兩個分別為秈稻與稉稻感病品種為背景,分別導入不同的抗病基因,以供水稻稻熱病病原生理小種的判別。但目前仍待解決的問題為,各 NIL 間農藝性狀並不完全相同,部份品系還有光敏感性,遺傳背景的純化工作仍有待加強,另希望能導入更多的抗病基因,以提供廣範的鑑別能力。

在稻熱病菌接種的病斑判別方法中,採行的是以病斑型態區分的 0-5 級制,0 級為無病斑、1 級為 0.5mm 以下病斑且未產孢、2 級為 0.5-1mm 的病斑且未產孢、3 級為 1-3mm 橢圓形病斑且中心灰色產孢、4 級為典型紡垂狀病斑,中間灰色產孢且大於 3mm、5 級病斑與 4 級同,但有多個病斑重疊發生。除介紹罹病等級的判別外,另外也指出,0-2 級判為抗病,4-5 級判為咸病,而 3 級的病斑則可能視不同抗病基因的抗病能力而可能被視為咸病或抗病。

(十)、病蟲害篩檢中心與病圃導覽

褐飛蝨會引發兩種病(Ragged stunt disease 與 grassy stunt disease),IRRI 收集菲律賓三個大島各地的褐飛蝨,進行生物性檢測水稻對於 Ragged stunt disease 與 grassy stunt disease 是否具有抗性。已點播的方式育苗,一排有六個品種,一個品種有 20 株,將感病對照品種 TN1(台中在來一號),抗病對照品種 IR64,前中後種植,估計大約株數,並放褐飛蝨於水稻植株上,每株水稻可分配約 10 隻褐飛蝨。育秧七天後,進行放置褐飛蝨,放置後七到八天後開始進行評分(主要還是依照感性品種),天氣也會影響抗感程度。

在病毒病害的介紹中,共介紹四種病毒造成的植株病徵,並就其傳播方式做進一步說明,四種病毒分別為 Rice Tungro Bacilliform(RTBV)、Rice Tungro Spherical(RTSV)、Rice Grassy Stunt(RGSV)、Ruce Ragged Stunt(RRSV)。Tungro virus disease 會造成植株發育不良,生長阻礙,葉片變黃。單獨感染 RTBV 的植株會有些微矮化、抽穗少之病徵,而單獨感染 RTSV 的水稻植株,其外表幾乎無病徵表現,與健康植株無異、穗數減少,所以在田間很難辨認出植株感染 RTSV。RTSV可以單獨經由媒介昆蟲在田間傳播,但 RTBV 無法單獨傳播,需要有RTSV的協助,意即昆蟲體內需要有 RTBV 及 RTSV 兩種病毒時,RTBV 才能被媒介昆蟲傳播,而帶有此兩種病毒的媒介昆蟲在取食植株時,可能單獨傳播 RTSV或 RTBV 或將兩種病毒皆傳入植物中。一般在田間,植株通常是先帶感染 RTSV,然後帶有 RTBV 的媒介昆蟲取

食帶有 RTSV 的植株後,昆蟲體內便帶有兩種病毒,當昆蟲至健康植株上取食時,使得植株獲得兩種病毒,複合感染 RTBV 及 RTSV 的水稻植株在株高上會嚴重矮化,此兩種病毒皆是由昆蟲經由半永續性傳播,主要的媒介昆蟲為黑尾葉蟬。黑尾葉蟬叮咬有病毒的植株,在兩小時內具有高傳染能力,且存在體內五至六天。感染 RGSV 之植株外型似草而名,而感染RRSV 會造成植株矮化,葉片有捲曲的現象,RGSV 和 RRSV 是藉由褐飛蝨傳播,褐飛蝨能永續性傳播此兩病毒。

稻熱病病圃於田間直接點播,周圍種植稻熱病感病品種,待植株長至一定高度時,將外圍已得稻熱病的感病品種,抓取幾株,放置試驗材料附近,間隔三到六行放一次,並以灑水系統保持田區高濕度。病害調查的方式則是依循 IRRI 訂定的 Standard evaluation system (0-9級)。除了針對葉稻熱病,該病圃也有進行穗稻熱病抗性品種的篩選。

