

## 行政院及所屬各機關出國報告

(出國類別：其他)

### 赴日本筑波參加「耐逆境糧食作物先進育種國際 研討會」報告

服務機關	職稱	姓名
行政院農業委員會科技處	技正	蔡偉皇
出國地點	日本	
出國期間	105年11月23日至105年11月26日	
報告日期	106年2月24日	

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## 行政院及所屬各機關出國報告提要

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報告名稱：赴日本筑波參加「耐逆境糧食作物先進育種國際研討會」報告

主辦機關：行政院農業委員會

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如封面所述相關單位及人員

出國類別：考察進修研究實習其他

出國期間：105 年 11 月 23 日起至 11 月 26 日

出國地點：日本筑波

報告日期：106 年 2 月 24 日

主題分類：農業

施政分類：農業科技

關鍵詞：耐逆境、糧食作物、育種

內容摘要：

本次蔡偉皇技正參加亞太糧食肥料技術中心(Food and Fertilizer Technology Center for the Asian and Pacific Region, FFTC)與日本國際農林水產研究中心(Japan International Research Center for Agricultural Sciences, JIRCAS)及日本農研機構(National Agricultural Research Organisation, NARO)，於 105 年 11 月 24 日與 25 日於日本筑波共同舉辦之「耐逆境糧食作物先進育種」國際研討會，會議邀請各國專家與演講及國家代表發表國情報告，期藉由本次會議達成下列目的，包括：一、提供作物抗逆境育種關鍵研究人員聚集討論場合；二、分享目前運用前瞻育種技術所得成果與知識；三、形成一個信息共享和協同合作的非正式研究網絡，除運用先進育種方法提升氣候變遷對糧食作物的抵禦能力，並能確保區域糧食安全。

本文電子檔已上傳至出國報告資訊網

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## 第一章 考察緣起及目的

氣候變遷預期將嚴重威脅農業生產力，進而使得糧食與營養安全受到挑戰。生活於亞洲地區之熱帶與亞熱帶靠雨水灌溉來種植稻作的區域約有 7 億人，估計因為氣候變遷所產生之高溫、乾旱、鹽害、洪水，以及病蟲害相的改變，將影響此區域之 30% 人口，雖然作物對於氣候變遷的抗性或耐受性除作物本身外，亦取決於作物生長之農業環境，隨著影響日趨嚴重，育種家選育能適應氣候變遷之作物，以承受非生物和生物逆境，是目前最經濟和持久因應氣候變遷的方式，並進而有助於提高作物生產力。

此外，近年來許多前瞻技術被應用於作物育種研究，例如透過分子標記輔助育種(marker-assisted selection, MAS)使作物育種進入新的領域，可將野生或傳統品種中具有抗性之性狀引入目標品種，以增強遺傳彈性應力和適應性。此外，運用高通量定序與表型平台(例如 genotype-by-sequencing)技術日漸成熟，已可提供大量數據資料，分析作物潛在抗性基因，利用這些先進的育種方法能更有效率解決複雜性狀。

因此，亞太糧食肥料技術中心(Food and Fertilizer Technology Center for the Asian and Pacific Region, FFTC)規劃與日本國際農林水產研究中心(Japan International Research Center for Agricultural Sciences, JIRCAS)及日本農研機構(National Agricultural Research Organisation, NARO)合作，於 105 年 11 月 24 日與 25 日於日本筑波辦理「耐逆境糧食作物先進育種」國際研討會，邀請各國專家與演講及國家代表發表國情報告，期藉由本次研討會達成下列目的，包括：一、提供作物抗逆境育種關鍵研究人員聚集討論場合；二、分享目前運用前瞻育種技術所得成果與知識；三、形成一個信息共享和協同合作的非正式研究網絡，除運用先進育種方法提升氣候變遷對糧食作物的抵禦能力，並能確保區域糧食安全。

另本會科技處蔡偉皇技正奉派擔任本次研討會觀察員，除參與研討會外，為加強後續臺日農業合作與交流，並於會議期間與日本農研機構同仁，研商雙方進行農業科技合作事宜。

## 第二章 活動行程與工作內容

一、時間：105 年 11 月 23 日至 11 月 26 日

二、地點：日本筑波

三、主辦單位：

(一) 亞太糧食肥料技術中心(FFTC)

(二) 日本國際農林水產研究中心(JIRCAS)

(三) 日本農業食品產業技術綜合研究機構(NARO)

四、本次研習及參訪目的：

(一) 參加「參加耐逆境糧食作物先進育種國際研討會」擔任觀察員

(二) 與 NARO 洽談國際農業科技合作事宜

五、活動行程如下表：

日期	行程	內容
11/23(三)	臺北出發至日本東京	13:30 抵達東京成田機場
	抵達筑波國際會議中心	自成田機場轉乘接駁巴士至筑波
11/24(四)	研討會 (會議資料如附件)	1. 報到 Registration 2. 開幕式 Opening Session 3. 專題演講 4. Session 1：非生物因子逆境— 鹽害與乾旱 5. Session 2：非生物因子逆境— 收穫前發芽(穗上發芽)
11/25(五)	研討會  研商本會與 NARO 農業 科技合作事宜	1. Session 3：非生物因子逆境— 高溫 2. Session 4：生物因子逆境(抗病 蟲害) 3. Session 5： Genotyping-by-Sequencing 4. Session 6：總結討論 5. 閉幕式  與 NARO 本部企劃調整部國際室 室長永井卓博士交換意見
11/26(六)	日本東京回程至臺北	12:40 抵達臺北桃園機場

## 六、會議專題報告紀要：

### (一) 專題演講：

#### 基因體輔助的基因探索並導入水稻育種

Genomics-Assisted Gene Discovery and Its Integration into Rice Breeding.)

了解核苷酸和表型變化之間的關聯性是分子遺傳學和水稻育種的一大挑戰，NARO 的作物研究所為了此一目標，進行了水稻天然表型變異的遺傳分離，到目前為止，已經確定了一些複雜性狀相關基因，包括抽穗時間，抗稻熱病和根系結構等。這些發現使研究人員能夠運用數量性狀基因座(Quantitative trait loci, QTL)開發出具有所需等位基因的新品種和品系，例如確定抗稻熱病 QTL, *pi21* 染色體中的基因位置，再透過分子標誌輔助技術(marker assisted selection, MAS)與其他品種的遺傳背景中的多個基因(QTL)相結合，進一步增強導入的抗性。另於根系架構部分，在水稻中所分離出數量性狀基因座 *DEEPER ROOTING 1(DROI)*，用來控制根生長角度，並獲得證明，*DROI* 參與細胞延伸，主要影響根系生長角，但其他根和芽性狀沒有顯著差異。使用這些 QTL 等位基因調整根系統結構將提高耐乾旱和改善其他如營養攝入和抗倒伏性的性狀。

### (二) 主題 1-非生物因子逆境—鹽害與乾旱

#### 1. 耐鹽基因 *Ncl* 的 Map-Base Cloning 及其在大豆耐鹽性方面的應用

Map-Based Cloning of a Salt Tolerance Gene *Ncl* and Its Utilization for Improvement of Salt Tolerance in Soybean.

大約有 7%的土地和 20%的灌溉面積受到鹽害影響，鹽漬土顯著降低土地價值和生產力。大豆(*Glycine max* (L.) Merr) 通常被認為是鹽敏感作物，耐鹽性的遺傳改良對鹽鹼地區可持續大豆生產至關重要。研究團隊鑑定了幾個具有高耐鹽性的大豆基因型，並將 QTL 分析衍生自這些耐受品種和敏感品種之間的四個映射群體，顯示大豆染色體 3 側面有 SSR 標記

Sat\_091, Satt255, Satt339 和 Satt237 的主要耐鹽 QTL，並使用圖位克隆策略，分離出了耐鹽性 QTL 的基因(Ncl)。為了確定 Ncl 在鹽脅迫田條件下的影響，研究團隊在三年內評估了鹽脅迫土壤中近等基因系的 Ncl。田間實驗表明，Ncl 可以在鹽田條件下將大豆籽粒產量提高 3.6-5.5 倍，並顯示通過基因轉移或 MAS 在大豆育種中使用 Ncl 將有助於易鹽地區的持續大豆生產。

## 2. 臺灣使用標記輔助育種發展水稻耐旱性

### Development of Rice Drought Tolerance Using Marker-assisted Breeding in Taiwan

臺灣節水稻種可以分為乾旱耐旱和抗旱兩種方式。為了提高水稻的產量和質量，在繁殖生長階段極大地需要在製備階段將有限的水用於早期分蘖期。通過將已知的耐旱 QTL 引入主栽品種，可以提高水稻營養期耐旱性。目前，透過 IRRI 遺傳資源交換，將耐旱 QTL 的遺傳物質種植在低地田間的耐旱篩選圖中，以監測其在乾旱條件下的有利生長。這些耐旱 QTL 通過分子標記輔助回交育種引入豎有品種，以減少水分供應而不犧牲產量和質量。基於產量穩定和水稻生產質量托，運用雨季調整移栽期是避免乾旱的水稻育種調適策略，用以減少灌溉需求，另開發早熟品種將減少旱季的水資源消耗，使用開發的分子標記和克隆基因控制水稻粒徑、每歲顆粒數或抽穗日期來調整生長期，提高水稻的水源和源的能力，以保持產量和質量。運用適當有效的育種策略，結合先進技術，開發新的水稻品種適應乾旱脅迫，增強作物對氣候變化的抵抗力，確保水稻生產穩定。

