Climate change and host plant resistance: effects of high temperature and drought on rice *R* genes' mediated resistance to bacterial blight

Von der Naturwissenschaftlichen Fakultät der Gottfried Wilhelm Leibniz

Universität Hannover

zur Erlangung des Grades

Doktor der Gartenbauwissenschaften (Dr. rer. hort.)

genehmigte Dissertation

von

M.Sc. Codjo Sylvestre Gerbert Dossa

geboren am 28.11.1986 in Totchangni /Benin

2016

Referentin   : Prof. Dr. Kerstin Wydra

Korreferentin   : Prof. Dr. Edgar Maiss

Tag der Promotion : 21.01.2016

Dedication

To God Almighty through whom everything is possible.

"Know that wisdom is such to your soul; if you find it, there will be a future, and your hope will not be cut off." Proverbs 24:14.

Abstract

Anthropogenic activities driven by demographic, economic and technologic changes affect the climate worldwide. As consequence, global warming, water scarcity and extreme weather events are predicted to occur like unprecedented. At the same time, pests and pathogens pose a significant threat to food security, and host-plant resistance to pathogens is likely to be affected by climate change. In this study, rice response to bacterial blight (BB) under high temperature and under drought stress was investigated. The effects of high temperature and drought stress on major rice *R* genes *Xa4* and *Xa7* mediated resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) were evaluated under field conditions and in screenhouse experiments, evaluating disease development and plant growth and analyzing the associated time course transcriptome profiles. Furthermore, the resistance of the cultivated African rice *Oryzae glaberrima* to bacterial blight was evaluated under high temperature in order to identify a new source of BB resistance. Thus, the resistance response of 19 *O. glaberrima* accessions and one genotype of *O. sativa*, variety Supa, to ten races of *Xoo* from The Philippines was enhanced under high temperature conditions. This finding suggests that *O. glaberrima* possesses traits that respond to combined stress of high temperature and bacterial blight. Interestingly, genotypic analysis using *Xa* gene markers indicates that *O. glaberrima* possesses *R* genes which are different to the to date known *Xa* genes.

For the first time it was shown that the effectiveness of the rice *R* gene *Xa4* was compromised under both high temperature and drought stress, while *R* gene *Xa7* benefited from abiotic stress and responded more efficiently to bacterial blight. The study shows that drought tolerant rice genotypes without suitable bacterial blight *R* genes are susceptible to the pathogen invasion and development under both irrigated and drought stress conditions. The benefit from drought stress in enhancing the resistance to bacterial blight of genotypes carrying *Xa7* suggests that the combination of

Xa7 with drought *qDTY* would provide a suitable way to combine both traits in rice genotypes resistant to bacterial blight and tolerant to drought stress. Time course transcriptome profiles of IR24 and IRBB67 show 4,683 differentially expressed genes across 3, 72 and 120 hpi under both temperature regimes. Our results further reveal that under low temperature the response to *Xoo* is triggered by protein kinase genes such as Leucine Rich Repeat (LRR) and Receptor like kinases (RLK) including wall associated kinases with significant up-regulation in the resistant genotype compared to the susceptible one. The plant cell wall constitutes the first barrier to pathogen invasion and out study shows that high temperature negatively affects the host plant cell wall, opening the door for pathogen invasion. However, the resistant genotype IRBB67 shows up-regulation of genes involved in the cell membrane sensor of stimuli. Moreover, catalytic activity is shown to be the major regulator in response to high temperature and to *Xoo* inoculation in the resistant genotype IRBB67. Our results also suggest that, under high temperature, molecular mechanism underlying the resistance to bacterial blight mediated by IRBB67 *R* genes *Xa4+Xa7* is manifested as cell membrane homeostasis through a low affinity cation transporter gene and through the regulation of glucose metabolism under expression of *OsTPP6*.

The genome sequences of two *Xoo* strains from the Philippines representing different races, strain PXO145 (race 7) and strain PXO86 (race 2), revealed close relatedness. The prediction of Transcription Activator Like (TAL) effectors in PXO145 provides additional information on the rice-*Xoo* pathosystem and suggests that prediction of host genes targeted by TAL effectors will reveal hidden threats posed by *Xoo* to rice. Finally, this study shows that evaluation of rice genotypes under combined stress (abiotic and biotic) provides a valuable insight into host plant resistance to pathogens under the conditions of climate change. Thus, breeding rice varieties for resilience to climate change is an urgent need, and requires the combination of abiotic and biotic stress

tolerance and resistance traits, respectively, in elite varieties. Our results lay the molecular basis as well as provide the information from field trials to select genotypes with enhanced resilience to climate change.

Keywords: Rice, *Xoo,* climate change

Zusammenfassung

Anthropogene Aktivitäten, verursacht durch demografische, ökonomische und technologische Veränderungen, beeinflussen das Klima weltweit. Folglich sind eine globale Erwärmung, Wasserknappheit und extreme Wetterereignisse in unbekanntem Ausmaß vorhergesagt. Gleichzeitig stellen Schädlinge und Pathogene eine bedeutende Bedrohung für die Nahrungssicherheit dar, und die Resistenz von Wirtspflanzen gegenüber Pathogenen könnte durch Klimawandel verändert werden. In dieser Studie wurde die Reaktion von Reis auf den Befall mit *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) unter hoher Temperatur und unter Trockenstress untersucht. Der Einfluss von hoher Temperatur und Trockenstress auf die durch die R-Gene *Xa4* und *Xa7* vermittelte Resistenz gegen *X. oryzae* pv. *oryzae* wurde im Feld und in Gewächshausexperimenten untersucht, Befallsentwicklung und Pflanzenwachstum wurden bewertet und ein über einen Zeitverlauf angelegtes Transkriptomsprofil erstellt. Des Weiteren wurde die Resistenz gegen *X. oryzae* in afrikanischem Reis (*Oryza glaberrima)* unter erhöhten Temperaturen untersucht, um eine neue Quelle einer BB-Resistenz zu identifizieren.

Die Resistenzreaktion von 19 *O. glaberrima* Herkünften und eines Genotyps von *O. sativa*, Sorte „Supa", auf 10 Rassen von *Xoo* von den Philippinen war unter erhöhten Temperaturbedingungen verstärkt. Dieses Ergebnis deutet darauf hin, dass *O. glaberrima* Eigenschaften besitzt, die zu einer Reaktion auf die Kombination aus Temperatur-Stress und *Xoo*-Befall rführen. Interessanterweise ergibt eine Genotypenanalyse, in der *Xa* Genmarker verwendet wurden, dass *O. glaberrima* R-Gene besitzt, die sich von den bisher bekannten *Xa*-Genen unterscheiden.

Zum ersten Mal konnte gezeigt werden, dass die Effektivität von R-Gen *Xa4* in Reis durch hohe Temperatur und Trockenstress beeinträchtigt wurde, wohingegen das R-Gen *Xa7* von abiotischem Stress profitierte und zu einer effizienteren Reaktion auf den *Xoo*-Befall führte. Die Studie zeigt, dass trockenstresstolerante Genotypen ohne

geeignete *Xoo*-R-Gene anfällig für Pathogenbefall und –entwicklung sowohl unter Bewässerung als auch unter Trockenstress sind. Der Vorteil von Trockenstress in der Verstärkung der Resistenz gegen *Xoo* von Genotypen, die das Xa7-Gen tragen, lässt vermuten, dass *Xa7* zusammen mit *qDTY* eine geeignete Kombination darstellen, um beide Eigenschaften (Resistenz gegen *Xoo* und Trockenstresstoleranz) in Reis-Genotypen zu vereinen. Transkriptomprofile von IR24 und IRBB67 über einen Zeitverlauf von 3, 72 und 120 hpi zeigen 4.683 unterschiedlich exprimierte Gene unter beiden Temperatur-Regimen. Unsere Ergebnisse zeigen des Weiteren, dass bei niedrigeren Temperaturen die Reaktion auf *Xoo*-Befall durch Gene, die Proteinkinasen wie z.B. Leucine Rich Repeat (LRR) und Receptor Like Kinases (RLK) codieren, gesteuert wird. Im resistenten Genotyp wurden Zellwand-Kinasen signifikant hoch reguliert im Vergleich zum anfälligen Genotyp. Die pflanzliche Zellwand stellt die erste Barriere gegen Pathogenbefall dar und unsere Studie zeigt, dass hohe Temperaturen die Zellwand negativ beeinflussen und somit den Türöffner für Pathogeninvasion darstellen. Der resistente Genotyp IRBB67 zeigt eine Hochregulierung von Genen, die in der Zellmembran Sensoren für Stimuli codieren. Außerdem wurde gezeigt, dass die katalytische Aktivität der Hauptregulator für die Reaktion auf hohe Temperatur und *Xoo*-Infektion im resistenten Genotyp IRBB67 ist. Unsere Ergebnisse zeigen ebenfalls, dass in die IRBB67-Resistenz gegen *X. oryzae* unter hohen Temperaturen die Zellmembranhomeostase Gene, die Affinitätstransporter codieren, und in den Glukosemetabolismus Gene unter *OsTPP6*-Regulierung involviert sind.

Die Genomsequenz von zwei *Xoo*-Stämmen von den Philippinen, die verschiedene Rassen repräsentieren, Stamm PXO145 (Rasse 7) und PXO86 (Rasse 2), zeigten enge Verwandtschaft miteinander. Die Vorhersage von Transcription Activator Like (TAL) Effektoren in PXO145 liefert zusätzliche Informationen über das Reis-*Xoo*-Pathosystem

und lässt vermuten, dass die Vorhersage von TAL-Effektoren, die auf Wirtsgene zielen, eine versteckte Bedrohung der Pflanze durch *Xoo* darstellen.

Unsere Studie gibt durch die Bewertung von Reis-Genotypen unter kombinierten Stressbedingungen (abiotisch und biotisch) einen wertvollen Einblick in die Wirtspflanzen-Resistenz gegen Pathogene im Klimawandel . So wurde die molekulare Basis für diese Resistenzinteraktionen gelegt und mit Ergebnissen aus Feldversuchen ergänzt, um Elitesorten mit erhöhter Widerstandsfähigkeit gegen Klimawandel zu selektieren, die eine Kombination aus abiotischer und biotischer Stresstoleranz besitzen.

Schlüsselwörter: Reis, *Xoo,* Klimawandel

List of Abbreviations

%	: Percentage
°C	: degree centigrade
BB	: Bacterial blight
CFU	: colony forming units
cm	: centimeter
das	: days after sowing
DEG	: Differentially expressed gene
DNA	: deoxyribonucleic acid
EBE	: Effector binding element
ha	: hectare
hpi	: Hour post inoculation
IPCC	: Intergovernmental Panel on Climate Change
MDST	: moderate drought stress
MiDST	: mild drought stress
MSU	: Michigan State University
NIL	: Near isogenic line
qDTY	: Drought yield qtl
qRT-PCR	: quantitative Real-Time PCR
QTL	: Quantitative Trait Loci
RNA	: ribonucleic acid
RNA-Seq	: RNA Sequencing
SMRT	: Single molecule, real-time
TAL	: Transcription Activator Like
WW	: well-watered
Xoo	: *Xanthomonas oryzae* pv. *oryzae*

Table of content

Chapter 5: *Xanthomonas oryzae* pv. *oryzae* genome sequencing and transcriptome changes in rice in response to combined high temperature and bacterial blight 119

List of Tables

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 1: Rice, bacterial blight and climate change

Abstract

Rice is staple food for half of the world's population and mostly grown and consumed in Asia. Rice like any other plants in their natural habitats faces challenges from multiple stress factors categorized as biotic and abiotic stresses. Single stress effects on plants have been largely studied, but plant reactions and adaptation to combined stress factors need more consideration. Abiotic stress such as high temperature and drought induce a range of biochemical, molecular and physiological changes and responses from cellular level to entire plant processes. Climate change accompanied by unexpected heat and drought periods is predicted to have significant impact on agriculture. Rice cultivation will face more challenges than a decade ago. Among biotic stress factors limiting rice yield, rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most important. A large number of *R* genes (to date 41 *R* genes) have been identified to confer bacterial blight resistance; however climate change influences the *rice-Xoo* pathosystem affecting *R* genes durability. *X. oryzae* pv. *oryzae* produces large candidate bacterial effectors that are injected into the host cell as virulence factor. Availability of omics data on the pathogen and also from *rice-Xoo* interaction provides opportunities' to study the complex interaction between rice and *Xoo* under different climate scenarios.

Keywords: Rice, climate change, bacterial blight, *R* genes

Introduction

Rice is the second largest worldwide cultivated cereal crop. As staple food for more than half the world, rice is cultivated mainly in Asia with 90.9% of world rice production. Rice remains the main source of calories, especially for people in Asia and also an important source of income. Rice is grown in different ecosystems and conditions, including irrigated rice cultivation and upland and rainfed lowland ecosystems. Rice dominates overall crop production when the rice area harvested is considered, and overall food consumption considering the total caloric intake from rice (Mohanty et al., 2013). Although the world's largest rice producers are found among the Asian countries, in Africa rice production has increased rapidly. African rice production accounts for 3.2% of the world production and the main part comes from West African countries accounting for more than 45% of African production. For the last decade, world rice production has increased from 530.90 million tons in 1993 to 745.172 million tons in 2013 (Figure 1). This growth is positively correlated to the increase of the area harvested which is expanded from 145.49 to 166.08 million of hectares between 1993 and 2013 (FAO, 2014). Rice ecosystems are dominated by irrigated cultivation followed by the upland rice culitvation. Rice under any of these ecosystems is subjected to a range of constraints which negatively affect the yield.

Rice plays important role in food security and, so far, rice production has met the population demands. However, there are more challenges that will affect its performance in future, where biotic and abiotic factors will play major roles. Rice like any other plant cannot escape the limitation imposed by these two factors. Unfavorable environmental conditions related to heat, water scarcity, salinity and cold reduce the average yield by more than 50% (Shao et al., 2008; Wang et al., 2003), and attack from a wide range of pests and pathogens including fungi, bacteria, viruses, nematodes and

herbivorous insects account for a further yield reduction (Hammond-Kosack and Jones, 2000).
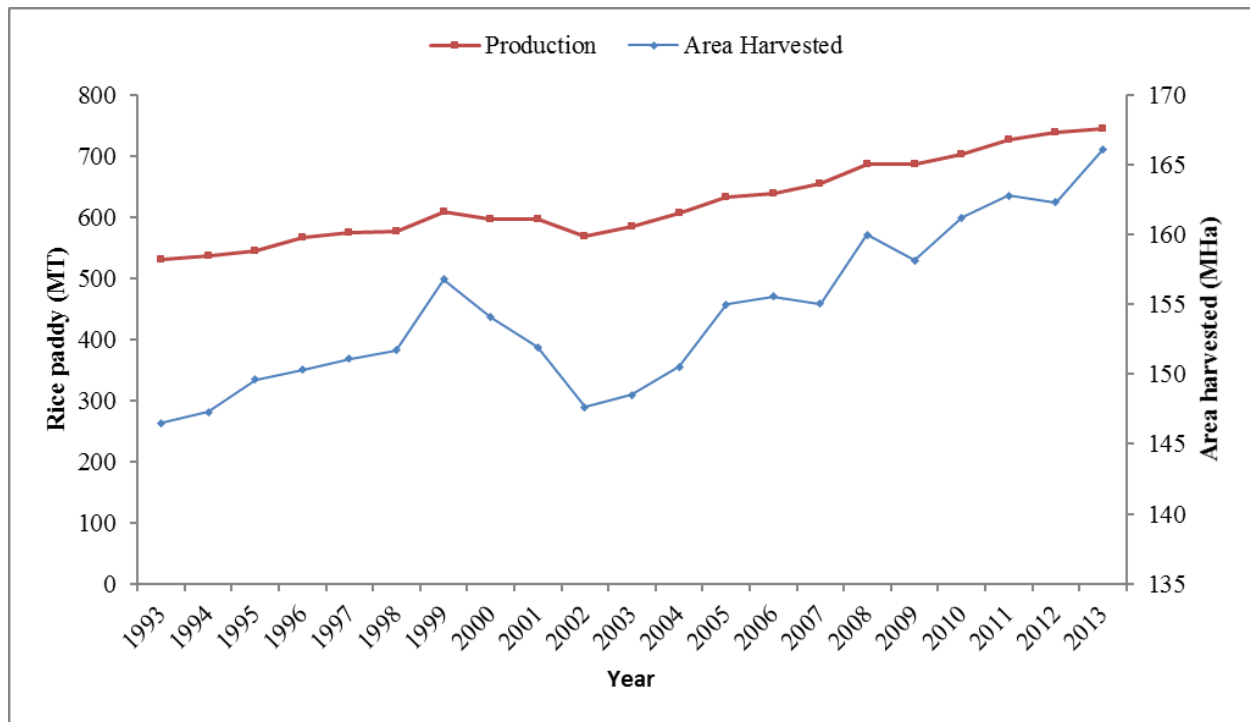
Figure 1: Evolution of rice paddy production and area harvested from 1993-2013, data derived from FAO, 2014.

IPCC (2013) reported that in the next century, the average surface temperatures will increase by 3-5°C, with an impact on the global agricultural system. As consequence, temperature rise will cause a reduction in the growing seasons in many regions, accompanied by unexpected weather events and shifts in the rainfall patterns. Also, an increase of sea level is predicted, with the consequence of salinization and decrease of agricultural land (IPCC, 2007; 2008; Easterling et al., 2000; Morison et al., 2008; Atkinson and Urwin, 2012). Thus, effects on the prevalence of pests and pathogens, their geographical expansion and reproductive capacity are expected, and plants are therefore likely to encounter more environmental stress than before, adding to it the more frequent occurrence of more virulent strains due to increased generation numbers.

Likewise, pests and pathogens will have more wild type species to colonize, as pests and pathogens will move poleward (Garrett et al., 2011; Bebber et al., 2013). To grow and develop under these challenging conditions, plants must develop sophisticated strategies of response to cope with multiple stress conditions.

1. Rice bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae*

Among the biotic factors affecting rice yield, bacterial blight is the major bacterial disease of rice. The causal agent, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a Gram-negative γ- proteobacterium belonging to the Xanthomonas genus. The length and width of individual cells vary between 0.7 μm to 2 μm and between 0.4 μm to 0.7 μm, respectively (Nino-Liu et al., 2006). *Xanthomonas oryzae* pv. *oryzae* has been reported in many rice growing regions including Asia, Africa, Northen Australia and Central and North America and causes economical losses to farmers. The pathogen invades its host through natural openings such as stomata, hydathodes or wounds, then multiplies in the intercellular space of the underlying epithelium and colonizes the plant through the xylem vessels. The bacterium in the leaf moves vertically through primary veins and spreads laterally to commissural veins (Nino-Liu et al., 2006). Few days after entering inside the host, the infected plant xylem vessels are filled with the bacterial cells and extracellular polysaccharides (EPS) which ooze from the hydathodes to form exudates on the leaf surface. The bacterial exudates on the leaf surface are a source of secondary inocula (Mew et al., 1993, Nino-Liu et al., 2006). Genetic diversity existing among *Xoo* strains has been revealed by molecular studies and genome sequencing. Gonzalez et al. (2007) reported existence of a genetic distance between African *Xoo* and Asian strains. Therefore, resistant rice varieties to control the pathogen in Asia may not be effective against African strains, and, so far effective *R* genes against African *Xoo* have not been identified. To date, three complete genome sequences of *Xoo* strains from Asia have been published. The Philippines strain PXO99A, a 5-azacytidine-resistant derivative of

PXO99 (Salzberg et al., 2008); Japanese strain MAFF311018 (Ochiai et al., 2005) and the Korean strain KACC10331 (Lee et al., 2005). In addition, eight draft genomes (PXO86, Philiipines; BAI4; BAI3, Burkina Faso; NAI8, Niger; CFBP1947, Cameroun; MAI1, Mali; X11-5a and X8-1a, United State) are also available to gain insight into the genetic diversity among the *Xoo* populations (Verdier et al., 2012). Genome sequence analyses revealed a genome size varying from 4.94 Mb to 5.24 Mb with an average G+C content of ~ 63.7%. The *X. oryzae* pv. *oryzae* genome harbors numerous IS elements, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) elements and Transcription Activator Like (TAL) effecotors (Salzberg et al., 2008) that the pathogen injects into the host cells via the Type 3 secretion system (T3SS). Multiple TAL effectors are delivered into rice cells from where they are translocated into the nucleus and bind to the corresponding Upregulated by TAL effector (UPT) box sequence in host DNA (Bosh and Bonas, 2010). Recent studies reveal how the TAL effectors in the host cells find their targets (Bogdanove et al., 2010; Scholze and Boch, 2011) and the binding specificity governed by repeat-variable diresidues (RVDs) which recognize different DNA base pairs (Boch et al., 2009; Moscou and Bodganove, 2009). Omics data from several *Xoo* strains/races will enable to get insight into the genetic diversity existing in *Xoo* population. This allows the use of genetic engineering as new molecular breeding strategies to identify promotor variant alleles of major susceptibility genes (Verdier et al., 2012), as shown by Hutin et al. (2015) with identification of *xa41(t)*, a variant allele of *OsSweet14* gene which has 18 bp deletion within several TAL effectors binding sites and cause resistance to BB.

Xanthomonas oryzae pv. *oryzae* causes different types of symptoms on the host plant, depending on the plant growth stage and level of resistance of the cultivars. Three major symptoms are reported on rice: leaf blight symptom, mostly on the susceptible cultivars, kresek or seedling wilt when infection occurs at seedling stage and can cause 100% losses, and the pale yellow leaf or pale yellow on mature plants. The infection on

plants usually occurs as green water soaked spots at the tips and margins of the fully developed leaves; it spreads along the veins, and symptoms merge and extend from the leaf tip down along leaf veins and margins (Figure 2A). However, depending on the entering point, the symptoms may extend from the entry point (e.g. leaf break) and prolong lengthwise. Under artificial inoculation, the pathogen can also spread to non-inoculated leaves on the susceptible cultivars even under drought stress conditions (Figure 2B).

Figure 2: Bacterial blight symptoms on rice leaves. A: Development of different types of bacterial blight symptoms on rice leaves; B: BB spread from artificial infection to non inoculated leaves under drought stress.

2. Bacterial blight resistance genes

Disease control strategies recommend several practices such as improved agricultural measures, chemicals' application, biological control agents and resistant cultivars. However, among these practices, the use of resistant cultivars reveals as the most environmental friendly and most effective approach. Resistance genes known to act in a gene-for gene manner have been identified and are the main source of rice resistance to *Xoo*. The avirulence protein injected into the host cell via T3SS by the pathogen is recognized by the host corresponding *R* gene and results in expression of resistance. According to Bhasin et al., 2012; Natraijkumar et al., 2012; Khan et al., 2014, Zhang et al., 2015; Suk-Man et al., 2015; Hutin et al., 2015) 41 *R* genes [*Xa1, Xa2, Xa3/Xa26, Xa4, xa5, Xa6, Xa7, xa8, xa9, Xa10, Xa11, Xa12, xa13, Xa14, xa15, Xa16, Xa17, Xa18, xa19, xa20, Xa21, Xa22(t), Xa23, xa24(t), xa25/Xa25(t), Xa25, xa26(t), Xa27, xa28(t), Xa29(t), Xa30 (t), xa31(t), Xa32(t), xa33(t), xa34(t), Xa35(t), Xa36(t), Xa38, Xa39, Xa40* and *xa41(t)*] are reported with a given prefix *Xa* as from Xanthomonas. These *R* genes comprise both dominant and recessive genes among which seven (*Xa1, xa5, xa13, Xa21, Xa25, Xa27 and Xa3/Xa26*) have been cloned with most of them encoding leucine-rich repeat domains. Due to the pathogen adaptation, a loss of function from monogenic line deployment has been reported; e.g. the predominance of virulent strains on rice varieties carrying the *Xa4 R* gene (Mew et al., 1992; Vera Cruz et al., 2000).

Disease resistance genes are comprised of two major classes: receptor kinase (RLK) and nucleotide-binding site leucine-rich repeat (NBS-LRR). Among rice *R* gene mediated resistance to bacterial blight, the majority of the cloned genes possesses LRR domain. Rice *Xa21* was the first clone member of RLK and confers a broad spectrum resistance to most the Philippines' *Xoo* races. NBS-LRR class is the largest *R* gene class that confers resistance against bacteria but also against fungi and viruses (Hulbert et al., 2001). Rice *Xa1* is the major gene encoding for NBS-LRR protein and confers highly specific

resistance to the Japanese *Xoo* race 1 (Yoshimura et al., 1998). The plant cell apoplast constitutes a physical barrier against pathogen attack and plays an important role in signaling and defense during the host-pathogen interaction. Rice *Xa27* resistance allele is expressed upon inoculation with *Xoo* strains harboring *avrXa27* and is reported to be localized in the xylem vessel, encoding a protein of 113 amino acids (Gu et al., 2005, Wu et al., 2008). Rice *xa5* and *xa13* are the two recessive genes that occur naturally among the cloned *R* genes. Rice *xa5* is a naturally occurring mutation and encodes for a small subunit of transcription factor IIA (TFIIAγ), and *xa13* is a recessive allele of Os8N3 rice susceptibility gene, which is a target of PthXo1 TAL effector. However, PthXo1 fails to induce *xa13*, therefore rice varieties carrying *xa13* reveal resistant to *Xoo* strains that rely only on the PthXo1 as virulence effector (Yang et al., 2006). *Xa26* known also as *Xa3* encodes for a LRR receptor kinase protein with broad spectrum resistance (Sun et al., 2004). The effectiveness of *Xa26* is more related to the genetic background and was found in cultivar Mingui 63 (Sun et al., 2004). Cao et al. (2007) reported that the *Xa26* expression level was much higher in japonica cultivars and increased from seedling to adult stage, suggesting that *Xa26* mediated resistance to *Xoo* is related to the development stage.

Some of the *R* genes have been widely used in rice breeding programs, and cultivars carrying them are deployed in many Asian countries. Among them, *Xa4*, *xa5* and *Xa7* are the major *R* genes. However, pathogen variability and adaptation lead to overcoming of the resistance as it was the case of rice *Xa4*. During the early 1970s, cultivars with *Xa4* after deployment became sensitive to the pathogen due to the pathogen adaptation and spread of new races that overcame *Xa4* resistance (Mew et al., 1992; Huang et al., 1997). Vera Cruz et al. (2000), after evaluating the pathogen fitness and predicting the durability of a disease resistance came to the conclusion that *xa5* and *Xa7* would be more durable than *Xa10,* and the combination of *xa5* and *Xa7* into the

same line would be more effective than the use of single *R* genes alone. Quantitative resistance governed by several genes provides partial resistance compared to qualitative (monogenic) resistance which confers effective race-specific resistance is more durable as pathogen populations' change frequently.

3. Host-pathosystem and climate change

Environmental factors such as biotic and abiotic stresses are limiting factors to plant productivity. Due to mainly anthropogenic factors, the climate is predicted to warm by an average of 2-5°C by end of the 21st century (Eitzinger et al., 2010; ICPP, 2013). Due to their sessile life style, plants are exposed to the increase of the global temperature, and high temperature and water scarcity are the major abiotic factors that constrain crop production. However, biotic stress occurs simultaneously with abiotic stress. Plants have developed specific mechanisms to detect environmental changes to survive and reproduce (Pieterse et al., 2009; Atkinson and Urwin, 2012), and when subjected to multiple stresses, plants respond in a non-additive manner (Rhizhsky et al., 2004; Mittler, 2006).

Although climate change affects crop yield potential, pests and pathogens also contribute to crop yield losses (Gregory et al., 2009). The disease development is the result of the interaction between a susceptible host plant, a virulent pathogen and the environment. The interaction between biotic and abiotic factors has been demonstrated by several reports about the effects of abiotic stress on many pathosystems. Plant defense can be affected after long-term abiotic stress, resulting in increased plant susceptibility to pathogens (Amtmann et al., 2008; Goel et al., 2008, Mittler and Blumwald, 20010; Atkinson and Urwin, 2012). For example wheat susceptibility to the fungus *Cochliobolus sativus* is correlated with high mean temperature, as observed over a six year period (Sharma et al., 2007); tobacco and Arabidopsis HR and R- gene response to *Pseudomonas syringae* and viral elicitors are compromised under high temperatures

(Wang et al., 2009). Sorghum and common bean plant show a higher susceptibility to *Macrophomina phaseolina* under drought stress (Diourte et al., 1995; Mayeke-Perez et al., 2002). Moreover, a recent study shows increase in susceptibility of Arabidopsis plants to virus infection under heat stress or heat and drought combined stress (Prasch and Sonnewald, 2013). Climate changes affecting plant growth and development will directly or indirectly also affect the microorganisms living on the plant leaf and root surfaces. Garrett et al. (2006) in their modeling study, showed evidence of the influence of climate change on pests and pathogens and point out a possible increase in their reproductive potential, geographical distribution and likely increasing number of pests and pathogens' hosts and numbers of virulent strains. Recent studies have confirmed the increased pathogen spread (Luck et al., 2011; Madgwick et al., 2011). Climate change most likely predisposes the host to pathogen colonization. Plant recognition of the pathogen effectors through gene-for- gene manner belongs to the NB-LRR protein family and occurs as result of effector-triggered immunity (ETI). However, innate immune responses activated by pattern-triggered immunity (PTI) and pathogen interception through ETI triggers systemic signals resulting in plant defense responses and limiting disease spread (Kissoudis et al., 2014). The study of Cheng et al. (2013) suggest that changes in ambient temperature lead to a switch of ETI to PTI signaling in plants with activation of ETI at low temperatures and PTI at moderately elevated temperatures. Temperature increase affects crop *R* genes' responses. For example, the wheat stripe rust R gene *Yr36* confers broad spectrum resistance to *Puccinia striiformis* f. sp. *tritici* at high temperature (25-35°C), but shows susceptibility to the pathogen at low temperature (15°C) (Uauy et al., 2005). Thus, climate change adds a complexity to food production and food security. Therefore, there is an urgent need to develop varieties with enhanced tolerance to combined stresses.

In rice, Peng et al. (2004) reported that for each increase of temperature by 1°C rice yield declines by 10% during the dry season. High temperature or drought stress affect rice yield and the combined stress of high temperature and drought showed greater effects on rice than high temperature or drought stress alone (Prasad et al., 2011). There are few reports on the effects of the combined biotic and abiotic stress on rice, particularly combined stress of pathogen and heat or drought. Rice IR26 and IR36 showed decreased trends in the resistance against brown planthopper (BPH), *Nilaparvata lugens*, when the temperature increased from 25 to 34°C (Wang et al., 2010). Contrarily, Webb et al. (2010) reported evidence on rice *Xa7* increased effectiveness against bacterial blight at high temperature. Other rice bacterial blight resistance genes (*Xa3*, *Xa4*, *xa5* and *Xa10*) studied by the authors were less effective under high temperature compared to low temperature, especially *Xa4* in variety IRBB4, which showed high disease increase under higher temperature. Rice bacterial blight r esistance gene durability is therefore in question under the climate change. Pathogen adaptability under climate change brings the use of new approaches for breeding tolerance/resistance to stresses into front since abiotic and biotic stresses often occur simultaneously. Since the combination of several traits might lead to antagonistic interactions between the expression of traits, omics data from different combinatorial stress experiments are therefore required to allow identification of major regulatory genes involved in multiple stresses signaling pathways.

Conclusion

Climate changes affect plants from physiological to molecular level. Plant defense responses to pathogens are influenced by abiotic stress factors such high temperature and drought. Hundreds of reports exist on traditionally a single stress factor on plants, however, with the climatic predictions, stress combinations are more likely to occur in agricultural systems. The single stress factor study model is no longer sufficient in

creating multiple stress-tolerant crops to face the challenges from climate changes. As rice is consumed widely in the world, the predicted climatic conditions will negatively influence the role played by this cereal crop in food security. There is urgent need of research to study stress combinatory effects and to come out with multiple stress tolerant/resistance varieties. Signaling pathways of individual stress responses point to interaction and antagonism mechanisms controlled principally by hormones. For example, Abscisic Acid (ABA) produced in response to abiotic stress induces downstream processes for suppression of biotic stress signaling pathways (Anderson et al., 2004; Asselbergh et al., 2008; Atkinson and Urwin, 2012; Atkinson et al., 2013). An antagonism exists also between jasmonic acid, salicylic acid and ethylene in response to biotic stress. Breeding new varieties that hold multiple stress tolerance/resistance will therefore depend on the stress regulatory network. Research will have to explore existing omics data and construct new data if needed to reveal common genes existing between the biotic and abiotic signalling pathways to allow the manipulation of stress tolerance/resistance. Improved varieties for abiotic stress tolerance and biotic stress resistance should therefore be evaluated under combined stress, rather than single stress (Atkinson and Urwin, 2012).

This study was conceived with the following objectives:

1. To determine the rice and rice bacterial blight resistance gene response to bacterial blight under drought stress conditions. Under this objective, rice lines with different background were evaluated under two drought stress levels. The effects of drought stress on the pathogen development *in planta* were further evaluated in the lab. Secondly, seventeen rice lines were evaluated under field conditions for their responses to combined drought and bacterial blight using two *Xanthomonas oryzae* pv. *oryzae* strains, and further under screenhouse conditions with four *Xoo* strains.

2. To evaluate the cultivated African rice *Oryza glaberrima* resistance to the combined bacterial blight and high temperature stress. Nineteen *O. glaberrima* accessions and Supa (*O. sativa*) were evaluated under greenhouse conditions for their response to ten races of the Philippines *Xoo* strains. Their resistance response was enhanced under high temperature conditions.

3. To understand the effects of high temperature on the defense response to bacterial blight of rice genotype IRBB67 carrying both *Xa4* and *Xa7 R* genes. In this part, time course transcriptome profiles using RNA sequencing technology under illumine platform were analysed on two rice genotypes under two temperature regimes. Finally, the whole genome sequence of *Xoo* strain PXO145 was established using Pacific Bio SMRT cell technology.

References

Amtmann, A., Troufflard, S., Armengaud, P. 2008. The effect of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum.*, 133: 682–691.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., Kazan, K .2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell.,* 16: 3460–3479

Asselbergh, B., De Vleesschauwer, D., Höfte, M. 2008. Global switches and fine-tuning-ABA modulates plant pathogen defense. *Mol Plant Microbe Interact.,* 21: 709–719

Atkinson, N.J. and Urwin, P.E. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany*, 63:3523–3544.

Atkinson, N.J., Catherine J.L, Urwin, P.E. 2013. Identification of Genes Involved in the Response of Arabidopsis to Simultaneous Biotic and Abiotic Stresses. *Plant Physiol.,* 162: 2028–2041.

Bebber, D.P., Ramotowski, M.A.T., Gurr, S.J. 2013. Crop pests and pathogens move polewards in a warming world. Nature Climate Change 3: 985–988.

Bhasin, H., Bhatia, D., Raghuvanshi, S., Lore, J. S., Sahi, G. K., Kaur, B., et al. 2012. New PCR-based sequence-tagged site marker for bacterial blight resistance gene Xa38 of rice. *Molecular Breeding,* 30: 607–611.

Boch, J., Bonas, U. 2010. Xanthomonas AvrBs3 family-type III effectors: discovery and function. *Annu. Rev. Phytopathol.,* 48: 419–436

Boch, J., Scholze, H., Schornack,S., Landgraf,A., Hahn, S., Kay,S., Lahaye,T., Nickstadt, A. and Bonas, U. 2009. Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors. *Science.* 326: 1509.

Bogdanove A.J., Schornack, S., Lahaye, T. 2010. TAL effectors: finding plant genes for disease and defense. *Curr. Opin. Plant Biol.,* 4: 394–401

Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp, doi:10.1017/CBO9781107415324.

Cao, Y., Ding, X., Cai, M., Zhao, J., Lin, Y., Li, X., Xu, C. and Wang, S. 2007. The Expression Pattern of a Rice Disease Resistance Gene Xa3/Xa26 Is Differentially Regulated by the Genetic Backgrounds and Developmental Stages That Influence Its Function**.** *Genetics,* 177**:** 523-533.

Cheng, C., Xiquan, G., Baomin, F., Jen, S., Libo, S., Ping, H. 2013. Plant immune response to pathogens differs with changing temperatures. *Nat Commun.* 4:2530

Diourte, M., Starr, J.L., Jeger, M.J., Stack, J.P., Rosenow, D.T. 1995. Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water-stress on disease development in sorghum. *Plant Pathology,* 44: 196–202.

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O. 2000. Climate extremes: observations, modeling, and impacts. *Science,* 289: 2068–2074.

Eitzinger, J., Orlandini, S., Stefanski, R., Naylor, R. E. L. 2010. Climate change and agriculture: introductory editorial. *Journal of Agricultural Science*, 148: 499–500

FAO, 2014. Food and Agriculture Organization of the United Nations, Statistics Division. **http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E**

Garrett, K. A., Forbes, G. A., Savary, S., Skelsey, P., Sparks, A. H., Valdivia, C., van Bruggen, A. H. C., Willocquet, L., Djurle, A., Duveiller, E., Eckersten, H., Pande, S., Vera Cruz, C. and Yuen, J. 2011. Complexity in climate-change impacts: an analytical framework for effects mediated by plant disease. *Plant Pathology,* 60: 15–30.

Garrett, K.A., Dendy, S.P., Frank, E.E., Rouse, M.N., Travers, S.E. 2006. Climate change effects on plant disease: genomes to ecosystems. *Annu Rev Phytopathol.,* 44:489–509

Goel, A.K., Lundberg, D., Torres, M.A., Matthews, R., Akimoto-Tomiyama, C., et al. 2008. The *Pseudomonas syringae* type III effector HopAM1 enhances virulence on water-stressed plants. *Mol. Plant-Microbe Interact,* 21:361–70.

Gonzalez, C., Szurek, B. , Manceau, C. , Mathieu, T. , Sere, Y. and Verdier, V. 2007. Molecular and pathotypic characterization of new *Xanthomonas* strains from West Africa. *Molecular Plant-Microbe Interactions*, 20: 534-546.

Gregory, P.J. Johnson, S.N., Newton, A.C., Ingram, J.S.I. 2009. Integrating pests and pathogens into the climate change/food security debate. *Journal of Experimental Botaby*, 60:2827-2837

Gu, K.Y., Yang, B., Tian, D.S., Wu, L.F., Wang, D.J., Sreekala, C., Yang, F., Chu, Z.Q., Wang, G.L., White, F.F. and Yin, Z.C. 2005. R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature,* 435 **:** 1122–1125.

Hammond-Kosack, K.E., Jones, J.D.G. 2000. Response to plant pathogens. In: Buchannan B, Gruissem W, Jones R, eds. Biochemistry and molecular biology of plants. Rockville, MD: *American Society of Plant Physiologists*, 1102–1157.

Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J., Khush, G.S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.,* 95: 313–320

Hulbert, S. H., Webb, C. A., Smith, S. M., & Sun, Q. 2001. Resistance genes complexes: evolution and utilization. *Annual Review of Phytopathology,* 39: 258–312.