(十一)、抗病種原開發與應用研究

一般進行育種的流程,要先找到目標的性狀以及調控此性狀的數量性狀基因座(QTLs)或基因,並針對 QTLs/gene 設計分子標誌,進行品種改良。育種學家在意的會是改良後的品種在自然環境中,該性狀的持久性和有效性。因此,我們必須要了解 R gene 的特性,包含 R gene 在染色體上的位置、基因和基因之間的交互作用、以及使否會影響到其他農藝性狀的表現。 R gene 的持久性,根據 Johson (1984)的定義,是指該抗性在大的區域內皆能有效表現。

GRiSP (Global Rice Science Partnership)是由 IRRI,AfricaRice 和 CIAT 主導,和 CIRAD, IRD,JIRCAS 還有世界上許多研究者合作的計畫。宗旨是為了減少飢餓和貧窮的問題、讓人類吃得更健康、降低對環境的傷害以及增加水稻產量。基本的流程架構從種源庫, heirloom rice, traditional rice, 和 land race 挑選合適品種進行基因型及表行的分析,並從中尋找目標的性狀進行品種改良。

以水稻抗白葉枯病相關研究為例,必須要對病原菌生理小種以及不同生理小種的毒性有所了解,才能以此做為育種的方向。在菲律賓,菌株依照接種 IRBB lines 的結果可分為六個生理小種,在 1972-1988 年間,Race 1 由高毒力轉為低毒力;Race 6 則是相反,而在田間的主流菌株,則是由早期的 Race 3 轉為 Race 9b。不同地區的菌株,也會因為地緣性,在親緣分析上被分在同一個 sub-population。這些現象顯示出了病原菌的多樣性,為了尋找對白葉枯病菌具廣效抗性的抗病基因,IRRI 提出了 Hotspot strategy: 要開發已知 R gene 的 NILs、每年定期收集菌株病進行病原性檢定、區域性的調查菌株族群。目前已經發現超過 35 個 R gene,另外也發現基因堆疊可增強植株抗性。有趣的事情是,同樣的 R gene,在不同的地區表現並不相同,且各地有效的 R gene 友會有所差異。但在白葉枯病抗性基因的部分,在西非、南亞

及東南亞,共同有效的 R gene 有 Xa4, xa5, Xa21。

最後我們必須要了解的是:沒有任何抗性基因是永遠有效的;沒有最好的抗性基因,但有些抗性基因是相對具有廣效性且能適應較多區域;基因堆疊、輪作、多重品系(multi-lines)等不同的策略提供了良好的病害管理策略;要對於病原族群結構有所了解,我們才有可能有聰明的防治策略!

(十二) 參訪米質中心

IRRI 米質中心的研究方向包括三個方面:物理性質、生化性質、食味與烹煮品質。在物理性質方面,量測的重點包括米粒顏色、白堊質、大小、透明度、形狀以及香味。在生化性質方面,包括澱粉與蛋白質的分析、營養成分、香味組成物質等,支鍊澱粉與直鍊澱粉的比例主要影響米粒的黏度與彈性,同時蛋白質的成分除了影響營養之外也會對黏度與烹煮性質造成影響,在米粒微量元素與香味分析的部分則是採用質譜儀進行分析。在食味分析的部分除了人工官能品評,亦採用質地分析儀,測量米飯的彈性、黏性與延展性。當我們提問如何擬定策略促進稻米加值,Dr. Nese Sreenivasulu表示,稻米產業鏈不只考量農民與消費者兩端,碾米加工業者扮演的角色也很關鍵,好的品種在生產端容易栽培、便於管理,在加工過程中米粒也應該維持良好的適用性,因此育種者應考量加工業者的需求,才能選育適合農民、工廠、市場三方面皆有應用價值的稻米。在米粒加工的部分,如彩色米或米的衍伸製品雖然是臺灣、日本、韓國等國家的創新加值方法,在IRRI的研究較少,IRRI主要還是以育種為研發方向。