## 3. QTL 對水稻土壤水分波動脅迫響應的根系可塑性分析

### QTL Analyses of Root Plasticity in Response to Soil Moisture Fluctuation Stress in Rice

靠雨水灌溉的低地(雨育低地)，稻田通常暴露於土壤水分波動(soil moisture fluctuation, SMF)壓力的影響下，乾旱與突發性淹水會交替發生。因此，在旱澇交替發生對根系發育和

功能會產生重大影響。為了找出根系發育與旱澇交替發生的相關性，以側根發育的可塑性作為條件，並將發芽能力作為重要指標。研究團隊使用 Nipponbare 和 Kasalath 雜交的染色體片段替代品系(CSSLs)，其側根發育可塑性相關的 QTL 已被鑑定並證實為作物適應 SMF 的重要性狀。這些根系篩選系統將用於評估攜帶特異性或組合的根系可塑性 QTL 以選育出因應不同 SMF 的水稻品系，系統如完成，即可以定義出實際的育種目標，並從而產生一種新的具有最適合特定表徵的兩養低地稻田的根系可塑性特徵的高產稻。

#### 4. 信息轉導和耐旱作物

##### Signal Transduction and Drought Stress Tolerance in Crops

環境壓力是全球作物生產的主要限制因素，約 70% 的產量損失是由諸如乾旱，淹水，土壤鹽漬化，土壤酸化以及過熱和過冷的非生物脅迫引起的。離層酸(ABA)具有調節植物抗旱性和耐旱性作用，並協調調節與降低水分利用度以及多種發育過程如種子休眠相關的複雜信號通路。在此基礎上，研究團隊重點投入 ABA 信號在乾旱逆境中的作用，以改善作物的耐旱性近年來，在理解 ABA 介導的轉錄調節反應乾旱脅迫信號成分的分機和作用方面已取得了重大進展。

#### 5. 轉基因方法提高水稻耐旱性

##### Transgenic Approaches to Improve the Drought Resistance of Rice

轉基因方法提供擴增基因庫超過常規育種系統可用的手段，提高耐旱性是包括水稻在內的農作物育種的重要目標。耐旱性可分為避旱和耐旱兩個主要機制。避旱在此定義為植物在輕度或中度乾旱脅迫條件下維持基本正常生理過程的能力，以及耐旱性作為植物在嚴重干旱脅迫條件下維持一定水平的生理活動的能力。通過轉基因方法產生耐旱性更好的水稻，需要三個步驟，即：(1)候選基因的發現；(2)水稻品種轉化；和(3)轉基因品系的評估。JIRCAS 正在與國際熱帶農業中心(CIAT)和國際稻米研究所(IRRI)合作，透過轉基因方法開



發具有改善耐旱性的水稻。

## 6. 使用嵌合抑制子產量身定做的多重壓力耐受植物

### Production of Tailor-Made Multi-Stress Tolerant Plants Using the Chimeric Repressor

在自然界中，所有的植物都受到環境引起的多重壓力，但大多數情況是複雜的。離層酸(ABA)應用於增強對乾旱脅迫的耐受性，但與抗病性相關的激素拮抗，如水楊酸，茉莉酸和乙烯。這表明 ABA 活性增加以提高耐旱性可能會消除耐藥性。研究團隊開發了使用“嵌合抑制子”命名為嵌合抑制子基因沉默技術(CRES-T)的新型基因沉默系統。使用這種 CRES-T，發現植物含有大量負調節劑，抑制了各種應力的耐受性。而且我們已經通過使用嵌合阻遏物來抑制這些陰性調節劑，成功地賦予了各種壓力的容忍。因此，通過使用嵌合阻遏物，賦予涉及拮抗作用的多重脅迫有幾種可能性。

### (三) 主題 2-非生物因子逆境—收穫前發芽(穗上發芽)

#### 1. 鑑定水稻的收穫前和種子休眠的遺傳和分子特徵

##### Towards Identification of Genetic and Molecular Characteristics for Pre-harvest Sprouting and Seed Dormancy in Rice

稻米收穫前發芽是收穫前生理成熟的萌發，其導致產量損失和籽粒質量差。在臺灣，稻米種植有兩季，由於雨季開始於第一季結束，第一季播種期通常出現收穫前發芽問題。強壯種子休眠育種是防止水稻早熟發芽的有效手段之一。然而，如果在第一季作物中收穫的種子被立即使用，則會導致不一致的種子發芽。研究團隊對收穫前發芽和種子休眠特徵的 182 個水稻品種進行了調查，發現種子休眠與收穫前發芽有很高的相關性。但也有例外。發現具有強烈抵抗萌芽前的四個品種具有弱的種子休眠(Chen *et al*，1980 年)。後者提出開發種子休眠較差的新水稻品種，但對收穫前發芽強烈抗性的可能性。

本研究項目的目標是確定控制對收穫前發芽的抗性的

QTL，並希望確定獨立控制收穫前發芽和種子休眠的分子機制。研究團隊計畫對過去八十年對台灣發展的一批水稻品種進行調查。對於預收穫發芽和種子休眠的表型，並開發一種新的生理指標，除了典型的萌發率測量之外，還包括  $\alpha$ -澱粉酶的活性。期望收集 RADseq 技術製作的表型數據和基因型數據，用於全基因組關聯研究。此外，我們還預計會發現種子休眠較差的品種和對收穫前發芽的強烈抗性。這些品種將用於開發未來父母本交叉遺傳群體進行 QTL 定位分析。

## 2. 遺傳與分子解剖前期收穫發芽抗性的水稻

### Genetic and Molecular Dissection of Pre-Harvest Sprouting Resistance of Rice

抗收穫前發芽(PHS)是水稻重要的農藝性狀，因為小麥籽粒大大降低了穀物質量。種子休眠的水平是抗性變化的重要組成部分。為了闡明分子機制，研究團隊一直在進行控制種子休眠的數量性狀基因座(QTL)的研究，研究團隊已使用 indica 和 japonica 間的天然等位基因變異產生了 Sdr1(Seed Dormancy 1)，Sdr4 和 Sdr7，並定義了候選區域，與確定了每個基因的互補方法。Sdr1 是 MAP-激酶級聯的頂部，MAPKKK，Sdr4 是未知的蛋白質基因，Sdr7 是擬南芥延遲發芽 1(DOG1)的同源基因。研究團隊推測，Sdr7 / Sdr4 的休眠途徑，誘導性根據成熟後被消除，可能在休眠狀態下抑制 Sdr1 的表達，並在成熟後釋放 Sdr1 的表達，達到發芽。

#### (四) 主題 3-非生物因子逆境—高溫

### 1. 運用來自水稻的清晨開花特徵的QTLs減輕水稻開花期間的熱誘導不稔

#### QTLs for the Early-Morning Flowering Character Derived from *Oryza officinalis* to Mitigate Heat-Induced Spikelet Sterility at Flowering in Rice, *O. sativa*

由於高溫引起的水稻(*Oryza sativa* L.)產量下降是未來氣候變化最大的隱憂之一。水稻最容易因開花時的高溫引起不

稔。早晨開花(EMF)性狀可透過在白天避免開花期的高溫誘導不稔。研究團隊試圖運用來自野生水稻(*O. officinalis*) (CC 基因組)的 EMF 基因座，在 indica 型遺傳背景下開發 EMF 的近等基因係(NILs)。研究顯示於 EMF 的 NILs 在升高的溫度條件下緩解高溫誘導的不稔，在於達到 35°C 之前完成開花，這是導緻不稔的一般閾值。在熱帶和亞熱帶受歡迎的品種的 FOT (flower opening time) 量化並沒有揭示任何所測試的品種的 EMF 性狀，這表明 qEMF3 有可能提高目前流行的品種的 FOT，以在未來較熱的氣候下開花時避免高溫逆境。這是運用野生稻作為遺傳資源的標記輔助育種來檢測具有 EMF 性狀的水稻的第一份報告

## 2. 研發在成熟期高溫條件下具有高品質稻穀之水稻新品種

Development of New Rice Varieties Producing Grains with High Appearance Quality under High Temperature Conditions during the Ripening Period

水稻成熟期如遭遇高溫環境，將導致白堊質豐度的增加，影響稻穀外觀與品質。白堊質顆粒被分類為基底白色核，白背仁，白莓仁，乳白色核或白核仁。特別是白背仁和白底仁在高溫條件下容易誘導產生。在日本稻米市場，糙米的價格是以完美穀物的百分比為基礎的質量等級決定的。因此，避免生產白堊是日本最重要的育種目標之一。Brown rice 1 (Apq1) 是影響水稻白堊質重要基因，研究團隊已經從越光米中選育出 Apq1-NIL (near isogenic line)，將與一些具有抗稻熱病基因(Pi-NILs)和半矮化基因(sd1-NIL)的越光米 NIL 進行雜交，並運用分子標記輔助育種。

## 3. 高溫對發育中穀物基因轉錄和組織代謝的影響

Impact of High Temperature on the Transcriptome and Metabolome in Developing Grains

在日本，預計下一個世紀稻米成熟季節的溫度上升約 3°C，暴露於 26°C 以上的高溫常會使穀物產生白堊質，並導致產品商業價值的損失。由於白堊外觀源於澱粉顆粒中保留

微空氣空間的胚乳組織中的不規則光反射，所以認為由於種子儲存材料如澱粉和蛋白質的不完全積累而導致穀物白垩。一直以來，許多研究指出，在高溫下成熟穀物的“下沉”能力下降。研究團隊透過使用生物電子實驗系統分析了高溫對澱粉合成和代謝相關基因表達和代謝產物的影響以及其他抗熱應激的防禦反應，並且顯示了澱粉生物合成的表達酶和澱粉代謝酶的誘導)引發澱粉數量和質量的損失，總結數據，提出討論了導致澱粉不足的可能因素，另研究團隊亦介紹了最近緩解高溫損壞的方法。