Hutin, M., Sabot, F., Ghesquière, A., Koebnik, R. and Szurek, B. 2015. A knowledge-based molecular screen uncovers a broad spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. *Plant J.* Accepted Author Manuscript. doi:10.1111/tpj.13042

IPCC. 2008. Climate change and water. In: Bates BC, Kundzewicz ZW, Palutikof J, Wu S, eds. Technical Paper of the IPCC. Geneva: IPCC Secretariat, 210.

IPCC. 2012. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change [Field, C.B., V. Barros, T.F. Stocker, D. Qin,

D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, UK, and New York, NY, USA, 582 pp.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental

Khan, M.A., Naeem, M., Iqbal, M. 2014. Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions**.** *Eur J Plant Pathol.,* 139:27-37.

Kissoudis, C., van de Wiel, C., Visser, R.G., van der Linden, G. 2014. Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. *Front Plant Sci.,* 5:207.

Lee, B.M., Park, Y.J., Park, D.S., Kang, H.W., Kim, J.G., Song, E.S., Park, I.C., Yoon, U.H., Hahn, J.H., Koo, B.S., *et al.* **2005.** The genome sequence of *Xanthomonas oryzae* pv. *oryzae* KACC10331 the bacterial blight pathogen of rice. *Nucleic Acids Res.* 33:577-586.

Luck, J., Spackman, M., Freeman, A., Trebicki, P., Griffiths, W., Finlay, K., Chakraboraty, S. 2011. Climate change and diseases of food crops. *Plant Pathol.,* 60: 113–121

Madgwick, J.W., West, J.S., White, R.P., Semenov, M.A., Townsend, J.A., Turner, J.A., Fitt, B.D.L. 2011. Impacts of climate change on wheat anthesis and fusarium ear blight in the UK. *Eur J Plant Pathol.,* 130: 117–131

Mayek-Perez, N., Garcia-Espinosa, R., Lopez-Castaneda, C., Acosta-Gallegos, J.A., Simpson, J. 2002. Water relations, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiological *and Molecular Plant Pathology.* 60:185–195.

Mew, T.W., Alvarez, A.M., Leach, J.E., Swings, J. 1993. Focus on bacterial blight of rice. *Plant Dis.,* 77: 5–12.

Mew, T.W., Vera Cruz, C.M., Medalla, E.S. 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. *Plant Dis.,* 76: 1029–1032

Mittler R, Blumwald E. 2010. Genetic engineering for modern agriculture: challenges and perspectives. *Annual Review of Plant Biology*, 61:443–462.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science,* 11: 15–19.

Mohanty, S., Wassmann, R., Nelson, A., Moya, P., Jagadish S.V.K. 2013. Rice and climate change: significance for food security and vulnerability. IRRI Discussion Paper Series No. 49. Los Baños (Philippines): *International Rice Research Institute.* 14 p.

Morison, J.I.L., Baker, N.R., Mullineaux, P.M., Davies, W.J. 2008. Improving water use in crop production. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 363: 639–658.

Moscou, M.J., Bogdanove A.J. 2009. A simple cipher governs DNA recognition by TAL effectors. *Science,* 326: 1501

Natrajkumar, P., Sujatha, K., Laha, G. S., Srinivasarao, K., Mishra, B., Viraktamath, B. C., et al. (2012). Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathology*, 102: 222–228.

Niño-Liu, D.O., Ronald, P.C. and Bogdanove, A. 2006. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Molecular Plant Pathology*, 7: 303–324.

Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A. and Kaku, H. 2005. Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. *Jpn Agric Res Q.,* 39**:** 275-287.

Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)].

Peng, S.B., Huang, J.L., Sheehy, J.E., Laza, R.C., Visperas, R.M., Zhong, X.H., Centeno, G.S., Khush, G.S., Cassman, K.G. 2004. Rice yields decline with higher night temperature from global warming. *Proc. Natl. Acad. Sci.*, 101:9971-9975.

Pieterse, C.M.J, Leon-Reyes, A., Van der Ent, S., Van Wees, S.C.M. 2009. Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, 5: 308–316.

Prasad, P. V. V., Pisipati, S. R., Momčilović, I., Ristic, Z. 2011. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. *Journal of Agronomy and Crop Science*, 197: 430–441.

Prasch, C.M., Sonnewald, U. 2013. Simultaneous application of heat, drought, and virus to Arabidopsis plants reveals significant shifts in signaling networks. *Plant Physiol.*162:1849-66.

Rizhsky, L., Liang, H.J., Shuman, J., Shulaev, V., Davletova, S., Mittler, R. 2004. When defense pathways collide, the response of Arabidopsis to a combination of drought and heat stress. *Plant Physiology*, 134: 1683–1696.

Salzberg, S.L., Sommer, D.D., Schatz, M.C., Phillippy, A.M., Rabinowicz, P.D., Tsuge S., Furutani, A., Ochiai, H., Delcher, A.L. and Kelley, D. 2008. Genome sequence and rapid evolution of the rice pathogen *Xanthomonasoryzae pv. oryzae* PXO99A. *BMC Genomics* 2008, 9: 204.

Scholze, H., Boch, J. 2011. TAL effectors are remote controls for gene activation. *Curr. Opin. Microbiol.,* 14: 47–53.

Shao, H.B., Chu, L.Y., Jaleel, C.A., Zhao, C.X. 2008. Water-deficit stress- induced anatomical changes in higher plants. *Comptes Rendus Biologies*, 331: 215–225.

Sharma RC, Duveiller E, Ortiz-Ferrara G. 2007. Progress and challenge towards reducing wheat spot blotch threat in the Eastern Gangetic Plains of South Asia: is climate change already taking its toll*? Field Crops Research,* 103: 109–118.

Suk-Man, K., Jung-Pil, S., Yang, Q., Tae-Hwan, N., Russell, F.R., Kshirod, K. J. 2015. Identification and fine-mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theor Appl Genet.* 128:1933-1943.

Sun, X., Cao, Y., Yang, Z., Xu, C., Li, X., Wang, S., Zhang, Q. 2004. *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv *oryzae* in rice, encodes an LRR receptor kinase like protein. *Plant J.,* 37: 517–527

Uauy, C., Brevis, J.C., Chen, X. *et al.* , 2005. High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1. Theoretical and Applied Genetics,* 112: 97–105.

Vera Cruz, C.M., Bai, J.F., Ona, I., Leung, H., Nelson, R.J., Mew, T.W., Leach, J.E. 2000. Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. Proc. *Natl. Acad. Sci. U.S.A.,* 97:13500–13505

Verdier, V., Vera Cruz, C., Leach, J.E. 2012. Controlling rice bacterial blight in Africa: Needs and prospects. *J. of Biotech.* 159: 320-328. DOI:10.1016/j.jbiotec.2011.09.020

Wang, B., Xu, H., Zheng, X. Fu, Q., Lu, Z. 2010. Effects of Temperature on Resistance of Rice to Brown Planthopper, *Nilaparvata lugens. Rice Science,* 17: 334–338.

Wang, W.X., Vinocur, B., Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* 218: 1-14

Wang, Y., Bao, Z.L., Zhu, Y., Hua, J. 2009. Analysis of temperature modulation of plant defense against biotrophic microbes. *Molecular Plant-Microbe Interactions* 22: 498–506.

Webb, K.M., Ona, I., Bai, J., Garrett, K.A,, Mew, T., Vera Cruz, C. M. and Leach, J.E. 2010. A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene. *New Phytologist.,* 185: 568–576.

Wu, L.F., Goh, M.L., Sreekala, C., Yin, Z.C. 2008. *XA27* depends on an amino-terminal signal-anchor-like sequence to localize to the apoplast for resistance *Xanthomonas oryzae* pv *oryzae. Plant Physiology,* 148: 1497–1509.

Yang, B., Sugio, A., and White F.F. 2006. *Os8N3* is a host disease susceptibility gene. *Proc. Natl. Acad. Sci. USA.* 103**:** 10503–10508.

Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z.X., Kono, I., Kurata, N., Yano, M., Iwata, N. and Sasaki, T. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc. Natl Acad. Sci. USA.* **95:** 1663–1668.

Chapter 2: Rice response to simultaneous bacterial blight and drought stress: Inverse responses of two major R genes under incompatible and compatible interactions.

Gerbert Sylvestre Dossa[1,3*], Rolando Torres[2], Amelia Henry[2], Ricardo Oliva[1], Edgar Maiss[3], Casiana Vera Cruz[1], Kerstin Wydra[3,4]

1: Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, Los Baños, Philippines

2: Crop and Environmental Sciences Division, International Rice Research Institute, Los Baños, Philippines

3: Department of Phytomedicine, Leibniz Universität Hannover, Hannover, Germany

4: Plant Production and Climate Change, Erfurt University of Applied Sciences, Erfurt, Germany

* Corresponding author: Gerbert S. Dossa; Email: c.dossa@irri.org

Abstract

Xanthomonas oryzae pv. *oryzae* (*Xoo*), the causal agent of rice bacterial blight (BB), is a common reason for severe economic yield losses in rice. Plant response to one type of stress can be affected by simultaneous exposure to a second stress, for example when abiotic and biotic stresses occur together. In this study, ten rice genotypes comprising those with BB resistance (*R*) genes, drought QTLs plus a BB *R* gene, and BB susceptible genotypes, were subjected to mild and moderate drought stress and plants were inoculated with two *Xoo* strains (PXO99 and PXO145) to simulate the challenges rice crops face under simultaneous stress of drought and BB. Plant height, dry shoot biomass and BB disease development were significantly reduced by drought stress treatments. The PXO99 population and spread *in planta* was higher compared to

PXO145 and generally decreased under mild drought stress. Rice IRBB7 (*Xa7*) showed less bacterial spread and a reduced *Xoo* population under drought stress compared to the well-watered control. In contrast, in genotypes with a different BB *R* gene and/or drought QTLs [IRBB4 (*Xa4*), IR87705-6-9-B (*Xa4+qDYT$_{2.2}$*), IR87707-445-B-B-B (*Xa4+qDYT$_{2.2}$+qDYT$_{4.1}$*) and IR87707-446-B-B-B (*Xa4+qDYT$_{2.2}$+qDYT$_{4.1}$*)] an inverse reaction of *Xoo* population and spread *in planta* was observed in which *Xoo* population and spread increased with drought stress. This study has shown the inverse responses of the two major BB *R* genes under drought stress. It is concluded that evaluating rice varieties under combined abiotic and biotic stresses will be the best strategy to evaluate biotic stress resistance durability under climate change.

Keywords: *Xanthomonas oryzae* pv. *oryzae*; bacterial blight; rice; drought stress; *Xa4*; *Xa7*.

Introduction

Plants are limited to a sessile life style and are often exposed to diverse environmental stresses. However, experiments concurrently testing the effect of multiple stresses are typically not performed when developing stress resistant or tolerant varieties. Under field conditions, rice (*Oryza* spp.) like other crops is often simultaneously exposed to a number of biotic and abiotic constraints. Among rice biotic stress factors, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major constraint causing substantial yield losses worldwide. The disease is prevalent in irrigated and rain-fed lowland systems and is favored by leaf surface wetness, high relative humidity, and high temperature (25-30°C). Initially, *Xoo* causes water-soaked leaf lesions and yellowing, and later colonizes the host xylem and turns systemic at an advanced stage of infection. Host resistance remains the most economically effective control measure against bacterial blight disease, and 39 rice resistance genes have been identified to control the disease in Asia (Natraijkumar et al. 2012; Khan et al. 2014; Zhang et al. 2015).

The occurrence of drought is the consequence of increasingly unexpected fluctuations in precipitation. Rice production in general requires a large quantity of water, and drought stress can limit rice production. Rice is a drought-sensitive crop and occurrence of drought stress at the reproductive stage leads to severe yield loss (Venuprasad et al. 2009a). Drought stress affects plant growth through its direct influence on plant water status (Anjum et al. 2011). Imposing drought stress on rice plants can decrease fresh and dry biomass, plant height, tiller number, and panicle number (Bhattacharjee et al. 1973; De Datta 1973; Rahman et al. 2002). Large efforts have gone into the identification of QTLs for rice yield under drought stress (Kumar et al. 2014), and several reports have highlighted the positive effect of drought QTLs on rice yield under drought stress in upland and rainfed lowland rice systems (Bernier et al. 2007; Kumar et al. 2008; Venuprasad et al. 2009b; Swamy et al. 2013).

Climate change is predicted to increase the simultaneous occurrence of abiotic and biotic stresses which may act synergistically in damaging the plant. Wright and Beattie (2004) reported that foliar pathogen growth is restricted by low water availability at an early stage of interaction, suggesting that leaf water content may be correlated with the host plant defense response to pathogens, and that lack of water may restrict the bacterial growth in intercellular spaces. Thus, Beattie (2011) observed that the apoplastic water availability for bacteria is reduced during effector-mediated defense and may have a negative impact on bacteria growth. Several physiological responses are related to water availability, such as stomata closure, increase of ABA, accumulation of compatible solutes and an increase in expression of aquaporin, a regulator of water flow across membranes to maintain the cell turgor (Bartels and Sunkar 2005). These physiological responses help the plant to economize water use, which may interact with pathogen response. For example, under drought stress, water limitation in the apoplast may affect bacterial growth and movement - bacterial movement inside the host plant is regulated by its flagellae, which is favored by water availability in the leaf apoplast. An early study on colonization and movement of *Pseudomonas syringae* on bean seedlings suggested that a greater spread of the bacterium was promoted by water (Leben et al. 1970), and an abundance of free water has been reported to favor phyllosphere tissue entry by bacteria (Beattie 2011). However, bacteria can still move by swarming motility under limited water content (Hattermann and Ries 1989; Kearns 2010; Beattie 2011).

Continued climate change is expected to have effects on *R* gene-mediated responses to pathogen invasions. Long-term abiotic stress can increase host susceptibility to pathogen attack (Amtmann et al. 2008; Atkinson and Urwin 2012). A higher susceptibility of sorghum and common bean to *Macrphomina phaseolina* under drought stress has been reported (Diourte et al. 1995; Mayeke-Perez et al. 2002). Arabidopsis exposed to drought showed a higher infection level of an otherwise avirulent

Pseudomonas syringae strain (Mohr and Cahill 2003). In rice, simultaneous effects of drought stress and BB on rice resistance (*R*) gene-mediated resistance is still unknown. Therefore, we hypothesized that combined stresses of drought and BB would affect rice *R* gene-mediated resistance to BB. Understanding this complex interaction will provide information on how current BB *R* genes will respond under future climate change conditions. In this study, two major rice *R* genes in different genotypes were evaluated for their effects on BB symptoms under vegetative-stage drought in terms of bacterial development, multiplication, and movement *in planta*. The most resistant rice genotypes currently available for BB and drought stress were included in order to understand the range of potential responses of rice to these combined stresses.

2- Materials and methods

2-1- Rice genotypes and bacterial strains

In order to study drought stress effects on bacterial blight development, ten rice genotypes were selected containing different combinations of *R* genes and *R* genes combined with drought QTLs (Table 1). The first group consisted of rice bacterial blight monogenic resistance lines IRBB4 (with *R* gene *Xa4*), IRBB7 (with *R* gene *Xa7*), IR64 (with *R* gene *Xa4*), and bacterial blight susceptible check IR24. The second group consisted of pyramided bacterial blight resistance lines IRBB61 (with *R* genes *Xa4*, *xa5*, *Xa7*), IRBB67 (with *R* genes *Xa4*, *Xa7*) and PSBRc82 (with *R* genes *Xa4*, *xa5*). The third group consisted of IR64 (with *R* gene *Xa4*) and IR64 introgression lines with drought QTLs *qDTY$_{2.2}$* and *qDTY$_{4.1}$* (IR87707-445-B-B-B and IR87707-446-B-B-B) (Kumar et al. 2014) and q*DTY$_{2.2}$* only (IR87705-6-9-B).

Two *Xoo* strains from the Philippines in the collection at the International Rice Research Institute (IRRI) were used. We used PXO145 (*avrXa4+avrxa5+avrXa7*) for the incompatible interaction and PXO99 for the compatible interaction.

2-2- PVC-tube cultivation and drought stress application

This study was conducted under greenhouse conditions at IRRI (14° 11'N, 121° 15'E). Two levels of drought stress were established: mild (MiDST; maintained at 70% of field capacity) and moderate (MDST; maintained at 50% of field capacity). The experiment was conducted in PVC cylinders of 10.8 cm diameter and 21 cm height following a split plot design with drought stress as the main factor, bacterial strains as a sub-factor, and genotype as a sub-sub factor with 3 replications for each strain per drought stress treatment and two plants per cylinder. The seeds of each genotype were pre-germinated for 4 days in the dark at 30 °C. Each cylinder was filled with 1.5 kg of dry soil mixed with 0.3 g of ammonium sulfate and watered to field capacity.

Progressive drought stress was initiated at 14 days after sowing (das) and continued until the end of the experiment by imposing a gradual reduction over a period of 7 days. The cylinders were weighed every two days and water was added when soil moisture dropped below the target level. At 21 das, the plants were maintained at the target soil moisture level until the end of the experiment. The well-watered control was maintained flooded for the whole experiment. Two different trials were performed: Trial 1 from March-April 2014 with average temperature 35.2°C, and Trial 2 from April to May 2014 with average temperature of 33.6°C. The average relative humidity was 54.4% in Trial 1 and 58.2% in Trial 2.

2-3- Bacterial blight inoculation and evaluation

X. oryzae pv. *oryzae* strains were grown on Modified Wakimoto's medium (Leach et *al.*, 1992) for 72h. Inocula were prepared by suspending the 72h-old culture in sterilized demineralized water and inoculum concentration was adjusted to 5×10^8 CFU/ml. Leaf-clip inoculation (Kauffmann et al. 1973) was performed at 21 das. Bacterial blight lesion lengths were measured at 32 das.

2-4- Bacterial multiplication *in planta*

To assess drought stress effects on *Xoo* development and spread *in planta* from both compatible and incompatible interaction, the second leaf of the main tiller of each inoculated plant from each soil moisture condition was collected. Each leaf was separated into segments of 5 cm in length. Each segment was crushed in phosphate buffered saline [Sodium Chloride (NaCl): 8.0g, Potassium Chloride (KCl): 0.2g, Sodium Phosphate (Na_2HPO_4): 1.44g, Potassium Phosphate (KH_2PO_4): 0.24g for 1 liter preparation of phosphate buffered saline] and dilution series were prepared from the homogenate. Fifteen (15) µl of each dilution were spotted on Suwa's medium [Sodium Glutamate: 2.0g; $MgCl_2$ ($6H_2O$): 1.0g; KH_2PO_4: 0.1g; Peptone: 17.0g; Sucrose: 5.0g; Agar: 17.0g and Fe-EDTA: 6.57g for 1 liter of medium] containing 0.8% of Cycloheximide antibiotic [8mL from stock solution (1g/100mL H_2O) for 1L of medium] and 0.2% of Cephalexin antibiotic [4mL from stock solution (0.5g/100mL H_2O) for 1 L of medium], with 3 spots per dilution and 2 replications. *Xoo* colonies were counted after 48h and the bacterial number was calculated in number of colony forming units (CFU) per ml for each segment. The whole experiment was replicated and the logarithm 10 of the mean values was used to evaluate BB spread along the length of the leaf.

2-5- Measurement of plant morphological traits

Plant height was measured on a single plant per cylinder at 32 das. The plant height was measured from soil surface to the tip of the most developed leaf of each plant. Shoots were harvested from all cylinders at 32 das. Shoot dry biomass was recorded after 72h of oven drying at 70°C.

2-6- Data analysis

The plant height, shoot dry biomass, and bacterial blight lesion length data were subjected to homogeneity of variance test using residual plots, followed by ANOVA. The homogeneity of variance test indicated no significant effect of the trial on plant

height and shoot dry biomass; therefore the data from the three replicates of each trial were combined for subsequent analyses. The bacterial blight lesion length data were analyzed separately by trial because a significant effect of trial was observed. Analyses of variance based on split plot design were performed on each trial using Statistical Tool of Agricultural Research (STAR v.2.0.1). Means differences of BB lesion lengths showed on each genotype were compared between drought stress treatment using Tukey Honest Significant Differences (HSD) test at α=0.05. For plant height, dry shoot biomass, Least Significant Differences (LSD) values were used to differentiate the mean values at the 95% confidence level.

3-Results

3-1-Plant height reduction with drought stress increased

All genotypes showed plant height reduction as drought stress increased (Fig. 1). An average plant height reduction of approximately 20 cm was generally observed between the well-watered mild drought treatments, and approximately 30 cm between well-watered and moderate drought stress treatments. Under well-watered and mild drought stress treatment, no differences in plant height were observed between genotypes (Figure 1). Genotypes IR87707-445-B-B-B and IR87707-446-B-B-B plant height were significantly different to other genotypes.

Drought stress has significant effects (p-value <2e-16) on shoot dry biomass with shoot dry biomass reduction under drought stress. No differences were observed between genotypes across the three treatments (Fig. 2)

3-2- Rice *R* genes *Xa4* and *Xa7* reactions to *X. oryzae* pv. *oryzae* under different drought stress

Bacterial blight lesion lengths were generally higher in plants inoculated with PXO99 compared to plants inoculated with PXO145. The BB lesion lengths under drought

stress varied according to rice genotypes and between trials. BB lesion lengths in IR87705-6-9-B from both mild and moderate drought stress were generally not significantly different compared to the well-watered treatment in both Trial 1 and Trial 2 (Fig. 3A, 3B, 3C, 3D). The susceptible genotype IR24 showed the greatest lesion length reduction with both strains by drought stress, although the lesion length remained higher compared to other genotypes.

3-2-1- Incompatible interaction

Rice *R* genes *Xa4*, *xa5* and *Xa7* confer resistance to PXO145 (*avrXa4+avrxa5+avrXa7*) strain. The average lesion lengths of less than 5 cm are clustered as resistant, and those higher than 5 cm as susceptible. In the well-watered treatments, all rice genotypes' average lesion lengths were below 5 cm, except for the susceptible genotype IR24, of which the average lesion length was higher than 5 cm (Fig. 3A, 3B). The effects of different drought stress conditions on BB disease development generally showed a decrease of BB lesion lengths with increased drought stress. After inoculation with strain PXO145, lesion lengths were reduced in four genotypes (IR24, IR64, IR87707-446-B-B-B, IRBB7) in the moderate drought stress treatment compared to the well-watered treatment, while a tendency of increase in lesions lengths was observed in six genotypes (IRBB4, IR64, IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B, IRBB67) in the mild drought stress treatment. IR64 and IR87707-446-B-B-B showed lesion length reduction under moderate drought stress compared to well-watered and mild drought stress treatments (Fig. 3A). In plants inoculated with PXO145 in the second Trial, four genotypes (IR64, IR87707-445-B-B-B, IR87707-446-B-B-B, PSBRc82) showed significant BB lesion lengths' increase under mild drought stress compared to well-watered treatment, with two genotypes IR87707-445-B-B-B and IR87707-446-B-B-B showing significant BB lesion lengths' increase under moderate drought stress in comparison to the well-watered treatment (Fig. 3B). Analyzing the reactions of genotypes carrying the

single *Xa4* gene inoculated with PXO145, lesion lengths were significantly higher in mild drought stress compared to the well-watered treatment in IRBB4 and IR87707-445-B-B-B, while the lesion lengths were not significantly different to well-watered in IR64 and IR87707-446-B-B-B (Fig. 3A). IR64, IR87707-445-B-B-B and IR87707-446-B-B-B showed significant BB lesion length increase under mild drought stress compared to the well-watered treatment, and lesion lengths in IRBB4 were not significantly different across the three water stress treatments (Fig. 3B). BB lesion lengths in genotype IRBB7 carrying *Xa7* under drought stress were generally reduced. Rice genotypes with a combination of *Xa4+xa5*, *Xa4+xa5+Xa7* and *Xa4+Xa7* in IRBB61, PSBRc82 and IRBB67, respectively, showed slight BB lesion length increase under mild drought stress after inoculation with PXO145, which was significant in PSBRc82 in Trial 2.

3-2-2- Compatible interaction

Strain PXO99 was generally virulent on all genotypes used in this study, since the average lesion length was always above 5 cm in the well-watered treatment. In drought stress treatments compared to well-watered treatment, in plants inoculated with strain PXO99, BB lesion lengths were generally reduced (Fig. 3C & 3D). Three (IR64, IR87707-446-B-B-B, IRBB67) of the ten genotypes showed BB lesion lengths' reductions when drought stress increased. Genotypes IR24, PSBRc82, IRBB7 showed a tendency of BB lesion length reduction under mild and moderate drought stress conditions – while genotypes IR64 and IR87707-445-B-B-B showed significant BB lesion lengths' reduction under moderate drought stress compared to well-watered treatment (Fig. 3C). In the second trial with inoculation with PXO99, BB lesion lengths were reduced in four genotypes (IR64, IR87707-446-B-B-B, PSBRc82 and IRBB7), when drought stress increased from mild to moderate drought stress. Genotypes IRBB4, IRBB61 and IRBB67 showed BB lesion length reduction under moderate drought stress compared to well-watered treatment (Fig. 3D). Drought QTL genotypes (IR87707-445-B-B-B and IR87707-

446-B-B-B) under moderate drought stress inoculated with PXO99 showed generally reduced BB lesion length in Trial 1 and in Trial 2.

3-3- *In planta Xoo* populations and spread increased in rice genotypes with the single *Xa4* gene under mild drought stress.

We investigated the possible role of drought stress on bacterial multiplication and movement *in planta* and found that *in planta Xoo* spread was reduced by drought stress, especially by moderate drought stress compared to the well-watered treatment, except in rice genotype with single *Xa4 R* gene (Fig. 4). Comparison of BB lesion length and *Xoo* spread *in planta* revealed that *Xoo* progressed beyond the symptomatic area in both drought stress and well-watered conditions (Figs. 4 & 5). Similarly to the drought stress effects on BB lesion length, *Xoo* numbers *in planta* generally did not show significant differences between water stress treatments.

3-3-1- Incompatible interaction

In the incompatible interaction PXO145 (*avrXa4*+*avrxa5*+*avrXa7*) spread less in IR87705-6-9-B with a lower *Xoo* number under the well-watered treatment than in mild drought and moderate drought stress treatments. Similar to IR87705-6-9B, genotype IR87707-445-B-B-B showed less *Xoo* spread *in planta* under the well-watered treatment. Genotypes PSBRc82 and IRBB67 showed *in planta Xoo* spread up to 20 cm (segment A to D) and 25 cm (segment A to E), respectively, under the well-watered treatment. Other genotypes such as IR24, IR64, IR87707-446-B-B-B, IRBB61 and IRBB7 showed highest *Xoo* spread *in planta* under the same treatment, and in IRBB4, *Xoo* spread up to 30 cm (Fig. 4).

Under drought stress (mild drought stress), *Xoo* spread increased in genotype IR24, IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B, IRBB4 and PSBRc82 while it decreased in genotypes IRBB67 and IRBB7. Genotypes IR64 and IRBB61 did not show

changes in *Xoo* spread under mild drought stress with PXO145. Under increased drought stress, *Xoo* spread *in planta* was reduced in IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B, IRBB61, IRBB67, IRBB7 and PSBRc82 (Fig. 4). Genotypes IR24 and IRBB4 showed *Xoo* spread up to 40 cm under moderate drought stress while *Xoo* spread in IR64 remained with no change (35 cm).

3-3-2- Compatible interaction

With *Xoo* strain PXO99, bacterial spread *in planta* was generally reduced with increasing drought stress. Genotypes IR64, IR87707-445-B-B-B, IRBB7 and PSBRc82 showed the highest (40 cm) *Xoo* spread under the well-watered treatment (Fig. 5). In IRBB4, it spread less (30 cm) under the same treatment. With drought stress, *Xoo* spread more in IRBB4 (40 cm) compared to well-watered treatment. Genotype IRBB7 showed the lower *Xoo* spread (20 cm) under moderate drought stress. In the compatible interaction (PXO99) *Xoo* numbers *in planta* were generally higher than *Xoo* numbers *in planta* in the incompatible interaction (PXO145). The maximum average lesion length was 17 cm, recorded on IR24 inoculated with PXO99 in the well-watered treatment (Fig. 3), while *Xoo* spread was up to 35 cm in the same plants (Fig. 5).

4- Discussion

In this study, BB lesion lengths in rice leaves varied by drought stress severity as well as genotype and the *Xoo* strain inoculated. Bacterial blight disease infection was generally reduced when drought stress increased in severity from well-watered to mild drought to moderate drought stress in compatible and incompatible interactions. The use of a range of rice genotypes with different combinations of BB *R* genes and drought QTLs in this study has shown that plant response to pathogens following a gene for gene interaction could be affected by drought stress.

In the compatible interaction, *Xoo* strain PXO99 virulence was reduced under drought stress. However, *Xoo* strain PXO145 induced longer BB lesion lengths - especially in mild drought stress, Trial 2 - in the susceptible genotype IR24 under drought stress compared to lesion lengths induced by PXO99. Foliar pathogen growth has been previously reported to be restricted by low water availability (Wright and Beattie 2004) and our results indicate that decrease in soil water content reduced BB lesion length, and additionally the different disease responses could be related to the genetic background of rice genotypes. Furthermore, the BB lesion lengths with PXO99 observed under well-watered conditions compared to lesion lengths under drought stress indicated that virulence of the strain was reduced by drought stress. Moreover, PXO145 had shown increase in both BB lesion length and *Xoo* numbers in IR64, IR87707-445-B-B-B, IR87707-446-B-B-B and PSBRc82 under mild drought stress compared to well-watered conditions while the lesion length with PXO99 was reduced under low drought stress, further highlighting the variable responses of different rice genotypes to *Xoo* inoculation.

Although lesion length was generally reduced with increasing drought stress, more bacteria were recorded from different segments of inoculated leaves under mild drought stress compared to the well-watered and moderate drought stress treatments, especially in rice genotypes with single *Xa4* gene. Moreover, the BB lesion lengths, although reduced under moderate drought stress, were higher on genotypes with the single *Xa4* gene compared to genotypes with the *Xa7* gene when inoculated with PXO145 (*avrXa4+avrxa5+avrXa7*), suggesting that *Xoo* multiplication and spread *in planta* and BB lesion lengths depended on the resistance gene. Mild drought stress may favor *Xoo* multiplication and spread *in planta*, possibly through negative effects on host immune responses leading to increased bacterial multiplication and allowing *Xoo* movement *in planta*. Therefore, we hypothesized that mild drought stress possibly

created a negative impact on those genotypes by lowering their resistance to bacterial blight. Also Webb et al. (2010) observed that rice *Xa4* gene mediated resistance to bacterial blight is compromised under high temperature as similarly our study revealed that *Xa4* resistance was compromised under drought stress, particularly under mild drought stress. Additional, rice genotype IRBB7 (*Xa7*) showed lesion length, *in planta Xoo* multiplication and spread reduced with drought stress increased, suggesting that *Xa7* resistance to bacterial blight increase was associated with leaf water loss. This result is consistent with the findings of Freeman and Beattie (2009) who reported that host resistance to bacteria is associated to leaf water loss in *Arabidopsis thaliana*.

Although the disease symptoms were less developed under drought stress, this study showed that *Xoo* spread *in planta* extended beyond the symptomatic area under both compatible and incompatible interactions. The increase in *Xoo* spread *in planta* in IRBB4, IR64, IR87707-445-B-B-B, IR87707-446-B-B-B, and IR87705-6-9-B under drought stress could be associated with BB *R* gene *Xa4* response. In contrary to *Xa4* response, rice genotype IRBB7 carrying BB *R* gene *Xa7* showed lower *Xoo* numbers and restricted *Xoo* spread *in planta* under drought stress. Since drought stress results in stomatal closure, drought stressed leaves are typically higher in temperature (Garrity and O'Toole 1995) than well-watered leaves. Therefore, our results appear to be consistent with the observation of less effectiveness of IRBB4 (*Xa4*), and increased effectiveness of IRBB7 (*Xa7*) under high temperature that has been previously reported (Webb et al. 2010). *Xa7* response to BB under drought stress compared to well-watered conditions may be synergistically linked with abiotic stress response genes. In contrast to lower *Xoo* numbers observed with IRBB4 under well-watered conditions (Fig. 4), IRBB7 showed high *Xoo* numbers. Collectively, these results suggest that resistance conferred by *Xa4* was less effective under drought and high temperature conditions, and that IRBB7 may respond more efficiently to BB under climate change situations.

Development of rice genotypes with tolerance and resistance to combined abiotic and biotic stress would be suitable under climate change conditions and could consequently contribute to addressing food security problems. Tippmann et al. (2006) hypothesized that simultaneous occurrence of abiotic and biotic stresses can lead to host susceptibility or resistance, and that the outcome may depend on the stress and pathogen. Our study further builds on this hypothesis by suggesting that the outcome of multiple stress interaction may also be influenced by host plant genetic background (Figure 6). Moreover, the microclimates in which the plants are growing can also influence the plant-pathogen interaction, as indicated by the longer disease lesion lengths in Trial 2 during which the relative humidity (58.2%) was higher compared to Trial 1 (54.4%).

Rice genotypes IRBB7 (*Xa7*), IRBB61 (*Xa4+xa5+Xa7*), IRBB67 (*Xa4+Xa7*) and IR87705-6-9-B (*Xa4/qDTY$_{2.2}$*) showed less disease development with strain PXO145 (*avrXa4+avrxa5+avrXa7*) while IRBB4 (*Xa4*), IR64 (*Xa4*), IR87707-445-B-B-B (*Xa4/qDTY$_{2.2}$+qDTY$_{4.1}$*), IR87707-446-B-B-B (*Xa4/qDTY$_{2.2}$+qDTY$_{4.1}$*) were less effective under simultaneous application of drought and bacterial blight stresses, demonstrating the genetic background effect on the interaction. Moreover, both increase and decrease of disease lesion length under drought stress revealed the complex interaction leading to physiological and molecular responses occurring in plants exposed to simultaneous abiotic and biotic stresses. The role that is played by different BB *R* genes and drought tolerance QTL*s* during stress combinations may also indicate which *R* genes are to be considered for variety improvement to cope with climate changes. Furthermore, Dossa et al. (2015) propose to customize bacterial blight resistance varieties deployment and in this study, the two major *R* genes (*Xa4* and *Xa7*) used demonstrated that *Xa4* effectiveness was compromised under drought stress while *Xa7* effectiveness was enhanced when inoculated with PXO145 (*avrXa4+avrxa5+avrXa7*) suggesting an inverse response to BB when drought stress increased (Figure 6) and suggesting that rice *R* gene

Xa7 should be taken into account for varietal improvement for combinations of abiotic and biotic traits expected under climate change. Rice genotypes with drought QTL*s* were not morphologically (plant height and dry biomass) distinct from varieties without these QTLs, suggesting that these specific drought QTL*s* responsiveness to water stress at seedling stage was minimal and may not be involved in plant water uptake under water stress conditions at seedling stage. This result is correlated to the report from Demirevska et al. *(*2009) who suggested that plant tolerance to water deficit depends on stress level, plant species, and also developmental stage. Moreover, Henry et al. (2015) reported no significant differences in shoot biomass, root architecture and anatomy in IR64 NILs at seedling stage. Tolerance of genotypes with drought QTL*s* to drought stress at advanced growth stage may be beneficial to BB disease development. Drought tolerant genotypes may have the capability to avoid water loss through stomata or develop deep roots for water uptake from deeper soil as reported in *Arabidopsis thaliana* (Yu et *al.,* 2008) and in transgenic rice (Yu et al. 2013) and a high root hydraulic conductivity might occur in IR87707-445-B-B-B and IR87707-446-B-B-B (Henry et al. 2015). This suggests that a combination of drought QTL*s* and suitable BB *R* genes could enhance rice resistance and tolerance to simultaneous stresses of drought and bacterial blight. More research at vegetative and reproductive stages is necessary to evaluate these possibilities.

In summary, BB lesion length development was generally reduced under drought stress conditions, but bacterial blight multiplication and spread varied according to rice genetic background. Rice BB single *R* gene *Xa4* in interaction with PXO145 under mild and moderate drought stresses failed to limit BB multiplication and spread compared to well-watered conditions. However, *Xa7* in IRBB7 showed less BB multiplication and restricted BB spread under moderate drought stress with *Xoo* strains PXO145, as well as with *Xoo* strain PXO99 which is virulent on IRBB7, suggesting that *Xa7* benefited from

the drought stress for its resistance to bacterial blight under incompatible interaction and response to drought stress reduced the BB multiplication and spread *in planta* with PXO99. Furthermore, mechanistic understanding gained on the impact of drought stress on BB *R* gene mediated resistance to bacterial blight would provide better insights into the rice and bacterial blight pathosystem for rice varieties' improvement under climate change.

References

1. Anjum, S., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., Wang, L., (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research, 6, 2026-2032.

2. Atkinson, N.J., & Urwin, P.E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany, 63, 3523-3544.

3. Bahattacharjee, D.P., Krishnayya, G.R., Ghosh, A.K., (1973). Analyses of yield components and productive efficiency of rice varieties under soil moisture deficit. Indian J. Agron., 16, 314-343.

4. Bartels, D., & Sunkar, R., (2005). Drought and Salt Tolerance in Plants. Critical Reviews in Plant Sciences, 24, 23-58.

5. Beattie, G.A., (2011). Water relations in the interaction of foliar bacterial pathogens with plants. Annu Rev Phytopathol., 49, 533-55.

6. Bernier, J., Kumar, A., Venuprasad, R., Spaner, D., Atlin, G., (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci, 47, 505-516. doi: 10.2135/cropsci2006.07.0495

7. De Datta, S.K., Abilay, W.P., Kalwar, G.N., (1973). Water stress effect on flooded tropical rice. 2. Water management in Philippines Irrigation System Research and Operation, pp: 16-36.

8. Demirevska, K., Zasheva, D., Dimitrov, R., Simova-Stoilova, L., Stamenova, M., Feller, U., (2009). Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. Acta Physiol. Plant., 31, 1129-1138.

9. Diourte, M., Starr, J.L., Jeger, M.J., Stack, J.P., Rosenow, D.T., (1995). Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water-stress on disease development in sorghum. Plant Pathology, 44, 196-202.

10. Dossa, S.G., Sparks, A., Vera Cruz, C., Oliva, R., (2015). Decision tools for bacterial blight resistance gene deployment in rice-based agricultural ecosystems. Front. Plant Sci., 6, 305. doi: 10.3389/fpls.2015.00305

11. Freeman, B.C., & Beattie, G.A., (2009). Bacterial growth restriction during host resistance to *Pseudomonas syringae* is associated with leaf water loss and localized cessation of vascular activity in *Arabidopsis thaliana*. Mol Plant Microbe Interact, 22, 857-867.

12. Garrity, D.P., & O'Toole, J.C., (1995). Selection for reproductive stage drought avoidance in rice, using infrared thermometry. Agron. J., 87, 773-779.

13. Hattermann, P.D., & Ries, S.M., (1989). Motility of *Pseudomonas syringae* pv. *glycinea* and its role in infection. *Phytopathol.,* 79, 284-289.