五、參訪心得

- 1. 目前藉由溫度、雨量等氣候因子進行抗病品種的選育效果預測,用來探討新穎抗病品種釋出後對於病害防治效果,初步模式結果顯示白葉枯病抗病品種的效果較為顯著,但是稻熱病的抗病效果則是比較薄弱。以台灣的水稻栽培模式而言,北部地區的施肥量與南部地區不同,而且過去栽培模式的施肥量與現行模式也不同,抗性品種釋出後可能會讓流行病原菌組成改變,但在農民取得抗性品種後,可能會投入更多肥料量換取高產量回饋,但高氮肥下會導致品種的分蘖數增加、葉色更加深綠,也增加病害風險,因此抗性品種釋出後,往往不僅會改變該地區的病原菌組成,也會讓農民的栽培模式隨之改變,或許施肥量應該考量在探討抗性品種的影響因子之一。
- 2. 另隨著全球氣候變遷、氣候暖化下,已有部分抗性基因的有效性隨之失效,未來應該要加強探討抗性品種對於氣候變遷下的穩定性與有效性。
- 3. 在IRRI來自各國的科學家以全球糧食安全為己任,十分令人非常佩服,在研究方法方面

有許多值得我們深入合作的項目,例如單一核苷酸多型性(SNP)的平台,IRRI已有高密度的水稻基因組分析工具,臺灣若開發自己的SNP平台要花費大量的人力與資源,若能與IRRI合作,將需要分析的水稻DNA樣品委託IRRI基因定型服務實驗室(GSL)進行分析,將可以支付合理費用的情況下獲得高解析度的資料,是非常值得應用的服務。

六、參訪建議

- 1. 隨著由IRRI引入抗性品種與部分相關分子標誌技術,在國內已經逐步抗性基因導入栽培 品種中,選育出帶有抗性基因的高世代回交品系,已經完成第一步抗性基因滲入試驗; 但隨著氣候變遷,部分栽培季節的氣候已有偏高趨勢,對於抗性基因的有效性與穩定性 應該加強探討。
- 2. 白葉枯病的宿主抗病基因研究方向已完成雜交育種與評估方法之建立,然而臺灣在病菌本身的基因型分析研究較為缺少,IRRI在這方面已經定序105個菌株Xanthomonas oryzae pv. oryzae 的基因組,若臺灣能將本土流行菌株進行全基因組定序,並將資訊與IRRI共享,不僅我國可以利用IRRI的分析能力將國內菌株定型與監控,IRRI也可以增加在全世界蒐集的菌株基因組多樣性,將會是一項對雙方皆有幫助的作為。

八、參訪照片



白葉枯病研發進度簡報

雙方議題討論





白葉枯病研究團隊簡報

水稻生物資料庫導覽





白葉枯病田間檢定導覽

溫室判別品種檢定標示



基因型分析應用實驗室簡介



IRRI水稻種原中心種子保存作業





種子收穫後於15°C、15%條件下進行低溫乾燥



稻熱病篩檢苗圃簡介與導覽



旱田式秧苗稻熱病檢定圃





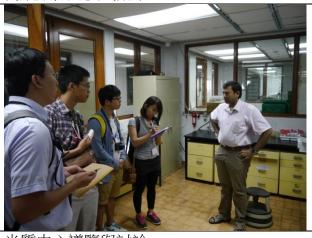
野生稻分類與應用簡介



稻屬物種溫室導覽



褐飛蝨檢定模式觀摩



稻作病毒病簡介與實體觀摩

米質中心導覽與討論

九、抗病研習小組英文回饋報告

Wu, Dong-Hong 吳東鴻

Assistant researcher, Taiwan Agricultural Research Institute

I'd like to share some of my thoughts:

First, during Dr. Adam presentation about the impact of using resistant varieties, the predicted result showed a better effect in bacterial blight than in blast. The environment factor mainly considered the rainfall and temperature in Philippines and Indonesia. I suggest the model can include the application of fertilizers as another factor. In Taiwan, the amounts and frequencies of fertilizer application can be very different in different locations. The farmers applied much more fertilizers in northern Taiwan than in southern Taiwan. The cultivation model possessed diversity in different areas. Furthermore, with the cultivation of a novel variety with more durable resistance, the farmer may apply much more fertilizers to get higher grain yield than before. But the overly-applied nitrogen would cause more tillers and dark green leaves, compared to the plants at a normal condition as suggested in the model. The resistant variety could be sensitive to such changes. So I think the resistant cultivars will not only push the pathology shift but also change the cultivation system.