#### 4. 運用分子標記輔助育種於產生具氣候適應力果樹

##### Marker-Assisted Breeding Applied for Generation of Climate Resilient Fruit Trees in Japan

根據 NARO 的一項調查顯示，全球變暖已經在日本發展，影響了日本的園藝產業。日本在過去 100 多年中，溫度升高了 1.1°C，高於全球平均水平。對果樹的重要影響是水果皮膚的劣質和延遲著色，果肉軟化和紊亂，極端高溫，萌芽和開花障礙導致果皮曬傷，並增加病蟲害發生率，對這種影響最有效的方法是通過標記輔助育種(MAB)來發展具有抗逆性的水果樹品種。研究團隊介紹了 MAB 試驗的三個主題，即耐痂和自相容的日本梨，鍊格孢病抗性蘋果和日本梨，以及葡萄漿中花青素的數量和組成。梨痂病(由 *Venturia nashicola* 引起)是最有害的真菌性疾病，全球變暖導致這種真菌病的損害增加。

#### (五) 主題 4-生物因子逆境(抗病蟲害)

##### 1. 使用標記輔助育種方法改善馬來西亞水稻品種對抗稻熱病

##### Improvement of Malaysian Rice Variety against Blast Disease using Marker-Assisted Breeding Approach

由 *Pyricularia oryzae* 引起的稻熱病是導致大米產量損失的主要生物脅迫之一。迄今為止，已在水稻中鑑定了 100 多種抗病基因。以前的研究顯示，Pi9 基因對馬來西亞稻熱病分

離株具有廣效抗性。Pi9 基因顯示出優勢的抗病性，並成功導入到第6號染色體上。研究團隊透過使用標記輔助育種(MAB)技術，將抗性品種 IRTP21683 的 Pi9 基因滲入馬來西亞高產易感品種 MR219。研究顯示，分子標記輔助育種方法提供了一種將 Pi9 基因引入親本商業水稻品種的有效方法。

## 2. 提高泰國東北部兩育低地水稻抗生物逆境之分子育種， Molecular Breeding to Improve Biotic Stress Resistance for Rainfed Lowland Rice in Northeast Thailand

泰國東北是兩育低地稻米面積最大的地區(約 600 萬公頃)，約佔全國稻米種植面積的一半以上。約 80%的地區種植品種以 KDML105 和 RD6 為主，該地區主要生物逆境是稻熱病與和褐飛蟲，由於農民生活習慣的變化，遺傳多樣性的缺乏和氣候變遷的影響，成為這些地區的主要害蟲。為了保持該地區水稻生產的產量穩定性，研究團隊已經開發了幾種具有與 KDML105 和 RD6 相似的穀物質量標準的品種，成為生物抗藥性的直接來源，將對害蟲爆發危險地區的產量穩定性產生影響。

## 3. 水稻生長和生產的改善

### Improvement on Rice Growth and Production

研究團隊運用農業試驗所 (TARI)收集的疊氮化鈉( $\text{NaN}_3$ )誘變的水稻突變體，鑑定水稻的對應基因和構建基因堆積性狀。水稻白葉枯病，XF89b 為(*Xanthomonas oryzae* pv *oryzae*, *Xoo*)最具毒性的菌株。研究團隊已經完成了 XF89b 整個基因組的定序，鑑定了主要的致病因子的編碼基因(TAL)，並發現，*Pseudomonas taiwanensis* 是對 *Xoo* 有效生物防治劑。研究團隊運用 Tn5 轉座子突變，確定了許多對抗 *Xoo* 活性至關重要的基因，包括參與 pyoverdine 生物合成的基因。並發現 6 種分泌系統涉及 pyoverdine 分泌。至於 pyoverdine 對生物防治劑對抗 *Xoo* 的可能用途目前正在評估中。

## (六) 主題 5-Genotyping-by-Sequencing

## 1. 應用基因分型測序發現水稻和瓜分子育種的SNP

### Application of Genotyping-by-Sequencing to Discover SNPs for Rice and Melon Molecular Breeding

DNA 測序技術的進步使作物育種領域發生了革命性變化，研究團隊分析成千上萬種自然變異，並對包括稻米和瓜在內的任何物種以合理的成本對野生親緣物種，地方品種 F2，重組近交系(RILs)進行測繪。結合表型數據，作物遺傳學家可以使用高密度標記物研究農藝性狀的遺傳結構。我們正在應用經濟有效(Genotyping-by-Sequencing, GBS)平台來對水稻和瓜類中的不同種質和圖譜進行排序，產生了數萬個 single nucleotide polymorphism (SNP)標記應用於遺傳分析，並對序列材料進行了水稻和水果性狀對甜瓜非生物脅迫的耐受性評估。

篩選出不同的水稻種質和重組近交系(RILs)對萌發階段淹水的反應。粳稻與 *Indica* 亞群間差異性厭氧反應。從我們的全基因組關聯研究(GWAS)鑑定的許多 SNP 與厭氧發芽相關，在以前的雙親 QTL 研究中也檢測到一些基因組區域。僅在我們的 RIL 群體中才發現了一個獨特而強大的信號，解釋了約 30%的表型變異。我們的研究表明，連鎖作圖和 GWAS 對於解剖水稻耐低氧脅迫的遺傳結構是互補的。我們預計這些結果可能有利於水稻直播種植的品種發展。

雖然 GBS 能夠有效和低成本地生成大量的標記，但是所得到的基因型的效用是有限的，因為它們是非常容易出錯的並且包含高比例的缺失數據。研究團隊生成了 109 個 SNP 標記使用 GBS 方法的瓜的 RIL，並根據它們在雙單倍體線 DHL92 基因組的草案上的物理位置進行排序。通過進行嚴格的質量控制和過濾和插補，使用平均失蹤數據為 0.281%的 22,933 個位點構建遺傳圖譜，跨 12 個染色體跨越 1,088.3cM，最大間距為 6.0cM。使用這種高質量的連鎖圖譜，可以確定已知可以控制水果性狀的幾個數量性狀位點(QTL)，並驗證了我們的方法。本研究強調了 GBS 標記在瓜中鑑定性狀相關

QTL 的作用，並有助於進一步研究基因組結構。

## 2. 基因組和表型組分析應用於水稻改良

### Genomes and Phenomes for Rice Improvement

據估計，世界各地的基因庫中保護了大約 78 萬份水稻，其中包括未經開發的遺傳多樣性來源，用於改良作物。目前在基因組水平上表徵這種多樣性的努力來自 3,000 個水稻基因組計畫中 5,000 多個品種和地方品種的序列。然而，遺傳變異的這個“數位字典”不能直接用於作物改良，除非排序的遺傳種群(GS)的特徵是在一系列環境，時間和發育階段的特徵，在這些環境，發育階段他們遭受不同的壓力和管理從分子到整個植物水平採樣。一旦這樣的「組學」數據可用，關聯和機器學習的分析將能夠鑑定參與性狀表達的基因和/或基因組區域，將基因型橋接到表型分裂。然後，通過優化分子育種策略，將這些知識整合到水稻改良育種計畫中。

表型組分析的關鍵是在受控和現場環境中採用高通量 (HTP) 表型組，這些方法可以快速表徵產量組分和其他性狀，這些特徵可以與更麻煩的手動測量性狀相關聯。例如，研究人員檢測到生物量和產量組分性狀相同的 QTL / 峰，與 HTP 傳感器/圖像衍生參數和手動測量性狀之間具有顯著相關性。

此外，由序列 GS 產生的新型種群(如 RILs, NAMs, MAGIC 和 BIL)的建設創造了對遺傳學和育種前期有用的快速反應遺傳資源(RR-GR)。國際稻米研究中心正與 Cirad, Ciat 和其他合作夥伴正在開發一個“Rice Array”，其中將在許多地點/環境中篩選多樣性面板，RR-GR，育種線和其他材料以檢測 G x E x M 效應。研究團隊規劃這些數據的核心是 SNP-seek 數據庫 <http://snp-seek.irri.org>，與國際稻米信息學聯盟(IRIC, <http://iric.irri.org>)一起為全球社區開發。將努力建立一個可擴展的平台，提高基因庫的價值，將新基因及其來源基因型轉移到育種計畫中。

## 3. 印尼沼澤地稻米育種方法

### Advance Breeding Methods for Indonesian Swampland Rice

印尼沼澤地景觀分為三個區域(沿海，潮汐和低地沼澤地)，具有典型的特徵，不同沼澤地區面臨水稻種植的挑戰。研究團隊應用了各種分子標記來推動印尼水稻育種計劃，這些標記用於評估遺傳多樣性和品種鑑定，性狀標記和標記輔助選擇，標題日期和產量組分的檢測試劑盒，以及進食質量，以及基於 DNA 指紋的品種鑑定/種子純度評估，利用基因組輔助育種方法，使用全基因組關聯研究(GWAS)鑑定與鐵毒性耐受性相關的許多標誌物。



### 第三章 考察心得與結論建議

本次研討會舉辦期間日本東京地區降下 54 年來 11 月的初雪，另本年初北半球的霸王級寒流，影響東亞的中國大陸、香港、澳門、臺灣、日本，還有北美的加拿大、美國等地，臺灣因霸王級寒流造成過往少有下列下雪紀錄的地區降雪，發生嚴重寒害與農損，均顯示氣候變遷造成之極端氣候已逐漸影響著我們的生活，農作物栽培更是面臨嚴峻挑戰，因此，耐逆境作物育種工作刻不容緩。本研討會邀請各國育種專家分享其耐逆境研發成果，希望提供資訊交流平臺，並進一步促進科研交流合作，所獲心得與建議簡述如下：