14. Henry, A., Swamy, M.B.P., Dixit, S., Torres, R.D., Batoto, T.C., Manalili, M., et al. (2015). Physiological mechanisms contributing to the QTL-combination effects on improved performance of IR64 rice NILs under drought. J. Exp. Bot, 66, 1787-1799. doi: 10.1093/jxb/eru506

15. Kauffman, H.E., Reddy, A., Hsieh, S.P.Y., Merca, S.D., (1973). An improved technique for evaluating resistance of varieties to *Xanthomonas oryzae* pv. *oryzae*. Plant Dis. Rep., 57, 537-541.

16. Khan, M.A., Naeem, M., Iqbal, M., (2014). Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. Eur J Plant Pathol., 139, 27-37.

17. Kumar, A., Bernier, J., Verulkar, S., Lafitte, H.R., Atlin, G.N., (2008). Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. Field Crop Res, 107, 221-231. doi: 10.1016/j.fcr.2008.02.007

18. Kumar, A., Dixit, S., Ram, T., Yadaw, R.B., Mishra, K.K., Mandal, N.P., (2014). Breeding high-yielding drought-tolerant rice: genetic variations and

conventional and molecular approaches. J. Exp. Bot., 65, 6265-6278. doi:10.1093/jxb/eru363

19. Leben, C., Schroth, M.N., Hildebrand, D.C., (1970). Colonization and movement of *Pseudomonas syringae* on healthy bean seedlings. Phytopathol., 60, 677-80

20. Mayek-Perez, N., Garcia-Espinosa, R., Lopez-Castaneda, C., Acosta-Gallegos, J.A., Simpson, J., (2002). Water relations, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiological and Molecular Plant Pathology, 60,185–195.

21. Mohr, P.G., & Cahill, D.M., (2003). Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to *Pseudomonas syringae* pv. *tomato* and *Peronospora parasitica*. Funct. Plant Biology, 30, 461-469. DOI: 10.1071/FP02231

22. Natraijkumar, P., Sujatha, K., Laha, G.S., Srinivasarao, K., Mishra, B., Viraktamath, B.C., et al. (2012). Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathology, 102, 222-228.

23. Rahman, M.T., Islam, M.T., Islam, M.O., (2002). Effect of water stress at different growth stages on yield and yield contributing characters of transplanted aman rice. Pak. J. Biol. Sci., 5, 169-172.

24. Ramegowda, V., & Senthil-Kumar, M., (2015). The interactive effects of simultaneous biotic and abiotic stresses on plants: Mechanistic understanding from drought and pathogen combination. Journal of Plant Physiology, 176, 47-54.

25. Swamy, B.P.M., Ahmed, H.U., Henry, A., Mauleon, R., Dixit, S., et al. (2013). Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. PLoS ONE, 8, e62795. doi:10.1371/journal.pone.0062795

26. Tippmann, H.F., Schlüter, U., Collinge, D.B., (2006). Common themes in biotic and abiotic stress signaling in plants. *In Floriculture, ornamental and plant biotechnology*, Middlesex, UK: Global Science Books; 2006, pp. 52-67.

27. Venuprasad, R., Bool, M.E., Dalid, C.O., Bernier, J., Kumar, A., et al. (2009a). Genetic loci responding to two cycles of divergent selection for grain yield under drought stress in a rice breeding population. Euphytica, 167, 261-269. doi: 10.1007/s10681-009-9898-3

28. Venuprasad, R., Dalid, C.O., Delvalle, M., Zhao, D., Espiritu, M., et al. (2009b). Identification and characterization of large-effect quantitative trait loci (QTL) for grain yield under lowland drought stress and aerobic conditions in rice using bulk-segregant analysis (BSA). Theor Appl Genet, 120, 177-190. doi: 10.1007/s00122-009-1168-1

29. Webb, K.M., Ona, I., Bai, J., Garrett, K.A., Mew, T., Vera Cruz, C.M., Leach, J.E., (2010). A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene. New Phytologist, 185, 568-576.

30. Wright, C.A., & Beattie, G.A., (2004). *Pseudomonas syringae* pv. *tomato* cells encounter inhibitory levels of water stress during the hypersensitive response of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA, 101, 3269-74.

31. Xu, P., Chen, F., Mannas, J.P., Feldman, T., Sumner, L.W., Roossinck, M.J., (2008). Virus infection improves drought tolerance. New Phytologist, 180, 911-921.

32. Yu, L., Xi, C., Zhen, W., Shimei, W., Yuping, W., Qisheng, Z., Shigui, L., Chengbin, X., (2013). Arabidopsis enhanced drought tolerance1/homeodomain glabrous11 confers drought tolerance in transgenic rice without yield penalty. Plant Physiology, 162, 1378-1391.

33. Yu, H., Chen, X., Hong, Y.Y., Wang, Y., Xu, P., Ke, S.D., Liu, H.Y., Zhu, J.K., Oliver, D.J., Xiang, C.B., (2008). Activated expression of an *Arabidopsis* HD-START

protein confers drought tolerance with improved root system and reduced stomatal density. Plant Cell, 20, 1134-1151.

34. Zhang, F., Zhuo, D-L., Zhang, F., Huang, L-Y., Wang, W-S., Xu, J-L., Vera Cruz, C., Li Z-K,, Zhou, Y-L., (2015). *Xa39*, a novel dominant gene conferring broad-spectrum resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. Plant Pathology, 64, 568-575.

Table1: List of rice genotypes used in this study and their corresponding *R* genes and QTLs

S/N	Genotypes	*Xa* genes/QTLs
1	IR24	*Xa18**
2	IRBB4	*Xa4*
3	IR64	*Xa4*
4	IR87705-6-9-B	*Xa4/qDTY$_{2.2}$*
5	IR87707-445-BBB	*Xa4/qDTY$_{2.2}$+qDTY$_{4.1}$*
6	IR87707-446-BBB	*Xa4/ qDTY$_{2.2}$+qDTY$_{4.1}$*
7	PSBRc82	*Xa4+xa5*
8	IRBB61	*Xa4+xa5+Xa7*
9	IRBB67	*Xa4+Xa7*
10	IRBB7	*Xa7*

*Not effective in most Asian countries, except Myanmar and Africa

Rice BB *R* gene is designated by *Xa* and drought QTL as *qDTY*

List of Figures

Figure 1: Plant height of ten rice genotypes under well-watered (WW), mild drought stress (MiDST; 70% soil moisture) and moderate drought stress (MDST; 50% soil moisture). The letters a, b, c and d indicate the significant differences of plant height between genotypes at each stress level as determined by Tukey HSD test ($p<0.05$).

Figure 2: Dry shoot biomass of 10 rice genotypes under different drought stresses. The shoot biomass was collected from 32 days-old plants. WW, MiDST and MDST represent well-watered (control), mild drought stress (70% soil moisture) and moderate drought stress (50% soil moisture), respectively. The letters a and b indicate the significant differences of the dry biomass between genotypes at each stress level as determined by Tukey HSD test ($p<0.05$). No significant differences between genotypes under mild drought stress.

Figure 3: Bacterial blight lesion length comparison from 10 rice genotypes at each level of stress. A) plants inoculated with *Xoo* strain PXO145 in Trial 1; B) plants inoculated with *Xoo* strain PXO145 in Trial 2; C) plants inoculated with *Xoo* strain PXO99 in Trial 1; D) plants inoculated with *Xoo* strain PXO145 in Trial 2. The letters a, b, c and d indicate the significant differences of lesion length between drought stress and at genotype level as determined by LSD test ($p<0.05$).

Figure 4: PXO145 numbers and spread *in planta* under different soil moisture conditions. *Xoo* colonies were counted from each segment after 48h of incubation following leaf sampling. WW: well-watered (control), MiDST: mild drought stress (drydown to 70% of field capacity), MDST: moderate drought stress (drydown to 50% of field capacity). *Xa18* is not effective in most Asian countries, except Myanmar and Africa. *Xa4*, *xa5* and *Xa7* are resistant to PXO145 strain.

Figure 5: PXO99 numbers and spread *in planta* under different soil moisture conditions. *Xoo* colonies were counted from each segment 48h after incubation. WW, MiDST and MDST represent well-watered (control), mild drought stress (70% soil moisture) and moderate drought stress (50% soil moisture), respectively. *Xa18* is not effective in most Asian countries, except Myanmar and Africa. PXO99 is virulent on *Xa4*, *xa5* and *Xa7*.

Figure 6: A proposed model of *Xa4* vs *Xa7* mediated responses to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) under drought stress. Plants with *Xa4* and *Xa7* showed an inverse response to BB under drought stress. *Xoo* numbers and spread *in planta* increased with increasing drought stress on plants with *Xa4*. Plants with *Xa7* in contrast had *Xoo* numbers and spread restricted when drought stress increased. The model proposed here suggests that *Xa7* resistance follows the drought stress gradient, while *Xa4* resistance decreases when stress increased. The green plant represents a healthy plant while the dark yellow and yellow plants are resistance reduced plants.
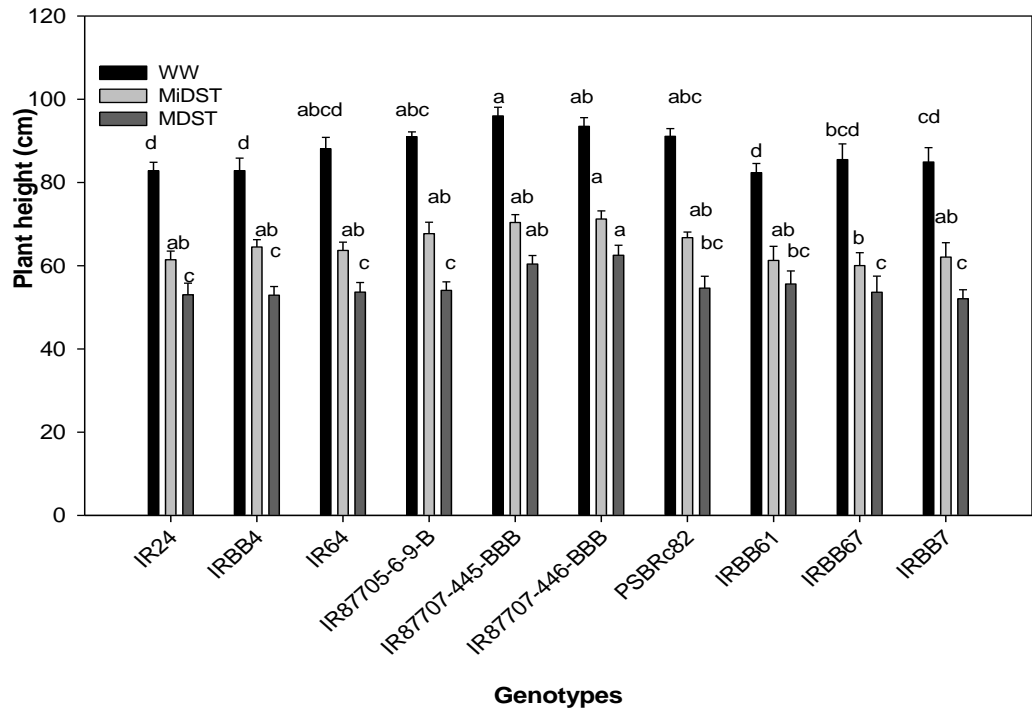
Figure 1
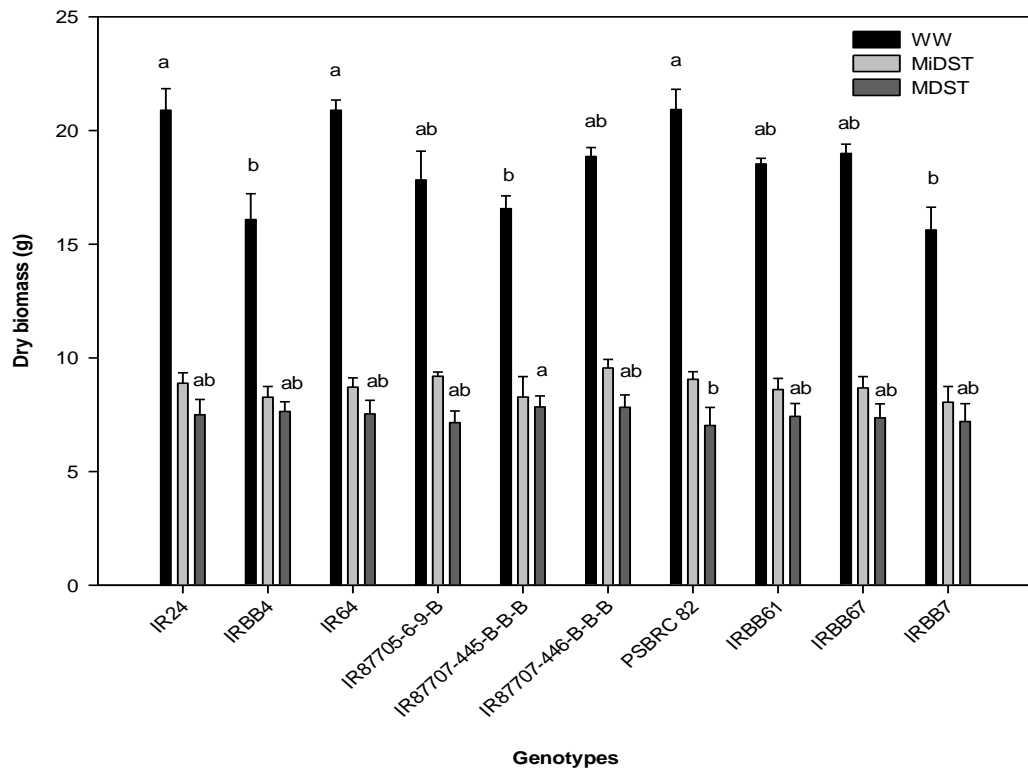
Figure 2
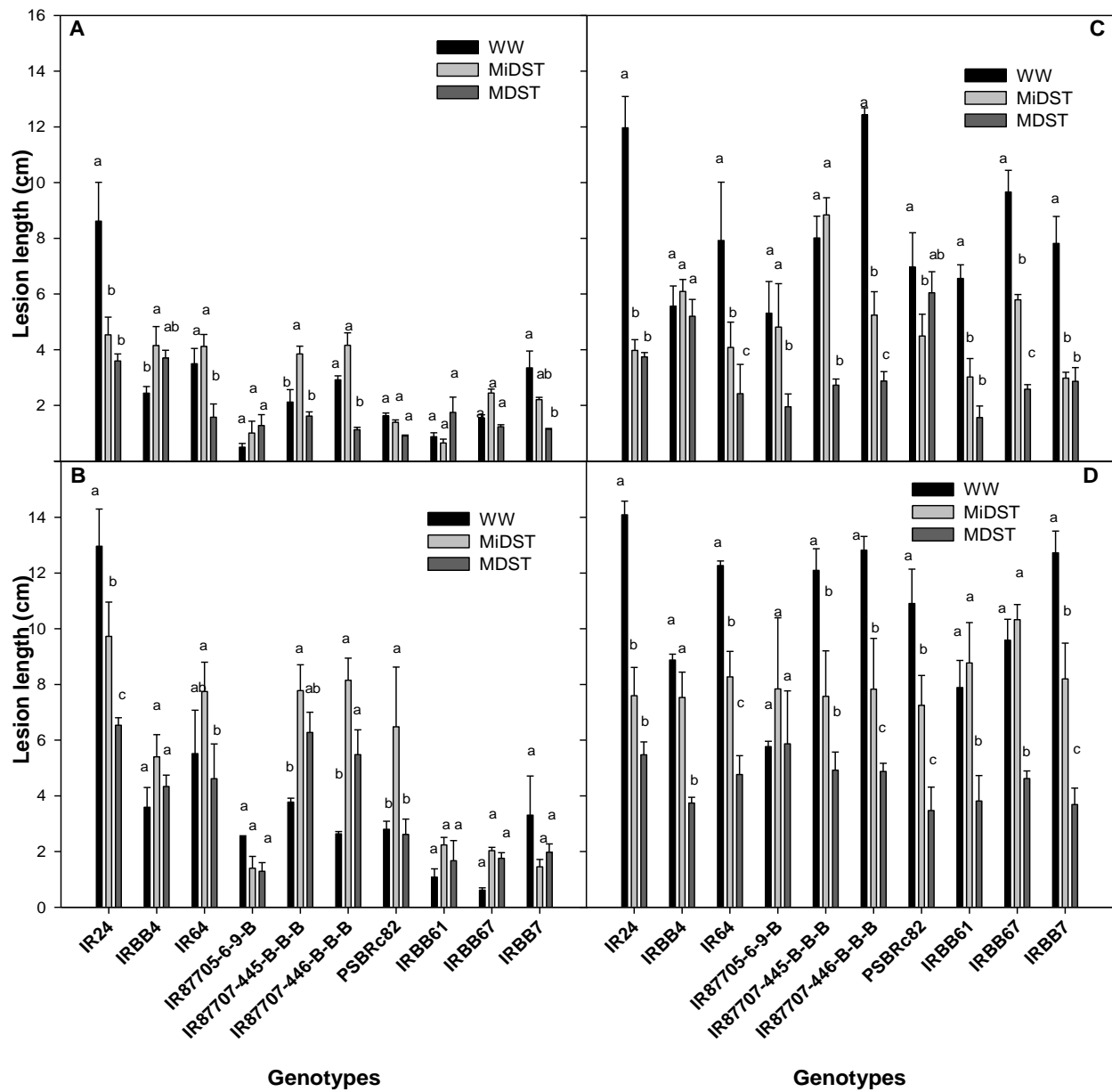
Figure 3
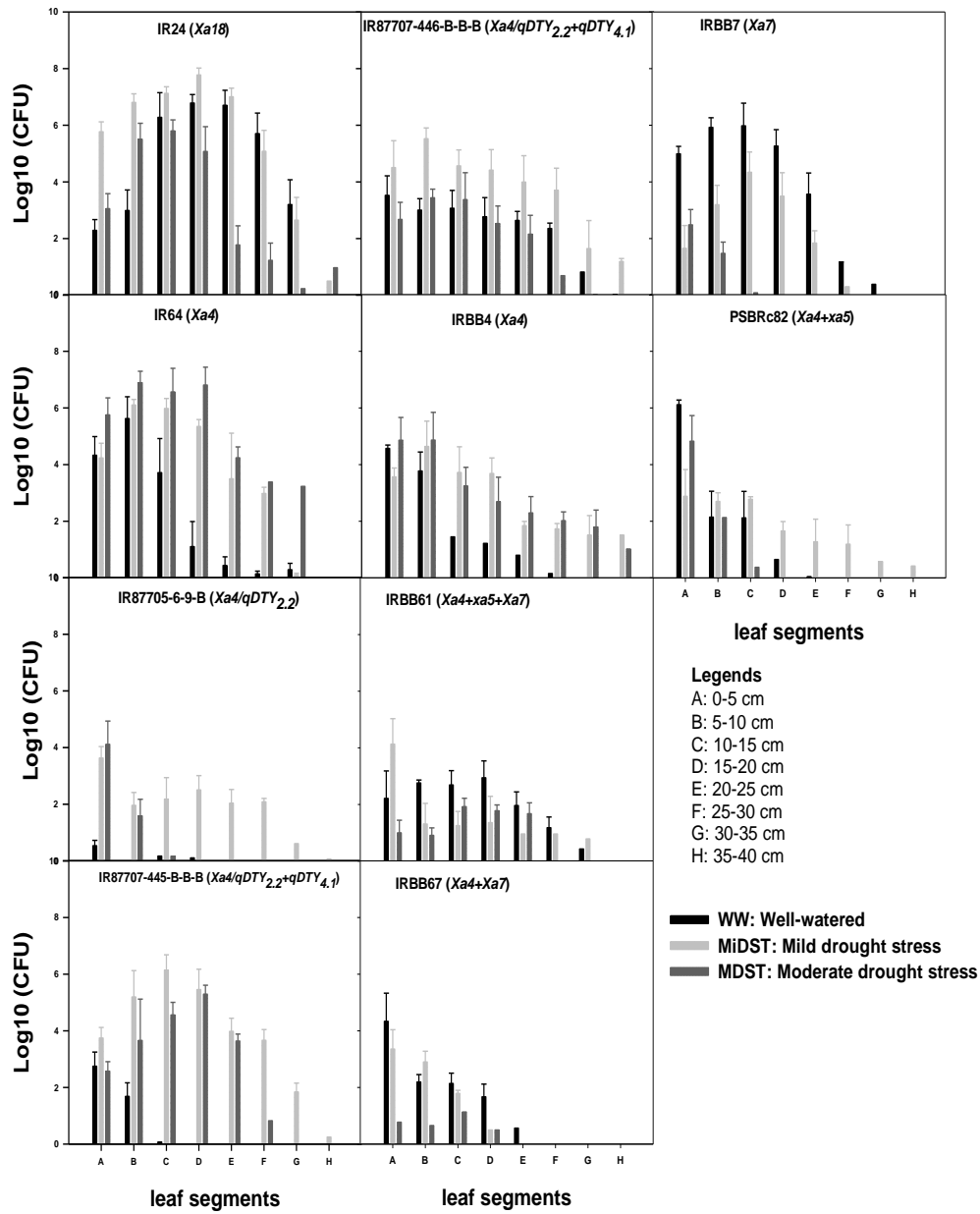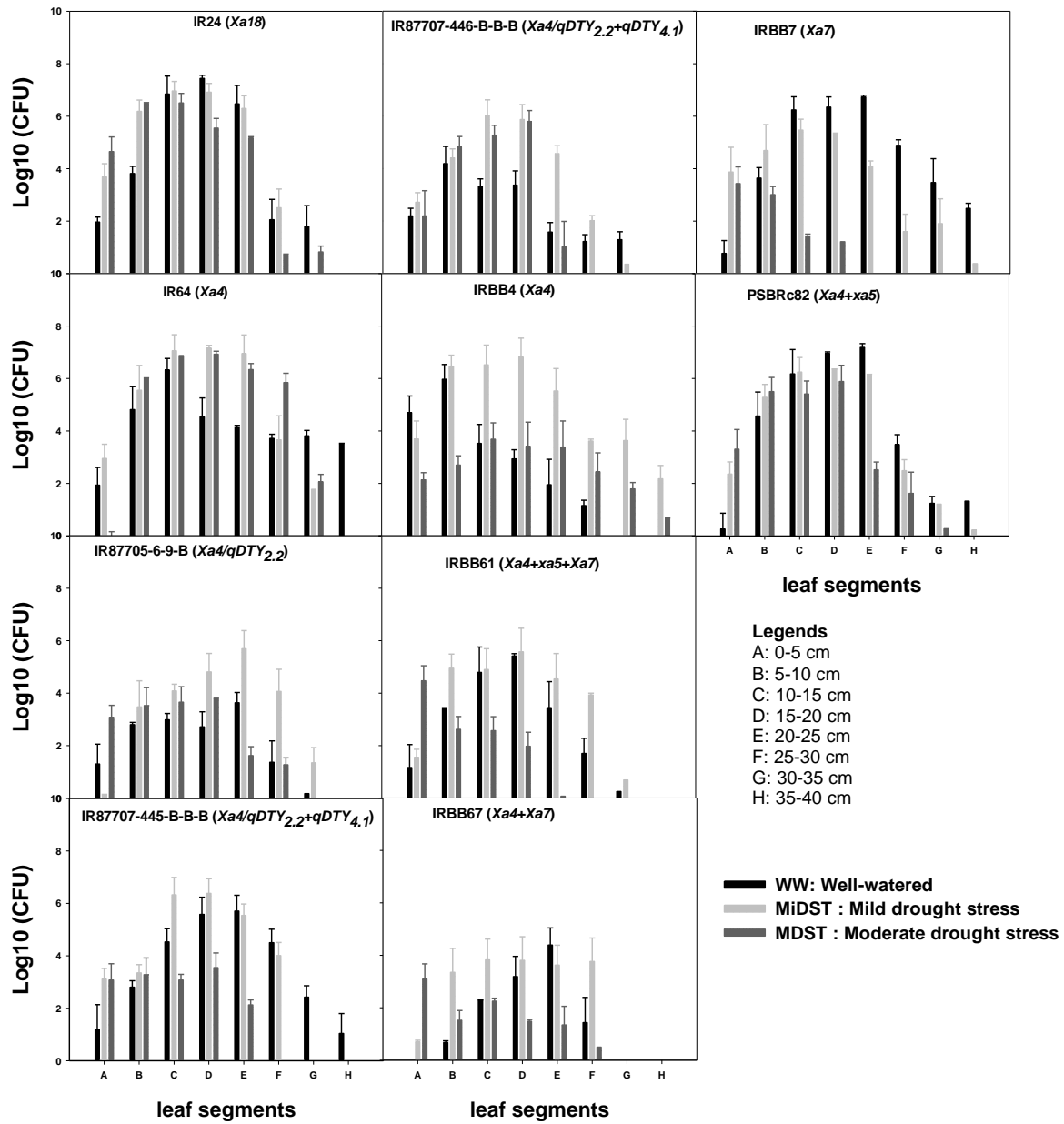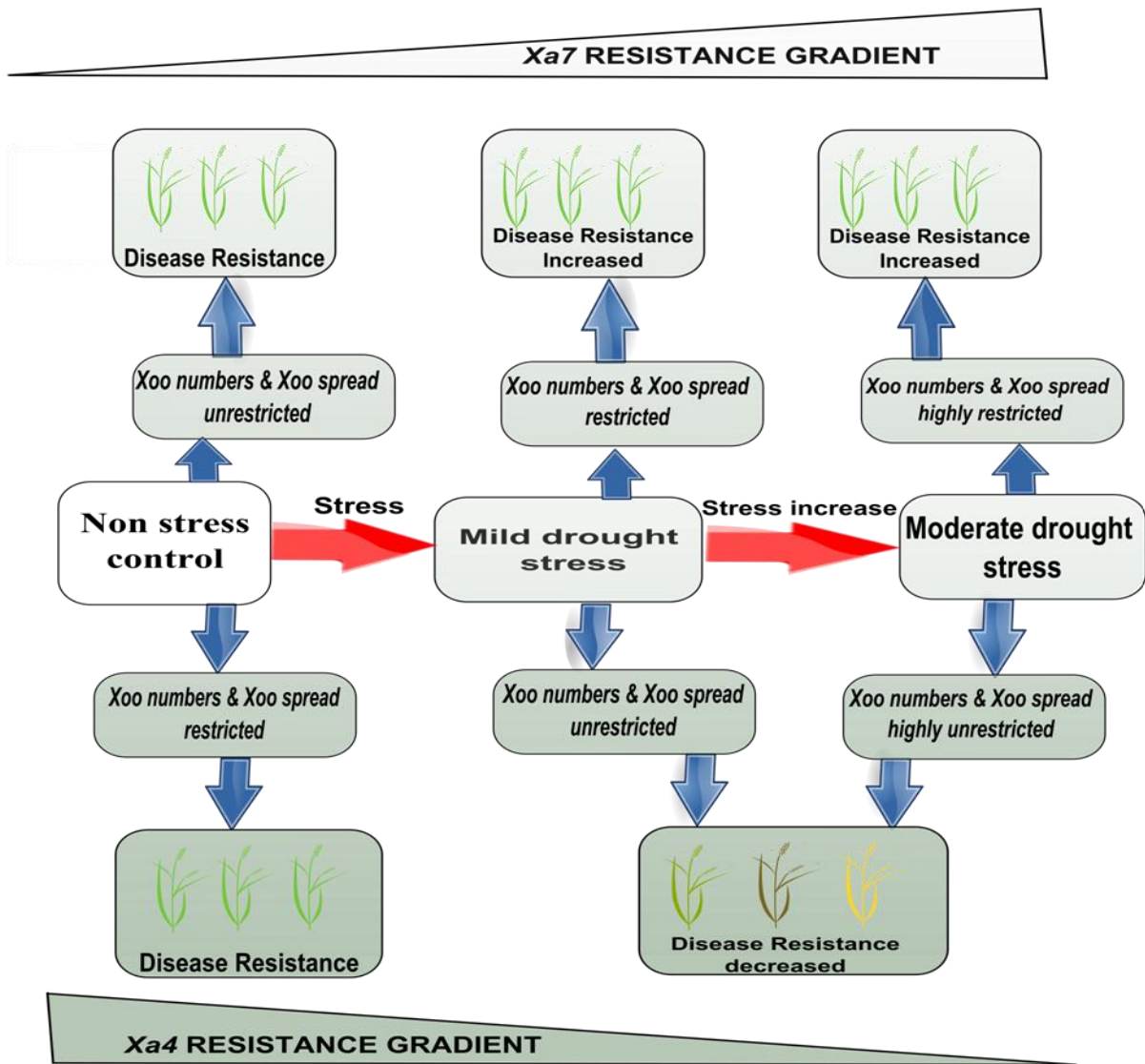
Figure 4

Figure 5

Figure 6

Chapter 3: Combining drought QTLs and bacterial blight Xa-genes to control bacterial blight disease under drought stress.

Gerbert Sylvestre Dossa[1,3*], Amelia Henry[2] , Ricardo Oliva[1], Edgar Maiss[3], Arvind Kumar[1], Casiana Vera Cruz[1], Kerstin Wydra[3,4]

1: Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, Los Banos, Philippines

2: Crop and Environmental Sciences Division, International Rice Research Institute, Los Banos, Philippines

3: Department of Phytomedicine, Leibniz Universitat Hannover, Hannover, Germany

4: Plant Production and Climate Change, Erfurt University of Applied Sciences, Erfurt, Germany

*: Corresponding author: Gerbert S. Dossa; Email: c.dossa@irri.org

Abstract

To control rice bacterial blight, near-isogenic lines carrying *Xa* genes were developed, while rice NILs with drought yield QTL (*qDTY*) were selected for rice yield improvement under drought conditions. Under climate change crops will be exposed to multi-stresses of biotic and abiotic nature simultaneously. In this study, the response of 17 rice lines to simultaneous bacterial blight and drought stresses were evaluated in screenhouse trials and field trials. Under drought stress, *qDTY* NILs with their shorter growing period were less affected, while IR24 and IRBB4 showed growth reduction and 0% flowering under field conditions. NILs with *Xa* gene alone showed resistance to *Xoo* strains carrying the corresponding avirulence gene, except in genotype IRBB4 with *Xa4 R* gene that, although resistant, showed significant increase in disease severity under

drought stress after inoculation with *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain PXO61 (*avrXa4*) compared to irrigated conditions. The combination of *Xa4 R* gene with *qDTY* in IR64 introgression lines (IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B) showed resistance to *Xoo* strains PXO61 (*avrXa4*) and PXO145 (*avrXa4*) under irrigated conditions but were less resistant under drought stress treatment. *qDTY* NILs were susceptible to all strains under both irrigated and drought stress conditions. These results highlight the different responses of rice lines to bacterial blight under drought stress and the advantage of traits' combination (*Xa+qDTY*) to confer drought tolerance and BB resistance under unfavourable future climate conditions.

Keywords: Rice, drought, bacterial blight, *Xa* gene, *qDTY*

Introduction

Climate change accompanied by unexpected heat and drought events is predicted to influence diseases development in crops. Increase of the night temperature by 1°C will affect rice and result in a 10% yield reduction (Welch *et al.*, 2010). Furthermore, the current climate situation and the predicted increases in global temperature and water scarcity conditions (IPCC, 2007) are expected to affect crop resistance against pathogens. Considering climate extremes and the looming water crisis for agriculture in the near future, drought is an important abiotic factor that affects crop growth and limits yield. Plants are continuously exposed to abiotic and biotic stresses which affect numerous of their physiological processes. While scientists face a challenge for developing resistant genotypes against biotic stress, the abiotic stress factors will bring about further threats for crop production. According to Prasad and Staggenborg (2008), high temperature and drought induce in plants a range of changes and biochemical, molecular and physiological responses from cellular level to entire plant processes. A long-term abiotic stress can cause host susceptibility to pathogen attack (Amtmann *et al.*, 2008; Goel *et al.*, 2008; Mittler and Blumwald, 2010; Atkinson and Urwin, 2012). Sorghum and common bean plant showed a higher susceptibility to *Macrphomina phaseolina* under drought stress (Diourte *et al.*, 1995; Mayeke-Perez *et al.*, 2002), and Arabidopsis exposed to drought showed a higher infection level to an avirulent *Pseudomonas syringae* strain (Mohr and Cahill, 2003).

To date, many studies have examined either the effect of a single stress on crops, or the identification of resistance genes under appropriate rice growth condition. Rice bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the economically most important bacterial disease in rice in both favorable and unfavorable rice growing areas. Webb et al. (2010) reported that rice genotype IRBB7 is effective under high temperature while IRBB3, IRBB4 and IRBB5 lost their resistance. A more recent study

revealed that triple stress (heat, drought and virus infection) reduced the expression of genes for resistance to disease, accompanied by an increase of cytoplasmic protein response in Arabidopsis plants (Prasch and Sonnewald, 2013). Garett *et al.* (2011) in their review reported that to predict pathogens response to climate variability, besides climate change, pathogen population shifts should be taken into account. According to Mittler (2006), determining plant responses to multiple stresses requires simultaneous application of stresses. The *rice-Xoo* pathosystem under drought conditions brought about by water limitation and increasing temperature is not yet well understood. Here we report about the impact of combined stress of drought and *Xoo* on rice *R* gene mediated response to bacterial blight and drought *qDTY* lines. We aim at an approach to control BB under drought conditions to help breeders and farmers to cope with the challenges of climate change.

2. Materials and methods

2.1. Plant materials

Seventeen rice lines (Table 1) were used for the field experiments (irrigated and drought stress). Due to low germination of IR87705-6-9-B, only 16 lines were considered for the first year experiment while all the lines were used for the second year. In order to have drought stress during flowering stage of all genotypes, BB near isogenic lines, IR64 and its introgressed lines (IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B) and Supa were seeded 21 days before seeding the genotypes with drought QTL alone (Vandana, IR90020:22-283-B-1, IR90020-22-283-B-4, IR84984-21-19-78-B, IR84984-83-15-481-B) during the second year.

2.2. Field experiment

Field experiments were conducted at the experimental station of the International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines, during the dry seasons of 2014 and 2015. IRRI is located at 21 m of elevation above mean sea level at 14°13′N latitude and 121°15′E longitude. The soil type is a Maahas clay loam; isohyperthermic mixed typic Tropudalf (Venuprasad *et al.* 2009).

2.3. Climate conditions

During the first trial in 2014 the average temperature varied between 24.3 °C and 28.7 °C. The total amount of rainfall ranged from 0.1 mm to 2.4 mm while the relative humidity ranged from 86.9 % in December 2013 to 77.9 % in April 2014. In 2015, the average temperature ranged from 24.9 °C to 29.5 °C between January 2015 and May 2015. The rainfall amount varied between 0.2 mm and 2 mm from January to May 2015 and the relative humidity ranged between 82.7 % and 80.1 % during the same period (Figure 1). In 2014, some rains were observed two weeks after *Xoo* inoculation. During 2015, some rains were also observed in a month of March and were less during April.

Experiment

Three week-old seedlings were transplanted in two fields, one for well-watered and the second for drought stress. The experimental design was a split-plot design for each treatment with 3 replicates, with a *Xoo* strain as main factor and the rice lines as sub-factor. The distance between plants was 20 cm with 25 hills in row and 6 rows per subplot giving in total 150 plants per subplot. N-P-K fertilizer in the rate of 40-40-40 kg.ha^{-1} was applied at transplanting and 40 kg.ha^{-1} of N in form of ammonium sulfate ($(NH4)_2SO_4$) three weeks after transplanting for irrigated conditions. The well-watered treatment was maintain under irrigated conditions until maturity stage of the plant and the drought field was maintained under irrigated conditions for 3 weeks post

transplanting. The drought stress was imposed by cessation of irrigation from 3 weeks post transplanting until end of the experiment.

BB strains and Inoculation

Plants were inoculated with Philippine *Xoo* strains PXO61 (race 1, *avrXa4*) and PXO86 (race 2, *avrXa7*) at 10^6 CFU/ml by the leaf clipping method (Kauffman *et al.* 1973). The strains were grown on solid modified Wakimoto's medium.

2.4. Simulating drought stress in screenhouse

Under screenhouse conditions, three week-old seedlings of the 17 rice lines (Table 1) were transplanted in a complete randomized bloc with 3 replicates for each treatment (irrigated and drought stress). The *Xoo* treatments were the main plots and the rice lines the subplots. The seedlings were planted in three rows of 5 hills. While the irrigated plot was maintained under well-watered condition until plant maturity, the irrigation was withheld from three weeks post transplanting in the drought stress plot until the end of the experiment. In order to maintain the plants under drought stress, any water underneath the plantings was collected through a canal and pumped out of the area.

Three weeks after the drought stress initiation, the plants were inoculated with *Xoo* strains PXO61 (race1, *avrXa4*), PXO86 (race 2, *avrXa7*), PXO99 (race 6) and PXO145 (race 7, *avrXa4+avrXa7*) following leaf clipping inoculation method of.

2.5. Data collection

Daily data of rainfall, temperature, and relative humidity were collected from IRRI Climate Unit weather station in 2014 and 2015 during the experimental period. The plant height was recorded for three randomized selected plants as distance from the soil surface to the tip of the last developed leaf of the main tiller or of the tallest panicle of each plant at maturity stage. The number of days to flowering of 50% of plants in each sub-plot was also recorded.

2.6. Disease evaluation

In the field experiment, BB lesion length was scored 2 weeks post inoculation. Five leaves were evaluated from each plant and 30 plants were evaluated from each sub-plot. However, in the screenhouse trials, 15 plants were evaluated at 14 days post inoculation. In order to compute BB severity, leaf length was also measured together with BB lesion length. BB severity was determined as percentage of BB lesion length per leaf length.

2.7. Plant biomass, height and flowering date

To evaluate drought stress effects on the rice genotypes, the shoot biomass weightof three plants per genotype and per replicate from both well-watered and drought stress treatments was determined after drying the samples in the oven at 70 °C for 3 days. Plant height was measured at maturity stage as height from the soil surface to the tip of the most developed leaf and the flowering date was recorded when 50% of the sub-plot had flowered.

2.8. Plant canopy temperature and leaf water potential

Canopy temperature is considered as indicator for plant water stress. Plants under water stress close their stomata to decrease transpiration, resulting in leaf temperature increase. We measured the canopy temperature using an infrared (IR) sensor (Apogee Instruments, Logan UT, USA) in each subplot and the average values from the three replicates were computed for each genotype. Leaf water potential was the second water stress indicator evaluated in this experiment. Leaf water potential (LWP) was determined at mid-day with a pressure chamber (3000HGBL Plant Water Status Console, Soil moisture Equipment Corp., CA, USA) using compressed N_2 at 14 dpi.

2.9. Statistics

Statistical analyses were performed in R (v3.1.0) to compare genotypes' responses to BB under drought stress. ANOVA under agricolae package were performed for each

response variable under each conditions (irrigated and drought stress) and Tukey HSD test was used for mean comparison.

3. Results

3.1. Plant morphology and agronomic characteristic under drought stress

In both trials (2014 and 2015), drought stress significantly affected plant height in all lines (Figure 2), with Supa, IR90020:22-283-B-4 and IR84984-83-15-481-B showing the highest plant height under drought stress in the 2014 trial, and Supa was the tallest genotypes under irrigated and drought stress conditions in year 2015.