Second, I will talk about the effectiveness of bacterial blight R-genes in different temperatures. High temperature will lead to loss of *Xa4* function. In rice cultivation in Taiwan, the major disease of the first season is rice blast and the bacterial blight usually occurs more seriously in the second crop season. The temperature dynamics is from low to high in the first season, and from high to low in the second season. However, the strong wind and high humidity raised the BB risk in the second season. But now the farmers sometime report the BB damage at his/her rice fields in the first season. Some Taiwan cultivars, such as indica Taichung sen No. 17 and japonica Kaohsiung 145, possess *Xa4* gene. Maybe those cultivars only possess a single *Xa4* R-gene, which is not effective against local isolates under climate change.

Chang, Ray Jui-Hsin 張瑞炘 Assistant Researcher, Taichung District Agricultural Research and Extension Station

This is the second time that I visited IRRI, I really appreciate your efforts and arrangement. I'd like to thank Dr. Leung, Dr. Vera Cruz and Dr. Chitra for all they have done in this visit. This time we entered the rice quality research lab and saw a lot of fine equipments. In Taiwan's rice quality lab, we focus on the consumers' preferences, especially some characters like texture, cooking and eating quality are mostly investigated. In IRRI, I found your lab has many equipments for analyzing the nutrition components and beneficial elements. I think this is what we can learn from you, because it could be a better direction to develop new varieties with functional elements than those just with good tastes.

We also visited the Genotyping Service Lab and talked with Dr. Michael Thomson. GSL provides several kinds of SNP genotyping systems, some of the them are suitable for Taiwan's breeders. Since we used the IRBB66 as donor parent for B.B. resistance breeding and now the current status is at generation BC₃F₁, the 6k SNP chip would be very helpful when we need to analyze the genomes of following generations. It will be more economic to use the GSL's service than designing our own SNPs, and we really look forward to sending DNA samples for 6k chip analysis someday.

T. T. Chang genetic resources center is really impressive to me. We walked into the -20°C

conservation space and were surprised to learn that the seeds could be conserved for more than 50 years. The world's half population take rice as principle source of energy, and that's why IRRI's genetic resources are so important for food security. We also learned how IRRI do introgression of functional genes by crossing wild rice and *Oryza sativa*. It is really important to broaden the sources of functional genes and I really appreciate Dr. K. K. for his fantastic embryo rescue technologies. I know that *Xa21* gene for B. B. resistance is from *Oryza longistaminata*, I believe we will get more and more stress-tolerance genes and disease-resistance genes by his efforts.

The collaboration between Taiwan and IRRI provides great opportunities for us to learn more knowledge from IRRI, and to use more technologies that you provide. I believe that we will develop some elite varieties by the collaboration in the near future, and I'd like to thank all scientists participate in this project. I hope the collaboration relationship and our friendship will last forever.

Shen, Wei-Chiang 沈偉強 Professor, National Taiwan University

IRRI, the leading international research institute of rice science, provides collaborative partnerships and aims to provide rice germplasms, technology, sustainable systems for rice farming and production through the scientific studies and application. Under the vision and leadership of Drs. Hei Leung, Huu-Sheng Lur and Men-Chi Chang, this IRRI-NTU-TARI international collaboration project is in its third year of first four-year grant. The aims of this project are to breed biotic and abiotic stress tolerance/resistance varieties for Taiwan rice production, to establish the platforms for studying major rice diseases including blast and bacterial blight, to train junior research scientists and stimulate materials and technology exchanges.

During November 2 and 8 in 2014, a group of 13 people, including 8 students, from National Taiwan University, National Chung Hsing University, Taiwan Agriculture Research Institute, and Taichung District Agricultural Research and Extension Station, Council of Agriculture, visited IRRI to learn and study the updated knowledge or researches related to rice major diseases and grain quality evaluation. Dr. Hei Leung and his colleagues organized a four and half days program for our visit and the program includes a series of lectures and guided tours to genotyping service laboratory, wild rice greenhouse, Genebank, biotic stress screening centers, blast disease nursery, and rice fields. Overall, Drs. Leung and Chitra had organized a very useful and impressed program and offered different lectures and tours from my last visit in 2013. We are grateful for their efforts and organization and I was impressed with some of the progresses they made within such short period of time.