#### 一、導入前瞻技術提高育種效率刻不容緩

基因體科技為近代生物科技的重要突破，隨著次世代基因體定序(next-generation sequencing, NGS)、分子標誌輔助育種選拔(marker-assisted selection, MAS) 與簡化基因體定序技術(genotype-by-sequencing, GBS) 等技術日益成熟、效率顯著提高且成本降低。基因體科技之應用範圍日益廣泛，不再只是決定基因序列而已，同時也包含基因的表現研究及蛋白質的功能分析等，在農業上的運用對象，包括農作物、畜產品、水產品與微生物等，具體應用方式則包含品種鑑定、品種改良、標示認證、病原檢測、疫苗開發等，特別是對農糧作物育種未來發展具有關鍵性的助益。

另評估選育品種於環境中之性狀表現，為品種選拔重要一環，為提升表型(phenotype)評估效率，國外大型種子種苗公司業導入高通量植物基因型-表型之育種模式(High Throughput Plant Genotyping- Phenotyping Breeding)並積極建構設施，以提升其育種速率，本次會議受邀之國際稻米研究中心(International Rice Research Institute, IRRI)專家，亦分享前開技術於該中心水稻育種工作之現況及成果。本會農業試驗所於 106 年度旗艦計畫「臺灣重要農作物核心種原基因體資源開發及快速育種平臺之建置」，亦著手導入建立我國核心種原育種平臺，協助我國種子種苗產業發展。

#### 二、加強國際農業科技交流與合作

為因應氣候變遷選育抗逆境品種，農委會近年來業積極與國

際研究組織合作，包括：國際稻米研究中心、亞蔬中心(AVRDC)、國際玉米與小麥改良中心(CIMMYT)，藉由技術交流合作進行種源交換，導入國際組織育出品種之優良特性，提升國內品種選育速度。另亦規劃與國際研究機構合作，藉由其國外分支機構進行海外品種測試，亦可協助我國種苗產業海外布局。本次會議除提供農業研究人員聚集討論場合，分享目前技術開發所得成果與知識，更開展原有與國際組織合作模式外，新型態之農業科技國際交流與合作方式。

本次會議主辦單位之一 NARO 是日本農業和食品研究和開發的核心研究所，其轄下共有 20 個機構/中心，該機構對雙方農業科技交流合作表達興趣，爰本次考察目的除參與研討會外，並與日本農研機構研商與臺灣農業研究機構之合作方式，期進一步促成臺日雙方研究機構實質合作。

#### 第四章 附圖



圖 1、研討會於日本國際農林水產研究中心舉行。



圖 2、研討會綜合討論情形。



圖 3、NARO 本部企劃調整部國際室室長永井卓博士。

# Advanced Breeding Methods for Developing Stress-tolerant Food Crops



## Program and Abstracts

24–25 November 2016

Headquarters of the Japan International Research Center for  
Agricultural Sciences, Tsukuba, Ibaraki, Japan





## Background and Rationale

Global climate change is predicted to impose a severe threat to agricultural productivity worldwide, and thereby challenge food security and nutritional security. Heat, drought, salinity, flooding, and changed status of diseases and pests impacted by climate change affect about 30% of the 700 million poor in tropical and sub-tropical Asia alone who live in rainfed rice-growing areas. Although, the impact of climate change on crop's resistance and/or tolerance is difficult to predict and is likely to be variable depending on the crop and its agro-environment, the situation has compelled plant scientists to develop climate change-resilient crops, which can withstand broad-spectrum abiotic and biotic stresses and is the most economic and durable way to adapt to climate change, thus helping to deliver increased crop productivity.

Recently, marker-assisted selection methods have unlocked a new realm in the breeding of genes conferring the desired trait into crop varieties, often with traits introduced from wild relatives or traditional varieties to enhance genetic resilience to stress and adaptability in food crop plants. And with advent of cost-effective high-throughput sequencing and phenotyping platforms (i.e., genotype-by-sequencing), it has been possible to generate data-sets and models for deciphering the stress responsiveness of crop species with adaptation traits or in wild relatives toward identifying underlying genes, alleles or quantitative trait loci for an efficient utilization in crop improvement. Consequently, taking some findings from genetic engineering (GE) research, these advanced breeding methods are more efficient to tackle complex traits, have fewer safety concerns compared to GE crops, and are more acceptable to the consumers.

In view of the aforementioned, the said seminar is for exchanging research information on the progress and prospects of advanced breeding methods for improving climate change resilience in food crops, which is likely to play an ever increasing role in the effort to reduce the impact of climate change on future cropping scenarios and ensure regional food security.

### **Objectives:**

- bring together key researchers on crop breeding for stress tolerance
- share the current state of knowledge of advanced crop breeding methods
- establish an informal network committed to information sharing and collaborative research





## PROGRAM

**November 24, 2016 (THURSDAY)**

09:00-10:00 **Registration**

10:00-10:10 **Opening Session**  
Emcee: Dr. Antonio Baltazar, NARO

**Welcome Remarks**  
Dr. Masa Iwanaga  
President, JIRCAS

**Opening Remarks**  
Dr. Akio Takenaka  
Deputy Director, FFTC

10:10-11:00 **Keynote Speech**  
**Genomics-Assisted Gene Discovery and Its Integration into Rice Breeding**  
Dr. Masahiro Yano, Institute of Crop Science, NARO , Japan

**Session 1: Abiotic Stress: Salt and Drought**  
Chairperson: Dr. Kazuo Nakashima, JIRCAS, Japan

11:00-11:30 **Map-Based Cloning of a Salt Tolerance Gene *Ncl* and Its Utilization for Improvement of Salt Tolerance in Soybean**  
Dr. Donghe Xu, JIRCAS, Japan

11:30-12:00 **Development of Rice Drought Tolerance Using Marker-assisted Breeding in Taiwan**  
Dr. Rong-Kuen Chen, Tainan District Agricultural Research and Extension Station, Taiwan

12:00-13:30 ---- Lunch Break and Group Photo ----



- 13:30-14:00 **QTL Analyses of Root Plasticity in Response to Soil Moisture Fluctuation Stress in Rice**  
Dr. Roel R. Suralta, Philippine Rice Research Institute, Philippines
- 14:00-14:30 **Signal Transduction and Drought Stress Tolerance in Crops**  
Dr. Yasunari Fujita, JIRCAS, Japan
- 14:30-15:00 **Transgenic Approaches to Improve the Drought Resistance of Rice**  
Dr. Takuma Ishizaki, JIRCAS, Japan
- 15:00-15:30 **Production of Tailor-Made Multi-Stress Tolerant Plants Using the Chimeric Repressor**  
Dr. Masaru Ohme-Takagi, Saitama University, Japan
- 15:30-16:00 ---- Coffee Break ----
- Session 2: Abiotic Stress: Pre-Harvest Sprouting**  
Chairperson: Dr. Takeshi Urao, JIRCAS
- 16:00-16:30 **Towards Identification of Genetic and Molecular Characteristics for Pre-harvest Sprouting and Seed Dormancy in Rice**  
Dr. Kai-Yi Chen, National Taiwan University, Taiwan
- 16:30-17:00 **Genetic and Molecular Dissection of Pre-Harvest Sprouting Resistance of Rice**  
Dr. Kazuhiko Sugimoto, NARO, Japan
- 18:30-20:30 Welcome Dinner at Restaurants ESPOIR, International Congress Center EPOCAL TSUKUBA

**November 25, 2016 (FRIDAY)**

- Session 3: Abiotic Stress: High Temperature**  
Chairperson: Dr. Hsu-Sheng Lur, National Taiwan Univ.



- 09:30-10:00 **QTLs for the Early-Morning Flowering Character Derived from *Oryza officinalis* to Mitigate Heat-Induced Spikelet Sterility at Flowering in Rice, *O. sativa***  
Dr. Hideyuki Hirabayashi, Institute of Crop Science, NARO, Japan
- 10:00-10:30 **Development of New Rice Varieties Producing Grains with High Appearance Quality under High Temperature Conditions during the Ripening Period**  
Dr. Kazumasa Murata, Toyama Prefectural Agricultural, Forestry and Fisheries Research Center, Japan
- 10:30-11:00 **Impact of High Temperature on the Transcriptome and Metabolome in Developing Grains**  
Dr. Hiromoto Yamakawa, Central Region Agricultural Research Center, NARO, Japan
- 11:00-11:30 **Marker-Assisted Breeding Applied for Generation of Climate Resilient Fruit Trees in Japan**  
Dr. Toshiya Yamamoto, Institute of Fruit Tree and Tea Science, NARO, Japan
- 11:30-13:00 ---- Lunch Break ----
- Session 4: Biotic Stress**  
Chairperson: Dr. Ikuo Ando, NARO
- 13:00-13:30 **Improvement of Malaysian Rice Variety against Blast Disease using Marker-Assisted Breeding Approach**  
Dr. Mohd Shahril Firdaus Ab Razak, Malaysian Agricultural Research and Development Institute, Malaysia
- 13:30-14:00 **Molecular Breeding to Improve Biotic Stress Resistance for Rainfed Lowland Rice in Northeast Thailand**  
Dr. Jirapong Jairin, Ubon Ratchathani Rice Research Center, Thailand
- 14:00-14:30 **Improvement on Rice Growth and Production**  
Dr. Kuo-Chen Yeh, Agricultural Biotechnology Research Center, Academia Sinica, Taiwan
- 14:30-15:00 ---- Coffee Break ----





**Session 5: Genotyping-by-Sequencing**

Chairperson: Dr. Takashi Matsumoto, NARO

15:00-15:30 **Application of Genotyping-by-Sequencing to Discover SNPs for Rice and Melon Molecular Breeding**