The number of days to flowering in rice lines with drought *qDTY* as well as rice lines without *qDTY* was affected by drought stress. The flowering of *qDTY* lines (IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B, IR90020:22-283-B-1, IR90020:22-283-B-4, IR84984-21-19-78-B, IR84984-83-15-481-B) including IR64, Vandana and Supa was less affected by drought stress compared to IRBB NILs. IR24 and IRBB4 which showed 25 days delay in days to flowering between the control (irrigated) and drought in 2014 trial did not flower during the 2015 trial.

Similarly, under screenhouse conditions, the plant height was generally reduced under drought stress conditions compared to irrigated conditions. Genotype Supa was the tallest genotype under both irrigated and drought stress conditions, while the height of IRBB NIL genotypes was below 100 cm under both conditions (Figure 3).

A significant biomass reduction was observed in all lines under drought stress. Also drought *qDTY* lines showed biomass reduction under drought stress, with IR87707-445-B-B-B ($Xa4$/$qDTY_{2.2}$+$qDTY_{4.1}$) and IR87707-446-B-B-B ($Xa4$/$qDTY_{2.2}$+$qDTY_{4.1}$) being less affected than other drought *qDTY* lines (Figure 2). The highest biomass under drought conditions was recorded on drought *qDTY* lines IR87707-445-B-B-B

($Xa4$/$qDTY_{2.2}$+$qDTY_{4.1}$), IR87707-446-B-B-B ($Xa4$/$qDTY_{2.2}$+$qDTY_{4.1}$) and IR84984-83-15-481-B in screenhouse trials (Figure 3).

3.3. Canopy temperature and leaf water potential under drought stress

High temperature has been reported to affect host plant resistance to pathogen and plant canopy temperature has been reported to be higher under drought stress compared to control (irrigated) conditions. The canopy temperature was not significantly different between genotypes and irrespective of watr conditions and pathogen inoculation (Figure 4). In 2014, the canopy temperature varied between 35.5 °C and 40.7 °C and ranged from 34.1 °C to 38.6 °C in 2015.

The leaf water potential (LWP) was not significantly different across genotypes treatments during both years. During 2014, the LWP ranged from -22.3 to -16.1, while in 2015, the LWP was generally higher than in 2014 (Figure 4). Rice genotype IR64 showed the highest LWP (-19.5) under inoculation with PXO86, and the lowest LWP (-36.2) was observed in genotype IR84984-83-15-481-B inoculated with PXO61.

3.4. *qDTY* lines reveal susceptible to bacterial blight

Disease scale ranges from 1, 3, 5, 7 and 9, where 1, 3, 5, 7 and 9 represent percentage of diseased leaf area, respectively, 1-5% (resistant), 6-12% (moderately resistant, 13-25% (moderately susceptible), 26-50% and above 50% (susceptible) was used to categorized different reactions of rice lines to BB.

3.5. Bacterial blight *R* genes under drought stress: which candidate for drought *qDTY* varieties' improvement?

Three BB *R* genes (*Xa4*, *xa5* and *Xa7*) in single or combination in ten rice lines were evaluated for their reaction to *Xoo* under drought stress and control (irrigated) conditions.

3.5.1. Bacterial blight disease severity evaluation under field conditions

With inoculation of PXO61 (*avrXa4*), a tendency of increase in average BB severity was observed during 2014 trial on seven BB *R* genes NILs (IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB61 (*Xa4+xa5+Xa7*), IRBB67 (*Xa4+Xa7*), IR64 (*Xa4*), IR87707-446-B-B-B (*Xa4/qDTY$_{2.2}$+qDTY$_{4.1}$*) under drought stress conditions compared to irrigated conditions (Figure 5). In IR24 and generally in all gentoypes with drought qtl, BB severity decreased under drought stress compared irrigated conditions. BB severity was high (above 13%) on IR24 under both irrigated and drought stress conditions, while it was above 13% on IRBB7 under irrigated conditions only. Similarly in the 2015 trial, IRBB4, IRBB5, IRBB61, IR87707-445-B-B-B showed significant BB severity increase under drought stress compared to irrigated conditions, while IR24, Vandana, IR90020:22-283-B-1, IR90020:22-283-B-4, IR84984-21-19-78-B and IR84984-83-15-481-B showed BB severity reduction with drought stress (Figure 5). The effect of drought on BB severity under inoculation with PXO61 was less prominent in IRBB67.

Under inoculation of PXO86 (*avrXa7*) in 2014 trial, NILs IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB61 (*Xa4+xa5+Xa7*), IRBB67 (*Xa4+Xa7*) showed tendency of average BB severity increase under drought stress compared to irrigated conditions. BB severity was higher than 13% on IR24 and IR87707-446-B-B-B under both drought stress and irrigated conditions while it was above 13% on IRBB4, IR64 and IR87707-445-B-B-B only under irrigated conditions. During 2015 trial, a general decrease in BB severity was observed on BB *R* genes lines including IR24, except, IR87705-6-9-B which showed increase in BB severity under drought stress compared to irrigated conditions. BB severity on IRBB5, IRBB7 and IRBB67 was less increased under drought stress than in year 2014 (Figure 5). In both years, bacterial blight was reduced under drought conditions.

3.5.2. Bacterial blight disease severity evaluation under screenhouse conditions

Under inoculation with *Xoo* strain PXO61 (*avrXa4*) under drought stress in 2014, BB severity of all genotypes with BB *R* genes was below 13%, except for IR24, IRBB4 (*Xa4*), PSBRc82 (*Xa4+xa5*) and IR64 (*Xa4*). IRBB4 showed significant BB severity increase of about 100% under drought stress. Drought *qDTY* combination with BB *R* gene lines did not show differences in BB severity between both irrigated and drought stress conditions (Figure 6A). All lines with drought stress QTLs showed a significant reduction in BB severity under drought stress conditions compared to irrigated treatments. IRBB67 (*Xa4+Xa7*) showed the lowest BB severity under drought stress (1.8%) and IRBB61 (*Xa4+xa5+Xa7*) the lowest disease severity under irrigated conditions (1.6%). During the 2015 trial, disease severity was generally increased under drought stress on all rice lines with BB *R* genes, except, IR24 and PSBRc82, which did not show significant differences between both irrigated and drought stress conditions, and IRBB7 and IR64, of which BB severities were reduced under drought.

With PXO86 (*avrXa7*) in 2014 trial, BB severity was generally reduced under drought stress on rice lines with BB *R* genes and IR24, except IR64 (*Xa4*) which showed no difference between both stress conditions. IRBB7 (*Xa7*) and IRBB67 (*Xa4+Xa7*) showed the lowest BB severity, 1.7% and 2.1%, respectively. Similarly to 2014 trial, the BB severity in 2015 trial was generally reduced under drought stress, except for IRBB67 (*Xa4+Xa7*) which showed BB severity increased under drought stress (Figure 6A, 6B).

Inoculated with PXO99, all genotpyes generally showed BB severity reduction under drought stress compared to irrigated conditions during the 2014 trial, except IR64, and Vandana. Generally, the BB severity was moderately severe (above 13%) under both irrigated and drought stress conditions, except in IRBB4 under drought stress in both years, with no significant differences between BB severity under both irrigated and drought treatments. In year 2015, in ten of the tested genotypes BB severity was higher

under drought conditions, except in IRBB4, IRBB67, IR87707-445- B-B-B, Vandana, IR90020:22-283-B-1, IR84984-21-19-78-B and IR84984-83-15-481-B.

Generally, under the inoculation of PXO145 (*avrXa4+avrxa5+avrXa7*), BB severity on BB *R* gene NILs including IR24 decreased under drought stress during the 2014 trial, except IR87707-446-B-B-B (*Xa4/qDTY$_{2.2}$+qDTY$_{4.1}$*), which showed significant BB severity increase under drought stress. BB severity was generally below 13% for genotypes with r-genes, except for IR24 under both conditions and PSBRc82 under irrigated conditions only. In 2015, IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB61 (*Xa4, xa5, Xa7*), IR64 (*Xa4*), IR87705-6-9-B (*Xa4/qDTY$_{2.2}$*) and IR877070446-B-B-B (*Xa4/qDTY$_{2.2}$+qDTY$_{4.1}$*) showed BB severity increase under drought stress compared to irrigated conditions. BB severity on IR24 was higher than 13% under both irrigated and drought stress conditions and above 13% on IRBB61 under drought stress only. The lowest BB severity was recorded on IRBB7 (*Xa7*) under irrigated conditions and on IRBB67 (*Xa4+Xa7*) under drought stress conditions (Figure 6A, 6B). The genotypes with drought resistance genes showed a reduction of bacterial blight under drought stress conditions compared to irrigated conditions.

3.6. Prolonged drought stress and bacterial blight

Generally, BB disease evaluation is performed two weeks post inoculation. In this study, in order to see BB disease progression under drought stress, disease severity at 21 dpi was also recorded. Disease severity evaluation at 21 dpi showed that prolonged drought did not stop bacterial blight growth on all lines. Under irrigated conditions as well as under drought stress conditions, BB severity increased on all rice lines at 21 dpi compared to 14 dpi evaluation. Under irrigated and drought conditions and inoculation of PXO61 (*avrXa4*) and PXO86 (*avrXa7*) four lines with *R-genes* IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB61 (*Xa4+xa5+Xa7*) and IRBB67 (*Xa4+Xa7*) showed BB severity below 13%, except

IRBB4 (*Xa4*) which disease severity was below 13% under irrigated conditions with PXO61 (*avrXa4*) (Figure 7).

Discussion

The projected future climate changes will have a negative impact on food production in the tropical and subtropical regions and, thus aggravate the existing discrepancies between food supply and food demand. Drought occurrence as consequence of climate changes is responsible for economic yield reduction of 60% at reproductive stage in rice (Venusprasad *et al.*, 2007). Among the biotic stress factors, rice bacterial blight is responsible for a substantial yield reduction up to 50% (Robert & Pamela, 1992). Determining the rice response to combined drought stress and bacterial blight has shown that rice response varied between rice genotypes and depended on the *Xoo* strain. Studying the response of BB NILs, drought tolerant lines carrying *Xa4 R* gene to BB and drought tolerant lines to combined BB and drought provided a differentiated picture on the interaction of BB and drought stress on plant growth and on the effect of drought on BB development. Combining tolerance to abiotic and biotic stress is recommended to improve resilience to climatic changes in rice varieties' development.

This study has shown genotypic differences between the tested rice lines in days to flowering, plant height and biomass as well as in response to bacterial blight. Short growing period allow plants to reproduce and escape the dry environment (Farooq *et al.*, 2009), and rice lines with drought *qDTY* showed a shorter life cycle compared to IRBB Nils. The delay in days to flowering caused by drought stress was lesser in drought *qDTY* lines compared to IRBB NILs allowing them to escape the severe drought stress. Furthermore, PSBRc82, IR64 and Supa also showed a shorter life cycle and were less affected by drought stress, suggesting that these rice lines could possess drought tolerance traits which enable them to cope with drought stress. Further studies are therefore required to investigate drought tolerance in these lines. Additionally,

plant height and biomass were significantly reduced by drought stress, demonstrating the effect of drought stress on all the rice lines. This is consistent with previous reports which show that drought stress decreased rice biomass, plant height, tiller number and panicle number (Bhattacharjee *et al.,* 1973; De Datta, 1973; Rahman *et al.,* 2002).

Plant responses to environment changes follow complex mechanisms. Simultaneous application of biotic and abiotic stresses can lead to a failure of host resistance reactions which would be effective against a single stress factor. Bacterial blight and BB *R* Nils' interaction varies according to presence and absence of *R* gene in the genotype and avirulence protein from *Xoo.* In this study, the *Xoo* strains varied in virulence *Xoo* strain PXO99 causing the most severe symptoms' across all genotypes. This strain is known as most virulent *Xoo* strain among the Philippines *Xoo* strains (Cottyn and Mew, 204).

Among the tested rice genotypes, the lines with drought *qDTY* alone and genotype Supa revealed susceptible to bacterial blight under drought stress as well as under irrigated conditions, remaining above the resistance threshold (13%). Our previous study showed Supa susceptible to Philippines *Xoo* strains (Dossa *et al.,* 2015).

These results suggest that a combination of abiotic and biotic traits such as drought *qDTY* and BB *R* gene will be necessary to cope with both stress factors under climate change conditions. Lines with drought *qDTY* and BB *R* gene, thought their resistance were reduced under drought stress, revealed more resistant to BB under drought stress compared to drought *qDTY* alone. Severe drought stress may result in significant BB disease reduction (Chapter 2). According to Amtmann *et al.* (2008), host susceptibility to pathogen attack can be increased by long term abiotic stress as shown by this study where the bacterial blight symptom progression was not significantly affected. This suggests that severe drought stress causes more effects to the host plant than to the pathogen. IRBB NILs response to BB follows a gene-for-gene interaction. Their reaction

to BB depends on presence and absence of the corresponding *R* gene. Here, our results showed that IRBB NILs remained resistant under combined drought and BB stresses, except when *Xa4 R* gene is present. IRBB4 carrying single *Xa4 R* gene has shown significant increase in BB severity with PXO61 (*avrXa4*) under drought stress compared to disease severity under irrigated conditions in both field and screenhouse trials. This finding corroborates previous studies of Webb *et al.* (2010), Dossa *et al.* (chapter 2) who also report about decreasing *Xa4* effectiveness under combined stress of high temperature and BB, drought stress and BB at seedling stage, respectively, when inoculated with PXO145 (*avrXa4*). Similar results have been reported in sorghum and common bean which show susceptibility to *Macrophomina phaseolina* under drought stress (Diourte *et al.*, 1995; Mayeke-Perez *et al.*, 2002). This suggests that the basal defense of IRBB4 is weakened by drought stress, allowing BB to cause more damage than under irrigated conditions. Other BB *R* genes in IRBB5 (*xa5*) and IRBB7 (*Xa7*) or in combination in IRBB61 (*Xa4+xa5+Xa7*), IRBB67 (*Xa4+Xa7*) and PSBRc82 (*Xa4+xa5*) showed decrease in BB severity or either no significant difference in BB severity under drought conditions, except IRBB61 which showed BB severity increase under drought stress with PXO145 (*avrXa4+avrxa5+avrXa7*) in the 2015 screenhouse trial. Moreover, the lowest BB severity was observed either on IRBB7 (*Xa7*) or IRBB67 (*Xa4+Xa7*), suggesting that *Xa7 R* gene response was not affected by drought stress and *Xa4 R* gene showed a negative effect on *Xa7* in their combination in IRBB67. The effectiveness of *Xa7* or *Xa7* in combination with *Xa4* under dual stresses of BB and two drought stress levels was also reported by Dossa *et al.* (Chapter 2), and *Xa7* resistance enhanced by high temperature compared to *Xa4* resistance by Webb *et al.* (2010).

Traits combination such as BB *R* gene and drought *qDTY* would allow rice plants to better cope with stress factors convened by climate change. Results from this study showed that *Xa4* and drought *qDTY* combination in IR87705-6-9-B (*Xa4/qDTY$_{2.2}$*),

IR87707-445-B-B-B (*Xa4*/*qDTY$_{2.2}$*+*qDTY$_{4.1}$*) and IR87707-446-B-B-B (*Xa4*/*qDTY$_{2.2}$*+*qDTY$_{4.1}$*) were resistant to *Xoo* strains with *avrXa4* (PXO61 and PXO145) under drought conditions. This result suggests that the combination of *Xa4*+*qDTY* response to BB is affected by drought stress leading to disease severity increase. The use of BB *R* genes with broad spectrum of resistance such as *Xa21* in IRBB21 (Kush *et al.*, 1990; Ideka *et al.*, 1990) would give more advantage compared to *Xa4*. Thus, it will be necessary to determine the effects of drought stress on the response of these lines to BB. Additionally, *Xa7* has shown resistance enhanced to BB under high temperature conditions (Webb *et al.*, 2010) and also under drought stress when inoculated with *Xoo* strain harboring *avrXa7*. Incorporating *Xa7 R* gene into drought *qDTY* lines would be more suitable compared to *Xa4*+*qDTY* lines.

In summary, investigating rice response to BB under drought stress under field and screenhouse conditions using 17 rice lines, rice lines harboring drought *qDTY* alone showed resistance to drought stress but were highly susceptible to BB under both irrigated and drought stress conditions, though under drought conditions BB was reduced, indicating a possible effect of elicited abiotic stress resistance against biotic stress factors. Rice lines with BB *R* gene alone were resistant to BB, except, lines with *Xa4* which showed significant BB severity increased under drought stress compared to irrigated conditions. However, rice lines with BB *R* gene *Xa4* and drought *qDTY* were resistant to both BB and drought with BB severity increased under drought stress. This study is the first of its kind of BB and drought stress combination under field and screenhouse conditions and may support breeders in achieving crop improvement to cope with climate change. Trait combinations (abiotic and biotic) showed promising results, however, biotic trait selection to improve varieties with drought QTLs will play an important role in this process.

Acknowledgements

References

Amtmann A, Troufflard S, Armengaud P, 2008. The effect of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum*. 133: 682–691.

Atkinson NJ, and Urwin, PE, 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany*. 63:3523–3544.

Bahattacharjee DP, Krishnayya GR, Ghosh AK, 1973. Analyses of yield components and productive efficiency of rice varieties under soil moisture deficit. *Indian J. Agron.*, 16, 314-343.

Cottyn B, Mew T, 2004. Bacterial blight of rice. In: Encyclopedia of Plant and Crop Science. Marcel Dekker, Inc., doi:10.1081/E-EPCS 120010586.

De Datta SK, Abilay WP, Kalwar GN, 1973. Water stress effect on flooded tropical rice. 2. Water management in Philippines Irrigation System Research and Operation, pp: 16-36.

Diourte M, Starr JL, Jeger MJ, Stack JP, Rosenow DT, 1995. Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water-stress on disease development in sorghum. *Plant Pathology*. 44: 196–202.

Dossa SG, Oliva R, Maiss E, Vera Cruz C, and Kerstin K, 2015. High temperature enhances the resistance of cultivated African rice, *Oryza glaberrima*, to bacterial blight. Plant Disease 00:ja. http://dx.doi.org/10.1094/PDIS-05-15-0536-RE

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA, 2009. Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, 29: 185-212

Garrett KA, Forbes GA, Savary S, Skelseya P, Sparksa AH, Valdiviad C, A. H. van Bruggen C, Willocquet L, Djurl A, Duveiller E, Eckersten H, Pandeh S, Vera Cruz C,

Yuen J, **2011.** Complexity in climate-change impacts: an analytical framework for effects mediated by plant disease. *Plant Pathology.* 60: 15–30

Goel AK, Lundberg D, Torres MA, Matthews R, Akimoto-Tomiyama C, et al. 2008. The *Pseudomonas syringae* type III effector HopAM1 enhances virulence on water-stressed plants. *Mol. Plant-Microbe Interact.* 21:361–70.

Ikeda R, Khush G, Tabien R, 1990. A new resistance gene to bacterial blight derived from *O. longistaminata. Jpn J Breed,* 40:280-281

IPCC, 2007. Climate change 2007: The physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva: IPCC Secretariat.

Kauffman HE, Reddy A, Hsieh SPY, Merca SD, 1973. An improved technique for evaluating resistance of varieties to *Xanthomonas oryzae* pv. *oryzae. Plant Dis. Rep.,* 57, 537-541.

Khush GS, Bacalangco E, Ogawa T, 1990. A new gene for resistance to bacterial blight from *O. longistaminate. Rice Genet Newslett,* 7:121-122.

Mayek-Perez N, Garcia-Espinosa R, Lopez-Castaneda C, Acosta-Gallegos JA, Simpson J, 2002. Water relations, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiological *and Molecular Plant Pathology.* 60:185–195.

Mittler R, and Blumwald E, 2010. Genetic engineering for modern agriculture: challenges and perspectives. *Annual Review of Plant Biology.* 61:443–462.

Mittler R, 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science,* 11:15-19

Mohr PG, and Cahill DM, 2003. Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to *Pseudomonas syringae* pv. *tomato* and *Peronospora parasitica*. *Functional Plant Biology.* 30: 461–469.

Prasad PVV, and Staggenborg SA, 2008. Impacts of Drought and/or Heat Stress on Physiological, Developmental, Growth, and Yield Processes of Crop Plants. In: Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes. *Advances in Agricultural Systems Modeling Series 1. ASA, CSSA, SSSA, 677 S. Segoe Rd., Madison, WI 53711, USA.*

Prasch CM, and Sonnewald U, 2013. Simultaneous application of heat, drought, and virus to Arabidopsis plants reveals significant shifts in signaling networks. *Plant Physiology*, 162:1849-1866. doi: http://dx.doi.org/10.1104/pp.113.221044

Rahman MT, Islam MT, Islam MO, 2002. Effect of water stress at different growth stages on yield and yield contributing characters of transplanted aman rice. *Pak. J. Biol. Sci.*, 5, 169-172.

Robert KW, Pamela SG, 1992. Foliar diseases: bacterial blight. Compendium of Rice Disease 1992; University of California, Davis. pp. 10-11.

Venuprasad R, Dalid CO, Del Valle M, Bool ME, Zhao D, Espiritu M, Sta Cruz MT, Amante M, Kumar A, Atlin GN, 2009. Identification and characterization of large-effect quantitative trait loci (QTL) for grain yield under lowland drought stress in rice using bulk-segregant analysis (BSA). *Theor Appl Genet*, 120:177–190

Venuprasad R, Lafitte HR, Atlin GN, 2007. Response to direct selection for grain yield under drought stress in rice. *CropSci* 47:285–293

Webb KM, Ona I, Bai J, Garrett KA, Mew T, Vera Cruz C M and Leach JE, 2010. A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene. *New Phytologist*. 185: 568–576.

Welch JR, Vincent JR, Auffhammer M, Moya PF, Dobermann A, Daw D, 2010. Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proc Natl Acad Sci USA* 107: 14562–14567. doi: 10.1073/pnas.1001222107

Table 1: List of rice lines and their corresponding BB *R* genes and/or drought resistance QTLs (*qDTY*)

S/N	Plant	*Xa* genes/Drought *QTLs*
1	IR24	*Xa18*
2	IRBB4	*Xa4*
3	IRBB5	*Xa5*
4	IRBB7	*Xa7*
5	IRBB61	*Xa4+xa5+Xa7*
6	IRBB67	*Xa4+Xa7*
7	PSBRc82	*Xa4+xa5*
8	IR64	*Xa4*
9	IR87705-6-9-B	*Xa4/qDTY$_{2.2}$*
10	IR87707-445-B-B-B	*Xa4/DTY$_{2.2}$+qDTY$_{4.1}$*
11	IR87707-446-B-B-B	*Xa4/DTY$_{2.2}$+qDTY$_{4.1}$*
12	VANDANA	*qDTY$_{2.3}$+qDTY$_{3.1}$*
13	IR90020:22-283-B-1	*qDTY$_{12.1}$*
14	IR90020:22-283-B-4	
15	IR84984-21-19-78-B	
16	IR84984-83-15-481-B	
17	Supa	Unknown

Table 2: Days to flowering of 17 rice lines under irrigated and drought stress field conditions

Genotypes	Trial 2014		Trial 2015	
	Irrigated	Drought	Irrigated	Drought
IR24	90	115	89	no flowering
IRBB 4	90	115	88	no flowering
IRBB 5	90	104	85	105
IRBB 7	90	109	86	105
IRBB 61	90	104	79	87
IRBB 67	90	104	86	104
PSB RC 82	73	93	76	84
IR64	73	82	73	82
IR87705-6-9-B	-	-	71	77
IR87707-445-B-B-B	73	82	74	82
IR87707-446-B-B-B	73	88	70	82
Vandana	67	73	65	66
IR90020:22-283-B-1	67	73	60	65
IR90020:22-283-B-4	65	73	58	64
IR84984-21-19-78-B	65	73	59	63
IR84984-83-15-481-B	63	73	59	64
Supa	67	73	69	69

IR24 and IRBB4 did not flower during the drought experiment in 2015. IR87705-6-9-B was not included in the field trial in 2014 due to low germination rate.

List of figures

Figure 6A: Bacterial blight severity under irrigated and drought stress conditions in the screenhouse trial in 2014. Rice lines were inoculated with PXO61 (*avrXa4*), PXO86 (*avrXa7*), PXO99 and PXO145 (*avrXa4+avrxa5+avrXa7*). The break at 13% indicates the level of susceptibility. NS and S represent irrigated and drought stress conditions, respectively.

Figure 6B: Bacterial blight severity under irrigated and drought stress conditions in the screenhouse trial in 2015. Rice lines were inoculated with PXO61 (*avrXa4*), PXO86 (*avrXa7*), PXO99 and PXO145 (*avrXa4+avrxa5+avrXa7*). The break at 13% indicates the level of susceptibility. NS and S represent irrigated and drought stress conditions, respectively.

Figure 7: Bacterial blight severity increased under irrigated and drought stress conditions between 14 and 21 dpi (day post inoculation) in the field trials in 2014and 2015. Rice lines were inoculated with PXO61 (*avrXa4*) and PXO86 (*avrXa7*). The break at 13% indicates the level of susceptibility. NS and S represent irrigated and drought stress conditions, respectively.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

A

Bacterial blight severity (%)

Legend:
- PXO61-NS / PXO61-S
- PXO86-NS / PXO86-S
- PXO99-NS / PXO99-S
- PXO145-NS / PXO145-S

Rice lines:
IR24, IRBB4, IRBB5, IRBB7, IRBB61, IRBB67, PSBRc82, IR64, IR87705-6-9-B, IR87707-445-B-B-B, IR87707-446-B-B-B, Vandana, IR900 20:22-283-B-4, IR900 20:22-283-B-1, IR84984-21-19-78-B, IR84984-83-15-481-B, SUPA