The lectures include marker-assisted selection for biotic and abiotic stresses, disease situation in Asia, pathogen populations analysis, new sources of resistance from wild rices, disease epidemiology-field survey and GIS zoning, NILs series for rice blast resistance, disease resistance germplasm for blast, blight, sheath blight and others, and neck blast resistance. Several lectures impressed me very much, and the first one is "new sources of resistance from wild rices". Dr. K. K. Jena gave a comprehensive presentation of wild rice species. He mentioned that in addition to cultivated rice, 22 wild *Oryza* spp. are categorized into primary, secondary, and tertiary gene pools, representing great resources for hunting stress resistance traits. Bacterial blight resistance gene Xa27 is one of the well-known examples from wild rice. He also mentioned that sheath blight disease is a worldwide important disease of rice and most, if not all, of commercial rice varieties

are susceptible to this disease. Recent studies at IRRI has identified a sheath blight resistance gene from wild rice species and shows promising results. This finding may provide a useful resistance source for sheath blight breading around the world including Taiwan. I will be pleased to check into the possibilities for further collaboration on this disease. We also had a chance to visit IRRI wild rice greenhouse and Dr. Jena's laboratory to see the collected and protected wild rice germplasms and techniques such as embryo rescue. It was a wonderful and exciting experience for wild rice studies in this trip, and I really appreciate Dr. Jena's presentation and arrangement. The second one is a lecture presented by Dr. Adam Sparks. Dr. Sparks is a junior researcher at IRRI, but he is really an expert in the epidemiological fields. This time, he described the implementation of GIS zoning system with smart phone by his colleagues in Philippines. Nancy and her colleagues had trained extension people for the diagnosis of major rice diseases including pathogens and insect pests. Using this software in the GIS system, the on-site field monitoring or surveillance data can be effectively recorded and uploaded into the IRRI database. The epidemiological team can real time monitoring the occurrence and spread of rice diseases. This is a very powerful system especially for developing countries with large territory. Although we have set up a surveillance system for rice blast and bacterial blight in Taiwan, the ideas for more effective and real time monitoring crop diseases with modern technology are really intriguing. I will be pleased to check into the possibilities for implementing such system for rice diseases in Taiwan.

I was also glad to learn that the SNP SEEK database is constructed to provide allele mining for the researchers involved in molecular breeding. IRRI bioinformatics team gave us a very comprehensive introduction for the database. I believe this database will benefit a lot of people in rice research fields. We also appreciate the lectures and meetings offered by Dr. Bo Zhou. Dr. Zhou spent a lot of time to discuss the issues related to blast resistance gene mapping, R gene structure and diagnostic method. We also discussed the evolution and genome structure of rice blast AVR genes. He is really an expert in blast resistance and avirulence genes. We were grateful for his time and future collaboration on the blast pathosystem in Taiwan was also discussed.

Overall, this trip is my second trip to IRRI within two years. It is still a very impressed and fruitful experience. Myself and the other members of this group have learned and enjoyed very much. We really appreciate Dr. Leung's and Chitra's organization and arrangement and we all wish to visit IRRI again in the near future.

Chung, Chia-Lin 鍾嘉綾 Assistant Professor, National Taiwan University

Thanks to Dr. Hei Leung, Chitra, Dr. Norie, and Yang for organizing such an intensive program. This was my second time being at IRRI, and I still found new things to learn, which is really great! I enjoyed all the topics, covering not only the research of disease resistance currently conducted in my lab but also many topics of general interest (eg. germplasm diversity, wild rice, disease monitoring and modeling, etc). It must have been uneasy to include so many IRRI scientists and experts to participate in the program during the week of our visit. We appreciate IRRI's great effort, and think that all the knowledge and experience will be beneficial for our projects.

I'd like to express my sincere thanks for allowing the participation of four NTU students (three from my lab), four NCHU students, and one NCHU postdoc in our visit. It was a great opportunity for them to learn new concepts and technologies, which can possibly be applied in

their own research studies in the near future. I also think it's important to expose the young generation to the open-minded and enthusiastic atmosphere at IRRI, from which they would learn how to work together to build something bigger and better! Hopefully students' participation can formally be part of the IRRI-TARI-NTU project.