Dr. Chih-Wei Tung, National Taiwan University, Taiwan

15:30-16:00 **Genomes and Phenomes for Rice Improvement**

Dr. Kenneth McNally, International Rice Research Institute, Philippines

16:00-16:30 **Advance Breeding Methods for Indonesian Swampland Rice**

Dr. Dwinita Wikan Utami, Indonesian Center for Agricultural Biotechnology and Genomic Resources Research and Development, Indonesia

**Session 6: Wrap-up Discussion**

16:30-17:30 Chairpersons: Kenneth McNally, IRRI, and Antonio Baltazar, NARO

17:30-17:35 **Closing Remarks**

Dr. Osamu Koyama

Vice-President, JIRCAS

# ABSTRACTS



## Genomics-Assisted Gene Discovery and Its Integration into Rice Breeding

Masahiro Yano, Toshio Yamamoto, Kiyosumi Hori, Yusaku Uga and Shuichi Fukuoka

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Elucidation of the association between nucleotide and phenotypic changes is a big challenge in molecular genetics and breeding of rice that has to be addressed inevitably. Toward this goal, we have been involved in the genetic dissection of natural phenotypic variations in rice and so far, we have identified several genes associated with complex traits including heading date, blast resistance and root architecture. These findings allowed us to develop new cultivars and lines with desirable allele at the QTL with major effect. However, we still have difficulty to identify minor effect QTLs, which can be more effectively used in the modern rice breeding. To enhance the power of genetic dissection of complex phenotypes, we have developed chromosome segment substitution lines using many genetically diverse accessions. These materials could be used for detection of QTLs with minor phenotypic effects, as well as major QTLs.

Quantitative trait loci (QTL)-mediated resistance contributes to durable resistance to blast, a devastating disease of rice (*Oryza sativa* L.) caused by the fungal pathogen *Magnaporthe oryzae*. We successfully identified the genes of diverse biological functions for this resistance. Determination of precise gene location in chromosome of the blast resistance QTL, *pi21*, allowed us to overcome linkage drag, co-introduction of undesirable agricultural traits from the donors. Furthermore, we demonstrated that combining multiple genes (QTLs) in genetic backgrounds of elite cultivars by MAS is an effective strategy to further enhance QTL-mediated resistance. We also focused on root system architecture to improve several traits with agricultural and economical values. Several QTLs for root length and root growth angle were identified by QTL analyses. We isolated quantitative trait locus *DEEPER ROOTING 1 (DRO1)*, which controls root growth angle, in rice. Based on the analysis of a near-isogenic line of *DRO1* in genetic background of IR64, it was clarified that *DRO1* is involved in cell elongation and that predominantly influences root growth angle, but shows no marked differences in other root and shoot traits. Identification of several other QTLs for root angle and length are under way by map-based strategy. Modulation of root system architecture using these QTL alleles will enhance improvement of drought avoidance and other traits, such as nutrition uptake and lodging resistance.



## Map-Based Cloning of a Salt Tolerance Gene *Ncl* and Its Utilization for Improvement of Salt Tolerance in Soybean

Do Tuyen Duc<sup>1</sup>, Huatao Chen<sup>1</sup>, Hien Thi Thu Vu<sup>1</sup>, Aladdin Hamwiah<sup>1</sup>, Tetsuya Yamada<sup>2</sup>, Tadashi Sato<sup>3</sup>, Yongliang Yan<sup>4</sup>, Hua Cong<sup>4</sup>, Mariko Shono<sup>1</sup>, Kazuhiro Suenaga<sup>1</sup> and Donghe Xu<sup>1</sup>

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Salt-affected soils significantly reduce land value and productivity. About 7% of the earth's land and 20% of the irrigated field is affected by salt stress. Soybean [*Glycine max* (L.) Merr.] is generally regarded as a salt sensitive crop compared with other crops such as wheat, rice, and cotton. Genetic improvement of salt tolerance is essential for sustainable soybean production in saline areas. Based on screening of a large number of soybean germplasm from different countries, we identified several soybean genotypes with high salt tolerance, such as the Brazilian soybean cultivar FT-Abyara, the Chinese soybean cultivar Jindou 6, and the Japanese wild soybean accession JWS156-1. Quantitative trait locus (QTL) analysis with four mapping populations derived from crosses between these tolerance accessions and sensitive cultivars revealed a major salt-tolerant QTL on soybean chromosome 3 flanked by SSR markers Sat\_091, Satt255, Satt339, and Satt237. The QTL accounted for 31.3–68.7% of the total variance for salt tolerance in the mapping populations. Using map-based cloning strategy, we isolated a gene (*Ncl*) underlying the salt tolerance QTL from the soybean cultivar FT-Abyara. *Ncl* could synchronously regulate the transport and accumulation of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>. Higher expression of the salt tolerance gene *Ncl* in the root resulted in lower accumulations of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> in the shoot under salt stress. Transfer of *Ncl* full-length cDNA driven by 35S promoter (*35S:Ncl*) with the *Agrobacterium*-mediated transformation method into a soybean cultivar Kariyutaka significantly enhanced its salt tolerance. Introgression of *Ncl* into a salt sensitive soybean cultivar Jackson, using DNA marker-assisted selection (MAS) and continues backcross, produced an improved salt tolerance line. To determine the effect of *Ncl* in salt stress field conditions, we evaluated the *Ncl* near isogenic lines in a salt-affected soil over three years. The field experiment showed that *Ncl* could increase soybean grain yield by 3.6–5.5 times in saline field conditions. Our study suggests that using *Ncl* in soybean breeding through gene transfer or MAS would contribute to sustainable soybean production in saline-prone areas.

## Development of Rice Drought Tolerance Using Marker-Assisted Breeding in Taiwan

Rong-Kuen Chen<sup>1</sup>, Sheng-Shan Wang<sup>2</sup>, Chi-Feng Lu<sup>1</sup> and Jen-You Jian<sup>3</sup>

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Global climate change has led to higher temperature and uneven rainfall worldwide, causing extreme changes in water resources and the ensuing droughts have become a universal problem. Crop farming is facing water shortage. Rice, the most important food crop in Taiwan, is cultivated mainly in the lowlands and requires a lot of water from irrigation. The irrigation facilities in Taiwan are well developed. With river water flowing into reservoirs, water stored during the rainy season is used for irrigation later on. However, if rainfall is limited or too few typhoons hit Taiwan, water shortage likely occurs in the following year. Thus, Taiwan should carry out rice breeding of water-saving varieties, in order to increase water use efficiency and improve yield stability.

Rice breeding for water-saving in Taiwan can be divided into two approaches: drought tolerance and drought avoidance. In order to improve the yield and quality of the rice, applying limited water in the preparation stage till the early tillering period is greatly needed during the reproductive growth stage. By introgressing known drought-tolerance QTLs into the main cultivars, the drought tolerance during vegetative phase of rice can be improved. Currently, through IRRI genetic resources exchange, the genetic materials with drought-tolerance QTLs were planted in drought-tolerance screening plots in the lowland field, so as to monitor their favorable growth under drought condition. These drought-tolerance QTLs were introgressed into cultivars by molecular marker-assisted backcross breeding, so as to reduce the supply of water without sacrificing yield and quality. Basing on stabilized yield and quality of rice production, the rice breeding strategy of drought avoidance is to adjust its transplantation period during the rainy season, in order to reduce the irrigation requirement. And developing early maturity cultivars will reduce the consumption of water resource during the dry season. The practice of using developed molecular markers and cloned genes with grain size, grains per spike and heading date is to adjust the growth period and enhance sink and source capacity of rice plant for maintaining the yield and quality.

In conclusion, through appropriate and effective breeding strategies, together with advanced technology, new rice varieties can be developed to adapt to drought stress and to enhance crop resistance to climate change, so as to ensure that rice production can be stabilized and sustainable management of the rice industry in the future.



## QTL Analyses of Root Plasticity in Response to Soil Moisture Fluctuation Stress in Rice

Roel R. Suralta<sup>1</sup>, Jonathan M. Niones<sup>1</sup>, Stella Owusu-Nketia<sup>2</sup>  
Yoshiaki Inukai<sup>2</sup> and Akira Yamauchi<sup>2</sup>

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Rainfed lowland rice fields are commonly exposed to soil moisture fluctuation (SMF) stress, which is the alternating recurrence of drought and sudden waterlogging conditions. Root responses to drought and waterlogging stresses are contrasting and thus, their alternating recurrences in the field significantly affect the root system development and functions. The inability of roots to adapt to SMF leads to negative effect on growth and yield of rice. Root plasticity, which is defined as the ability of a genotype to change its root phenotypes in response to changing soil moisture conditions is important for crop adaptation. Under the conditions prone to SMF stress, the plasticity in the development of the entire root system as a function of the plasticity in lateral root development is important under waterlogged-to-drought (W-D) conditions, while plasticity in lateral root development that is associated with aerenchyma development is important under transient drought-to-waterlogged (D-W) conditions. Using a population of chromosome segment substitution lines (CSSLs) from a cross between Nipponbare and Kasalath, the quantitative trait loci (QTLs) associated with the plasticity in lateral root development under W-D and aerenchyma development under D-W conditions have been identified and validated as key traits for crop adaptations to SMF. Furthermore, the QTLs associated with root plasticity expressions at different soil depths in response to SMF have been identified by using the double haploid lines (DHLs) from a cross between CT9993 and IR62266. Currently, several crosses have been made in order to separate the QTLs, do fine mapping analyses and identify specific gene(s) involved in the expression of each root plasticity trait and develop corresponding perfect markers for introgression of the root plasticity traits in marker assisted breeding. Since rainfed lowland rice environments are widely differed in their locations in the toposequence, soil depths, presence or absence of hardpan, etc., the target rice production fields should be properly characterized in order to design an ideal functional root systems suited for each environment. Examples of different screening systems for root plasticity that can simulate different rainfed lowland rice fields will be discussed. These root screening systems will be used to evaluate different promising rice lines carrying specific or combined root plasticity QTL(s) in response to SMF and validate their functional roles for increased soil water uptake and maintenance of greater dry matter production and yield. Once done, an actual breeding target can be defined and consequently, generate a new high yield rice with root plasticity traits best suited to a particular well-characterized rainfed lowland rice fields.