Rice lines

Figure 6A

83

B
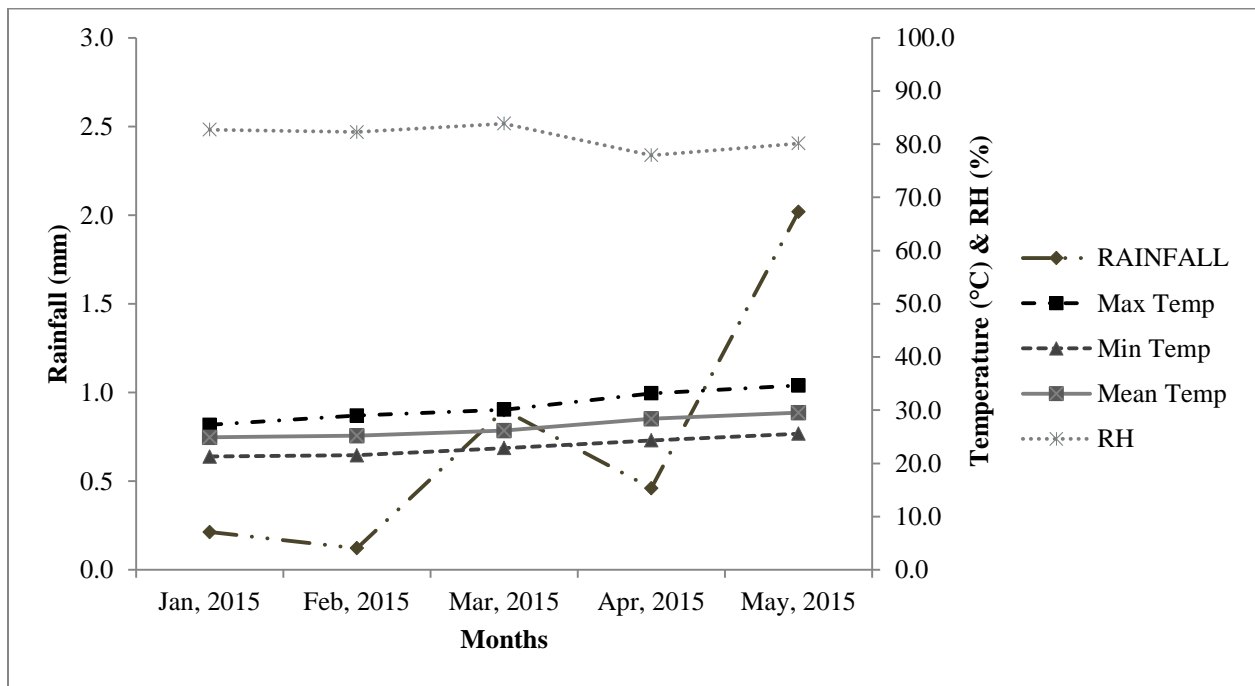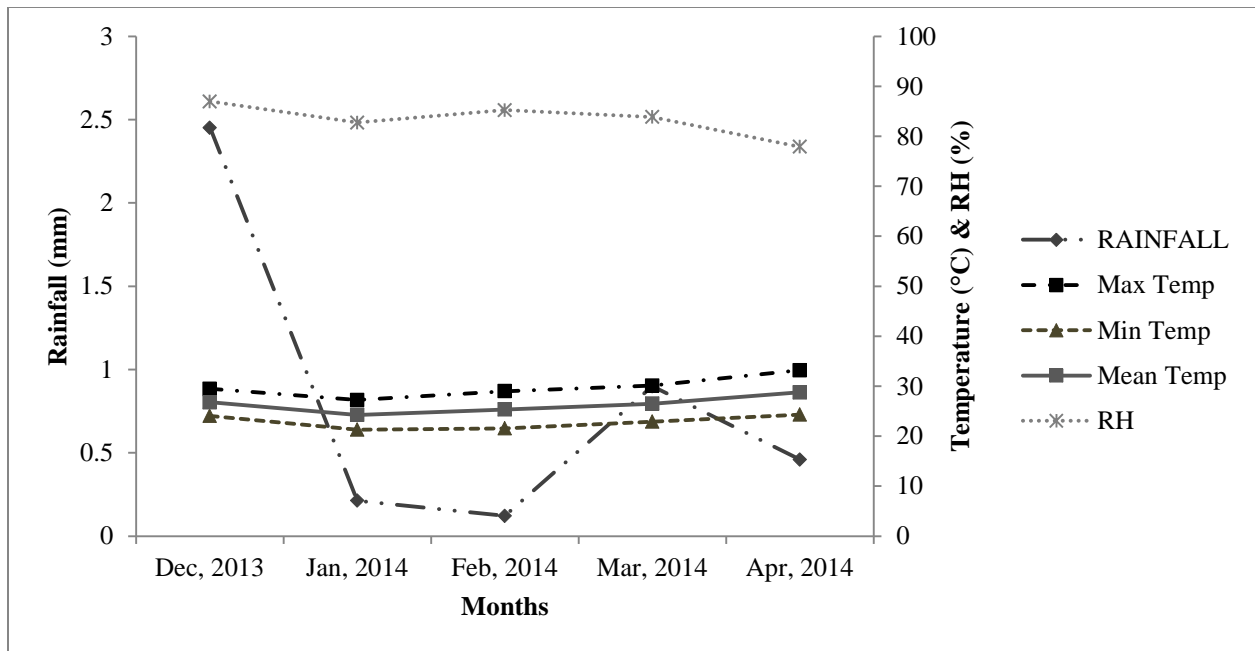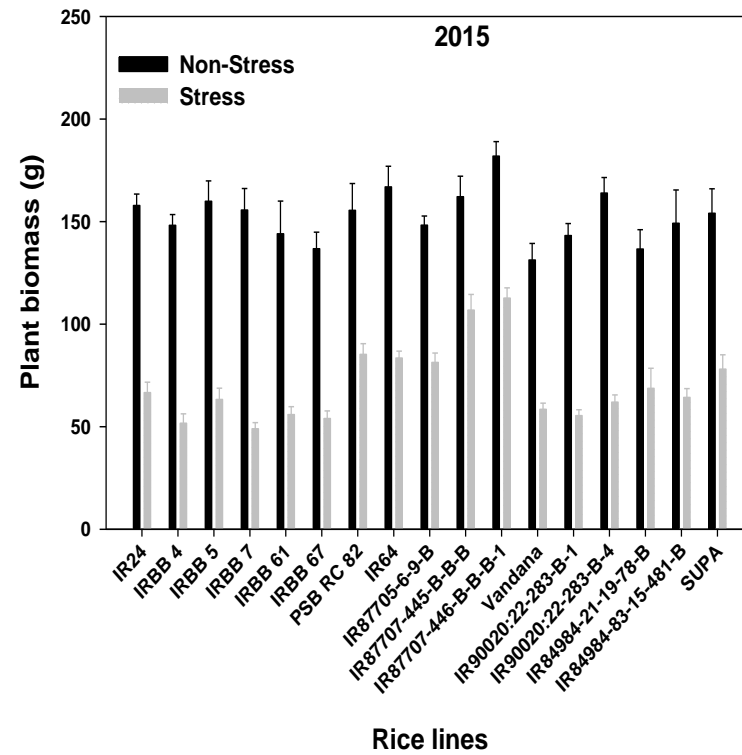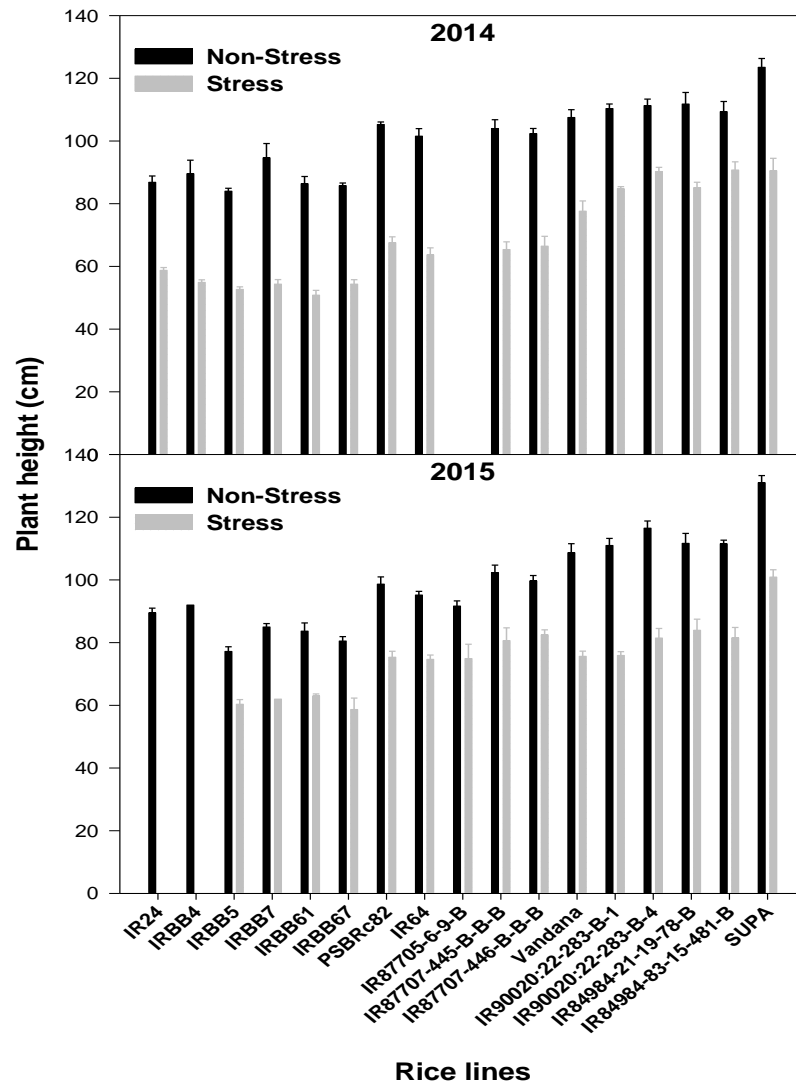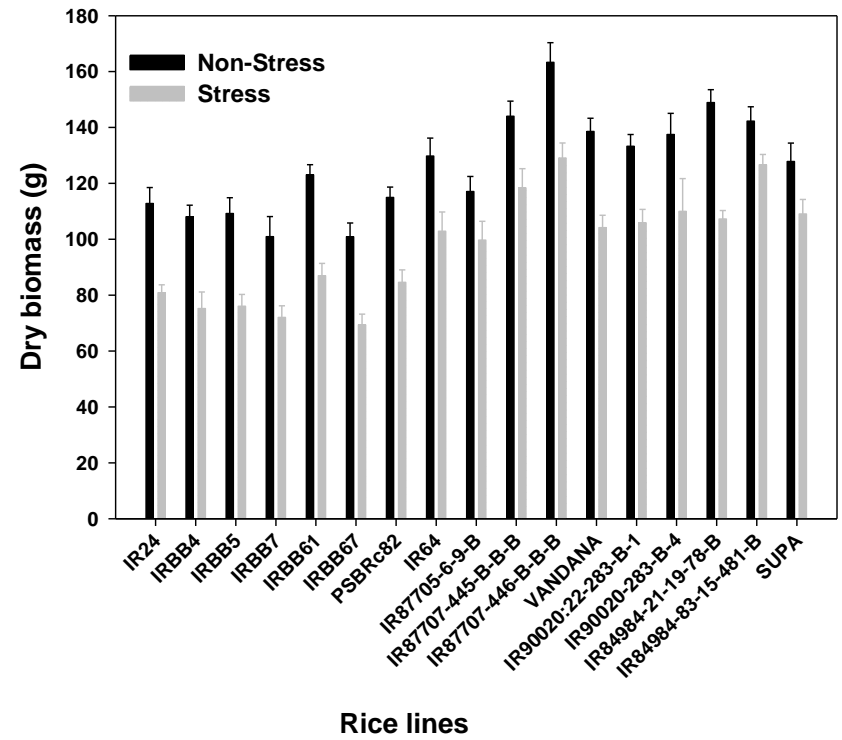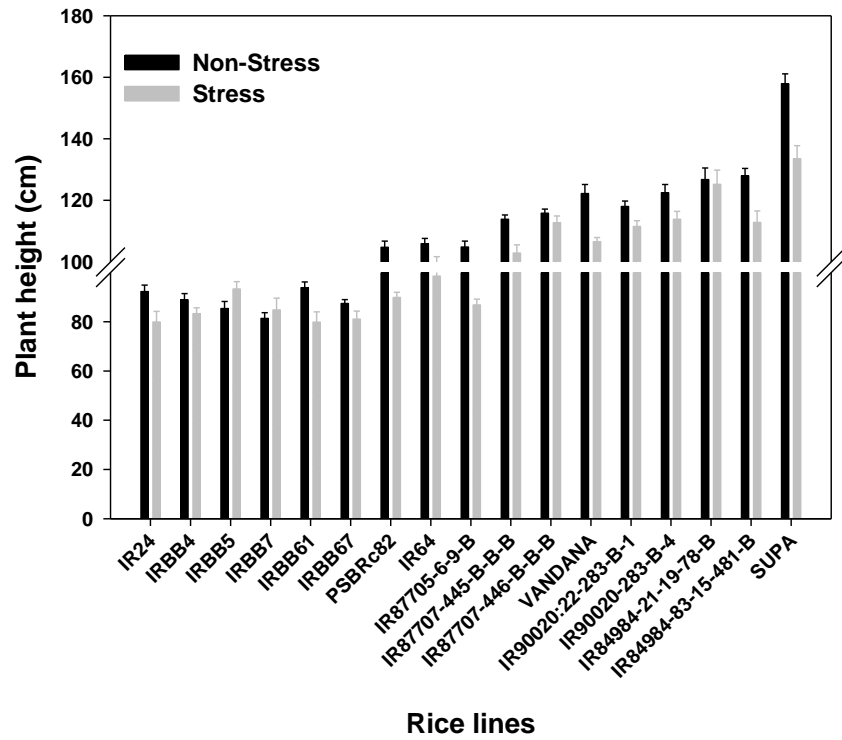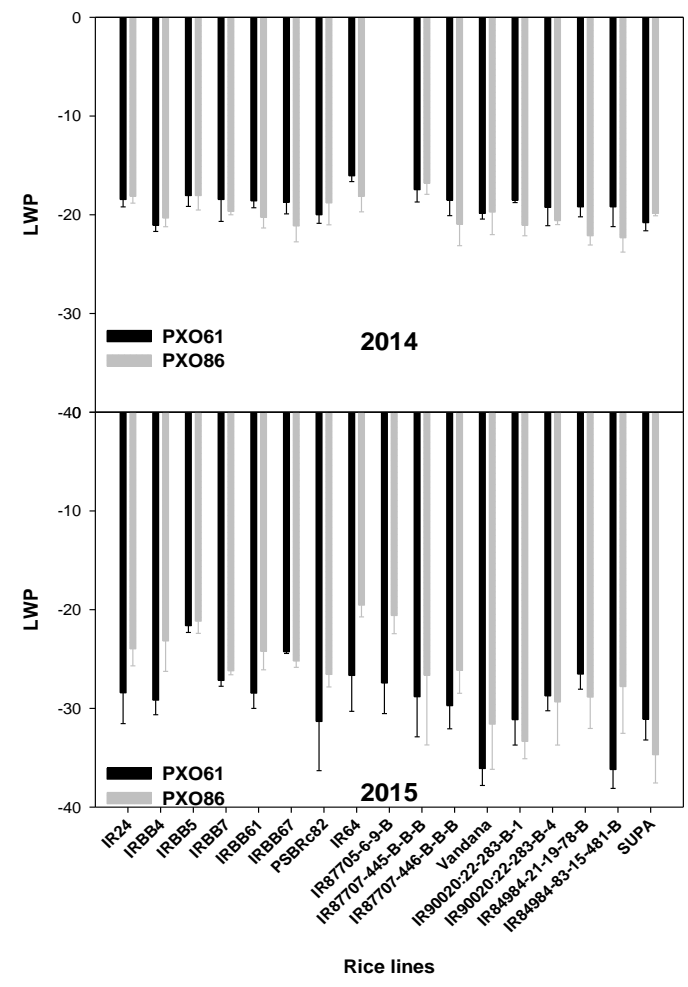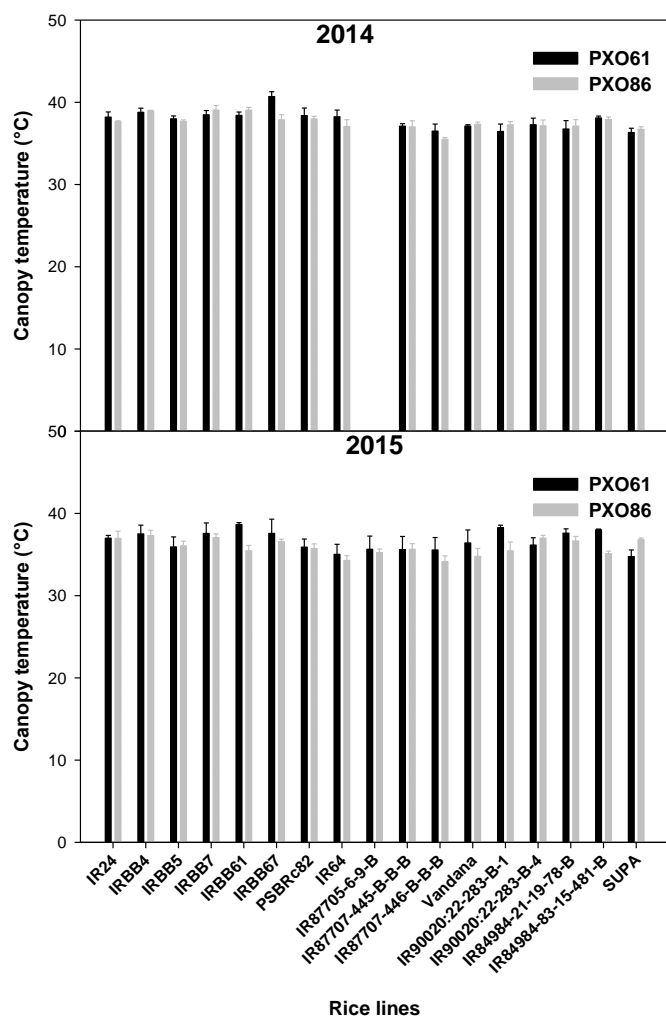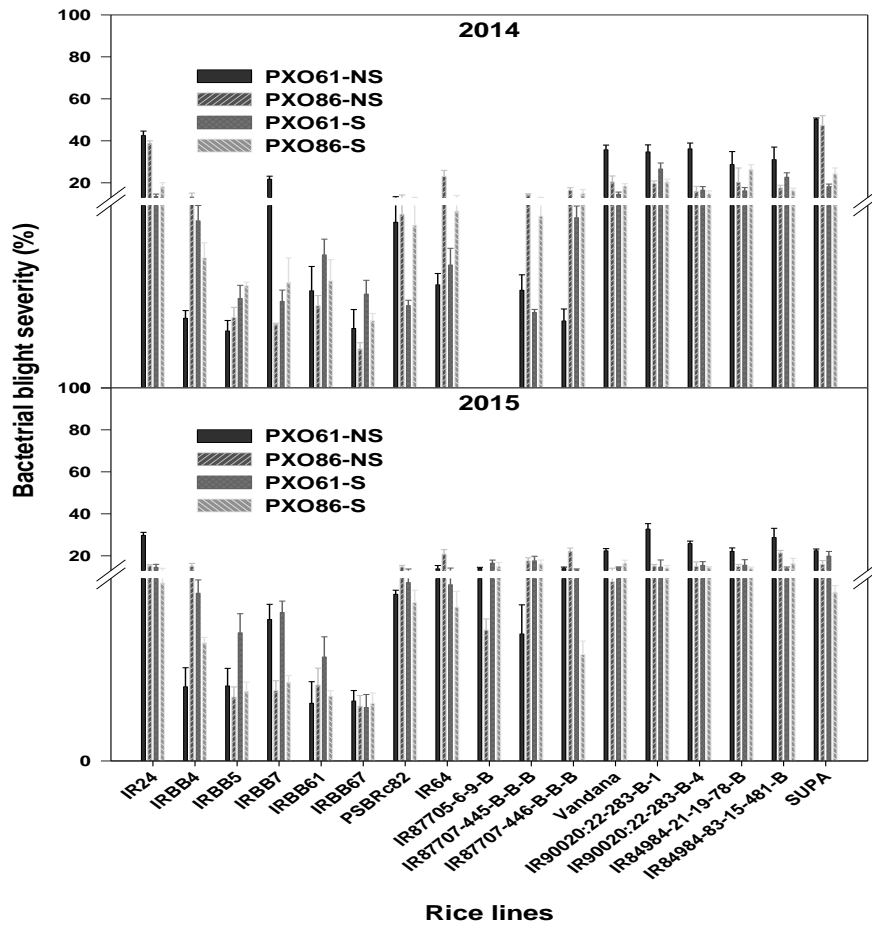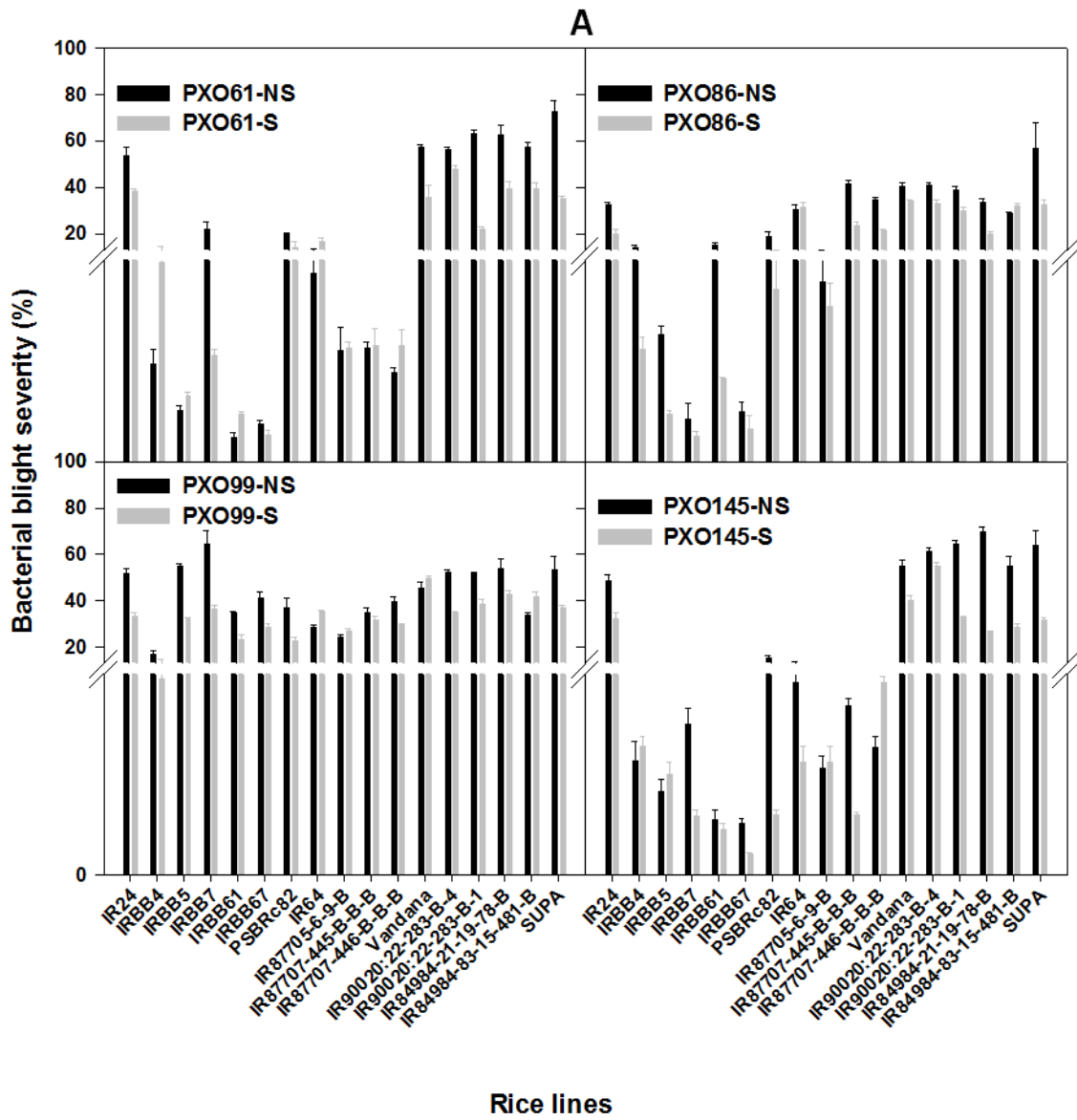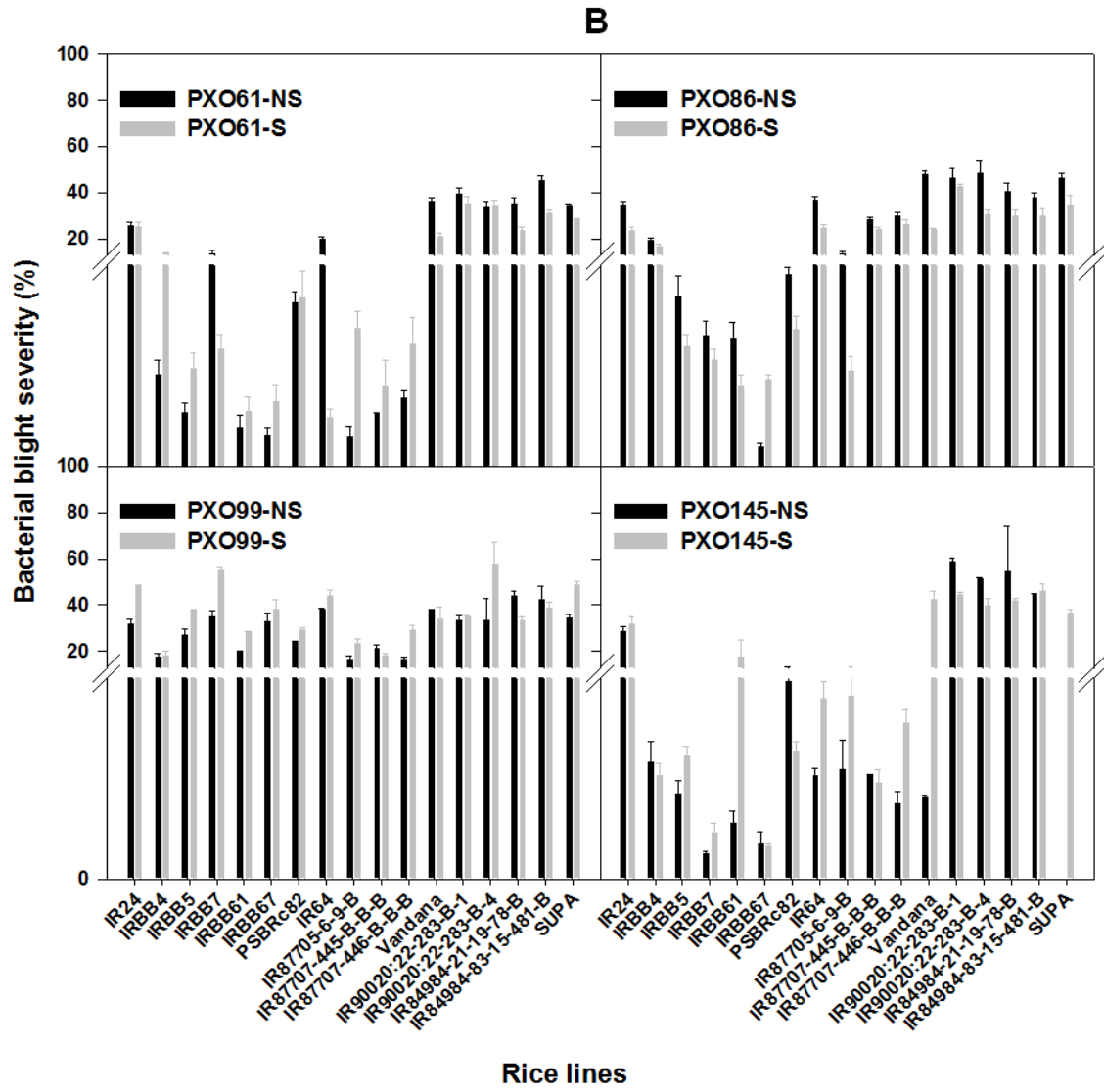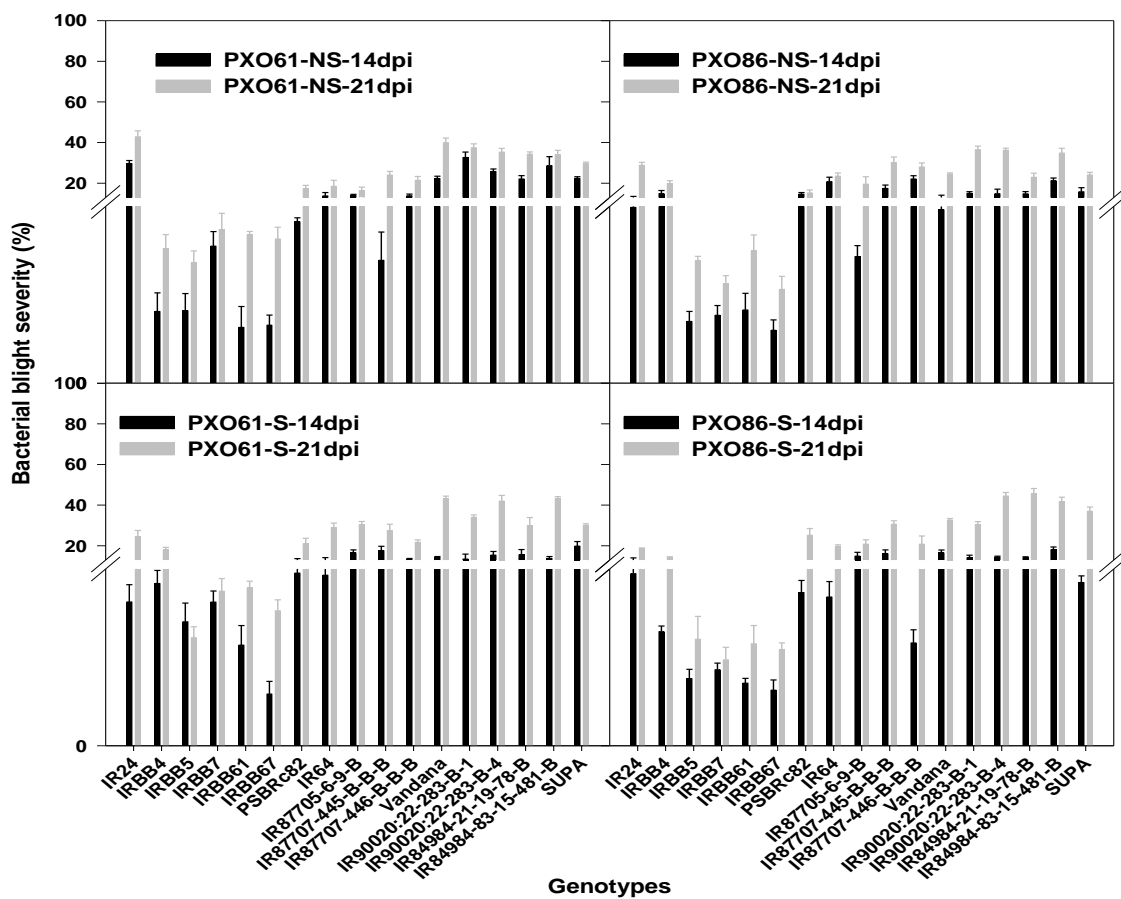
Figure 6B

Figure 7

Chapter 4: High temperature enhances the resistance of cultivated African rice, Oryza glaberrima, to bacterial blight

Gerbert Sylvestre Dossa[1,2*], Ricardo Oliva[1], Edgar Maiss[2], Casiana Vera Cruz[1], Kerstin Wydra[2,3]

1: Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, Los Baños, Philippines

2: Department of Phytomedicine, Leibniz Universität Hannover, Hannover, Germany

3: Plant Production and Climate Change, Erfurt University of Applied Sciences, Erfurt, Germany

* Corresponding author: Gerbert S. Dossa c.dossa@irri.org

Abstract

Rice bacterial blight (BB) is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and is responsible for substantial yield loss worldwide. Host resistance remains the most feasible control measure. However, pathogen variability leads to the failure of certain resistance genes to control the disease, and climate change with high amplitudes of heat predisposes the host plant to pathogen invasion. Due to pressure in natural selection, landrace species often carry a wide range of unique traits conferring tolerance of stress. Therefore, exploring their genetic background for host resistance could enable the identification of broad-spectrum resistance to combined abiotic and biotic stresses. Nineteen *Oryza glaberrima* accessions and *O. sativa* rice variety SUPA were evaluated for bacterial blight resistance under high temperature (35/31°C day/night temperatures) using 14 *Xoo* strains originated from the Philippines. Under normal temperature, most of the accessions showed resistance to 9 strains (64.3%) and accession TOG6007 showed

broad-spectrum resistance to 12 strains (85.7%). Under high temperature, most accessions showed a reduction in BB disease, whereas, accession TOG5620 showed disease reduction from all the *Xoo* strains under high temperature. Molecular characterization using gene-based and linked markers for BB resistance genes *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa2*1 revealed the susceptible alleles of *Xa4*, *xa5*, *xa13* and *Xa21* in *O. glaberrima*. However, no allele of *Xa7* was detected among *O. glaberrima* accessions. Our results suggest that *O. glaberrima* accessions contain a BB resistance different from the *Xa* gene type. Genome-wide association mapping could be used to identify quantitative trait loci (QTL) that are associated with BB resistance or combined BB resistance and high-temperature tolerance.

Keywords: Bacterial blight, *Xoo*, Rice, *Oryza glaberrima*

Introduction

Rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most important bacterial diseases in rice. Reported in several rice-producing countries in Asia and Africa, rice bacterial blight affects rice production worldwide. Thus, rice-*Xoo* interaction leads to severe yield losses in major rice ecosystems. Host resistance is still the most economically effective control measure against the disease, and, so far, 39 rice resistance genes have been identified against Asian *Xoo* populations (Natrajkumar et al. 2012; Khan et al. 2014; Zhang et al. 2014). However, in Africa, up till now, there has been no resistance gene identified to control this disease. The effectiveness or durability of host-plant resistance may be affected by climate change (Reddy 2015).

Climate change, with air temperature increase and water scarcity, will affect world agricultural production. Several reports have shown the effects of global warming on pathogen and host plant alike. Increase in temperature predisposes the host plant to pathogen colonization. Webb et al. (2010) reported an increase in BB disease on rice near-isogenic lines carrying single *Xa3*, *Xa4*, *xa5* and *Xa10,* with *Xa4* resistance genes mostly under high temperature; however, the effectiveness of bacterial blight resistance gene *Xa7* was enhanced under the same conditions. Similarly, the wheat stripe rust *R* gene, *Yr36*, confers broad-spectrum resistance to *Puccinia striiformis* f.sp *tritici* at high temperature (25-35°C) and shows susceptibility to the pathogen at low temperature (15°C) (Uauy et al. 2005). Therefore, there is an urgent need to develop cultivars with resistance to/tolerance of BB to sustain rice production under changing climate conditions. *Oryza glaberrima* is known to possess many important traits.

Oryza glaberrima (2n=14, AA) is the second most important cultivated rice worldwide. It was domesticated in West Africa in the Niger River Delta more than 3,500 years ago (Yves et al. 2012). Because of its low-yielding characteristic, *O. glaberrima* was progressively abandoned for *O. sativa*, the Asian cultivated rice, which has a higher

yield (Jones et al. 1997). However, because of its adaptation to African climate conditions, *O. glaberrima* is still grown in some West African countries. Although the high-yielding cultivars of *O. sativa* are preferred, their adaptation to the adverse conditions of the local environment is not satisfactory. *O. glaberrima* is well known to possess many useful traits, such as resistance to blast, rice yellow mottle virus (RYMV), sheat blight, nematodes, stem borer, hispa, stalk-eyed fly, the African gall midge for weed competitiveness, and bacterial blight (Alam and Efron 1986; Albar et al. 2006; Baggie et al. 2002; Djedatin et al. 2011; Lorieux et al. 2003; Maji et al. 2001; Ndjondjop et al. 1999; Nipah et al. 1997; Nwilene et al. 2002; Sahrawat and Sika 2002; Silue and Notteghem 1991; Thélémé et al. 2010; Wang et al. 1996), and tolerance of drought, submergence, soil acidity, salt, iron and aluminum. Interestingly, notable traits from *O. glaberrima* have been combined with high-yielding traits of *O. sativa* to generate the New Rice for Africa (NERICA) (Jones et al. 1997). Vikal et al. (2007) have identified 13 *O. glaberrima* accessions with resistance to Indian *Xoo* pathotypes, and a narrow genetic base for resistance to *Xoo* among 107 accessions of *O. glaberrima* was reported (Djedatin et al. 2011).

Natural selection often generates wild species with diverse traits, such as disease resistance, that could be inherited by wild relatives (Das et al. 2014). Thus, resistance genes were identified from wild rice. For example, BB resistance gene *Xa21*, which confers resistance to several Philippine *Xoo* races, was identified from African rice, *O. longistaminita.* Identification of resistance genes from African rice would enable breeding of resistance cultivars not only against the African *Xoo* population, but also against the Asian *Xoo*. Discovery of new, large-spectrum resistance genes will contribute to the control of rice bacterial blight disease in the future. To our knowledge, there is no report available on the combination of high temperature and bacterial blight

stress on *O. glaberrima*. This study aims to evaluate selected accessions of the cultivated *O. glaberrima* to a combination of high temperature and bacterial blight stresses.

Materials and methods

Plant materials

Nineteen (19) accessions of *O. glaberrima* (Table 1) were obtained from the Africa Rice Center gene bank (Cotonou, Benin), with the help of Dr. Drissa Silue. The 19 accessions, which originated from West African countries (Ghana, Liberia, Mali, Nigeria and Senegal), were selected according to their resistant reaction to African *Xoo* strain Mai1 and Asian *Xoo* race 2 strain PXO86 (Djedatin et al. 2011). We also included *O. sativa* variety SUPA (accession IRGC 69789) as it is one of the most preferred varieties in East Africa. One set of plants were grown under greenhouse conditions (12h day length). To evaluate the combination of high temperature and *Xoo*, the plants were grown under greenhouse conditions for 21 days and then transferred into a growth chamber (12h light and 12h dark, 35/31°C day/night temperatures and 80% relative humidity). Rice genotypes IR24, IRBB4, IRBB5, IRBB7, IRBB13 and IRBB21 were used as control for Xa-gene allele analysis.

Bacterial blight inoculation and evaluation

Fourteen strains (Table 1) representative of the Philippines' 10 *Xoo* races (Cottyn and Mew, 2007) were used in this study. Strains were grown on Modified Wakimoto's medium as described by Leach et al. (1992) for 72 hours. The inocula were prepared by suspending the harvested bacterial cells in demineralized sterile water. Approximately 10^8 CFU/ml of each inoculum were used to inoculate the plants with the use of the leaf clipping inoculation method (Kauffman et al. 1973) 4 weeks after sowing, under greenhouse conditions and in growth chamber under 35/31°C (day/night temperatures). Disease assessment was done by measuring lesion length 14 days after inoculation. Two replicated trials were performed, each trial consisting of 3 replicates with 6 plants per

strain. The average lesion length was classified as follows: resistant (lesion length 0-5 cm), moderately resistant (lesion length 5.1-10 cm), moderately susceptible (lesion length 10.1-15 cm) and susceptible (lesion length >15 cm). Ten accessions, including SUPA, were selected for resistance to bacterial blight under high temperature after evaluating 20 rice accessions in the greenhouse.

O. glaberrima **genotyping using *Xa* gene markers**

All the accessions were genotyped for the presence or absence of the resistance alleles of *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*. Rice genotypes IRBB4, IRBB5, IRBB7, IRBB13 and IRBB21 with resistance alleles *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*, respectively, were used as control. IR24 was used as a susceptible allele control. Genomic DNA was extracted from each sample and control using the CTAB DNA extraction protocol. The genomic DNA was purified with RNase. The primer pairs used for *Xa* gene detection and their corresponding product size are listed in Table 2.

For *Xa4* detection, 50-100 ng of genomic DNA were used to detect resistance and susceptible alleles in 10 μl PCR reaction according to the following cycles: initial denaturation at 94°C for 4 min; 32 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min; and a final extension step at 72°C for 8 min.

For *xa5* allele detection, 10-20 ng genomic DNA were used in 10 μl PCR reaction according to the following cycles: initial denaturation at 94°C for 3 min; 34 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 1 min, and extension at 72°C for 1 min; and a final extension step at 72°C for 4 min.

For *Xa7*, 50-100 ng of genomic DNA were used in 10 μl PCR reaction according to the following cycles: initial denaturation at 94°C for 4 min; 30 cycles of denaturation at 94°C

for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 2 min; and a final extension step at 72°C for 8 min.

xa13 alleles were detected with 10-20 ng genomic DNA in 10 μl PCR reaction according to the following cycles: initial denaturation at 94°C for 4 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min; and a final extension step at 72°C for 8 min.

Some 50-100 ng of genomic DNA were used for *Xa21* alleles in 10 μl PCR reaction according to the following cycles: initial denaturation at 94°C for 4 min; 32 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 2 min; and a final extension step at 72°C for 8 min.

The PCR products were separated in 1% agarose gel for *Xa7*, *xa13* and *Xa21* and 2% for *Xa4* and *xa5*, and then visualized under UV light.

Data analysis

The mean value from two replicated experiments was used for variance analysis (ANOVA) of bacterial blight lesion length. The significant difference ($p < 0.001$) in lesion length between accessions was assessed using the F test. Statistical analysis was performed using the R package.

Results

Identification of *O. glaberrima* accessions with broad-spectrum resistance to Philippine *Xoo* strains

O. glaberrima accessions and SUPA showed different reactions in response to the 14 *Xoo* strains (Figure). *O. sativa* variety SUPA was susceptible to all the strains (Table 3, Supplementary table). For the *O. glaberrima* accessions, all of them showed resistance to moderate resistance to PXO86 (race2), PXO79 (race3B), PXO347 (race9c) and PXO341 (race10). The majority of accessions were resistant to moderately resistant to PXO340

(race3C), PXO112 (race5), PXO145 (race7), PXO339 (race9A) and PXO349 (race9B). Strains PXO61, PXO71, PXO99, PXO363 and PXO280 were the most virulent ones with 18, 16, 19, 12 and 10 accessions being moderately susceptible to susceptible to them, respectively (Table 3, Supplementary table). *Xoo* strains PXO86, PXO79, PXO347 and PXO341 were less virulent on *O. glaberrima* accessions. Race 3 strains (PXO79, race 3B and PXO340, race 3C) induced different reactions on *O. glaberrima*. All the accessions were resistant and moderately resistant to PXO79.

O. glaberrima accessions TOG5953 and TOG6007 showed broad-spectrum resistance to 12 *Xoo* strains and TOG5810, TOG5566 and TOG7173 were resistant to 11 *Xoo* strains. *O. glaberrima* accessions TOG5458 and TOG5523 were susceptible to 6 *Xoo* strains (Table 3).

Accessions TOG5989 and TOG5473 were moderately susceptible and susceptible, respectively, to PXO340. Among race 9 strains, PXO363 (race 9d) was the most virulent with only 7 accessions showing resistance. Accessions CG17 and TOG5447 were moderately susceptible to race 9a, and accession TOG5523 was moderately susceptible to race 9b. All the accessions were resistant to moderately resistant to race 9c. Significant differences in lesion length were observed among all the accessions and differences were also significant among strains ($p < 2.20E - 16$), (Table 4).

High temperature enhances *O. glaberrima* resistance to *Xanthomonas oryzae* pv. *oryzae*
O. glaberrima resistance to *Xoo* was found to be enhanced under high temperature (HT) (35/31°C). Bacterial blight resistance was evaluated in 9 *O. glaberrima* accessions and SUPA. Majority of the accessions showed disease lesion length reduction and HT compared to the greenhouse study (Table 5). Accession TOG5620 showed broad-spectrum resistance to all *Xoo* strains, with moderate resistance to strains PXO71 and PXO99 under HT. Increase in lesion length under HT was observed in accession × strain—CG17 × PXO71; TOG5464 × PXO339, PXO341 and PXO347; TOG5953 × PXO71,

PXO112 and PXO339; TOG7173 × PXO339, PXO340 and PXO341; and SUPA × PXO61 and PXO145. Although most of the accessions showed lesion length reduction under high temperature, average lesion length induced by *Xoo* strains PXO61, PXO71 and PXO99 were generally higher compared to lesion lengths induced by other strains.

The lesion lengths under each treatment were significantly different among treatments and among accessions and strains, $p = 3.44E - 11$ (Table 5). Accession TOG5523 showed the strongest BB disease reduction under HT and TOG5620 was the most resistant to majority of the *Xoo* strains under HT.

Rice *Xa* gene detection from 10 *O. glaberrima* accessions

All the *O. glaberrima* accessions—SUPA, IR24, IRBB4, IRBB5, IRBB7, IRBB13 and IRBB21—were genotyped for the presence and absence of the *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* alleles. All the *O. glaberrima* accessions carried the susceptible alleles of *Xa4*, *xa5*, *xa13* and *Xa21*. No *Xa7* alleles were detected among *O. glaberrima* accessions and SUPA (Table 6). The *Xa4* resistant allele was detected in SUPA. As expected, IRBB4, IRBB5, IRBB7, IRBB13 and IRBB21 showed resistant alleles *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*, respectively. The susceptible alleles were detected in IR24.

Discussion

As resistance loss can occur anytime because of pathogen adaptation and variability, a continuous search for new resistance traits is necessary. Continuous exploring of existing BB *R* genes or, alternatively, the search among cultivated and wild rice genotypes to discover new *R* genes with broad-spectrum resistance would enable containing the disease in the future, particularly, under climate change. In this study, 19 cultivated African rice accessions evaluated for reaction to BB and combined BB and heat stresses showed different responses to BB. One accession with broad-spectrum resistance to at least 12 Philippine strains was identified, suggesting that more *O.*

glaberrima accessions should be evaluated to identify more accessions with broad-spectrum resistance to BB. Nevertheless, 4, 5 and 6 accessions were identified as having resistance to moderate resistance to 11, 10 and 9 *Xoo* strains, respectively, among the 14 strains. Our results are supported by previous reports which showed *O. glaberrima* accessions with broad-spectrum resistance to 7 Indian *Xoo* strains (Vikal et al. 2007) and resistance to West African *Xoo* strains and the Philippine strain, PXO86 (Djedatin et al. 2011). Moreover, the *O. glaberrima* accessions with broad spectrum resistance reported by Vikal et al. (2007) were not included in this study, suggesting that *O. glaberrima* could be a store of resistance to several BB populations. Future studies are therefore required to explore *O. glaberrima* gene pool for the development of new resistance cultivars. A new resistance gene for controlling BB in Asia, particularly in the Philippines, could be identified among *O. glaberrima* accessions. Furthermore, the use of broad-spectrum BB resistance genes would reduce the development of new virulent strains, and broad-spectrum resistance traits among *O. glaberrima* could be introduced into *O. sativa* elite varieties through hybridization. Although majority of the accessions ranged from moderately susceptible to susceptible to PXO61, accession TOG6007 showed susceptibility to only PXO71, PXO99 and PXO363.

Rice BB *R* gene *Xa4* confers resistance to IRBB4 against PXO61 and has been widely used in Asia; however, the occurrence of new virulent strains affects the durability of *Xa4* (Vera Cruz et al. 2000). We suggest that accession TOG6007 could be useful in areas dominated by Philippine race 1 strain. Majority of the accessions were susceptible to PXO61, PXO71, PXO99, PXO280 and PXO363. This could be explained by the: (a) presence of conserved transcription activator-like (TAL) effectors in these strains that activate susceptibility genes among the selected *O. glaberrima* accessions (4), and (b) lack of resistant genes among these accessions that could recognize the avirulence protein of

these strains. TAL effectors play an important role in the successful colonization of the host plant.

In this study, high temperature had generally shown positive effects on *O. glaberrima* resistance to BB. The lesion lengths induced by different *Xoo* strains were generally reduced under high temperature in comparison to the greenhouse study, suggesting that *O. glaberrima* tolerance of abiotic stress enhanced its biotic stress response under HT. Resistance to abiotic and biotic stresses has been reported in *O. glaberrima* (Alam and Efron 1986; Albar et al. 2006; Baggie et al. 2002; Djedatin et al. 2011; Lorieux et al. 2003; Maji et al. 2001; Ndjondjop et al. 1999; Nipah et al. 1997; Nwilene et al. 2002; Sahrawat and Sika 2002; Silue and Notteghem 1991; Thélémé et al. 2010; Wang et al. 1996), however, none of the previous reports had shown a dual stress (abiotic and biotic) response. Thus, we suppose that *O. glaberrima* possesses traits that respond to combined stresses and could be useful for *O. sativa* varieties improvement. Although reproductive barrier between *O. glaberrima* and *O. sativa* has been a major constraint in order to explore the gene pool offers by *O. glaberrima*, further studies involving development of chromosome segment substitution lines (CSSLs) to reduce hybrid sterility (Lorieux et al. 2013) are recommended to identify quantitative trait loci (QTL) responsible for abiotic and biotic stress in *O. glaberrima* to be used for development of interspecific hybridization between *O. glaberrima* and *O. sativa* (Ghesquière et al. 1997).

Conversely, a few combinations of strains and four accessions (CG17, TOG5464, TOG5953 and TOG7173), including SUPA, showed an increase in BB disease with high temperature, which suggests that more attention should be given for resistance durability under combined stresses through abiotic and biotic factors. This result was more often observed with *Xoo* strains PXO71, PXO339, PXO341 andPXO347.

Previous reports have shown temperature effects on plant responses to pathogens. In rice, Webb et al. (2010) reported an increase in BB disease on IRBB4 and a reduction on IRBB7 under high temperature. A wheat variety with *R* gene *Yr36* showed broad-spectrum resistance to the wheat stripe rust (*Puccinia striiformis* f.sp *tritici*) at high temperature (25-35°C), but susceptibility at low temperature (15°C) (Uauy et al. 2005). This suggests that, identification of accessions that increase plant resistance and thus reduce pathogen aggressiveness under high temperature conditions could play an important role in breeding rice varieties for combined stresses.

In this study, molecular characterization of *O. glaberrima* accessions and SUPA revealed that none of the *O. glaberrima* accessions possesses the resistance alleles of *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*. Interestingly, *Xa7* alleles were not detected in any of the accessions, suggesting that *O. glaberrima* lacks *Xa7*. Moreover, resistance to PXO86 (*avrXa7*) may be conferred by a different type of resistance gene in *O. glaberrima*. The absence of resistance alleles of *Xa4*, *xa13* and *Xa21* correlate with the phenotype data, since all the accessions were susceptible to PXO61 (except TOG6007) and PXO99. Although SUPA was susceptible to all *Xoo* strains, the molecular characterization of SUPA was revealed homozygous for the *Xa4* resistant allele. This suggests that the *Xa4* resistant allele in SUPA is defective and, moreover, the *Xa4* marker used in this study is not a linked marker.

The use of resistant cultivars remains the most effective control measure. Thirty-nine *R* genes have been identified in rice (Natrajkumar et al. 2012; Khan et al. 2014; Zhang et al. 2014), among which some of them, such as *Xa21*, derived from wild rice, originates from *O. longistaminata* (Khush et al. 1990; Song et al. 1995), *Xa23* from *O. rufipogon* (Zhang et al. 1998), *Xa27* from *O. minuta* (Gu et al. 2004, 2005) and *Xa30(t)* from *O. nivara* (Cheema et al. 2008). Wild rice genotypes are highly diverse due to natural selection and possess several traits that could be used for introgression of disease resistance in

rice cultivars. Thus, mutations due to selection pressure could be transmitted from the ancestor to various cultivated accessions. More attention should therefore be given to African cultivated rice and its ancestor, *O. longistaminata*, and to other wild rice species, to identify traits of combined BB resistance and abiotic stress tolerance. PCR-based detection of *Xa* genes revealed no resistance alleles of *R* genes studied here that could be associated to BB resistance. Therefore, we suggest the use of genome-wide association mapping to identify possible QTLs that are associated with BB resistance observed from *O. glaberrima*.