A lot of major genes and QTLs have been identified for resistance to rice blast. Great to have Dr. Bo Zhou help clarifying the locations and genetic structures of some important but complex R gene loci in rice genome. In a private meeting with Dr. Zhou, we discussed the strategy for fine-mapping of a candidate QTL in a Taiwan cultivar. We appreciate Dr. Zhou's kindness of sharing his expertise and providing the diagnostic primers for *Pi2/Pi9* and *Pik*. It was also very nice to have Jeanie talked about the development and application of IRBL differential lines, and the tips on determination of compatible and incompatible reactions. All of these will definitely be helpful for our current work in marker-assisted selection and the identification of the R gene loci in selected Taiwan cultivars.

I was very impressed by the 3000 genome project and the SNP-Seek database! IRRI must have put so much effort on it and I'm happy to see that the resource is now open to the rice community! Thanks for Chitra's demonstration on how to use the SNP-Seek to assist association mapping, haplotype analysis, and gene discovery. The database and germplasm will be very useful for candidate gene/QTL validation.

Lin, Heng-An 林珩安 MS student, National Taiwan University Advisor: Chung, Chia-Lin

I have heard about IRRI since I joined Dr Chung's lab and started to do rice-related project. I have been working on three projects, which were about bacteria leaf blight and rice blast disease. So, this trip to IRRI, it's kind of like a pilgrimage for me, I was eager to know what this place would look like. When we arrived IRRI, I was impressed by the vast rice field that extends as far as eye can see. In the first day, we also hung out with Dr. Wu and Ray, to visit UP and some shops nearby.

On 11/2, each student had to give a short presentation on his/her project. It was a special experience for me to present in this way. My project is about genome-wide association mapping of rice blast resistance genes in rice diversity panel and Taiwan rice cultivars. I also shared some problems we face while conducting experiments. Thanks to Dr. Chitra and Dr. Nollie for giving me really useful advices.

The rest of days in IRRI, I learned a lot from different topics. Not only useful for my master projects, but also got plenty of new knowledge about rice research. For example, regarding to Dr. Adam's talk, I think it's cool to teach farmers to use smart phone for disease investigation and to upload the data to cloud. Instead of going to the infested fields by themselves, researchers can easily get real-time information in the lab.

Another thing that I'm interested about is wild rice. From Dr K K Jena's talk, we know more about MAGIC and how to use the resources in wild rice. In Taiwan, the only wild rice is *Oryza rufipogon*, I only saw pictures of them on the internet. It was quite exciting to see all the wild rice species in the greenhouse!

For my own project, I also got useful information and advices about rice blast disease rating and the symptom recognition. I know that there is a standard evaluation system for rating, however, sometimes it's still difficult to define the lesion type or scale only by description. After discussing with people working in the IRRI blast nursery, I will be more confident running the inoculation experiments in the future. Also, thanks to Dr. Bo for spending time with us to discuss data on-hands. I can feel his passion on discovering new things about blast *R* and *Avr* genes.

I think the most impressive thing in this trip is that, I saw so many scientists, which are working so hard and full of interest in their own fileds, and hope the results can be shared with people all around the world or make some changes in people's life. I think that is the purpose to do research, not just sitting in the lab and apart from the reality.

Finally, I would like to thank IRRI's arrangement of the schedule. Thanks to Chitra and Yang for taking care of us. Also, thanks to Dr. Chung for giving me this chance to visit IRRI. It's really a wonderful experience.

Chen, Szu-Yu 陳思聿 MS student, National Taiwan University Advisor: Chung, Chia-Lin

I really learned a lot of new knowledge and got many special experiences in these days in IRRI. Each lecture and visit broadened my horizons. In the first day, I had the opportunity to present my research topic. Thanks to Nollie and Chitra's advice and questions, letting me know what I should pay more attention to in my research. I also mentioned the difficulty I currently face: How to choose candidate QTLs from the results of association mapping. Chitra gave me some advices and used her research topic as an example to explain the concept in the fourth day. It was really helpful for me to know what I should do in the next step. In addition, the SNP-Seek database was useful for my research in validation or to know more detail information of candidate QTLs.

We seldom have the chance to do field trials. In field trial, the experimental materials are in great amount. Each experiment needs to be planned carefully. People in charge of field trials have lots of experiences and knowledge to answer our questions. I saw people at rice blast nursery and greenhouse always introduce their experimental contents professionally with confidence. It let me know that it requires the accumulation of time and experiences to become an expert.