## Signal Transduction and Drought Stress Tolerance in Crops

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Environmental stresses are the major constraints to crop production worldwide. Indeed, about 70% of yield losses are caused by abiotic stresses such as drought, flooding, soil salinization, soil acidification, and excess heat and cold. These abiotic stresses directly or indirectly reduce crop yield and quality. Especially, in these years, global climate change has increased the frequency and severity of various abiotic stresses, particularly drought and excess heat, causing serious yield losses in major crops such as soybean, maize, and wheat. Drought is by far the most devastating abiotic stress affecting plant growth and productivity. More than half of the terrestrial region, including the vast majority of arable lands, is vulnerable to drought. The phytohormone abscisic acid (ABA) plays a central role in the regulation of response and resistance to drought stress in plants, and cooperatively regulates complicated signaling pathways implicated in the response to reduced water availability as well as in multiple developmental processes such as seed dormancy. Cellular dehydration, both in seeds and the post-germination growth stage, enhances endogenous ABA levels, which activate a large number of ABA-mediated dehydration-responsive genes to protect cellular activity and vitality from dehydration-induced damage and to regulate plant growth to ensure survival. Here we focus on the role of ABA signaling in drought stress, based on our findings obtained from basic to applied research to improve drought tolerance in crops. ABA binds to the Pyrabactin resistance1/PYR1-like/regulatory components of ABA receptor (PYR/PYL/RCAR) proteins functioning as ABA receptors to impede the activity of group A protein phosphatases 2C (PP2Cs), which are negative regulators of ABA signaling that inhibit subclass III Sucrose non-fermenting-1 related protein kinase 2 (SnRK2) protein kinases. In the presence of ABA, the subclass III SnRK2s (SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1, and SRK2I/SnRK2.3) released from PP2C-mediated negative regulation phosphorylate AREB/ABFs, which then activate the expression of many ABA-responsive genes in an ABRE-dependent manner. Such ABA-mediated gene expression is directly controlled by transcription factors (TFs) that bind to *cis*-acting elements in the promoter regions of their target genes. These TFs are regulated by ABA signaling components such as receptors, secondary messengers, protein kinases/phosphatases, and chromatin remodeling factors. In addition, other regulatory components, including protein degradation, mRNA maturation and stability, and miRNA targeting, modulate ABA-mediated transcriptional regulation. Substantial progress has been made in recent years in understanding the molecular mechanisms and roles of signaling components in ABA-mediated transcriptional regulation in response to drought stress. Furthermore, we will discuss perspectives and applications in crop protection to help facilitate the link between basic and applied studies.





## Transgenic Approaches to Improve the Drought Resistance of Rice

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Transgenic approaches provide the means of expanding the gene pool beyond what has been available to conventional breeding systems. Genetically modified crops, which are the products of transgenic approaches, can therefore act as powerful complements to the crops produced by conventional breeding systems. Improving drought resistance is a major goal of breeding of crops including rice. Drought resistance can be divided into two main mechanisms: drought avoidance and drought tolerance. Drought avoidance is defined here as the capability of plants to maintain fundamental normal physiological processes under mild or moderate drought stress conditions, and drought tolerance as the ability of plants to sustain a certain level of physiological activities under severe drought stress condition. To generate rice with improved drought resistance by transgenic approaches, three steps are required, namely: 1) discovery of candidate genes; 2) transformation of rice cultivars; and 3) evaluation of transgenic lines. The candidate genes for drought resistance that have been discovered by molecular biologists in the past years are mostly related to the mechanism of drought tolerance. There are reports demonstrating improved drought tolerance of rice by introduction of the candidate genes identified in other organisms, i.e. *Arabidopsis*, tomato, maize, barley, *Datura*, pea, *Xanthomonas* and *Agrobacterium*. These successful examples show the potential of transgenic approaches to improve drought tolerance of rice and to expand available gene pool. Most of the studies showing improved drought tolerance of rice by transgenic approaches used cultivars with high transformation efficiencies but are not commercially cultivated in the target regions. For the practical application, commercial rice cultivars that have adapted to the target regions have to be used as host cultivars. *Agrobacterium*-mediated transformation systems using immature embryos are effective to transform a number of commercial rice cultivars. The important concern in transgenic approaches is position effects: variation of expression patterns of transgenes caused by genomic integration sites. For the fair evaluation of the functions of transgenes, several numbers of lines for each transgene have to be generated and phenotyped, and then an elite line that shows the best performance has to be selected. The transgenic lines have to be evaluated not only by survival under drought, which is the most expected trait from the functions of the drought-tolerant genes, but also by other factors determining performance of the rice under drought. Evaluation under field condition is essential. JIRCAS is now collaborating with International Tropical Agriculture Center (CIAT) and International Rice Research Institute (IRRI) aiming to develop rice with improved drought resistance by transgenic approaches.

## **Production of Tailor-Made Multi-Stress Tolerant Plants Using the Chimeric Repressor**

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A large number of genetic factors have been identified that could confer tolerance to various environmental stresses. But most of them are tolerant to a single stress, not to multiple ones. In natural field, almost all plants suffer multiple stresses that are caused by environment. For example, heat stress is often associated with drought and salinity, and acid stress induces aluminum toxicity and phosphate starvation. In addition, flood, which also leads to diseases, and drought are major environmental stresses to plants; and they often occur alternatively from year to year. Thus environmental stress is not simple, but most cases it is complex. However, a few studies have been conducted to the mechanism and tolerance of multiple and complex stresses.

Abscisic acid (ABA) acts to enhance tolerance to drought stress but does antagonistically to hormones that are involved in disease resistance such as salicylic acid, jasmonic acid and ethylene. This suggests that increase of ABA activity for the enhancement of drought tolerance may dismiss disease tolerance. On this line of thoughts, we have developed novel gene silencing system using the “chimeric repressor” named Chimeric Repressor Gene-Silencing Technology (CRES-T). With this CRES-T, we found that plants contain large number of negative regulators that suppress tolerances to various stresses. And we have succeeded conferring tolerances to various stresses by suppressing such negative regulators using the chimeric repressor. Thus there are several possibilities for conferring to multi-stresses that involve antagonistic effects by using the chimeric repressors.



## Towards Identification of Genetic and Molecular Characteristics for Preharvest Sprouting and Seed Dormancy in Rice

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Preharvest sprouting in rice is germination of physiologically ripe kernel before harvest, which leads yield loss and poor grain quality. In Taiwan, there are two rice cropping seasons and the problem of preharvest sprouting usually occurs in the first cropping season because the rainy season begins about the end of the first cropping season. Breeding for strong seed dormancy is one of effective means to prevent preharvest sprouting in rice. Nevertheless, it can result in inconsistent seed germination if harvested seeds in the first cropping season are used immediately. A survey of 182 rice varieties for the characteristics of preharvest sprouting and seed dormancy revealed that there is a high correlation between seed dormancy and preharvest sprouting. But there are exceptions. Four varieties having strong resistance to preharvest sprouting are found to have weak seed dormancy (Chen et al. 1980). The latter raises the possibility to develop new rice varieties with weak seed dormancy but strong resistance to preharvest sprouting.

The objectives of this research project aim to identify QTLs controlling resistance to preharvest sprouting, and hopefully to identify molecular mechanisms independently controlling preharvest sprouting and seed dormancy. We plan to survey a panel of rice varieties that were developed in Taiwan in the past 80 years. For phenotyping preharvest sprouting and seed dormancy, we plan to develop a new physiological indicator, the activity of alpha-amylase, in addition to the typical measurement of germination rate. We expect to gather phenotypic data and genotypic data made by the RADseq technology for the genome-wide association study. Furthermore, we also expect to find few varieties with weak seed dormancy and strong resistance to preharvest sprouting. These varieties will be used to develop a bi-parental crossed genetic population for QTL mapping analyses in the future.

## Genetic and Molecular Dissection of Pre-Harvest Sprouting Resistance of Rice

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Utako Yamanouchi<sup>1</sup> and Masahiro Yano<sup>1</sup>

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Preharvest sprouting (PHS) resistance is an important agronomic trait of rice, since PHS largely reduces grain quality. The level of seed dormancy is an important component of variation in the resistance. To elucidate the molecular mechanisms, we have been conducting cloning of quantitative trait loci (QTLs) controlling seed dormancy. Previously, we cloned Sdr1 (Seed Dormancy 1), Sdr4 and Sdr7 by using natural allelic variation between indica cultivar and japonica. Map-based approaches defined small candidate region, and complementation approaches identified each gene. Sdr1 is a top of the MAP-kinase cascade, MAPKKK, Sdr4 is unknown protein gene, and Sdr7 is an orthologue of Arabidopsis Delay Of Germination 1 (DOG1).

To understand the genetic and molecular interaction between Sdr7 and Sdr4, we have stacked Kasalath allele of Sdr4 showing dormant phenotype and Sdr7, by crossing nearly isogenic line (NIL) Sdr4 and Sdr7. The stacked line, NIL(Sdr4/Sdr7), showed deeper dormancy than that of recurrent parent, 'Koshihikari', NIL(Sdr4) and NIL(Sdr7). We next examined gene expression of Sdr4 and Sdr7 on stacked line. Under the Sdr7 back ground, Sdr4 induced after imbibition, which was not found under the sdr7 back ground. In contrast, under the Sdr4 background, inducibility of Sdr7 was not affected. Thus, we speculated that Sdr7 may be located upstream of Sdr4 and a mediator of environmental condition such as rain, to Sdr4 and amplitude and/or elongate seed dormancy.