References

1. Alam, M. S., and Efron, Y. 1986. Resistance to stalk eyed fly, *Diopsis macroptholma*. Dalman. Int. Rice Commun. Newsl. 31:40-45.

2. Albar, L., Bangratz-Reyser, M., Hébrard, E., Ndjiondjop, M.-N., Jones, M., and Ghesquière, A. 2006. Mutations in the eIF (iso) 4G translation initiation factor confer high resistance of rice to *Rice yellow mottle virus.* Plant J. 47:417-426.

3. Baggie, I., Zapata, F., and Sangina, N. 2002. Genotypic response to aluminium toxicity of some rice. Int. Rice Res. Notes. 27:42.

4. Cheema, K. K., Grewal, N. K., Vikal, Y., Sharma, R., Lore, J. S., Das, A., Bhatia, D., Mahajan, R., Gupta, V., Bharaj, T., and Singh, K. 2008. A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 kb region on chromosome 4L and transferred to *Oryza sativa* L. Genet. Res. 90:397- 407.

5. Cottyn, B., Mew, T. 2007. Bacterial blight of rice. In Encyclopedia of Plant and Crop Science, Marcel Dekker, Inc (2007) http://dx.doi.org/10.1081/E-EPCS120010586

6. Das, B., Samik, S., Manoj P., and Tapas, K. G. 2014. Genetic diversity of the conserved motifs of six bacterial leaf blight resistance genes in a set of rice landraces. BMC Genetics. 2014, 15:82

7. Djedatin, G., Ndjiondjop, M.-N., Mathieu, T., Vera Cruz, C. M., Sanni, A., Ghesquière, A., and Verdier, V. 2011. Evaluation of African cultivated rice *Oryza glaberrima* for resistance to bacterial blight. Plant Dis. 95:441-447.

8. Ghesquière, A., Séquier, J., Second, G. and Lorieux, M. 1997. First steps towards a rational use of African rice, *Oryza glaberrima*, in rice breeding through a 'contig line' concept. Euphytica. 96:31–39.

9. Gu, K. Y., Yang, B., Tian, D. S., Wu, L. F., Wang, D. J., Sreekala, C., Yang, F., Chu, Z. Q., Wang, G. L., White, F. F., and Yin, Z. C. 2005. R gene expression induced by a type-III effectors triggers disease resistance in rice. Nature 435:1122-1125.

10. Gu, K., Tian, D., Yang, F., Wu, L., Sreekala, C., Wang, D., Wang, G. L., and Yin, Z. 2004. High-resolution genetic mapping of *Xa27(t),* a new bacterial blight resistance gene in rice. *Oryza sativa* L. *Theor.* Appl. Genet. 108:800-807.

11. Jones, M. P., Ding Kuhn, M., Aluko, G. K., and Semon, M. 1997. Interspecific *Oryza sativa* L. × *O. glaberrima* Steud. progenies in upland rice improvement. Euphytica 94:237-246.

12. Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., and Merca, S. D. 1973. An improved technique for evaluating resistance to rice varieties of *Xanthomonas oryzae*. Plant Dis. Rep. 57:537-541.

13. Khan, M.A., Naeem, M., and Iqbal, M. 2014. Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions**.** *Eur.* J. Plant Pathol. 139:27-37.

14. Khush, G. S., Bacalangco, E., and Ogawa, T. 1990. A new gene for resistance to bacterial blight from *O. longistaminata.* Rice. Genet. Newsl. 7:121- 122

15. Leach, J.E., Rhoads, M.L., Vera Cruz, C.M., White, F.F., Mew, T.W., and Leung, H. 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element. Appl. Environ. Microbiol. 58:2188-2195.

16. Lorieux, M., Reversat, G., Garcia Diaz, S. X., Denance, C., Jouvenet, N., Orieux, Y., Bourger, N., Pando-Bahuon, A., and Ghesquiere, A. 2003. Linkage mapping of Hsa-1Og, a resistance gene of African rice to the cyst nematode, *Heterodera sacchari*. Theor. Appl. Genet. 107:691-696.

17. Lorieux, M., Garavito, A., Bouniol, J., Gutiérrez, A., Ndjiondjop, M. N., Guyot, R., Pompilio Martinez, C., Tohme, J., Ghesquière, A. 2013. Unlocking the Oryza glaberrima treasure for rice breeding in Africa. Pages 130-143: In Realizing Africa's rice promise. Eds Wopereis, M. C. S., Johnson, D. E., Ahmadi, N., Tollens, E., Jalloh, A. (CABI), Wallingforg, UK.

18. Maji, T., Singh, B. N., and Akenova, M. E. 2001. Vegetative stage drought tolerance in *O. glaberrima* Steud and *O. sativa* L. and relationship between drought parameters. Oryza. 38:17-23.

19. Natrajkumar, P., Sujatha, K., Laha, G.S., Srinivasarao, K., Mishra, B., Viraktamath, B.C., et al, 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathology. 102: 222-228.

20. Ndjiondjop, M. N., Albar, L., Fargette, D., Fauquet, C., and Ghesquiere, A. 1999. The genetic basis of high resistance to yellow mottle virus (*RYMV*) in cultivars of the two cultivated rice species. Plant Dis. 83:22-24.

21. Nipah, J. O., Jones, M. P., Singh, B. N., Kantaka, O. S., and Sahrawat, K. L. 1997. Screening for tolerance for iron toxicity. Int. Rice Res. Notes. 22:26- 27.

22. Nwilene, F. E., Williams, U. T., Ukwungwu, M. N., Dakouo, D., Nacro, S., Hmadoun, A., and Kamara, S. I. 2002. Reactions of differential genotypes to African gall midge in West Africa. Intell. Pest Manage. 48:195-201.

23. Reddy, P.P. 2015. *Impacts on Crop Protection*. In Climate Resilient Agriculture for Ensuring Food Security. P 107-113, Spinger, New Delhi, India.

24. Sahrawat, K. L., and Sika, M. 2002. Comparative tolerance of *O. sativa* and *O. glaberrima* rice cultivars for iron toxicity in West Africa. Int. Rice Res. Notes. 27:30-31.

25. Silue, D., and Notteghem, J. 1991. Resistance of 99 *Oryza glaberrima* varieties to blast. Int. Rice Res. Newsl. 16:13-14.

26. Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, L. Y., Holsten, T., Gardner, J., Wang, B., Zhai, W. X., Zhu, L. H., Fauquet, C., and Ronald, P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science. 270:1804-1806.

27. Thiémélé, D., Boisnard, A., Ndjiondjop, M-N., Chéron, S., Séré, Y., Aké, S., Ghesquière, A., and Albar, L. 2010. Identification of a second major resistance gene to Rice yellow mottle virus, *RYMV2*, in the African cultivated rice species, *O. glaberrima.* Theor. Appl. Genet. 10:1300-1320.

28. Uauy, C., Brevis, J.C., Chen, X., Khan, I., Jackson, L., Chicaiza, O., Distelfeld, A., Fahima, T., and Dubcovsky, J. 2005. High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. Theoretical and Applied Genetics. 112: 97–105.

29. Vera Cruz, C. M., Bai, J. F., Ona, I., Leung, H., Nelson, R. J., Mew, T. W., and Leach, J. E. 2000. Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. Proc. Natl. Acad. Sci. USA. 97:13500-13505.

30. Vikal, Y., Das, A., Patra, B., Goel, R. K., Sidhu, J. S., and Singh, K. 2007. Identification of new sources of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in wild Oryza species and *O. glaberrima*. Plant Genet. Res. 5:108-112.

31. Wang, G. L., Song, W. Y., Ruan, D. L., Sideris, S., and Ronald, P. C. 1996. The cloned gene, *Xa21*, confers resistance to multiple *Xanthomonas oryzae* pv. *oryzae* isolates in transgenic plants. *Mol. Plant-Microbe Interact*. 9:850- 855.

32. Webb, K.M., Ona, I., Bai, J., Garrett, K.A., Mew, T., Vera Cruz, C.M., and Leach, J.E. 2010. A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene. New Phytologist. 185:568-576.

33. Yves A., Biaou, S. S. H., Sié, M., Vodouhè, R. S., and Ahanchédé, A. 2012. The African Rice *Oryza glaberrima* Steud: Knowledge Distribution and Prospects. Inter. J. of Biol. 4:15-180.

34. Zhang, F., Zhuo, D-L., Zhang, F., Huang, L-Y., Wang, W-S., Xu, J-L., Vera Cruz, C., Li, Z-K., and Zhou, Y-L. 2014. *Xa39*, a novel dominant gene conferring broad-

spectrum resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. Plant Pathology**.** doi: 10.1111/ppa.12283

35. Zhang, Q., Lin, S. C., Zhao, B. Y., Wang, C. L., Yang, W. C., Zhou, Y. L., Li, D. Y., Chen, C. B., and Zhu, L. H. 1998. Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) from *O. rufipogon*. Rice. Genet. Newsl. 15:138-142.

Table 1: List of rice accessions *Oryza glaberrima* and *Xanthomonas oryzae* pv. *oryzae* strains

S/N	Acc Name*	Acc No**	Origin
1	CG17[h]	IRGC 86741	Senegal
2	RAM90	na	Mali
3	RAM98	na	Mali
4	SUPA[h]	IRGC 69789	Tanzania
5	TOG5293	IRGC 96725	Nigeria
6	TOG5447	IRGC 96756	Nigeria
7	TOG5458	na	Mali
8	TOG5464[h]	IRGC 96760	Nigeria
9	TOG5473[h]	IRGC 104534	Nigeria
10	TOG5523[h]	IRGC 86757	Nigeria
11	TOG5566[h]	IRGC 96779	Nigeria
12	TOG5620[h]	IRGC 86764	Ghana
13	TOG5650	IRGC 104543	Nigeria
14	TOG5675	IRGC 96791	Nigeria
15	TOG5810	IRGC 86784	Liberia
16	TOG5953[h]	IRGC 96811	Nigeria
17	TOG5989[h]	na	Nigeria
18	TOG6007	IRGC 96824	Nigeria
19	TOG6231	IRGC 96854	Mali
20	TOG7173[h]	IRGC 96893	Nigeria
	Strain name	Race	
1	PXO61	Race1	Philippines
2	PXO86	Race2	Philippines
3	PXO79	Race3b	Philippines
4	PXO340	Race3c	Philippines
5	PXO71	Race4	Philippines
6	PXO112	Race5	Philippines
7	PXO99	Race6	Philippines
8	PXO145	Race7	Philippines
9	PXO280	Race8	Philippines
10	PXO339	Race9a	Philippines
11	PXO349	Race9b	Philippines
12	PXO347	Race9c	Philippines
13	PXO363	Race9d	Philippines
14	PXO341	Race10	Philippines

**Accession number according to IRRI (Los Baños, Philippines) germplasm data base, na: not available; *Accession name according to AfricaRice (Cotonou, Benin). [h]Accessions selected for combined high temperature and bacterial blight experiment.

Table 2: *Xa* gene-based and linked markers and size of their PCR products

Gene	Primer name	Sequence (5′ to 3′)	Type of marker	PCR Products
Xa4[a]	MP1_F	ATCGATCGATCTTCACGAGG	linked	S= 120bp
	MP2_R	dTG CTA TAA AAG GCA TTC GGG	linked	R= 150bp
Xa5[b]	Xa5_F2_Sus	GCTCGCCATTCAAGTTCTTGTC	Gene-based	
	Xa5-F2-Res	GCTCGCCATTCAAGTTCTTGAG	Gene-based	198bp
	Xa5_R2	CCTTGATAGAAACCTTGCTCTTGAC	Gene-based	
Xa7[a]	M5_F	CGATCTTACTGGCTCTGCAACTCTGT	linked	S= 1170bp
	M5_R	GCATGTCTGTGTCGATTGGTCCGTACGA	linked	R= 294bp
Xa13[a]	Xa13F_130-140	CCT GAT ATG TGA GGT AGT	Gene-based	S= 1326bp
	xa13R_1678-1662	GAG AAA GGC TTA AGT GC	Gene-based	R= 1523bp
Xa21[a]	Xa21 Forward	ATA GCA ACT GAT TGC TTG G	Gene-based	S= 1200bp
	Xa21 Reverse	CGA TCG GTA TAA CAG CAA AAC	Gene-based	R= 1400bp

[a] co-dominant primers

[b] Dominant primers

Table 3: *Oryza glaberrima* **reaction to 14 strains of** *Xanthomonas oryzae* **pv.** *oryza***e from the Philippines under greenhouse conditions**

Strains	R	MR	MS	S
PXO61 (Race 1)		TOG6007	TOG5293, TOG5620, TOG7173	CG17, RAM90,RAM98, SUPA, TOG5447, TOG5458,TOG5464, TOG5473,TOG5523,TOG5566, TOG5650,TOG5675,TOG5810, TOG5953,TOG5989, TOG6231
PXO86 (Race 2)	TOG7173	TOG6007,CG17,RAM90,RAM98,TOG544, TOG5458,TOG5464,TOG5473,TOG5523, TOG5566,TOG5650,TOG5675,TOG5810, TOG5953,TOG5989, TOG6231, TOG5293, TOG5620		SUPA
PXO79 (Race 3B)	TOG5650, TOG5810,	CG17, RAM90, RAM98, TOG5293, TOG5447, TOG5458, TOG5464,TOG5473, TOG5523, TOG5566, TOG5620,TOG5675, TOG5953, TOG5989,		SUPA,

		TOG6007,TOG6231, TOG7173		
PXO340 **(Race 3C)**	TOG5566, TOG5953, TOG6231	CG17, RAM90, RAM98, TOG5293, TOG5447, TOG5458, TOG5464,TOG5523, TOG5620, TOG5650, TOG5675,TOG5810, TOG6007, TOG7173	TOG5989,	SUPA, TOG5473,
PXO71 **(Race 4)**		CG17, RAM90, TOG5810, TOG5953,	TOG5523,TOG5566, , TOG5620,TOG5675, TOG5989, TOG6231	RAM98, SUPA, TOG5293, TOG5447, TOG5458, TOG5464, TOG5473, TOG5650, TOG6007, TOG7173
PXO112 **(Race 5)**	RAM90, TOG5566,TOG5675, TOG5810,TOG5953,TOG5989, TOG6007, TOG6231, TOG7173	CG17, TOG5293, TOG5447, TOG5458, TOG5473, TOG5523, TOG5620,TOG5650,	TOG5464,	RAM98, SUPA,
PXO99 **(Race 6)**				CG17, RAM90, RAM98, SUPA, TOG5293, TOG5447, TOG5458, TOG5464, TOG5473, TOG5523, TOG5566, TOG5620, TOG5650, TOG5675, TOG5810, TOG5953,

				TOG5989, TOG6007, TOG6231, TOG7173
PXO145 (Race 7)	TOG5650, TOG5810, TOG7173	CG17, RAM90, RAM98, TOG5293, TOG5464, TOG5473, TOG5523,TOG5566, TOG5620, TOG5675, TOG5953,TOG5989, TOG6007, TOG6231	SUPA, TOG5447,	TOG5458
PXO280 (Race 8)		RAM98, TOG5293, TOG5464, TOG5566, TOG5675, TOG5810, TOG5953, TOG6007, TOG7173	TOG5447,TOG5473,TOG5523,TOG5620,TOG5650,TOG5989, TOG6231	CG17, RAM90, SUPA, TOG5458
PXO339 (Race 9a)	TOG5293,TOG5464,TOG5473, TOG5566,TOG5810,TOG5953, TOG7173	RAM90, RAM98, TOG5458, TOG5523, TOG5620, TOG5650, TOG5675, TOG5989, TOG6007, TOG6231	CG17, TOG5447	SUPA
PXO349 (Race 9b)	TOG5293,TOG5464,TOG5473, TOG5620,TOG5810,TOG6231, TOG7173	CG17, RAM90, RAM98, TOG5447, TOG5458, TOG5566, TOG5650,TOG5675, TOG5953, TOG5989, TOG6007	TOG5523	SUPA
PXO347	TOG5953, TOG6231,	CG17, RAM90, RAM98, TOG5293,		SUPA

	R	MR	MS	S
(Race 9c)	TOG7173	TOG5447, TOG5458, TOG5464,TOG5523, TOG5566, TOG5620, TOG5650,TOG5675, TOG5810, TOG5989, TOG6007		
PXO363 (Race 9d)	TOG5464,TOG5473,TOG5566, TOG5953, TOG5989, TOG7173	RAM98	TOG5810, TOG6007	CG17, RAM90, SUPA, TOG5293, TOG5447, TOG5458, TOG5523, TOG5620, TOG6231, TOG5650, TOG5675
PXO341 (Race 10)	TOG5566,TOG5675,TOG5953, TOG7173	CG17, RAM90, RAM98, TOG5293, TOG5447, TOG5458, TOG5464,TOG5473, TOG5523, TOG5620, TOG5650,TOG5810, TOG5989, TOG6007, TOG6231		SUPA

Bacterial blight lesion lengths were evaluated under greenhouse conditions at two weeks after inoculation. Lesion lengths were scored 14 days after inoculation. Resistant (R): < 5 cm lesion length, moderately resistant (MR): > 5-10 cm lesion length, moderately susceptible (MS): > 10-15 cm lesion length, and susceptible (S): > 15 cm lesion length.

Table 4: Variance analysis of average lesion length from 20 rice accessions inoculated with 14 *Xoo* strains under greenhouse conditions

	Sum sq	Df	F value	Pr(>F)
Accession	10029.7	19	68.5506	<2.20E-16***
Strain	20340.7	13	203.1897	<2.20E-16***
Accession × strain	9691.4	247	5.0953	<2.20E-16***
Residuals	4312.3	560		

Sum of square (Sum sq); Degrees of freedom (Df): 14 *Xoo* strains (Df: 13) were used to inoculate 20 rice accessions (Df: 19) under greenhouse (GH) conditions. *** indicates the level of significance of each factor or between factors at p-value of 0.001.

Table 5: Bacterial lesion length on 10 rice accessions evaluated under greenhouse and high temperature conditions

Strains	CG17	SUPA	TOG5464	TOG5473	TOG5523	TOG5566	TOG5620	TOG5953	TOG5989	TOG7173
PXO61	25.5±2.43	25.05±2.7	19.4±2.02	17.6±0.98	16±1.49	18.7±1.88	14.3±1.74	17.3±2.26	19.04±1.83	13.81±0.75
(Race 1)- GH	(S)	(S)	(S)	(S)	(S)	(S)	(MS)	(S)	(S)	(MS)
PXO61	9.97±0.21	27.03±2.03	4.31±0.50	9.37±0.98	12.24±0.84	10.33±0.95	5.31±0.89	9.70±0.40	16.07±2.53	11.53±0.78
(Race 1)-HT	(MR)	(S)	(R)	(MR)	(MS)	(MS)	(MR)	(MR)	(S)	(MS)
PXO86	7.11±0.79	19.04±2.11	6.43±1.19	7.15±1.42	6.59±1.43	5.42±0.96	8.04±0.74	6.28±0.96	5.88±0.84	4.7±1.8
(Race 2)-GH	(MR)	(S)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)	(R)
PXO86	2.40±0.1	11.14±0.92	3.66±0.41	6.42±0.02	2.91±0.31	3.22±0.53	2.98±0.25	2.83±0.53	2.29±0.47	5.04±0.07
(Race 2)-HT	(R)	(MS)	(R)	(MR)	(R)	(R)	(R)	(R)	(R)	(MR)
PXO79	5.09±0.25	16.11±1.02	7.08±0.93	6.12±0.23	7.17±0.55	7.31±1.47	6.31±0.62	6.49±1.53	5.74±0.76	6.5±1.01
(Race 3B)-GH	(MR)	(S)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)
PXO79	2.88±0.77	7.63±0.88	4.35±0.13	3.13±0.83	6.44±1.41	3.61±0.85	2.63±0.48	2.74±0.59	3.14±0.66	2.85±0.78
(Race 3B)-HT	(R)	(MR)	(R)	(R)	(MR)	(R)	(R)	(R)	(R)	(R)
PXO340	7.17±1.57	21.17±1.76	5.31±0.93	17.13±0.23	8.15±0.84	4.53±0.36	7.24±0.59	4.93±0.73	13.05±0.5	5.03±1.66
(Race 3C)-GH	(MR)	(S)	(MR)	(S)	(MR)	(R)	(MR)	(R)	(MS)	(MR)
PXO340	2.18±0.58	11.48±1.23	4.08±0.48	7.56±1.31	2.53±1.13	2.48±1.47	5.44±0.32	2.27±0.61	4.55±0.13	15.37±1.79
(Race 3C)-HT	(R)	(MS)	(R)	(MR)	(R)	(R)	(MR)	(R)	(R)	(S)
PXO71	7.68±0.89	27.99±0.71	21.65±0.84	18.14±2.33	12.95±2.12	11.63±2.98	14.47±1.4	9.91±2.35	12.52±0.58	15.38±1.58
(Race 4)-GH	(MR)	(S)	(S)	(S)	(MS)	(MS)	(MS)	(MR)	(MS)	(S)
PXO71	14.74±0.74	15.75±1.99	13.75±0.13	15.06±1.11	6.11±0.36	13.15±0.97	13.01±0.15	14.84±0.86	3.84±0.29	10.63±0.25
(Race 4)-HT	(MS)	(S)	(MS)	(S)	(MR)	(MS)	(MS)	(MS)	(R)	(MS)
PXO112	6.01±2.52	24.91±0.24	13.46±0.65	5.91±2.27	5.44±1.97	4.18±1.44	5.21±0.95	4.82±1.65	4.16±1.25	3.84±1.35
(Race 5)-GH	(MR)	(S)	(MS)	(MR)	(MR)	(R)	(MR)	(R)	(R)	(R)
PXO112	2.61±0.69	18.56±2	3.99±0.57	8.43±0.93	3.26±0.59	3.12±0.39	3.28±0.63	7.70±0.80	4.78±0.77	3.56±0.44
(Race 5)-HT	(R)	(S)	(R)	(MR)	(R)	(R)	(R)	(MR)	(R)	(R)
PXO99	25.81±2.78	28.92±1.12	25.16±1.93	25.19±0.74	27.45±2.45	17.29±0.5	24±2.49	20.15±2.37	24.44±3.57	19.24±1.8
(Race 6)-GH	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
PXO99	5.19±0.34	26.55±0.30	12.33±0.21	10.48±1.25	3.45±0.21	6.81±0.27	12.08±1.98	9.86±0.7	5.08±0.7	14.05±0.98

(Race 6)-HT	(MR)	(S)	(MS)	(MS)	(R)	(MR)	(MS)	(MR)	(MR)	(MS)
PXO145 (Race 7)-GH	7.89±1.66	13.76±1.5	6.6±1.36	5.58±0.45	8.63±0.1	7.31±2.44	8.5±1.77	6.53±1.02	8.73±0.93	4.88±0.75
	(MR)	(MS)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)	(MR)	(R)
PXO145 (Race 7)-HT	2.47±0.76	17.01±1.08	3.55±0.65	5.26±0.25	4.64±0.16	4.39±1.02	3.42±0.12	4.15±0.0	7.28±0.48	4.56±0.48
	(R)	(S)	(R)	(MR)	(R)	(R)	(R)	(R)	(MR)	(R)
PXO280 (Race 8)-GH	17.88±3.32	28.26±2.42	5.99±0.56	14.5±2.46	10.95±2.75	6.96±1	10.17±1.45	9.1±0.84	13.13±1.75	7.3±0.5
	(S)	(S)	(MR)	(MS)	(MS)	(MR)	(MS)	(MR)	(MS)	(MR)
PXO280 (Race 8)-HT	5.68±0.68	13.97±0.92	4.62±0.74	4.49±1.04	4.06±0.01	1.94±0.19	4.10±0.85	13.92±1.62	3.89±0.47	4.89±0.54
	(MR)	(MS)	(R)	(R)	(R)	(R)	(R)	(MS)	(R)	(R)
PXO339 (Race 9a)-GH	10.75±1.66	29.22±2.81	4.1±0.41	4.94±0.65	7.1±0.69	4.93±1.11	5.86±0.86	5±1.25	6.4±0.78	3.65±0.8
	(MS)	(S)	(R)	(R)	(MR)	(R)	(MR)	(R)	(MR)	(R)
PXO339 (Race 9a)-HT	2.31±0.55	23.27±1.76	13.87±1.09	7.4±0.87	7.49±1.21	2.99±0.16	5.75±0.35	2.82±0.67	4.03±0.63	7.63±0.57
	(R)	(S)	(MS)	(MR)	(MR)	(R)	(MR)	(R)	(R)	(MR)
PXO349 (Race 9b)-GH	7.25±2.79	37.56±3.43	4.78±1.03	4.87±0.97	12.34±1.65	7.22±1.14	4.68±0.27	6.38±2.41	5.76±0.56	3.45±0.73
	(MR)	(S)	(R)	(R)	(MS)	(MR)	(R)	(MR)	(MR)	(R)
PXO349 (Race 9b)-HT	3.10±0.88	24.99±1.01	3.31±0.44	5.48±0.55	4.51±0.33	2.04±0.17	5.27±0.34	1.92±0.82	1.86±0.66	2.49±0.36
	(R)	(S)	(R)	(MR)	(R)	(R)	(MR)	(R)	(R)	(R)
PXO347 (Race 9c)-GH	7.35±1.96	28.46±2.45	5.27±0.97	4.92±0.80	5.32±1.41	6.22±0.84	5.31±0.10	4.75±1.13	6.2±0.80	4.53±0.59
	(MR)	(S)	(MR)	(R)	(MR)	(MR)	(MR)	(R)	(MR)	(R)
PXO347 (Race 9c)-HT	1.92±0.58	19.02±1.87	23.62±0.37	3.97±0.7	3.27±0.84	6.0±0.11	5.29±0.53	16.07±2.04	7.17±1	5.36±0.63
	(R)	(S)	(S)	(R)	(R)	(MR)	(MR)	(S)	(MR)	(MR)
PXO363 (Race 9d)-GH	20.85±1.66	17.15±1.2	3.87±1.71	3.57±1.03	16.89±2.55	4.56±1	19.45±2.59	3.53±1.23	4.96±1.33	4.76±1.65
	(S)	(S)	(R)	(R)	(S)	(R)	(S)	(R)	(R)	(R)
PXO363 (Race 9d)-HT	6.79±0.92	9.22±2.03	3.81±0.19	4.44±0.24	3.93±0.96	7.62±1.34	4.96±0.2	2.86±0.05	3.01±0.92	2.64±0.67
	(MR)	(MR)	(R)	(R)	(R)	(MR)	(R)	(R)	(R)	(R)
PXO341 (Race 10)-GH	9.1±2.23	23.13±2.62	5.53±0.95	5.58±0.67	6.02±0.51	4.39±0.33	6.18±1.18	3.8±0.64	6.31±1.47	4.58±0.41
	(MR)	(S)	(MR)	(MR)	(MR)	(R)	(MR)	(R)	(MR)	(R)
PXO341 (Race 10)-HT	2.38±0.48	23.71±0.22	8.43±1.13	3.66±0.68	7.16±0.76	3.91±0.29	3.98±0.82	2.07±0.52	5.78±0.28	5.63±0.14
	(R)	(S)	(MR)	(R)	(R)	(R)	(R)	(R)	(MR)	(MR)

	Sum Sq	Df	F value	Pr(>F)
Strain	5078.7	13	54.82	<2.20E-16***
Stress	5799.2	1	813.808	<2.20E-16***
Variety	7167.2	9	111.7523	<2.20E-16***
Strain × stress	1025.6	13	11.0705	<2.20E-16***
Strain × accessions	3321.7	117	3.9841	<2.20E-16***
Stress × accessions	685.1	9	10.6821	3.10E-16***
Strain × stress × accessions	2097.0	117	2.5151	3.44E-11***
Residuals	1995.3	280		

Bacterial blight lesion lengths were evaluated under greenhouse (GH) and high temperature (HT) conditions at two weeks after inoculation. Lesion lengths were scored 14 days after inoculation. Each value is the mean ±standard error.
[a]The mean values are followed by the standard error. [b]The letters in bracket are reaction categories based on lesion length. Resistant (R): < 5 cm, moderately resistant (MR): > 5-10 cm, moderately susceptible (MS): > 10-15 cm, and susceptible (S): > 15 cm.

Sum of square (Sum sq); Degrees of freedom (Df): 14 *Xoo* strains (Df: 13) were used to inoculate 10 rice accessions (Df: 9) under greenhouse (GH) and high temperature (HT) conditions (Df: 1). *** indicates the level of significance of each factor or between factors at p-value of 0.001.

Table 6: Allele analysis of *Oryza glaberrima* genotype using *Xa4, xa5, Xa7, xa13* and *Xa21* markers[a]

Acc.	*xa4* (S)	*Xa4* (R)	*xa5* (R)	*Xa5* (S)	*xa7* (S)	*Xa7* (R)	*xa13* (R)	*Xa13* (S)	*xa21* (S)	*Xa21* (R)	Genotype[b]				
											Xa4	*xa5*	*Xa7*	*xa13*	*Xa21*
CG17	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
RAM90	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
RAM98	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
SUPA	-	+	-	+	+	-	-	+	+	-	*Xa4/Xa4[c]*	*Xa5/Xa5*	*xa7/xa7*	*Xa13/Xa13*	*xa21/xa21*
TOG5293	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5447	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5458	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5464	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5473	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5523	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5566	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5620	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5650	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5675	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5810	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5953	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG5989	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*

TOG6007	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG6231	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
TOG7173	+	-	-	+	-	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	-	*Xa13/Xa13*	*xa21/xa21*
IRBB4	-	+	-	+	+	-	-	+	+	-	*Xa4/Xa4*	*Xa5/Xa5*	*xa7/xa7*	*Xa13/Xa13*	*xa21/xa21*
IRBB5	+	-	+	-	+	-	-	+	+	-	*xa4/xa4*	*xa5/xa5*	*xa7/xa7*	*Xa13/Xa13*	*xa21/xa21*
IRRB7	+	-	-	+	-	+	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	*Xa7/Xa7*	*Xa13/Xa13*	*xa21/xa21*
IRBB13	+	-	-	+	+	-	+	-	+	-	*xa4/xa4*	*Xa5/Xa5*	*xa7/xa7*	*xa13/xa13*	*xa21/xa21*
IRBB21	+	-	-	+	+	-	-	+	-	+	*xa4/xa4*	*Xa5/Xa5*	*xa7/xa7*	*Xa13/Xa13*	*Xa21/Xa21*
IR24	+	-	-	+	+	-	-	+	+	-	*xa4/xa4*	*Xa5/Xa5*	*xa7/xa7*	*Xa13/Xa13*	*xa21/xa21*

[a] + and – indicate presence or absence, respectively, of resistance and susceptible alleles

[b] – indicates absence of resistance and susceptible alleles

[c] Defective resistance allele in SUPA
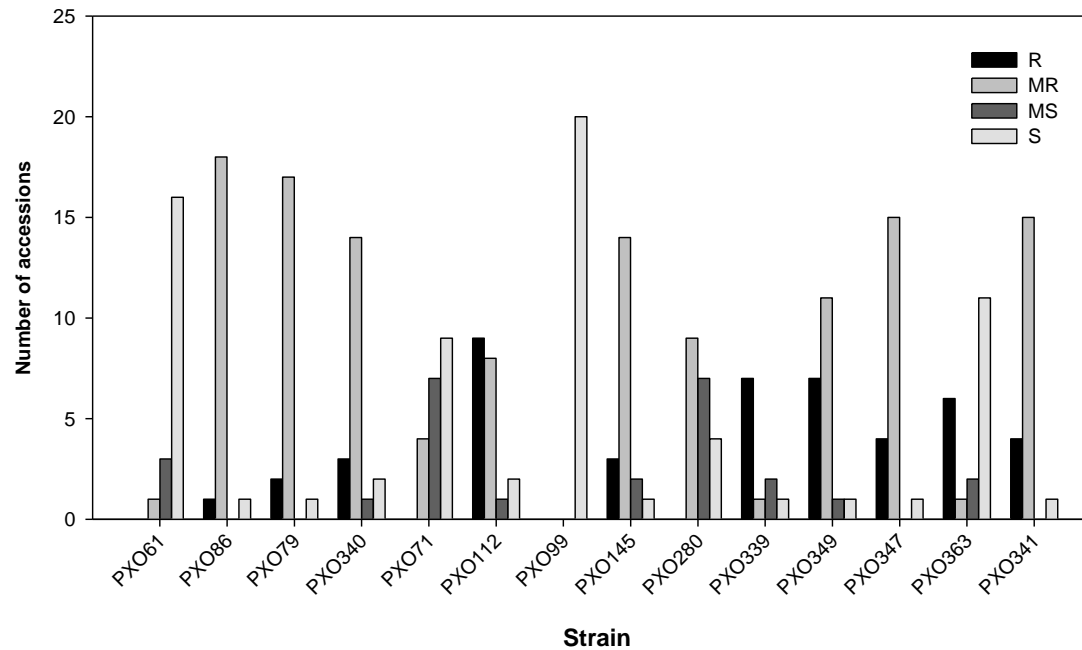
Figure: Reaction of 20 rice accessions to 14 strains of *Xanthomonas oryzae* pv. *oryza*e from the Philippines.

Bacterial blight lesion lengths were evaluated under greenhouse conditions at two weeks after inoculation. Lesion lengths were scored 14 days after inoculation

Resistant (R): < 5 cm lesion length, moderately resistant (MR): > 5-10 cm lesion length, moderately susceptible (MS): > 10-15 cm lesion length, and susceptible (S): > 15 cm lesion length.

Supplementary Table: Bacterial blight average lesion length of 14 *Xoo* strains from the Philippines

Accessions	PXO61 Race 1	PXO86 Race 2	PXO79 Race 3B	PXO340 Race 3C	PXO71 Race 4	PXO112 Race 5	PXO99 Race 6	PXO145 Race 7	PXO280 Race 8	PXO339 Race 9a	PXO349 Race 9b	PXO347 Race 9c	PXO363 Race 9d	PXO341 Race 10
CG17	25.5±2.43 (S)	7.11±0.79 (MR)	5.09±0.25 (MR)	7.17±1.57 (MR)	7.68±0.89 (MR)	6.01±2.52 (MR)	25.81±2.78 (S)	7.89±1.66 (MR)	17.88±3.32 (S)	10.75±1.66 (MS)	7.25±2.79 (MR)	7.35±1.96 (MR)	20.85±1.66 (S)	9.1±2.23 (MR)
RAM90	17.5±3 (S)	8.86±2.37 (MR)	5.33±0.65 (MR)	8.08±0.83 (MR)	8.17±0.25 (MR)	4.16±1.04 (R)	24.79±2.91 (S)	6.02±0.5 (MR)	17.06±1.21 (S)	8.22±0.32 (MR)	8.83±1.92 (MR)	8.98±1.9 (MR)	20.68±2.93 (S)	8.59±1.18 (MR)
RAM98	19.21±1.48 (S)	7.83±1.11 (MR)	6.64±0.98 (MR)	8.69±1.78 (MR)	17.25±1.44 (S)	23.2±0.55 (S)	20.02±2.35 (S)	5.47±0.4 (MR)	8.35±2.67 (MR)	6.42±2.07 (MR)	6.6±0.54 (MR)	5.68±0.49 (MR)	5.74±1.99 (MR)	7.62±0.98 (MR)
SUPA	25.05±2.7 (S)	19.04±2.11 (S)	16.11±1.02 (S)	21.17±1.76 (S)	27.99±0.71 (S)	24.91±0.24 (S)	28.92±1.12 (S)	13.76±1.5 (MS)	28.26±2.42 (S)	29.22±2.81 (S)	37.56±3.43 (S)	28.46±2.45 (S)	17.15±1.2 (S)	23.13±2.62 (S)
TOG5293	13.6±2.27 (MS)	8.15±1.55 (MR)	5.48±1.52 (MR)	6.4±0.57 (MR)	18.25±0.62 (S)	9.93±2.24 (MR)	22.06±0.36 (S)	5.44±0.17 (MR)	5.4±0.46 (MR)	4.72±1.15 (R)	4.22±0.86 (R)	6.44±1.04 (MR)	20.84±2.48 (S)	6.61±0.2 (MR)
TOG5447	15.4±2.28 (S)	7.83±1.4 (MR)	6.5±1.16 (MR)	9.74±2 (MR)	15±0.89 (MS)	6.27±2 (MR)	22.95±2.01 (S)	14.66±2.08 (MS)	14.78±0.59 (MS)	11.3±4 (MS)	9.15±3.55 (MR)	7.57±0.09 (MR)	18.23±3.36 (S)	5.84±1.58 (MR)
TOG5458	25.5±3.10 (S)	9.18±1.71 (MR)	7.77±0.80 (MR)	8.59±0.80 (MR)	19.08±2.16 (S)	7.89±1.92 (MR)	25.19±2.54 (S)	17.59±1.35 (S)	16.42±0.29 (S)	8.41±0.17 (MR)	7.11±0.18 (MR)	5.47±1.33 (MR)	18.05±2.31 (S)	7.11±2.25 (MR)
TOG5464	19.4±2.02 (S)	6.43±1.19 (MR)	7.08±0.93 (MR)	5.31±0.93 (MR)	21.65±0.84 (S)	13.46±0.65 (MS)	25.16±1.93 (S)	6.6±1.36 (MR)	5.99±0.56 (MR)	4.1±0.41 (R)	4.78±1.03 (R)	5.27±0.97 (MR)	3.87±1.71 (R)	5.53±0.95 (MR)
TOG5473	17.6±0.98 (S)	7.15±1.42 (MR)	6.12±0.23 (MR)	17.13±0.23 (S)	18.14±2.33 (S)	5.91±2.27 (MR)	25.19±0.74 (S)	5.58±0.45 (MR)	14.5±2.46 (MS)	4.94±0.65 (R)	4.87±0.97 (R)	4.92±0.80 (R)	3.57±1.03 (R)	5.58±0.67 (MR)
TOG5523	16±1.49 (S)	6.59±1.43 (MR)	7.17±0.55 (MR)	8.15±0.84 (MR)	12.95±2.12 (MS)	5.44±1.97 (MR)	27.45±2.45 (S)	8.63±0.1 (MR)	10.95±2.75 (MS)	7.1±0.69 (MR)	12.34±1.65 (MS)	5.32±1.41 (MR)	16.89±2.55 (S)	6.02±0.51 (MR)
TOG5566	18.7±1.88 (S)	5.42±0.96 (MR)	7.31±1.47 (MR)	4.53±0.36 (R)	11.63±2.98 (MS)	4.18±1.44 (R)	17.29±0.5 (S)	7.31±2.44 (MR)	6.96±1 (MR)	4.93±1.11 (R)	7.22±1.14 (MR)	6.22±0.84 (MR)	4.56±1 (R)	4.39±0.33 (R)
TOG5620	14.3±1.74 (MS)	8.04±0.74 (MR)	6.31±0.62 (MR)	7.24±0.59 (MR)	14.47±1.4 (MS)	5.21±0.95 (MR)	24±2.49 (S)	8.5±1.77 (MR)	10.17±1.45 (MS)	5.86±0.86 (MR)	4.68±0.27 (R)	5.31±0.10 (MR)	19.45±2.59 (S)	6.18±1.18 (MR)
TOG5650	22.3±1.63 (S)	5.94±0.45 (MR)	4.4±0.29 (R)	7.65±0.58 (MR)	19.37±3.2 (S)	5.26±1.58 (MR)	27.06±2.76 (S)	4.53±0.73 (R)	11.96±2.81 (MS)	7.35±0.78 (MR)	9.66±2.47 (MR)	6.01±1.33 (MR)	21.31±0.79 (S)	7.34±2.14 (MR)

TOG5675	22.14±3.57 (S)	5.04±0.42 (MR)	5.77±1.56 (MR)	5.51±0.87 (MR)	12.15±1.49 (MS)	3.02±0.41 (R)	25.34±2.29 (S)	6.1±0.99 (MR)	7.28±1.52 (MR)	5.94±0.71 (MR)	6.45±1.42 (MR)	5.2±1.21 (MR)	18.21±1.74 (S)	4.52±0.62 (R)
TOG5810	18.1±2.67 (S)	6.24±0.81 (MR)	3.73±0.67 (R)	6.62±1.5 (MR)	7.7±1.03 (MR)	4.41±1.38 (R)	24.83±2.39 (S)	4.48±0.32 (R)	7±0.68 (MR)	4.65±0.64 (R)	3.8±0.95 (R)	5.6±1.45 (MR)	12.62±0.73 (MS)	5.4±2.03 (MR)
TOG5953	17.3±2.26 (S)	6.28±0.96 (MR)	6.49±1.53 (MR)	4.93±0.73 (R)	9.91±2.35 (MR)	4.82±1.65 (R)	20.15±2.37 (S)	6.53±1.02 (MR)	9.1±0.84 (MR)	5±1.25 (R)	6.38±2.41 (MR)	4.75±1.13 (R)	3.53±1.23 (R)	3.8±0.64 (R)
TOG5989	19.04±1.83 (S)	5.88±0.84 (MR)	5.74±0.76 (MR)	13.05±0.5 (MS)	12.52±0.58 (MS)	4.16±1.25 (R)	24.44±3.57 (S)	8.73±0.93 (MR)	13.13±1.75 (MS)	6.4±0.78 (MR)	5.76±0.56 (MR)	6.2±0.80 (MR)	4.96±1.33 (R)	6.31±1.47 (MR)
TOG6007	7.8±1.02 (MR)	6.1±0.94 (MR)	8.31±0.88 (MR)	6.87±0.63 (MR)	18.84±2.03 (S)	4.52±1.44 (R)	22.17±2.05 (S)	6.43±0.76 (MR)	7.79±0.57 (MR)	7.17±0.48 (MR)	7.59±0.8 (MR)	9.43±1.83 (MR)	13.80±1.32 (MS)	5.11±0.82 (MR)
TOG6231	17.53±0.98 (S)	5.79±0.2 (MR)	6.33±0.24 (MR)	4.94±0.26 (R)	10.7±1.38 (MS)	4.89±1.58 (R)	17.61±0.5 (S)	7.11±1.6 (MR)	12.28±1.84 (MS)	5.27±0.7 (MR)	4.81±0.23 (R)	4.87±0.48 (R)	16.35±2.41 (S)	8.25±2.48 (MR)
TOG7173	13.81±0.75 (MS)	4.7±1.8 (R)	6.5±1.01 (MR)	5.03±1.66 (MR)	15.38±1.58 (S)	3.84±1.35 (R)	19.24±1.8 (S)	4.88±0.75 (R)	7.3±0.5 (MR)	3.65±0.8 (R)	3.45±0.73 (R)	4.53±0.59 (R)	4.76±1.65 (R)	4.58±0.41 (R)

[a]The mean values are followed by the standard error. [b]The letters in bracket are reaction categories based on lesion length.