Chitra said: we should accurately describe the specific condition at which the QTL were identified. I would never forget this concept in my mind. For our own research, to record our experiment carefully is needed. The more accurately we can describe our research, the more helpful and useful our data can be for others. In these days, I saw many presentations of the diseases occurring throughout many places. I not only deeply knew the difficulties encountered in rice currently, but also learned how to design the strategies to control disease, such as the use of smart phones for real-time monitoring of the situations of diseases and pests, the understanding of pathogen populations in local region, and the utilization of wild rice germplasm resources. Every study in IRRI is for practical application to solve problems currently encountered in rice. I hope I can keep in mind that the purpose of research is to be able to solve practical problems.

In these days, every speech was very exciting for me. Sometimes, I heard some consideration or thoughts in the experiment, I would try to think about how to apply the concept in my research. I was stimulated by new knowledge every day and got a lot of inspiration. There were many ideas in my mind and I got more motion to apply these ideas in the study. I also liked the atmosphere in IRRI. Everyone put much effort to solve problems together. People communicate and discuss with each other with open minds to make concepts and ideas more clearly. Each question we asked was answered in detail. I really feel lucky and glad to have the chance to come to IRRI. I returned with my mind enriched with knowledge and enthusiasm. Thanks for everything that IRRI did for us.

Chang, Wei-Bin 張為斌

MS student, National Taiwan University

Advisor: Chung, Chia-Lin

Thanks to Dr. Leung and Chitra for organizing the program. Also thanks to IRRI for providing the resources and places. This is my first time to IRRI. I got a lot of useful information from this trip. For example, I have been using the IRBL differential lines in my QTL mapping project. I was happy to learn more in-depth information about the breeding history and background of the monogenic lines and near-isogenic lines. We visited greenhouse facilities and disease nurseries, and learned about the artificial inoculation and screening systems for rice blast and bacterial blight diseases in IRRI. We also saw many high technology tools in the genotyping service center. It was nice to learn all the most updated knowledge from this program, including data collection with smartphone for epidemiological research, breeding and application of MAGIC populations, operation of SNP database, and the utilization of wild rice germplasm in resistance breeding. I got a lot of useful information on blast resistance genes from Dr. Bo Zhou. Through Dr. Hei Leung's and Dr. Nollie Vera Cruz's introduction, now I have an idea of the role and the areas of interest of IRRI in rice community.

Kuan-Lin Lo 駱冠霖

Postdoc and Research assistant, National Chung Hsing University

Advisor: Wang, Chang-Sheng

I would like to thank Dr. Hei Leung and all his colleagues during our visit in IRRI. You all are very kind and I learned a lot from this visit.

- I learned the knowledge about the phenotyping of bacterial blight disease in greenhouse and rice blast in the field. It would be very helpful for me to establish the screening system in our lab.
- 2. Dr. Bo Zhou provided very useful information about the key points in the processing of mapping blast R genes.
- 3. Chitra mentioned that we should always clearly define which stage of materials we used for the phenotyping in QTL mapping. I think that I had never seriously considered this issue before.
- 4. As a post-doc in the lab, I can initiate from organizing our thousands of sodium-azide mutants and screening them for pathogenic study. The mutant library is a treasure and I should be able to find something new.

Wei-Chia Hung 洪偉嘉

MS student, National Chung Hsing University

Advisor: Wang, Chang-Sheng

This is my second time to visit to the IRRI. IRRI is the most important research center for rice, and I learned useful information and experience that can be applied in my study. In addition to the programs for rice disease resistance, I am very interesting in the breeding programs in IRRI. Rice SNP-seek database is a really useful tool for breeders. The whole genome sequences of 3000 rice varieties are useful for identifying causative SNPs of disease resistance and other traits in rice. The genotyping data combined with the phenotyping data can help breeders to analyze the genes. After finding the major genes or QTLs, functional markers can be developed and applied for

marker-assisted selection, which allows the breeder to advance the breeding process more efficiently. The wild rice genome pool is also an interesting class. There's great level of diversity in disease resistance and agronomic traits in wild rice. Genes of interest can be introduced into commercial cultivars, by crossing and embryo rescue. Thanks to all the people I met this time in IRRI. All of you were really nice to help us. I also learned and saw a lot of interesting things and the operation in the field and lab. I hope I will have a chance to come back to IRRI again in the future.