Recently, isolated Sdr1 encodes MAPKKK, and was highly induced by imbibition. The over-expresser, genomic complementation lines showed higher germinability and RNAi lines showed more dormant phenotype. These results suggested that Sdr1 may be a positive regulator of germination or negative regulator of seed dormancy. The Sdr1 protein can interact with a MAPKK in yeast and phosphorylate. We also prepared the stacked line, NIL(Sdr1/Sdr4). NIL(Sdr1/Sdr4) showed deeper dormancy than that of recurrent parent, 'Nipponbare', NIL(Sdr1) and NIL(Sdr4). We also examined gene expression of Sdr1 and Sdr4. Under the Sdr4 background, the inducibility upon imbibition was diminished.

Together, we speculated that dormancy pathway involving Sdr7/Sdr4, inducibility was erased according to after-ripening, may inhibit Sdr1 expression during dormant state, and release Sdr1 expression during after-ripening and reaches germination.



## QTLs for the Early-Morning Flowering Character Derived from *Oryza officinalis* to Mitigate Heat-Induced Spikelet Sterility at Flowering in Rice, *O. sativa*

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A decline in rice (*Oryza sativa* L.) production caused by heat stress is one of the biggest concerns resulting from future climate change. Rice spikelets are most susceptible to heat stress at flowering. The early-morning flowering (EMF) trait mitigates heat-induced spikelet sterility at the flowering stage by escaping heat stress during the daytime. We attempted to develop near-isogenic lines (NILs) for EMF in the indica-type genetic background by exploiting the EMF locus from wild rice, *O. officinalis* (CC genome). A stable quantitative trait locus (QTL) for flower opening time (FOT) was detected on chromosome 3. A QTL was designated as *qEMF3* and it shifted FOT by 1.5–2.0 h earlier for cv. Nanjing 11 in Japan. NILs for EMF mitigated heat-induced spikelet sterility under elevated temperature conditions completing flower opening before reaching 35°C, a general threshold value leading to spikelet sterility. Quantification of FOT of cultivars popular in the tropics and subtropics did not reveal the EMF trait in any of the cultivars tested, suggesting that *qEMF3* has the potential to advance FOT of currently popular cultivars to escape heat stress at flowering under future hotter climates. This is the first report to examine rice with the EMF trait through marker-assisted breeding using wild rice as a genetic resource.

## Development of New Rice Varieties Producing Grains with High Appearance Quality Under High Temperature Conditions During the Ripening Period

Kazumasa Murata and Takeshi Ebitani

Toyama Prefectural Agricultural, Forestry and Fisheries Research Center, Toyama, Japan

High temperature conditions during the rice ripening period caused significant deterioration in appearance quality of grains. This deterioration is due to increases in the abundance of chalky grains which decrease milling quality. Chalky grains were sub-classified as basal-white kernels, white-back kernels, white-berry kernels, milky-white kernels or white-core kernels. Especially white-back kernels and white-basal kernels are easily induced to produce by high temperature conditions. In Japanese rice market, the price of brown rice is determined by quality grade based on the percentage of perfect grains. Therefore, to avoid producing the chalky grains is one of the most important breeding targets in Japan.

The grain appearance quality of an *indica* variety, 'Habataki', is very low because of lots of chalky grains including white-berry kernels and milky-white kernels. However, 'Habataki' produces little basal-white kernels and white-back kernels. We performed a QTL analysis for the grain appearance quality of brown rice using chromosome segment substitution lines of 'Habataki', in a *japonica* variety 'Koshihikari' genetic background that is a leading cultivar with good palatability in Japan. A line carrying a 'Habataki' segment on chromosome 7 showed a high percentage of perfect grains produced under high temperature conditions. To verify the role of this segment, and to narrow down the region containing the useful allele, substitution mapping was performed using multiple paired lines. As a result, the chromosomal location of a gene that we named Appearance quality of brown rice 1 (*Apq1*) was delimited to a 48 kb region. In addition, we developed an *Apq1*-near isogenic line of Koshihikari (*Apq1*-NIL) to evaluate the effect of *Apq1* on various agronomic traits. Under high temperature conditions, the *Apq1*-NIL produced significantly higher percentages of perfect grains than 'Koshihikari'. Other agronomic traits, including yield and palatability, were similar between the *Apq1*-NIL and 'Koshihikari'. Therefore, the 'Habataki' allele of *Apq1* would be useful in breeding programs aimed at improving the quality of grain ripened under high temperature conditions.

Furthermore, we have developed some NILs of Koshihikari, which possess a blast resistance gene to each of pathogen races (Pi-NILs) and a semi-dwarf gene, *sd1* (*sd1*-NIL). Among progenies derived from crosses of *Apq1*-NIL with Pi-NILs, and also, the next crosses with *sd1*-NIL, we selected the promising NILs of Koshihikari pyramided *Apq1*, a blast resistance gene and *sd1*, using marker assisted selection. These promising NILs are designed to spread as new types of Koshihikari.





## Impact of High Temperature on the Transcriptome and Metabolome in Developing Rice Grains

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Ongoing global warming is losing the quality of crop products. In Japan, approximately 3°C rise in the temperature of the rice ripening season is predicted for the next-coming century. Grain filling is one of the most heat-vulnerable period in the life of rice plants, and the exposure to elevated temperatures above 26°C often produces chalky and fragile grains, leading to the loss of commercial value of the products. Since the chalky appearance is originated from irregular light reflectance in endosperm tissue that remains micro air spaces among starch granules, the grain chalkiness is thought to occur due to imperfect accumulation of seed storage materials such as starch and proteins. Consistently, many studies pointed out the reduction in 'sink' ability of ripening grains at high temperature. In this presentation, we analyze the impact of high temperature on gene expression and metabolites related to synthesis and metabolism of starch as well as other defense responses against heat stress by using the biotron experiment system, and show that the changes (reduced expression of starch biosynthetic enzymes and induction of starch metabolizing enzymes) trigger the loss of starch quantity and quality. Through summarizing the data, possible factors leading to the shortage of starch are discussed. Finally, we also introduce recent approaches to mitigate the damage by high temperature.



## Marker-Assisted Breeding Applied for Generation of Climate Resilient Fruit Trees in Japan

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Global warming is already progressing and has affected horticultural industry in Japan, revealed by a survey by NARO. Japan has witnessed temperature increase of 1.1°C, higher than the global mean, over the past 100 years. The important impacts on fruit trees are the poor and delayed coloring of fruit skin, the fruit flesh softening and disorders, increasing sunburn for fruit skin by extreme high temperatures, budding and flowering disorders, and increasing incidence of pests and diseases. Furthermore, freezing and frost damages increase in fruit trees in spite of an increase in mild winters. The most effective approach of adaptation technologies to such impacts is to develop climate change-resilient fruit tree cultivars by means of marker-assisted breeding (MAB). In this paper, three topics of MAB trials are introduced, i.e., scab resistant and self-compatible Japanese pear, *Alternaria* disease-resistant apple and Japanese pear, and the quantity and composition of anthocyanins in grape berry. Pear scab (caused by *Venturia nashicola*) is the most harmful fungal disease, and global warming resulted in increased damage of this fungal disease. Since all commercially cultivated Japanese pears show susceptibility to *V. nashicola*, the *Vnk* gene of an indigenous cultivar 'Kinchaku' showing resistance to the disease has been introgressed by tightly linked DNA markers. Self-compatibility (self-fertility) is also a very important trait to keep most profitable fruit set in commercial pear cultivation. Japanese pear sometimes encounters low fruit set caused by self-incompatibility, cold temperature during flowering, and reduced activity and diminishing population of honey bees. The  $S_4^{sm}$ , self-compatible stylar-part mutant allele, was identified for self-compatible mutant Osa-Nijisseiki. DNA marker designed based on the 236-kbp deletion including  $S_4$ -RNase and other genes is used for MAS in pear breeding programs. Both *Alternaria* blotch disease in apple and black spot disease in pear are caused by *Alternaria alternata*. Apple pathotype of *Alternaria* produces AM-toxin and Japanese pear pathotype produces AK-toxin, which could be interacted with dominant responsible genes of host plants. A fine map of linkage group 11 of susceptible 'Kinchaku' was constructed and the *Aki* locus was identified within a 1.5-cM genome region between SSR markers Mdo.chr11.28 and Mdo.chr11.34, which corresponded to the 250 Kb in the 'Golden Delicious' apple genome and 107 Kb in the 'Dangshansuli' Chinese pear genome. Color variation in grape skin (black, red or white) is one of the most important fruit traits selected in the breeding program for new grape cultivars. Skin color is mainly determined by the quantity and composition of anthocyanins, which are controlled by the MYB transcription factor and anthocyanin O-methyltransferase loci. Functional MYB haplotypes are successfully used for MAS of dark-colored berry skin in table grape breeding programs.