Resistant (R): < 5 cm, moderately resistant (MR): > 5-10 cm, moderately susceptible (MS): > 10-15 cm, and susceptible (S): > 15 cm.

Bacterial blight lesion lengths were evaluated under greenhouse conditions at two weeks after inoculation. Lesion lengths were scored 14 days after inoculation

Chapter 5: Xanthomonas oryzae pv. oryzae genome sequencing and transcriptome changes in rice in response to combined high temperature and bacterial blight

Gerbert Sylvestre Dossa[1,2*], Ian Quibod[1], Ricardo Oliva[1], Edgar Maiss[2], Casiana Vera Cruz[1], Kerstin Wydra[2,3]

1: Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, Los Baños, Philippines

2: Department of Phytomedicine, Leibniz Universität Hannover, Hannover, Germany

3: Plant Production and Climate Change, Erfurt University of Applied Sciences, Erfurt, Germany

* Corresponding author: Gerbert S. Dossa c.dossa@irri.org

Abstract

Rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* implies substantial yield loss to rice. In times of climate change, increasing temperatures are observed and further acceleration is expected worldwide. Increasing temperature often turns into inhibition of host plant defense to pathogens. Recently, a reduced resistance in rice IRBB4 carrying *Xa4*, but an increase in resistance in IRBB7 carrying *Xa7* resistance by increasing temperature has been reported. Influence of high temperature on both *R* genes (*Xa4+Xa7*) combined in IRBB67 was analyzed under growth chamber conditions and transcriptomic analysis performed. The pyramided line IRBB67 showed no differences in lesion length between both temperature regimes, demonstrating that non-effectiveness of *Xa4* at high temperature did not affect IRBB67 resistance. Moreover, *Xa4* complements *Xa7* resistance with no *Xoo* spread *in planta* beyond the symptomatic area under both temperature regimes in IRBB67. Time course transcriptomic analysis revealed that temperature enhanced IRBB67 resistance to combined heat and *Xoo*. Our

findings highlight altered cellular compartment involved in *Xoo* resistance and heat stress tolerance in both susceptible (IR24) and the resistant (IRBB67) genotypes. Interestingly, up-regulation of trehalose-6-phosphatase gene and low affinity cation transporter in IRBB67 suggest that IRBB67 maintained a certain homeostasis under high temperature which may have enhanced its resistance. The interplay of both heat stress and *Xoo* responses as determined by up-regulated and down-regulated genes demonstrates how resistant plants cope with combined biotic and abiotic stresses.

Genomic analysis of *Xoo* PXO145 showed close relatedness of PXO145 to *Xoo* PXO86 as revealed by MAUVE alignment. TAL effector prediction from PXO145 genome revealed 18 TAL effectors including *avrXa7* which activates *Os11N3* and *avrXa27* which binds to the executor *R* gene *Xa27*. *Os11N3* was seen down-regulated in IRBB67 in comparison to IR24.

Keywords: Rice, *Xoo, Xa4, Xa7*, IRBB67, TAL-effector, High temperature

Background

The world population is projected to reach 9.7 billion by 2050 and half the world population growth is expected in developing countries (UN DESA, 2015). This world population growth coupled with the impact of climate change on agricultural production in those countries demands a rapid growth in food supply, animal feed and biomass for fuels (Naylor and Falcon, 2008; Battisti and Naylor, 2009). Previous reports have shown that an increase of seasonal temperature by 1°C results in decline of major grains yield in the range of 2.5 to 16% in the tropics and subtropics (Peng et al. 2004; Lobell et al. 2008). Unfortunately, due to their sessile life style, plants have no chance to escape this environment (biotic and abiotic stresses) and must respond and adapt (Hua, 2013). Abiotic stress may imply positive or negative effects on plant defense responses (Atkinson and Urwin, 2012). According to the latter authors, the outcome of the interaction depends on the timing, nature and the severity of the stress. Temperature, water, relative humidity, light and circadian rhythm significantly influence plant defense and pathogen invasion (Hua, 2013). A small variation in temperature can affect plant growth, but also plants' responses to pests and pathogens (Long et al. 1988; Garett et al. 2006). Thus, plant immunity is often compromised under high temperature (Dropkin, 1969). Most studies on plant responses to environmental changes were carried out under single stress and are therefore unsuccessful in explaining plant responses to more than one stress factor (Atkinson and Urwin, 2012). High temperature affecting host resistance to pathogens has been reported in tobacco with infected with Tobacco mosaic virus (Kiraly et al. 2008). Increased disease resistance to stripe rust (*Puccinia striiformis* f.sp *tritici*) was observed in wheat under high temperature (25-35°C) and is likely caused by the significant expression of resistance gene *Yr36* which is not effective under low temperature (15°C) (Uauy et al. 2005; Fu et al. 2009). Similar reactions were reported in Arabidopsis which shows resistant to virulent *Pseudomonas syringare* pv. *tomato* (*Pst*) strain DC3000 at 22°C and susceptible to the same strain at

28°C (Wang et al. 2009). In rice, Webb et al. (2010) reported high temperature reducing the resistance of rice IRBB NILs carrying the *Xa4* resistance gene to *Xantomonas oryzae* pv. *oryzae* (*Xoo*). According to these authors, an inverse response was observed in IRBB7, a NIL with *Xa7* resistance gene. This inverse response raised the question about the *R* gene durability under climate change conditions. However, the pyramided lines such as IRBB67 (*Xa4+Xa7*) may be an alternative as pyramided lines are more durable and have broad-spectrum of resistance than monogenic lines (For review: Suh et al. 2013). Therefore, there is a need to study the molecular mechanisms underlying the IRBB67 response to the pathogen under high temperature conditions. The inhibition of plant resistance to pathogens under high temperature is often associated to an enhanced activity of RNA-silencing mediated resistance and inhibition of effector triggered immunity under which the pathogen effector is normally recognized by the host *R* gene (Martin et al. 2003; Liu et al. 2015). The resistance induced by the NB-LRR class of *R* gene is reduced by temperature increase due to less nuclear accumulation of SCN1 (Zhu et al. 2010). In this study, both *R* genes *Xa4* and *Xa7* are not yet cloned and their putative function is still unknown. The reaction of NIL IRBB4 carrying *Xa4* under high temperature (Webb et al. 2010) suggests that this *Xa4 R* gene might be an NB-LRR type of *R* gene which is less expressed under high temperature conditions.

The hypothesis here is to determine the effects of *Xa4* on *Xa7* in the pyramided line IRBB67 carrying both *R* genes and to understand how this pyramided line responds to the combination of *Xoo* and heat stress. Combinations of high temperature and drought stress alter gene expression by activation of specific programs, revealing that plant response to multiple stresses differs to the one to single stress (Rizhsky et al. 2004). Moreover, the study of Rasmussen et al. (2013) has shown that 61% of transcripts were not predictable under double stress compared to single stress treatment. Understanding

how resistant plants respond to *Xoo* and heat stress will provide information to be used for developing double stress tolerant rice varieties.

Genome sequencing has contributed to receive insight into the rice-*Xoo* pathosystem. Since the first *Xoo* genome sequence (Korean strain KACC10331) published in 2005 by Lee et al (2005) and followed by genome sequencing of the Japanese *Xoo* strain MAFF311018 (Ochiai et al. 2005) and the Philippines' strain PXO99A, a 5-azacytidine-resistant derivative of PXO99 (Salzberg et al. 2008), a lot of efforts were made to sequence more *Xoo* strains from Philippines, Africa and United State of America (for review: Verdier et al. 2012). Availability of these genome sequences has shown the diversity existing among *Xoo* strains. Moreover, Gonzales et al (2007) demonstrate existence of a genetic distance between African and Asian *Xoo* strains. Genome sequencing of *Xoo* has allowed understanding its interaction with rice through identification of effectors which are injected into the host cell via type 3 secretion systems known as important virulence factor in bacterial blight (Nino-Liu et al, 2006). The effectors identified so far are based on available genome sequences and will certainly significantly increase with additional genome sequences.

Transcription activator-like (TAL) effectors have recently gained a lot of attention as they function like transcription activators of plant genes by binding to the gene promoters (Römer et al. 2010). TAL effectors can bind to host susceptible genes and promote disease or activate the host resistance gene and trigger defense (Boch et al. 2014). For example, PXO99A TAL effector *PthXo1* activates the rice susceptible gene *Os08N3*, a sugar transporter gene (Yang et al. 2006), however, when this *Os08N3* gene lacks the effector binding site (EBE), it confers resistance as it is the case of IRBB13 carrying a recessive gene *xa13* (Yang et al. 2006, Yuan et al. 2009). Another susceptible gene, *Os11N3*, is targeted by several TAL effectors: *PthXo3* (Yang and White, 2004), *avrXa7* (Anthony et al. 2010), *Tal5* (Streubel et al. 2013) and *TalC* (Yu et al. 2011),

resulting in increase in disease lesion development. Some of the rice genes targeted by TAL effectors' are executor genes. These genes have EBEs in their promoter and are transcriptionally activated by TAL effectors. As an example, *Xa27* is activated by *AvrXa27* leading to resistance to *Xoo* (Römer et al. 2009). This approach could be used as a trap by engineering an *R* gene with EBE site that the TAL effector binds to (Wilkins et al. 2015). Mutation in the TAL effector binding site of the susceptible gene leads to expression of resistance in IRBB13, while genome editing with removal of the TAL effector binding site of the sugar transporter genes (*OsSweet*) promoters also results in resistance (Li et al. 2012). Therefore, it becomes highly important to identify TAL effector containing *Xoo* and their target genes in rice for development of resistance to rice bacterial blight. TAL effectors and the prediction of their targets will only be possible with availability of *Xoo* genome sequences and here we report about *rice-Xoo* pathosystem under high temperature conditions and predicted the TAL effectors in PXO145.

Materials and Methods

Plant growth conditions

Rice genotypes' IR24 (susceptible), IRBB4 (*Xa4*), IRBB7 (*Xa7*) and IRBB67 (*Xa4+Xa7*) seeds were pre-germinated for 4 days at 37°C and transferred to pots for further growth under greenhouse conditions (12h light, 12h dark). Two week-old healthy plants were then transferred into indoor growth chambers under two temperature regimes (29/21 °C and 35/31 °C; day/night temperatures) and 70% of relative humidity. Inoculation was conducted on 21 day-old plants.

Plant inoculation

Philippines' *Xoo* race 7, strain PXO145 (*avrXa4+avrXa7*), inoculum was prepared from a 3 day-old culture. Twenty-one day-old seedlings of IR24, IRBB4, IRBB7 and IRBB67 were inoculated by the leaf clipping method (Kauffman et al. 1973) while IR24 and

IRBB67 were inoculated with syringes with PXO145 and with sterilized demineralized water (mock) for RNA-Seq gene expression analysis. The leaf at the second position of the main tiller was inoculated and disease assessment was performed daily from 4 days post inoculation to 11 dpi. Syringe inoculated leaves were sampled at 3, 72 and 120 hours post inoculation (hpi). One leaf from 5 plants each for each rice genotype and treatment were taken at each time point and immediately frozen in liquid nitrogen, and stored at -80°C.

Total RNA extraction, library construction and RNA sequencing

Total RNA was extracted from syringe inoculated leaves using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Total RNA was then treated with DNase (Promega) and quantified using NanoDrop. RNA integrity was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). For each time point and condition, two biological replicates were prepared. A single-end fragment library of 100bp length was generated from cleaned total RNA following instructions of the TruSeq RNA Sample Preparation kit. Cluster generation of the produced libraries was performed using Illumina_ TruSeq SR Cluster Kit v3 - cBot - HS, and sequenced on a HiSeq 2000 platform (Illumina) with single-end 100-bp reads and submitted to the Microarray and Deep-Sequencing Core Facility of the University Medical Center Göttingen (Germany). Sequence images were transformed with Illumina software BaseCaller to bcl files, which were demultiplexed to fastq files with CASAVA v1.8.2.

Transcriptome data analysis

The reads of each sample were mapped to the rice genome version 7 of the Rice Genome Annotation Project (RGAP) at MSU using clcbiogenomics workbench v7.0.4 with the following parameters: 2 for mismatch cost, 3 for insertion cost and deletion cost, 0.5 for length fraction and 0.8 for similarity fraction. The expression data for each

sample was exported from clcbiogenomics workbench and analyzed in the R/Bioconductor environment loading DESeq2 (Love et al. 2014), gplot, ggplot2 packages. The differentially expressed genes were analyzed based on a generalized linear model likelihood ratio test assuming negative binomial data distribution via DESeq2. Candidate genes were filtered to a minimum of 4X fold change and FDR-corrected p-value <0.05. Functional association enrichment analysis was conducted following the methodology of Du et al. (2010).

Time course quantitative Real-Time PCR validation

Quantitative RT-PCR using SYBR Green detection reagents in Step One Plus (Applied Biosystems, USA) was used to validate the expression of ten candidate genes shown to be differentially regulated from RNA-Seq data. Total RNA from the third biological replicate was used according to each time point. The sequence of each gene was obtained from the Rice Genome Annotation Project database, RGAP7 (http://rice.plantbiology.msu.edu/), and the sequences of genes were used for primers design using qPCR Assay Design tool of Integrated DNA Technology (IDT, http://sg.idtdna.com/site). Rice actin gene was selected as a reference gene in qRT-PCR. $2^{-\Delta\Delta Ct}$ method as described by Schmittgen and Livak (2008) was used to determine the relative expression and the log2 transformation of the relative expression was used to compare with the RNA-Seq data. All samples were studied in triplicate PCR. The primers and corresponding sequences are listed in Supplementary Table1.

PXO145 genome sequence and TAL effectors prediction

Single molecule, real-time (SMRT) technology was used to sequence *Xoo* strain PXO145 as described by Eid et al. (2009). *De novo* genome assembly was performed using HGAP version 3 (Chin et al., 2013, Wilkins et al., 2015), Pacific Biosciences, Menlo Park, CA) and the PBX toolkit (https://github.com/njbooher/pbx). Two (02) SMRT cell libraries were used for sequencing. Whole genome alignment with other publicly available *Xoo*

genome sequences was performed using MAUVE v2.3.1 (Darling et al., 2010). TAL effector prediction was conducted using PBX toolkit as described in https://github.com/njbooher/pbx and Wilkins et al. (2015).

Type III effectors were identified using tBlastn in clcbiogenomics workbench v.7.0.4. A database with all Xanthomonas type III effectors from *Xoo* and *Xoc* was built and used to blast against the whole genome sequence of PXO145.

Results

High temperature affects plant morphology

Plant morphology change due to temperature rise was determined by measuring the plant height under both temperature regimes. All rice genotypes were taller with narrower leaves under high temperature compared to normal temperature (Figure 1A). Under low temperature, the plant height ranged from 47.8 cm to 50.8 cm, and under high temperature from 55.5 cm to 63 cm. The highest plant height under low temperature was recorded on IRBB7, and under high temperature on IRBB4.

Rice near isogenic line with *R* genes *Xa4* and *Xa7* combination confers strong resistance to BB under high temperature

Rice bacterial blight *R* genes *Xa4* and *Xa7* are among the major *R* genes mediating resistance to BB. In order to determine the high temperature effect on the combination of the two *R* genes (*Xa4* and *Xa7*) responses to BB, rice NIL IRBB67 carrying *Xa4* and *Xa7*, along with IR24 (susceptible) and IRBB4 carrying *Xa4* and IRBB7 carrying *Xa7* were used. Plants were inoculated under both temperature regimes in the growth chamber with PXO145 (*avrXa4+avrXa7*). Disease evaluation after 11 days showed an inverse response between *Xa4* and *Xa7*, with *Xa7* resistance enhanced under high temperature. However, the gene combination in IRBB67 did not show a significant difference in disease lesion length between high and low temperatures. Under low

temperature the average lesion length was 1.5 cm, under high temperature 1.3 cm on IRBB67 (Figure 1B).

Evaluation of bacterial blight lesion length development recorded from 4 dpi to 11 dpi showed that IRBB67 resistance follows a similar pattern to that of IRBB7 under high temperature (Supplementary Figure 1). IRBB67 resistance was high compared to IRBB7 resistance under low temperature with an average lesion length of 2.6 cm for IRBB7 at 11 dpi. Additionally, *in planta* bacterial counts showed evidence of complementation of *Xa4* to *Xa7* under high temperature compared to low temperature (Figure 1C).The leaf segment plating beyond the visible lesion length showed no significant differences in the bacterial count in IR24 leaves under both temperature regimes. IRBB4 showed similar results at high temperature while significant differences were observed at low temperature. However, in IRBB7, the bacterial count between leaf segments was significantly different at high temperature compared to low temperature, suggesting that *Xoo in planta* spread less in IRBB7 under high temperature compared to the spread in IRBB4. Unexpectedly we did not detect bacterial growth beyond the symptomatic area under both temperature regimes in the pyramided NIL IRBB67.

Gene expression profiling of IR24 and IRBB67, *Xoo* and mock inoculated under normal and high temperature

Total RNA from each sample was single end sequenced using Illumina Hiseq technology. The read length of 100 bp in the range of 25.2-82.1 millions was generated (Supplementary Table 2). Percentage of mapped reads ranged from 97% to 98.6%. The mapped reads were used for further analysis.

To identify differentially expressed genes (DEGs), we used the DESeq2 package (Love et al. 2014) under R/bioconductor. The significant DEGs were identified based on the dales discovery rate (FDR) of 0.05 and log-2 fold change. Using these criteria, we

identified 4,683 transcripts differentially expressed comparing *Xoo* inoculated samples to mock inoculated samples from both IR24 and IRBB67 under both temperature regimes at 3, 72 and 120 hpi. In IR24, under low temperature most of the transcripts were differentially expressed at 3 hpi (2,202 DEGs), 160 ones at 72 hpi and 232 DEGs at 120 hpi. In the resistant NIL IRBB67, 2,296, 222 and 76 DEGs were identified at 3, 72 and 120 hpi, respectively, at low temperature following the same comparison. Similar to low temperature, *Xoo* inoculated and mock inoculated sample comparisons revealed 3,110, 62 and 521 DEGs in IR24 at 3, 72 and 120 hpi, respectively, while in IRBB67, 2,967, 91 and 96 DEGs were identified at the same points (Figure 2). The temperature increase showed significant effects on DEG numbers, especially at 3 hpi in both IR24 and IRBB67. IRBB67 showed a decrease in DEG numbers with increase in the incubation period (hour post inoculation), suggesting that this rice NIL responds sufficiently to combined stresses of BB and high temperature at an early stage of infection. Both NILs showed significant reduction in DEGs at 72 hpi, however, IR24 showed more DEGs induced at 120 hpi compared to 72 hpi time points under both temperature regimes, with more DEGs (521) induced at high temperature.

Functional classification using of the 4,683 transcripts differentially expressed using Pageman revealed late (120 hpi) up-regulation of hormone metabolism in IR24 at high temperature while it was induced at 3 and 120 hpi under low temperature. In IRBB67, the hormone metabolism was up-regulated from 3 to 72 hpi under low temperature and up-regulated across the three time points under high temperature. Jasmonate metabolism did not show significant differences in expression between both rice NILs and between both temperature regimes. Abscisic acid (ABA) negatively regulates host plant resistance to the pathogen by negative antagonistic effects on salicylic acid (SA) mediated resistance (Yasuda et al. 2008; Fan et al. 2009; Cao et al. 2011; Xu et al. 2013). In this study, ABA and an ABA induced response were shown to be up-regulated only in

129

IR24 under low temperature. Under temperature increase, no differences were seen between *Xoo* inoculated and mock inoculated samples. SA was seen to be up-regulated at 3 and120 hpi in IR24 under low temperature and only induced at 72 hpi under high temperature. In IRBB67, SA up-regulation was observed at 72 hpi under low temperature and from 72-120 hpi under high temperature (Figure 3). Cell wall plays several roles such as physical barriers against insects and pathogens, shape and structure, but also cell-cell communication and osmotic regulation. In this study, the cell wall was generally affected by high temperature combined with *Xoo*, especially in IRBB67. Similarly, the cell wall proteins such as AGPs (arabinogalactans-proteins) which link the cell wall with the plasma membrane and the cytoskeleton (Ellis et al. 2010; Lyuben et al. 2014) were shown to be affected by high temperature and *Xoo* in both rice NILs (IR24 and IRBB67) at an early stage of inoculation (Figure 3). Cell wall precursor synthesis-sugar kinases and cell wall precursor synthesis-sugar kinases-galacturonic acid kinase were seen to be up-regulated at low temperature at 72 hpi in both rice NILs.

High temperature affects rice membrane enclosure

To determine the effects of high temperature on rice, DEGs from mock inoculated plants at high and low temperature were compared at each time point and within genotype. A total of 332 DEGs were expressed in both mock inoculated genotypes. Several DEGs were down-regulated in both genotypes in response to high temperature. Differences in rice transcript accumulation between high and low temperatures were observed at 3 hpi at which most of the DEGs were down-regulated (Supplementary Table 3). Besides, 05 DEGs (LOC_Os07g34520.2; LOCOs01g12490.1; LOC_Os08g30020.3; LOC_Os07g34520.3; LOC_Os11g46850.1) were up-regulated in IR24 at 3 hpi and LOC_Os08g04500.2 encoding for terpene synthase was up-regulated at 72 hpi. In the resistant genotype IRBB67, 9 DEGs were up-regulated (Supplementary Table 3)

including MYB family transcription factor (LOC_Os02g53670.1) and MYB transcription factors, which are involved in plant development, but also in defense responses to hormone or stress treatments (Yanhui et al. 2006). Additionally, flavin mono-oxygenase (LOC_Os01g12490.1) which plays important role in n-tryptophan (Trp)-dependent indole-acetic acid (IAA) biosynthesis in plants and regulates plant growth and development was among the nine up-regulated DEGs in IRBB67.

Functional analysis of the DEGs revealed enrichment in three functional groups (Figure 4). Twelve DEGs were enriched, deriving from the external encapsulating structure (GO:0030312) and the cell wall (GO:0005618), with down-regulation in IR24 at 72 hpi. The third functional group was nucleus (GO:0005634) and showed overall up-regulation in the resistant reaction at 120 hpi. The DEGs, 32 in total enriched in nucleus at 120 hpi in IRBB67 were significantly down-regulated at 3 hpi.

High temperature during pathogen infection affects rice cellular compartments

With mock inoculation, 332 transcripts were differentially expressed between high and low temperatures from both genotypes. In order to determine the effects of high temperature on rice during pathogen infection, transcript accumulation was compared between high and low temperatures of inoculated samples from both IR24 and IRBB67. A total of 156 DEGs (Supplementary Table 4) were induced from both IRBB67 and IR24 genotypes, demonstrating the repression of several DEGs in response to *Xoo* inoculation between high and low temperatures. Besides, only 19 DEGs were shared between mock and *Xoo* inoculated samples (Supplementary Figure 2).

At 3 hpi, no significant DEG was found to be up or down-regulated, except LOC_Os08g39850.2 encoding for lipoxygenase, a chloroplast precursor involved in the programmed cell death pathway and biotic and abiotic stress response in plants (Pavan, 2011), which was down-regulated in IRBB67 (Supplementary Table 4). At 72 hpi, 14

DEGs were expressed in IR24 with down-regulation of 9 DEGs and up-regulation of 5 DEGs. In the resistant genotype, the difference in the response to *Xoo* between high and low temperature was generally significant at 72 hpi compared to the susceptible response at the same time point. Fifty four and 39 DEGs were down-regulated and up-regulated, respectively, in IRBB67 at 72 hpi. A late response was observed in the susceptible genotype at 120 hpi, where 63 DEGs were expressed with 7 down-regulated DEGs and 56 up-regulated. Only 5 significant DEGs were expressed in IRBB67 at 120 hpi and all were up-regulated. These 5 DEGs included LOC_Os08g04500.1, LOC_Os08g04500.2, LOC_Os05g01140.1, LOC_Os12g1440.1, LOC_Os01g01840.1 and LOC_Os12g02470.1 (Supplementary Table 4).

Functional analysis of the 156 DEGs showed down-regulation of plasma membrane (GO:0005886) and membrane (GO:0016020) genes across the three time points in IR24 and at 72 and 120 hpi in IRBB67 (Figure 4). Genes of the cell wall (GO:0005618), extracellular region (GO:0005576), and external encapsulated structure (GO:0030312) were significantly down-regulated at 72 hpi in both genotypes while transferase activity (GO:0016740) was down-regulated in IR24 at 120 hpi, and vacuole (GO:0005773) was down-regulated in IRBB67 at 72 hpi. The late response observed in the susceptible genotype IR24 at 120 hpi was correlated with up-regulation of the following GO terms: transcription regulator activity (GO:0030528), transcription factor activity (GO:0003700), DNA binding (GO:0003677) and nucleic acid binding (GO:0003676) in IR24 at 120 hpi (Figure 4).

IRBB67 mediated resistance to bacterial blight under low temperature conditions.

To determine the difference in transcript accumulation after *Xoo* inoculation between the resistant (IRBB67) and the susceptible (IR24) genotypes, selected DEGs in the IRBB67 and IR24 were compared at each time point. A total of 145 DEGs were differently induced in IRBB67 and IR24 after *Xoo* inoculation under low temperature

(Supplementary Table 5). Among them, 102 DEGs (51 up-regulated and 51 down-regulated) were induced at 3hpi, 40 (six up-regulated and 34 down-regulated) at 72hpi and 85 (37 up-regulated and 48 down-regulated) at 120hpi. DEG numbers decreased at 72hpi, however, a late response to *Xoo* was observed at 120hpi with induction of 85 DEGs. Additionally, 51 DEGs were specifically induced at 3hpi and there were 24 DEGs in common between the 3 time points.

GO enrichment analysis of the DEGs revealed no significant GO terms at 5% of p-value. However, DEGs (LOC_OS02g40130 and LOC_Os02g40190) which belong to the group of protein kinases related genes, involved in cell death, response to biotic stimulus and response to stress were up-regulated in the resistance genotype (IRBB67). Protein kinases play important roles in activation of plant defense mechanisms and signal transduction. Among the 145 DEGs which are differentially induced in IRBB67 and IR24, 21 DEGs were related to protein kinases. Four (04) LRR type receptor like kinase genes were induced among which three (LOC_Os11g29110, LOCOs_02g40130 and LOC_Os11g29090) were significantly up-regulated in IRBB67 at 3hpi and LOC_Os05g46090 was down-regulated at 120hpi. One DEG encoding for cysteine-rich receptor-like protein kinase (LOC_Os02g12130) was down-regulated in IRBB67 (Supplementary Table 5). Other types of receptor like kinase such as LOC_Os02g40180, LOC_Os06g38760, LOC_Os06g16300, LOC_Os09g18594, LOC_Os11g07170 and LOC_Os09g19500 were down-regulated in IRBB67. Additionally, protein kinase genes (LOC_Os05g41950, LOC_Os11g44250) were specifically up-regulated in IRBB67 at 3hpi and a DEG (LOC_Os09g18159) which encodes for light repressible receptor protein kinase, putative expressed was only up-regulated at 120 hpi. Two DEGs encode for wall associated kinases (LOC_Os11g47140 and LOC_Os11g46860) and LOC_Os07g03920 which encodes for lectin-like receptor kinase, and LOC_Os06g38650 which encodes for RLKs were significantly up-regulated at 3 and 120hpi in IRBB67 in comparison to IR24

after *Xoo* inoculation under low temperature conditions. LOC_Os11g46850 which encodes for wall associated kinase was significantly up-regulated in IRBB67 only at 3hpi as well as receptor kinase (LOC_Os02g40190) and cyclin-dependent kinase G-1 (LOC_Os02g39010) in IRBB67 (Supplementary Table 5).

In addition to protein kinases related genes, LOC_Os07g05400 encoding for Ferredoxin--NADP reductase, chloroplast precursor, putative expressed, LOC_Os06g38120 which encodes for low-affinity cation transporter, and LOC_Os06g38110, encoding for expressed protein, were significantly up-regulated at 3hpi in IRBB67. Uncharacterized glycosyl hydrolase Rv2006/MT2062, putative expressed, encoded by LOC_Os09g20390, LOC_Os11g44950 (glycosyl hydrolase family 3 protein) showed significant up-regulation in IRBB67 at 120 hpi, and LOC_Os07g46660, encoding ubiquitin carboxyl-terminal hydrolase domain containing protein expression was up-regulated in IRBB67 from 72-120 hpi, while glycine-rich cell wall protein (LOC_Os03g07270) showed up-regulation at 120 hpi (Supplementary Table 5).

High temperature enhances IRBB67 resistance to bacterial blight

In order to understand the mechanisms by which the resistant rice varietyIRBB67 harboring *Xa4* and *Xa7* resistance genes, responds to the combined stress of high temperature and bacterial blight, DEGs from the resistant genotype IRBB67 and the susceptible IR24 after inoculation with *Xoo* under high temperature were compared. A total of 188 transcripts were differentially expressed between IRBB67 and IR24 at 3, 72 and 120 hpi under high temperature conditions. At 3 hpi, 113 DEGs were expressed with 56 down-regulated and 57 up-regulated. At 72 hpi, 99 transcripts were differentially expressed (44 down-regulated and 55 up-regulated) and 145 DEGs at 120 hpi with 77 down-regulated and 68 up-regulated (Supplementary Table 6).

Functional analysis of the differential DEGs between IRBB67 and IR24 after inoculation with *Xoo* under high temperature conditions revealed no functional enrichment at 3 hpi, which suggested no response to both high temperature and *Xoo* at an early stage of inoculation from both rice NILs. Examination of GO terms at 72 and 120 hpi suggested that under combined stress of *Xoo* and high temperature, the rice transcriptome is largely devoted to catalytic activity. At 72 hpi, 65 DEGs were functionally enriched in catalytic activity (Figure 5). Catalytic activity (GO:003824), transferase activity (GO:0016740), kinase activity (GO:0016301) and transferase activity, transferring phosphorus-containing groups (GO:0016772) were the most significant functional groups at 120 hpi. Catalytic activity was shown to be a major regulator in the response to high temperature and *Xoo* in IRBB67, as well as kinase activity (24 DEGs), transferase activity (35 DEGs) and transferase activity and transferring phosphorus-containing groups (24 DEGs) which belong also to catalytic activity groups (Figure 5).

Besides these four functional groups, DEGs encoding wall associated kinases together with DEGs encoding for low-affinity cation transporter (LOC_Os06g38120.1) and expressed proteins (LOC_Os06g38110.1, LOC_Os06g38210.1, LOC_Os06g38210.2) were significantly up-regulated in IRBB67. Additionally, IRBB67 preferentially responds to the pathogen infection with up-regulation of NB-ARC/LRR disease resistance protein (LOC_Os11g29090.1) and NB-ARC domain containing protein (LOC_Os11g44990.1) and down-regulation of stress response genes such as DEGs encoding for NB-ARC domain containing protein (LOC_Os07g02570.1, involved in stress response, LOC_Os01g24820.1, and LOC_Os11g46210.1, involved in protein binding and plasma membrane). Receptor like kinases function like cell membrane sensors of stimuli and lectin-like receptor kinase 7 (LOC_Os07g03920.1 and LOC_Os07g03970.1) were up-regulated in IRBB67 compared to IR24 in response to high temperature and *Xoo* (Supplementary Table 6).DEG LOC_Os09g20390 encoding for uncharacterized glycosyl

hydrolase Rv2006/MT2062, putative, expressed involved in trehalose 6 phosphate metabolism, also showed significant up-regulation to combined *Xoo* and high temperature in IRBB67. Additionally, the rice bacterial blight pathogen during its interaction with the host plant injects effector proteins that bind to host susceptibility genes (*OsSweet*), a sugar transporter gene. Induction of *OsSweet14* caused by TAL effectors *PthXo3* (Yang and White, 2004), *avrXa7* (Anthony et al. 2010), *Tal5* (Streubel et al. 2013) and *TalC* (Yu et al. 2011) results in increase in *Xoo* growth and lesion development. In the resistant plant IRBB67, *OsSweet14* (LOC_Os11g31190.1) was down-regulated, and more significantly at 120 hpi (Supplementary Table 6).

RNA-Seq validation by RT-PCR

To validate gene expression pattern determined by RNA-Seq, qRT-PCR was performed on 10 candidate genes (Figure 6). Expression pattern of the candidate genes determined by qRT-PCR data was in consistent with their expression pattern in RNA-Seq (Figure 6), although some smaller variation can be seen.LOC_Os11g44250.1, LOC_Os06g38110.1 and LOC_Os06g38120.1 were not induced in IR24 under both temperature regimes confirming the RNA-seq data. The expression of *OsSweet14* (LOC_Os11g31190.1) was much higher in IR24 as determined by qRT-PCR compared to RNA-seq data.

PXO145 genome comparison reveals similarity to PXO86 genome sequences

PXO145 genome was assembled in a single circular chromosome of 5,053,846 bp of size and 63.7% of GC content. Analysis of the circular representation of PXO145 showed more similarity to *Xoo* strain PXO86 than to other *Xoo* strains and *Xoc* strain BLS256. TAL effector locations seemed to be conserved between *Xoo* strains and *Xoc* BLS256. The circular schematic representation of PXO145 and other *Xoo* strains and *Xoc* BLS256 is provided in Figure 7.

Phylogenetic relationship between PXO145, PXO86, PXO99A, MAFF, KACC and BLS256 was assessed after aligning all the genomes using MAUVE 2.3.2 (Darling et al. 2010). Guide tree data were generated to build the phylogenetic relationship tree. Philippines' *Xoo* strains (PXO145, PXO99 and PXO86) appeared to be distant from other *Xoo* strains (MAFF and KACC). The similarity between PXO145 and PXO86 was also confirmed by the phylogenetic relationship tree where both strains group together (Supplementary Figure 3).

Whole genome alignment using progressive MAUVE (Darling et al., 2010) showed existence of rearrangement between *Xoo* strains. Several local blocks of 3,000,000 bp length were inverted in PXO86 in comparison to PXO145. Genome rearrangement was more significant in PXO99, MAFF and KACC (Figure 8). All the strains showed conserved in 4 locally blocks, and all blocks are more conserved in strains MAFF and KACC.

Type 3 effectors and TAL effectors' identification in PXO145

To identify Type 3 (T3) effectors, 29 T3 effectors from PXO99, MAFF, and BLS256 were blast to the PXO145 genome sequence using tblastn in clcbiogenomics workbench v.7.0.4. The results from tBlastn showed hit of 25 T3 effectors in PXO145. After applying the following filtering parameters; 97% of identity, 0.5 % of gaps and 10E-20 for E-value cut off, 21 T3 effectors were identified to be present in PXO145 (Table 1).

In order to identify TAL effector containing PXO145, the PBX toolkit was used as described in https://github.com/njbooher/pbx and by Wilkins et al. (2015). Eighteen TAL effectors were identifiedincluding*avrXa7*and *avrXa27* (Table 2). Among the 18 TAL effectors, eight Tal effectors are identical to known TAL effectors in PXO99A (Table 2). *Tal4, Tal5, Tal7a, Tal7c and Tal11a* show high similarity to *PthXo6, Tal3a, Tal4, avrXa10* and *avrXa23*, respectively. *Tal11c* which corresponds to *Tal9e* in PXO99 has the shortest

RVDs sequence (12.5) length. The longest TAL in PXO145 was *Tal11a* which showed high similarity to *avrXa23* and *Tal9* with 26.5 RVDs. Predicted TAL effectors from PXO145 are identical to those of PXO86 as shown by RVDs comparison (data not shown).