Pua Chai Ying 潘彩雲

MS student, National Chung Hsing University

Advisor: Wang, Chang-Sheng

I would like to express my sincere gratitude to Dr. Hei Leung and Dr. Chitra for your time and great effort to organize this program. It was both enjoyable and informative. The excellent planning and scheduling for the meeting, discussion and hands-on activities covered most of our interest and that was relevant and very helpful. Thank you for all the IRRI researchers for their patience and willingness to share their expertise and experience. It was a worthwhile and the most fruitful learning program that I have ever participated.

- 1. The sequence and phenotype of all the 3, 000 rice varieties from 89 countries help to centralize the information and data. I can see the huge effort and investment to unveil the genes-trait association by effectively utilized the gene bank assets. This is extremely useful information to accelerate the rice breeding by looking very specifically on the gene and variety of interest.
- 2. The development of Multi-parent Advanced Generation Inter-Cross populations (MAGIC) in rice was a super brilliant idea that provide an immortal mapping population and combined many different traits in a single line over a relatively short period of time. Before visiting to IRRI, I really wonder how the genotyping and so many crossing combinations were done. Now, I have a clearer envision on development of MAGIC population and wish to try it out!
- 3. The genotyping and sequencing laboratory showed the leaf sampling kits, Fluidigm and ion torrent sequencing technology. Both the leaf sampling and Fluidigm were something new to me, it was such a convenient and time-saving ways compared to the 'classical' genetic analysis. However, it is too expensive and more than we can afford. But, it really inspired me by how creative the human mind is.
- 4. Dr. Kshirod Jena gave a wonderful presentation on wild rice and showed his great enthusiasm to find hidden traits and genes that can help breed new rice varieties better at thriving and producing food in difficult environments. This was the first time I have seen so many wild rice in my life. Words cannot tell how wonderful it was!
- 5. Dr. Bo, Dr. Vera Cruz and the pathology group gave an excellent presentation on the blast and bacterial blight disease. Lectures on the R genes, Avr genes and their co-evolution provide a useful information for the breeding program to improve the rice resistant. The availability of differential lines for bacterial blight and blast are important tools to determine the pathotypes present in Taiwan. This can help the breeders to determine which resistant genes are actually effective and monitor the pathogen frequency from time to time.

Last but not the least, many thanks again to everyone from IRRI and Taiwan members for this memorable and fruitful learning program. I really have a great time!

Wei-Jhan Siao 蕭偉展 MS student, National Chung Hsing University

Advisor: Wang, Chang-Sheng

This is my first time to come to IRRI. I would like to show my appreciation to everyone we met during the week. IRRI is one of the most important research organizations in the world. I'm so glad to learn so many novel things there. There are two ways, pathology or breeding, to go through. The topic I was most interested in is the introgression of genes from wild species. In my opinion, the source of genes of stress tolerance and resistance need to be expended, and one of the natural sources is the genes from the wild species. If we can improve the skill of introgression, we can handle more environment stress due to climate change in the future. However, one week is very short. I wish I could take more time there to learn more, and hopefully that IRRI can provide the information of workshops to the universities with department of agronomy in Taiwan.

Zong-Yan Bai 白宗晏 MS student, National Chung Hsing University Advisor: Wang, Chang-Sheng

This is my first time visiting IRRI. I have read some papers from IRRI about rice and really admire every single researcher in this institution.

- 1. My research is mainly on 'Breeding of bacterial blight resistance in rice'. For the bacterial blight screening in the greenhouse, the rice plants are planted in a rectangular plastic tray submerged in a big water container. Every tiller was labelled and inoculated with different isolates to determine the resistant reaction of plants as well as the virulence of the isolates. This is very important information for breeding of disease resistant rice.
- 2. Visit to the gene bank and wild rice was very interesting. The long-term preservation of rice seeds for traditional and modern varieties as well as wild rice showed the importance of sustainable diversity of rice genes.
- 3. It was a great opportunity to learn more about other biotic diseases of rice. The experience helped build my capability of identifying disease symptoms in the field, which is important for conducting right control methods to reduce yield loss. Moreover, I wish to develop a new disease resistant variety in the future.

It was my pleasure to look around in IRRI to gain knowledge. I've got a lot of helpful information to make my research more interesting and profound.