## Improvement of Malaysian Rice Variety Against Blast Disease Using Marker-Assisted Breeding Approach

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Blast disease, which is caused by *Pyricularia oryzae*, is one of the major biotic stress which results in yield losses in rice. To date, more than 100 major blast resistance genes have been identified in rice. The previous study demonstrated that the *Pi9* gene conferred broad spectrum resistance against Malaysian *P. oryzae* isolates. The *Pi9* gene showed dominant resistance against blast disease and was successfully mapped on chromosome 6. Therefore our present study is focused on introgression of *Pi9* gene from resistant variety IRTP21683 into Malaysian high yielding susceptible variety MR219, by using marker-assisted breeding (MAB) technology. A set of marker which consisted of tightly linked markers, flanking markers and background markers to *Pi9* gene were used in order to perform foreground, recombinant and background selection, respectively. Background analysis using evenly distributed single nucleotide polymorphisms (SNP) markers showed the similarity percentages of recurrent genome were varied from 33.3% to 63.3% for BC<sub>1</sub> progenies, 58.0% to 89.1% for BC<sub>2</sub> progenies and 80.4% to 97.1% for BC<sub>3</sub> progenies. Preliminary assessment of yield and morphological character of MR219-Pi9 advanced breeding lines showed successful recovery of the MR219 traits with the yield ranged from 6.5 to 8.0 t/ha. The yield and morphological character of MR219-Pi9 advanced breeding lines did not showed any significant difference except their resistance reaction against blast disease. These improved advanced MR219-Pi9 breeding lines are potential to replace the current MR219 variety due to their high yielding blast resistant characteristics. This study demonstrated that marker-assisted breeding approach provides an efficient and effective way to introgress *Pi9* gene from the donor parent into susceptible commercial rice variety.

## **Molecular Breeding to Improve Biotic Stress Resistance for Rainfed Lowland Rice in Northeast Thailand**

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Northeastern (NE) Thailand has the largest area of rainfed lowland rice (approximately 6 million hectares), which account for more than half of the country's total rice-growing area. About 80% of the area dominated by KDML105 and RD6 varieties. Biotic stresses i.e. rice blast disease (BL) and brown planthopper (BPH), become major pests in the areas due to the influence of changing farmer practices, lack of genetic diversity and heightened effects of climate change. There is ample reason for concern about severe outbreaks of BL and BPH that will wildly spreading throughout the NE because KDML105 and RD6 are susceptible to these pests. To maintain yield stability of rice production in the area, germplasm improvement is one of our main focuses. The concept of utilizing resistance genes has been considered as an outstanding approach to deal with the biotic problems. Recent advances in rice genomic research have enabled us to identify various rice pest resistance genes and provided DNA markers for marker-assisted selection (MAS). MAS has been used to transfer and pyramid BPH and BL resistance QTLs into KDML105 and RD6 and it has been applied as a routine basis in Thai breeding programs. Several promising lines with similar grain quality standards with KDML105 and RD6 have been developed. The improved lines have been directly developed into varieties and it have been also served as immediate sources of biotic resistance to improve good grain quality in breeding programs, which will have an impact on the yield stability in the pest outbreak risk areas. Increasing yield potential with good cooking quality and multiple resistant to biotic and abiotic stresses rice varieties for the rainfed lowland areas is our next challenge. Advances associated with next generation sequencing technology open the opportunity to redesign genotyping strategies for more effective genetic mapping and genome analyses in rice. This technology is now applied for identifying importance genes/QTLs and developing new rice varieties in Thai breeding programs. This review gives an overview on marker-assisted breeding in the past, present and future of rice breeding programs to improve biotic stress resistance for rainfed lowland in NE Thailand.



## Improvement on Rice Growth and Production

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Climate changes could affect agriculture through its effects on environmental conditions, such as drought, extreme temperature and flooding. In Taiwan, there were reports in recent years that environmental changes have resulted in reduction in rice production and grain quality, and acceleration in emergence of virulent rice pathogens. By screening of sodium azide (NaN<sub>3</sub>)-mutagenized rice mutants collected by Taiwan Agricultural Research Institute (TARI), our team have obtain various mutants with tolerance to bacterial blight disease and better mineral nutrient utilization. Genetic approaches are employed for identifying the responsible genes and composing gene stacked traits in rice. For rice bacterial blight disease, XF89b has been reported as one of the most virulent *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains in Taiwan. We have completed sequencing of the entire genome of XF89b. A number of major virulent factor encoded genes, TAL genes, were identified. We also found that *Pseudomonas taiwanensis* is an effective biocontrol agent against *Xoo*. Using Tn5 transposon mutagenesis, we have identified a number of genes that are essential for anti-*Xoo* activity, including genes involved in pyoverdine biosynthesis. In addition, we found that Type 6 secretion system is involved in pyoverdine secretion. The possible usage of pyoverdine for the biocontrol agent against *Xoo* is under evaluation.

## Application of Genotyping-by-Sequencing to Discover SNPs for Rice and Melon Molecular Breeding

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Advances in DNA sequencing technology have revolutionized the field of crop breeding, we can analyze thousands of natural variations and mapping population such as wild relatives, landraces,  $F_2$ , recombinant inbred lines (RILs) at reasonable cost for any species including rice and melon. Combining the phenotypic data, crop geneticists could investigate the genetic architecture of the agronomic traits using high-density markers. We are applying an economical and efficient genotyping platform (Genotyping-by-Sequencing, GBS) to sequence diverse accessions and mapping populations in rice and melon, over ten thousands of single nucleotide polymorphism (SNP) markers are generated and used for genetic analysis such as estimation of population structure, decay of linkage disequilibrium (LD), distribution of recombination breakpoints etc. The sequenced materials were evaluated for their tolerance to abiotic stresses in rice and fruit traits for melon.

Diverse rice accessions and recombinant inbred lines (RILs) were screened for their responses to submergence at germination stage. Differential anaerobic response was observed between *Japonica* and *Indica* subgroups. Many SNPs identified from our genome-wide association studies (GWAS) were associated with anaerobic germination, some genomic regions were also detected in previous bi-parental QTL studies. A unique and strong signal explaining ~30% of the phenotypic variation was only detected in our RIL population. Our study demonstrated that linkage mapping and GWAS are complementary for dissecting the genetic architecture of seedling tolerance to low oxygen stress in rice. We expect these results could benefit the variety development for direct seeding cultivation.

Although GBS enables the efficient and low-cost generation of large numbers of markers, the utility of resultant genotypes are limited, because they are enormously error-prone and contain high proportions of missing data. In this study, we generated SNP markers for 109 RILs of melon using the GBS approach and ordered them according to their physical position on the draft double haploid line DHL92 genome. By performing rigorous quality control and filtering and imputation, 22,933 loci that have an average rate of missing data of 0.281% were used to construct a genetic map, which spans 1,088.3 cM across 12 chromosomes and has a maximum spacing of 6.0 cM. Use of this high-quality linkage map enabled the identification of several quantitative trait loci (QTL) known to control traits in fruit and validated our approach. This study highlights the utility of GBS markers for the identification of trait-associated QTLs in melon and facilitates further investigation of genome structure.



## Genomes and Phenomes for Rice Improvement

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Over 780,000 accessions of rice are estimated to be conserved in gene banks worldwide, comprising a largely untapped source of genetic diversity for crop improvement. Current efforts characterizing this diversity at the genomic level has resulted in sequences of upwards of 5,000 varieties and landraces from the 3k Rice Genome Project and others. Yet, this ‘digital dictionary’ of genetic variation is not immediately useful for crop improvement unless the sequenced genetic stocks (GS) are characterized for traits across a range of environments, times and developmental stages, where they have been subjected to different stresses and management regimes and sampled from the molecular to the whole plant level. Once such omics data is available, analyses for association and machine learning will enable the identification of the genes and/or genomic regions involved in trait expression, bridging the genotype to phenotype divide. Then, this knowledge can be integrated into breeding programs for rice improvement using optimized molecular breeding strategies.

Crucial to the phenotyping effort is the adoption of high throughput (HTP) phenomics in controlled and field environments. These methods enable rapid characterization of yield-components and other traits that can be correlated to the more laborious, manually measured traits. For example, we have detected the same QTL/peaks for biomass and yield-component traits with significant correlations between HTP sensor/image derived parameters and manually measured traits.

Additionally, construction of novel populations, such as RILs, NAMs, MAGIC, and BILs derived from sequenced GS, creates rapid-response genetic resources (RR-GR) useful for both genetics and pre-breeding. IRRI along with Cirad, Ciat and other partners are developing a ‘Rice Array’ where diversity panels, RR-GR, breeding lines and other materials will be screened at many locations/environments to detect G x E x M effects. Central to our curation of these data is the SNP-seek database <http://snp-seek.irri.org>, developed with the International Rice Informatics Consortium (IRIC, <http://iric.irri.org>) for the global community. Together, these efforts will establish an extensible platform for enhancing the value of gene banks to deliver novel genes and their source genotypes into breeding programs.

## Advance Breeding Methods for Indonesian Swampland Rice

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One of the bases of the modern agriculture is the improved cultivars, which are much higher yield than ancient ones. It could not be denied that the significant food production worldwide is contributed by plant breeding technology. Nowadays, agriculture has new and huge challenges, due to population growth, the pressure on agriculture liability on the environmental conservation, and climate change. To cope with these new challenges, many plant breeding programs have reoriented their breeding scope to stress tolerance in the last years. Development of breeding technology as the advance breeding methods is needed to support food security. Indonesian plant breeding presents the most recent advances and discoveries applied to abiotic stresses. Unfortunately, diverse swampland areas in Indonesia are challenged for rice paddy farming and the landscape of swampland is divided into three zones (coastal, tidal and lowland swampland) with their typical properties. Various molecular markers have been applied to support rice breeding program in Indonesia. These markers were used to assess genetic diversity and varietal identification, trait tagging and marker-assisted selection, detection kit for heading date and yield component, and eating quality, and varietal identification/seed purity assessment based on DNA fingerprint. With the benefit of genomic-assisted breeding approach, a number of markers associated with tolerance to Fe toxicity were identified using genome-wide association study (GWAS). Overall, the new physiological concepts, breeding methods, and modern molecular biological approaches facilitate to develop improved cultivars tolerant to abiotic stress, especially on tolerance to Fe toxicity.



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