Discussion

Increasing temperature affects rice response to bacterial blight. Plants have evolved mechanisms to respond to external stimuli such as biotic and abiotic stresses. Host plant immunity allows plants to counter-attack the invading pathogen, following pathogen recognition mediated by resistance *R* proteins (Martin et al. 2003). Our results on the effects of high temperature on the response of rice NIL IRBB67 which pyramids bacterial blight resistance genes (*Xa4+Xa7*) to bacterial blight, revealed a complementation effect of *Xa4* to *Xa7*. *Xa4* appears to be among the most widely used in rice breeding programs in Asia (Khan et al. 2014, Dossa et al. 2015). As previously reported; *Xa4* resistance to *Xoo* decreases with temperature increase while the inverse trend is seen with *Xa7* (Webb et al. 2010). In this study, we confirmed this inverse response between the two *R* genes. Moreover, the pyramiding NIL IRBB67 harboring the two *R* genes showed resistance to PXO145 (*avrXa4+avrXa7*) with no significant differences between both temperature regimes. Also, disease progression recorded on IRBB67 under both temperature regimes is similar to that of IRBB7 under high temperature, suggesting that *Xa4* may not loose completely the resistance and is complemented by *Xa7* in IRBB67. High temperature altered *Xa4* response in IRBB4 to *Xoo*. The mechanisms by which the two *R* genes respond to *Xoo* is still unclear although it is known that *Xa7* resistance is mediated by *avrXa7* and gives pathogenic fitness cost to the pathogen (Vera Cruz et al. 2000). Hence, we hypothesized that these two *R* genes use different resistance mechanisms to mediate resistance to *Xoo*. The mechanisms by which *Xa4* triggers the response to the pathogen seems to be affected by temperature

rise. In contrast, *Xa7* resistance mechanisms may confer an abiotic tolerance component which enhances its reaction to *Xoo* under temperature rise, therefore, *Xa4* resistance mechanism may not be affected by temperature when it is combined with *Xa7* in IRBB67. This corroborates an early report that high temperature can negatively impact the temperature sensitive resistance to stem rust in oat cultivars harboring Pg3 and Pg4 genes (Martens et al. 1967).

In planta Xoo spread beyond the symptomatic area suggested that *Xa4* complements *Xa7* at low temperature in IRBB67 thereby reducing *Xoo* spread *in planta*. The predominance of *Xa7* over *Xa4* in IRBB67 mediates strong resistance to PXO145 under high temperature with no *Xoo* spread *in planta* beyond the symptomatic area. High temperature promoting higher bacterial multiplication in the host was reported to possibly depend on the type of *R* gene mediating resistance to the pathogen (Chen et al. 2013) as we observed in the current study as inverse reaction between *Xa4* and *Xa7*. Moreover, it is plausible that these two genes may belong to two different classes of gens coding *R* proteins and are modulated by temperature. Consistently, SNC1 gene, a NB-LRR type of *R* gene, does not confer resistance at high temperature when activated compared to low temperature in Arabidopsis (Yang and Hua, 2004), suggesting that *Xa4 R* gene may belong to this type of *R* gene. Further study will have to investigate this hypothesis since both *R* genes (*Xa4* and *Xa7*) are not cloned and the putative proteins are not known. Wang et al. (2009) suggest that high temperature inhibiting plant defense to pathogens may be regulated by some defense signalling components or by a combination of multiple factors. Bacteria spread in IRBB4 under high temperature suggests that recognition of corresponding *avrXa4* by *Xa4 R* gene might be compromised by high temperature while effector recognition increased in IRBB7 leads to resistance increase. These results are in contrast to Chen et al. (2013), whose findings

suggest that high temperature promotes vigorously bacteria multiplication compared to low temperature.

Examining the gene expression in IR24 and IRBB67 in response to PXO145 and two temperature (high and low) regimes, we found that the differentially expressed genes at early time triggered efficient response to the pathogen under both temperature regimes in IRBB67. However, in IR24, the pathogen progression *in planta*, enabled by late expression of genes in IR24 under both temperature regimes with more differentially expressed genes under high temperature (Figure 2). In response to environment changes, cells initiate a gene expression program to adjust its physiology and metabolism to the new environment, preventing it for damage or death (for review: Lopez-Mauray et al. 2008). In this study, it appears that at 3 hpi, high temperature and *Xoo* modulate more DEGs in both resistant and susceptible genotypes compared to that of low temperature. Phytohormones involved in host immunity and response to environmental stimuli (Pieterse et al. 2009; Santner et al., 2009; Jaillais and Chory, 2010; Denance et al. 2013) showed induction in this study, especially SA which is up-regulated in the resistant genotype from 72-120 hpi under high temperature. One of the major SA signaling regulators (*OsNPR1*, LOC_Os01g09800.1) was significantly up-regulated by the pathogen under high temperature. Expression of SA related genes in both rice genotypes appears to be a general response to pathogens which gets enhanced by high temperature in the resistant plant. Salicylic acid has numerously been reported to play an important role in resistance against pathogens (Cao et al. 1997; Alvarez, 2000; Desveaux et al. 2004; Garcion and Metraux, 2006; Ciokowski et al. 2008; Vlot et al. 2009; Sugano et al. 2010; De los Reyes et al. 2015).

To invade the host organism, pathogens need to encounter the host cell wall, the first physical defense barrier. In case of *Xoo*, it enters the host through hydathodes or wounds (Ou, 1985; Nino-Liu et al. 2006). In this study, the infiltration allowed the

bacteria to gain access directly to the host xylem allowing the pathogen to interact directly with the host parenchyma cells (Hillaire et al. 2001; Nino-Liu et al. 2006). It appeared that high temperature increased host colonization by the pathogen as the host cell wall is affected allowing the pathogen to move easily *in planta* when no *R* gene is present. Moreover, the cell wall is a source of nutrients to the pathogen as the cell wall is composed of cellulose and hemicellulose layers which the pathogen degrades during the infection process (Bellincampi et al. 2014). The general down-regulation of the cell wall pathway in the resistant plants as shown by pageman analysis (Figure 3) is in contrast to resistance mediated by this genotype under high temperature. However, the comparison between low temperature and high temperature after mock inoculation showed that high temperature generally affects the membrane enclosure with down-regulation of cell wall genes, external encapsulating structure and plastid in the susceptible genotype IR24, while high temperature in combination *Xoo* repressed the entire cellular compartment in both rice genotypes (Figure 4). Dahal et al. (2010) also reported suppression of cell wall metabolic proteins in susceptible tomato plant inoculated with *Ralstonia solanacearum*. A change caused by high temperature stress on cell wall metabolism is an important physiological mechanism for heat stress tolerance (Le Gall et al. 2015) suggesting that the plant responds by remodeling its cell wall architecture under abiotic stress, such as high temperature. This hypothesis could explain the fact that no bacterial spread was detected beyond the symptomatic area in IRBB67.

Plant cell wall is a battle ground between the pathogens and its host, but after the pathogen won this battle, it is still confronted to molecular resistance responses (Jones and Dangl, 2006) as the information of the foreign invasion proceeds to the nucleus. With the mock inoculation, the nucleus is down-regulated in both IR24 and IRBB67 at 3 and 72 hpi while a significant up-regulation was seen in IRBB67 at 120 hpi. However,

with pathogen stress combined to high temperature, the up-regulation of this pathway across the three time points suggests that the host plant stays under permanent alert after pathogen invasion. To sustain the response to this combined stress, lipid metabolism was significantly up-regulated in IRBB67 from 72 to 120 hpi while the significant expression was observed only at 72 hpi in IR24.

Under low temperature, although no significant enrichment was seen between both rice genotypes, up-regulation of defense related genes in the resistance genotype suggests an early recognition of PXO145 in IRBB67 that triggered the defense reaction. Moreover, the up-regulation of the defense genes in IRBB67 in comparison to the susceptible genotype IR24 may suppress pathogen growth and spread *in planta* (Kottapalli et al. 2007). Similarly, low-cation affinity transporter (LOC_Os06g38120) mediated cadmium transport into rice grains (Uraguchi et al. 2011) but also is involved in cellular homeostasis (Conde et al., 2011) was seen to be up-regulated in response to *Xoo* in IRBB67. Given that trehalose is a universal stress molecule, the up-regulation in the resistant genotype IRBB67 of LOC_Os09g20390 compared to the susceptible suggests that it may be involved in the defense response to *Xoo*.

High temperature modulates resistance to the pathogen in IRBB67 suggests the existence of a shared pathway between biotic and abiotic stresses. Environmental changes induce plant cells to trigger several events that start with perception of the stimuli at the membrane level (Tuteja and Mahajan, 2007). According to the same authors, receptor sensors located in the cell membrane activate several signal transductions that triggered calcium mobilization and other secondary signals to induce stress responsive genes. Calcium acts as second messenger in various stresses (Snedden and Fromm, 1998; Snedden and Fromm, 2001; DeFalco et al. 2010; Conde et al. 2011) and calcium signaling was seen to be up and down regulated in both genotypes. However, calcium transport genes (LOC_Os05g02940, LOC_Os03g27960) were up-

regulated in IRBB67 at 3 hpi. Additionally, wall associated kinase genes LOC_Os07g03920 and LOC_Os07g03970 were significantly up-regulated in the resistant reaction. Cell wall sensing pathogen invasion and high temperature triggering the plant response with up-regulation of receptor kinase gene LOC_Os07g03920 was also reported by Narsai et al. (2013) who observed a similar response of the resistant cultivar to bacterial blight. Response to combined high temperature and *Xoo* showed that the resistant genotype devoted large parts of its transcriptome to catalytic activity (Figure 5). Physiological mechanisms may contribute to IRBB67 resistance to *Xoo* under high temperature. Up-regulation of trehalose phosphate phosphatase gene (LOC_Os09g20390) which dephosphorylates trehalose phosphate synthase to release free trehalose (Yadav et al. 2014), recently reported to be involved in anaerobic germination in rice (Kretzschmar et al. 2015) may also play an important role in IRBB67 tolerance or adaptation to high temperature to trigger resistance to *Xoo*. Interestingly, up-regulation of the trehalase gene LOC_Os10g37660 was seen in the resistant genotype, suggesting that conversion of trehalose to glucose for carbohydrate metabolism may contribute to high temperature tolerance in IRBB67. Similarly, a low-affinity cation transporter gene (LOC_Os06g38120) was also up-regulated in the resistant genotype compared to IR24 in response to high temperature and *Xoo*, suggesting a possible role of this gene in maintaining a certain homeostasis of the resistant genotype cell membrane under high temperature. Given that, the resistance increased in IRBB7 under high temperature could possibly relate to these genes which are involved in alleviating the high temperature effects on rice cell membrane by maintaining homeostasis during the stress. Further studies are required to prove the possible role of trehalose 6 phosphate and low-affinity cation transporter genes' functions in IRBB7 and IRBB67. Stress tolerance accompanied with up-regulation of resistance gene such as NB-ARC/LRR (LOC_Os11g44990) and NR-ARC domain containing protein (LOC_Os11g44990) may contribute to enhance resistance to *Xoo*

143

under high temperature in IRBB67 and possibly in IRBB7 while their activities might be reduced in IRBB4 under high temperature. Total resistance activity below the threshold under high temperature results in no defense (Zhu et al. 2010).

Availability of *Xoo* genome sequences supported the determination of the pathogen diversity and the understanding of the host-pathogen interaction. Genome comparison between PXO99, PXO86, PXO145, MAFF and KACC and *Xanthomonas oryzae* pv. *oryzicola* strain BLS256 showed variation among strains and demonstrates a diversity existing within *Xoo* population and between *Xoo* and *Xoc*. Strain MAFF and KACC shared more common features (Salzberg et al. 2008) than with PXO strains. The comparative analysis revealed close relatedness of PXO145 to PXO86 with a large inversion in PXO86 compared to that of PXO145. Additionally, type 3 effectors determined from PXO145 were similar to those in PXO99A and MAFF. White et al. (2009) reported that type 3 effectors are similar among *Xoo* strains.

The genome sequence leads to the prediction of several bacterial effectors. Bacterial effectors are injected into the host plant through the type 3 secretion system and once inside the host bind to the host genes and initiate their activation (Kay et al. 2007; Römer et al. 2010, Wilkins et al. 2015). In PXO145, 18 TAL effectors were predicted among which *avrXa7* that bind to *Os11N3* (Antony et al., 2010) was also predicted. *AvrXa27* which is recognized by the *R* gene *Xa27* (Gu et al. 2005) was also predicted to be present in PXO145. An allele of *avrXa23*, *avrXa10* and *PthXo6* were also predicted in PXO145. The resistance reaction observed on IRBB23 and IRBB10 with PXO145 suggests that the presence of an allele of these TAL effectors does not affect the defense response in both IRBB23 and IRBB10. Up-regulation of *Os11N3* in the susceptible genotype during the time course transcriptome profile confirmed the prediction of *avrXa7* in PXO145. Host genes activated by TAL effectors can be either susceptible (S) gene which results in disease development or resistance (*R*) gene and contribute to host defense

(Boch et al. 2014, Wilkins et al. 2015). Moreover, sRNA biogenesis gene (*OsHEN1*, LOC_OS07g06970) was also up-regulated in the susceptible genotype IR24. *OsHEN1* is a target of PXO99A TAL effector *TAL9A* (Moscou and Bogdanov, 2009) which is also predicted in PXO145. Prediction of TAL effectors repertoire in PXO145 opens the door to determine the host targeted genes and the use of biotechnology application to develop rice with *R* genes that could recognize multiple TAL effectors.

Conclusion

In the light of our overall results, high temperature affects the host response to *Xoo*. Time course transcriptome profiles revealed evidence that the resistance of genotype IRBB67 is enhanced under high temperature as several physiological changes were observed in comparison to the susceptible genotype IR24. The response to high temperature with regulation of cell membrane homeostasis might confer high temperature stress tolerance in IRBB67. Complementation effects of *Xa4* in *Xoo* spread *in planta* as observed in IRBB67 under both temperature regimes suggest that this *R* gene may not be completely lost under high temperature, and that stress (high temperature) tolerance failure could possibly explain the decrease in *Xa4* effectiveness compared to that of *Xa7* under temperature increase. Thus, further understanding of how IRBB67 mediates resistance to *Xoo* under temperature will reveal insight into crosstalk in abiotic and biotic stress regulatory pathways.

Finally, we predicted 18 TAL effectors in PXO145 among which *avrXa7* and *avrXa27* were identified, which correlate with the resistance reaction observed on IRBB7 and IRBB27. Additionally, the PXO145 genome showed close relatedness to PXO86 and reveals the existence of genome rearrangement between *Xoo* strains from the Philippines and MAFF and KACC.

Acknowledgment

References

Alvarez ME (2000) Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant Mol Biol 44:429-442.

Antony G, Zhou J, Huang S, Li T, Liu B, White F. et al. (2010) Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*. Plant Cell 22: 3864-3876. doi: 10.1105/tpc.110.078964

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63: 3523–3543. doi: 10.1093/jxb/ers100

Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C (2014) jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 15:293.

Battisti D, Naylor RL (2009) Historical Warnings of future food insecurity with unprecedented seasonal heat. Science 323: 240. doi: 10.1126/science.1164363

Bellincampi D, Cervone F, Lionetti V (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front Plant Sci 5: 228. doi: 10.3389/fpls.2014.00228

Boch J, Bonas U, Lahaye T. (2014) TAL effectors-pathogen strategies and plant resistance engineering. New Phytologist 204: 823-832. doi: 10.1111/nph.13015

Cao F, Yoshioka K, Desveaux D (2011) The roles of ABA in plant-pathogen interactions. J Plant Res 124: 489-499.

Cao H, Glazebrook J, Clarke JD, Volko S, Dong X (1997) The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88: 57-63.

Cheng C, Gao X, Feng B, Sheen J, Shan L, He P (2013) Differential temperature operation of plant immune responses. Nat. Commun. 4:2530. 10.1038/ncomms3530

Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J (2013) Nonhybrid, finished microbial

genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. 10.1038/nmeth.2474.

Ciolkowski I, Wanke D, Birkenbihl RP, Somssich IE (2008) Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function. Plant Mol Biol 68:81-92.

CLC Genomics Workbench 7.0.4 (http://www.clcbio.com)

Conde A, Chaves MM, Gerós H (2011) Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant Cell Physiol 52: 1583-1602.

Dahal D, Pich A, Braun HP, Wydra K (2010) Analysis of cell wall proteins regulated in stem of susceptible and resistant tomato species after inoculation *with Ralstonia solanacearum*: a proteomic approach. Plant Mol Biol 73: 643–658.

Darling AE, Mau B, Perna NT (2010) Progressive Mauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS ONE 5:e11147. doi: 10.1371/journal.pone.0011147

De los Reyes BG, Mohanty B, Yun SJ, Park M-R, Lee D-Y (2015) Upstream regulatory architecture of rice genes: summarizing the baseline towards genus-wide comparative analysis of regulatory networks and allele mining. Rice 8:14.doi:10.1186/s12284-015-0041-x.

DeFalco T, Bender K, Snedden W (2010) Breaking the code: Ca2+ sensors in plant signaling. Biochem. J 425:27-40.

Denancé N, Sánchez-Vallet A, Goffner D, Molina A (2013) Disease resistance or growth: The role of plant hormones in balancing immune responses and fitness costs. Front Plant Sci. 4:155.

Desveaux D, Subramaniam R, Despres CA (2004) 'Whirly' transcription factor is required for salicylic acid-dependent disease resistance in Arabidopsis. Dev Cell 6:229-240.

Dossa GS, Sparks A, Vera Cruz CM, Oliva R (2015) Decision tools for bacterial blight resistance gene deployment in rice-based agricultural ecosystems. Front Plant Sci 6:305. doi:10.3389/fpls.2015.00305.

Dropkin V (1969) The necrotic reaction of tomatoes and other hosts resistant to Meloidogyne: reversal by temperature. Phytopathology 59:1632-1637.

Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res 38:W64-W70. doi: 10.1093/nar/gkq310.

Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, et al. (2009) Real-time DNA sequencing from single polymerase molecules. Science 323:133-138. 10.1126/science.1162986.

Ellis M, Egelund J, Schultz CJ, Bacic A (2010) Arabinogalactan-proteins: key regulators at the cell surface? Plant Physiology 153: 403-419.

Fan J, Hill L, Crooks C, Doerner P, Lamb C (2009) Abscisic acid has a key role in modulating diverse plant-pathogen interactions. Plant Physiol 150: 1750–1761.

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, et al. (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323: 1357-1360. 10.1126/science.1166289

Garcion C, Métraux JP (2006) Salicylic acid. Plant Hormone Signal 24:229-255.

Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE (2006) Climate change effects on plant disease: genomes to ecosystems. Annu Rev Phytopathol 44:489-509.

Gonzalez C, Szurek B, Manceau C, Mathieu T, Sere Y, Verdier V (2007) Molecular and pathotypic characterization of new *Xanthomonas oryzae* strains from West Africa. Mol Plant Microbe Interact 20:534-546.

Gu KY, Yang B, Tian DS, Wu LF, Wang DJ, Sreekala C, Yang F, Chu ZQ, Wang GL, White, FF, Yin ZC (2005) *R* gene expression induced by a type-III effector triggers disease resistance in rice. Nature 435: 1122-1125.

Hilaire E., Young SA, Willard LH, McGee JD, Sweat T, Chittoor JM, Guikema JA Leach JE (2001) Vascular defense responses in rice: peroxidase accumulation in xylem parenchyma cells and xylem wall thickening. Mol. Plant–Microbe Interact 14: 1411-1419.

Hua J (2013) Modulation of plant immunity by light, circadian rhythm, and temperature. Curr Opin Plant Biol 4: 406-413. doi: 10.1016/j.pbi.2013.06.017

Jaillais Y, Chory J (2010) Unraveling the paradoxes of plant hormone signaling integration. Nat. Struct. Mol. Biol 17: 642-645.

Jones JD, Dangl JL (2006) The plant immune system. Nature 444: 323–329. 10.1038/nature05286

Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae.* Plant Disease Reporter 57: 537-541.

Kay S, Hahn S, Marois E, Hause G, Bonas U (2007) A bacterial effector acts as a plant transcription factor and induces a cell size regulator. Science 318: 648-651.

Khan MA, Naeem M, Iqbal M. 2014. Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. *Eur. J. Plant Pathol.*139: 27–37. 10.1007/s10658-014-0377-x

Király L, Hafez YM, Fodor J, Király Z (2008) Suppression of Tobacco mosaic virus-induced hypersensitive-type necrotization in tobacco at high temperature is associated with downregulation of NADPH oxidase and superoxide and stimulation of dehydroascorbate reductase. J Gen Virol 89: 799-808.

Kottapalli KR, Rakwal R, Satoh K, Shibato J, Kottapalli P, Iwahashi H, Kikuchi S (2007) Transcriptional profiling of indica rice cultivar IET8585 (Ajaya) infected with bacterial leaf blight pathogen *Xanthomonas oryzae* pv.*oryzae*. Plant Physiol Biochem 45:834-850.

Kretzschmar T, Pelayo MAF, Trijatmiko KR, Gabunada LFM, AlamR,Jimenez R, MendioroMS,Slamet-Loedin IH, Sreenivasulu N, Bailey-Serres J, Ismail AM, Mackill, David J, Septiningsih EM (2015) A trehalose-6-phosphate phosphatase enhances

anaerobic germination tolerance in rice. Nature Plants 1:15124. doi:10.1038/nplants.2015.124

Le Gall H, Philippe F, Domon J-M, Gillet F, Pelloux J, Rayon C (2015) Cell Wall Metabolism in Response to Abiotic Stress. Plants 4: 112-166.

Lee BM, Park YJ, Park DS, Kang HW, Kim JG, Song ES, Park IC, Yoon UH, Hahn JH, Koo BS, et al. (2005) The genome sequences of *Xanthomonas oryzae* pathovar *oryzae* KACC10331 the bacterial blight pathogen of rice. Nucleic Acids Res 33: 577-586.

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat. Biotechnol 30: 390-392. 10.1038/nbt.2199

Liu J, Feng L, Li J, He Z (2015) Genetic and epigenetic control of plant heat responses. Front. Plant Sci 6:267. doi: 10.3389/fpls.2015.00267

Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL (2008) Prioritizing climate change adaptation needs for food security in 2030. Science 319: 607-610. doi: 10.1126/science.1152339

Long SP, Woodward FI (1988) Plants and temperature. **In Cambridge, England: Society for Experimental Biology; 1988**. Plants and temperature; Long SP, Woodward FI, editors.

López-Maury L, Marguerat S, Bähler J (2008) Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat Rev Genet 9: 583-593. doi: 10.1038/nrg2398

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15: 550.

Lyuben Z, Plamena K, Mariela O (2014) The Role of Plant Cell Wall Proteins in Response to Salt Stress, The Scientific World Journal 764089-764097. doi:10.1155/2014/764089

Martens JW, McKenzie RIH, Green GJ (1967) Thermal stability of stem rust resistance in oat seedlings. Can. J. Bot 45:451-58.

Martin GB, Bogdanove AJ, Sessa G (2003) Understanding the functions of plant disease resistance proteins. Annual Review of Plant Biology 54:23-61.

Moscou MJ, Bogdanove AJ (2009) A simple cipher governs DNA recognition by TAL effectors. Science 326:150.

Narsai R, Wang C, Chen J, Wu J, Shou H, Whelan J (2013) Antagonistic, overlapping and distinct responses to biotic stress in rice (*Oryza sativa*) and interactions with abiotic stress. BMC Genomics 14:93. doi: 10.1186/1471-2164-14-93.

Naylor R, Falcon W (2008) "Our Daily Bread: A Review of the Current World Food Crisis", Boston Review September/October, 13–18.

Niño-Liu DO, Ronald PC, Bogdanove AJ (2006) *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Molecular Plant Pathology 7: 303-324.

Ochiai H, Inoue Y, Takeya M, Sasaki A, Kaku H. 2005. Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. Jpn Agric Res Q., 39:275-287.

Ou SH. 1985. Rice Diseases. Kew, Surrey: Commonwealth Agricultural Bureau.

Pavan U (2011) Genome-wide analysis of lipoxygenase gene family in Arabidopsis and rice. *Plant* Signaling & Behavior 6: 335-338, DOI: 10.4161/psb.6.3.13546

Peng SB, Huang JL, Sheehy JE, Laza RC, Visperas RM, Zhong XH, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. Proc. Natl. Acad. Sci. USA 101:9971-9975.

Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol 5: 308-316.

Rasmussen S, Barah P, Suarez-Rodriguez MC, Bressendorff S, Friis P, Costantino P, et al. (2013) Transcriptome responses to combinations of stresses in Arabidopsis. Plant Physiol 161: 1783-1794. 10.1104/pp.112.210773

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiology 134: 1683-1696.

Römer P, Recht S, Lahaye T (2009) A single plant resistance gene promoter engineered to recognize multiple TAL effectors from disparate pathogens. Proc. Natl. Acad. Sci. USA 106:20526-31.

Römer P, Recht S, Strauss T, Elsaesser J, Schornack S, Boch J, Wang S, Lahaye T (2010) Promoter elements of rice susceptibility genes are bound and activated by specific TAL effectors from the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae.* New Phytologist 187: 1048-1057.

Salzberg SL, Sommer DD, Schatz MC, Phillippy AM, Rabinowicz PD, Tsuge S, Furutani A, Ochiai H, Delcher AL, Kelley D et al. (2008) Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. BMC Genomics 9: 204.

Santner A, Calderon-Villalobos LIA, Estelle M (2009). Plant hormones are versatile chemical regulators of plant growth. Nat. Chem. Biol 5: 301-30.

Schmittgen TD, Livak KJ (2008). Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101-1108. doi: 10.1038/nprot.2008.73.

Snedden WA, Fromm H. 2001. Calmodulin as a versatile calcium signal transducer in plants. New Phytol 151:35-66.

Streubel J, Pesce C, Hutin M, Koebnik R, Boch J, Szurek B (2013) Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae.* New Phytol 200: 808-819. doi: 10.1111/nph.12411

Suh JP, Jeung JU, Noh TH, Cho YC, Park SH, Park HS, Shin MS, Kim CK, Jena KK (2013) Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice 6:5.

Tuteja N, Mahajan S (2007). Further characterization of calcineurin B-like protein and its interacting partner CBL-interacting protein kinase from *Pisum sativum*. Plant Signal Behav 2 : 358-361.

Uauy C, Brevis JC, Chen X, Khan I, Jackson L, Chicaiza O, Distelfeld A, Fahima T, Dubcovsky J (2005) High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticumturgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus Gpc-B1. Theoretical and Applied Genetics 112: 97-105.

UN DESA (United Nations, Department of Economics and Social Affairs) (2015) World population projected to reach 9.7 billion by 2050. Report, 29 July 2015, New York. http://www.un.org/en/development/desa/news/population/2015-report.html

Uraguchi S, Kamiya T, Sakamoto T, Kasai K, Sato Y, Nagamura Y, Yoshida A, Kyozuka J, Ishikawa S, Fujiwara T (2011) Low-affinity cation transporter (OsLCT1) regulates cadmium transport into rice grains. Proc. Natl. Acad. Sci. USA 108: 20959–20964.

Usadel B, Nagel A, Steinhauser D, Gibon Y, Blaesing OE, Redestig H, Sreenivasulu N, Krall L, Hannah MA, Poree F, Fernie AR, Stitt M (2006) PageMan an interactive ontology tool to generate, display, and annotate overview graphs for profiling experiments, BMC Bioinformatics 18:7:535.

Vera Cruz CM, Bai JF, Oña I, Leung H, Nelson RJ, Mew TW, Leach JE (2000) Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. Proc. Natl. Acad. Sci. U.S.A 97:13500-13505.

Verdier V, Vera Cruz C, Leach JE (2012) Controlling rice bacterial blight in Africa: needs and prospects. J Biotechnol 159:320–328

Vlot AC, Dempsey DMA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47:177-206.

Wang Y, Bao Z, Zhu Y, Hua J (2009) Analysis of temperature modulation of plant defense against biotrophic microbes. Mol Plant Microbe Interact 22: 498–506.

Webb KM, Oña I, Bai J, Garrett KA, Mew T, Vera Cruz CM, Leach JE (2010) A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene. New Phytol 185:568-576.

White FF, Potnis N, Jones J.B., Koebnik R (2009) The type III effectors of *Xanthomonas*. Mol. Plant Pathol 10:749-766.

Wilkins KE, Booher JN, Wang L, Bogdanove AJ (2015) TAL effectors and activation of predicted host targets distinguish Asian from African strains of the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* while strict conservation suggests universal importance of five TAL effectors. Frontiers in Plant Science 6.

Xu J, Audenaert K, Hofte M, De Vleesschauwer D (2013) Abscisic acid promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae* pv.*oryzae* by suppressing salicylic acid-mediated defenses. PLoS One. 8: doi: 10.1371/journal.pone.0067413

Yadav UP, Ivakov A, Feil R, Duan GY, Walther D, Giavalisco P, Piques M, Carillo P, Hubberten HM, Stitt M, et al. (2014) The sucrose-trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signalling by Tre6P. J Exp Bot 65: 1051-1068.

Yang B, and White FF (2004) Diverse members of the AvrBs3/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. Mol. Plant Microbe Interact 17:1192-1200. doi: 10.1094/MPMI.2004.17.11.1192

Yang B, Sugio A, White FF (2006) *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. Proc. Natl. Acad. Sci. USA 103: 10503-10508.

Yang S, Hua J (2004) A haplotype-specific Resistance gene regulated by BONZAI1 mediates temperature-dependent growth control in Arabidopsis. Plant Cell 16: 1060-1071.

Yasuda M, Ishikawa A, Jikumaru Y, et al. (2008) Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. Plant Cell. 20:1678-1692. doi: 10.1105/tpc.107.054296.

155

Yu YH, Streubel J, Balzergue S, Champion A, Boch J, Koebnik R. et al. (2011) Colonization of rice leaf blades by an African Strain of *Xanthomonas oryzae* pv. *oryzae* depends on a new TAL effector that induces the rice nodulin-3 *Os11N3* gene. Mol. Plant Microbe Interact 24:1102-1113. doi: 10.1094/MPMI-11-10-0254

Yuan M, Chu Z, Li X, Xu C, Wang S. (2009) Pathogen-induced expressional loss of function is the key factor in race-specific bacterial resistance conferred by a recessive R gene xa13 in rice. Plant Cell Physiol 50: 947-955.

Zhu Y, Qian W, Hua J. 2010. Temperature Modulates Plant Defense Responses through NB-LRR Proteins. PLoS Pathogens 6: e1000844.

Table 1: List of Type 3 effectors identified from PXO145 using tBlastn

T3 effectors	% of Identity	% of gaps	E-value
AvrBs2	99.39	0.0	0.0
XopC	99.76	0.0	0.0
XopF	99.82	0.0	0.0
XopK	100	0.0	0.0
XopK	100	0.0	0.0
XopN	99.46	0.0	0.0
XopP	99.38	0.0	0.0
XopQ	99.49	0.0	0.0
XopR	99.31	0.0	0.0
XopU	98.03	0.0	0.0
XopX	98.87	0.32	0.0
XopZ	99.93	0.0	0.0
XopAA	97.70	0.0	0.0
XopAD	99.23	0.0	0.0
XopAE	100	0.0	0.0
XopV	100	0.0	0.0
XopT	98.95	0.0	3.55E-128
XopAB	98.96	0.0	8.78E-122
XopI	99.47	0.0	7.88E-119
XopW	100	0.0	1.53E-66
XopI	100	0.0	6.42E-24

Table 2: list of TAL effectors and their corresponding RVDs sequences identified from PXO145 genome

Tal ID	Size	Repeats	RVDs*	Identity*	*R* genes
tal1a	3411	16.5	NI N* NI NS NN NG NN HD HD HD NG HD NS HD N* NS NG		
tal1b	3855	20.5	NI N* NI HG NI NI NS HD NN HD NS NG SS HD NI NI NN NI NN NI NG		
tal2a	3297	15.5	NI NS HD HG NS NN HD H* NG NN NN HD HD NG HD NG	Tal5a	
tal2b	3755	19.5	NI HG NS HG HG HD NS NG HD NN NG HG NG HD HG HD HD NI NN NG	Tal7b	
tal3	3717	19.5	HD HD HD NG N* NN HD HD N* NI NI NN HD HI ND HD NI HD NG NG	Tal9A	
tal4	4020	22.5	NI H* NI NN NN NN NN NN HD NI NN HG HD NI N* NS NI NI HD N* NS NI NG	Similar to PthXo6	
tal5	2977	17.5	NS HD NG NG! NG NG NG HD HD HD NN HD NG HD NI HD NN N*	Similar to Tal3a	
tal6	3720	19.5	NI NG NN NG NK NG NI NN NI NN NI NN NS NG NS NN NI N* NS NG	Tal2a	
tal7a	3315	15.5	NI NN NN NI NI NS HD NS HG NN NN NN NI NI NG HD	Similar to Tal4	
tal7b	3519	17.5	NI HG NI NI NI NN HD NS NN NS NN HD NN NI HD NN NS NG	Tal7a/Tal8a	
tal7c	3306	15.5	NI H* NI HG NI NI NN HD NI HD NN HG NS N* HD N*	Similar to AvrXa10	*Xa10*
tal8	2246	17.5	NS NG NG NG! NG NG NG HD HD HD NN HD NG HD HD HD HD H*		
tal9	4431	26.5	NI HG NI NI HG HD NN HD HD HD NI NI NN NI HD HD HD HG NN NN HD NS NN HD N* NS N*		
tal10	4341	25.5	NI HG NI NI NS HD NN HD HD HD NS N* N*! HD HD NS NS NN NN NI NG NN NI N* NS N*	AvrXa7	*Xa7*
tal11a	4449	26.5	HD HD NN NN NS NG HD S* HG HD NG N* HD HD HD N* NN NI! NN HD HI ND HD HG NN HG N*	Similar to AvrXa23	*Xa23*
tal11b	3411	16.5	NI NN N* NG NS NN NN NN NI NN NI N* HD HD NI NG NG	AvrXa27	*Xa27*
tal11c	3009	12.5	NI NN NI HG HG NV HG HD HG HD HD HD NG	Tal9d	
tal11d	4127	23.5	NN HD NS NG HD NN N* NI HD NS HD NN HD NN HD NN NN NN NN NN NN NN HD NG	Tal9e	

* All the TAL effectors predicted from PXO145 are identical to those of PXO86

List of Figures

Figure 1A: Plant height under low and high temperature regimes conditions. Plant height was recorded at 32 days old by measuring the length from soil surface to the tip of the most expanded leaf. Plant under high temperature conditions were significantly higher compared to low temperature. The letters a, b represent the significant differences between low and high temperature on each genotype as determined by least significant differences means (LSD means) at α<0.05.

Figure 1B: Bacterial blight lesion length under low and high temperature regimes conditions. Bacterial blight lesion length was recorded at 32 days old (11dpi).

Figure 1C: PXO145 spread *in planta* in four rice NILs under two temperature regimes. Data were collected from inoculated leaves at 21 days old by leaf clipping and collected 11 days after inoculation. A, B and C represent leaf segments of 5 cm length beyond bacterial blight symptomatic area.

Figure 2: Venn diagram showing the distribution of DEGs between *Xoo* and Mock inoculated samples under both temperature regimes. (2A) DEGs at 3 hpi, (2B) DEGS at 72 hpi and (2C) DEGS at 120 hpi. The venn diagrams were created using jvenny (Bardou et al., 2014)

Figure 3: Pageman analysis showing the overall overview of the DEGs from both rice genotypes IR24 (susceptible) and IRBB67 (resistant) under low and high temperature across the three time points. Pageman is integrated program in MapMan version 5 (Usadel et al., 2006). The red and green mean up and down-regulation, respectively.

Figure 4: GO enrichment in DEGs from high vs Low after mock inoculation and after *Xoo* inoculation. The enriched GO terms across the three time points and from both genotypes (IR24 and IRBB67) as predicted using AgriGO tools Parametric Analysis of Gene Set Enrichment (PAGE) at p-value 5% (Du et al., 2010). The red and green mean up and down-regulation, respectively.

Figure 5: GO enrichment analysis of 188 DEGs expressed between IRBB67 and IR24 under high temperature after *Xoo* inoculation as predicted using AgriGO tools Parametric Analysis of Gene Set Enrichment (PAGE) at p-value 5% (Du et al., 2010). The red and green mean up and down-regulation, respectively.

Figure 6: Validation of RNA-Seq data by qRT–PCR

Figure 7: PXO145 circular map comparison to *Xoo* strains PXO99, PXO86 and *Xoc* strain BLS256. PXO145 genome sequence was used as seed.

Figure 8: Genome alignment using MAUVE v3.2.1. Linear genomes were aligned in MAUVE v3.2.1 and comparisons were performed using PXO145 as reference.
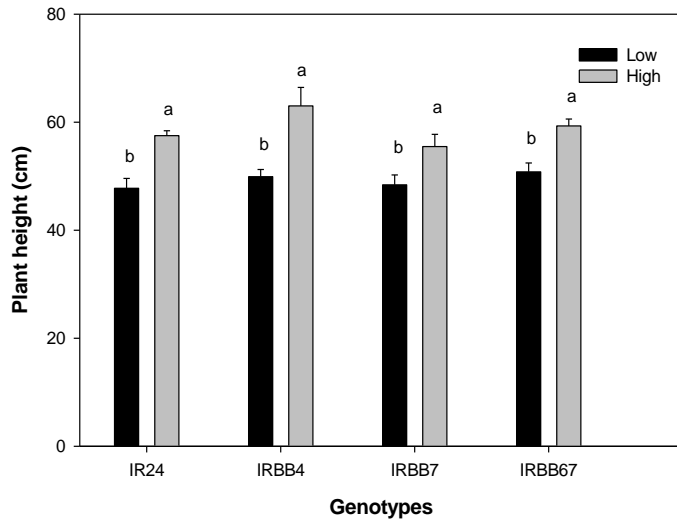
Figure 1A: Plant height under low and high temperature regimes conditions
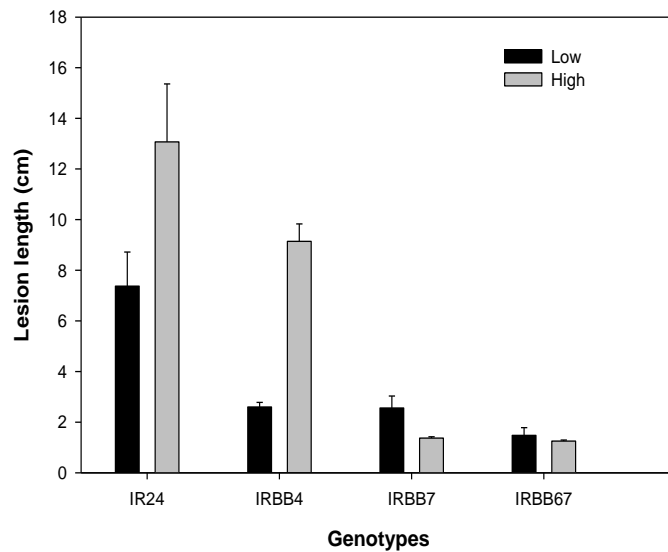
Figure 1B: Bacterial blight lesion length under low and high temperature regimes conditions
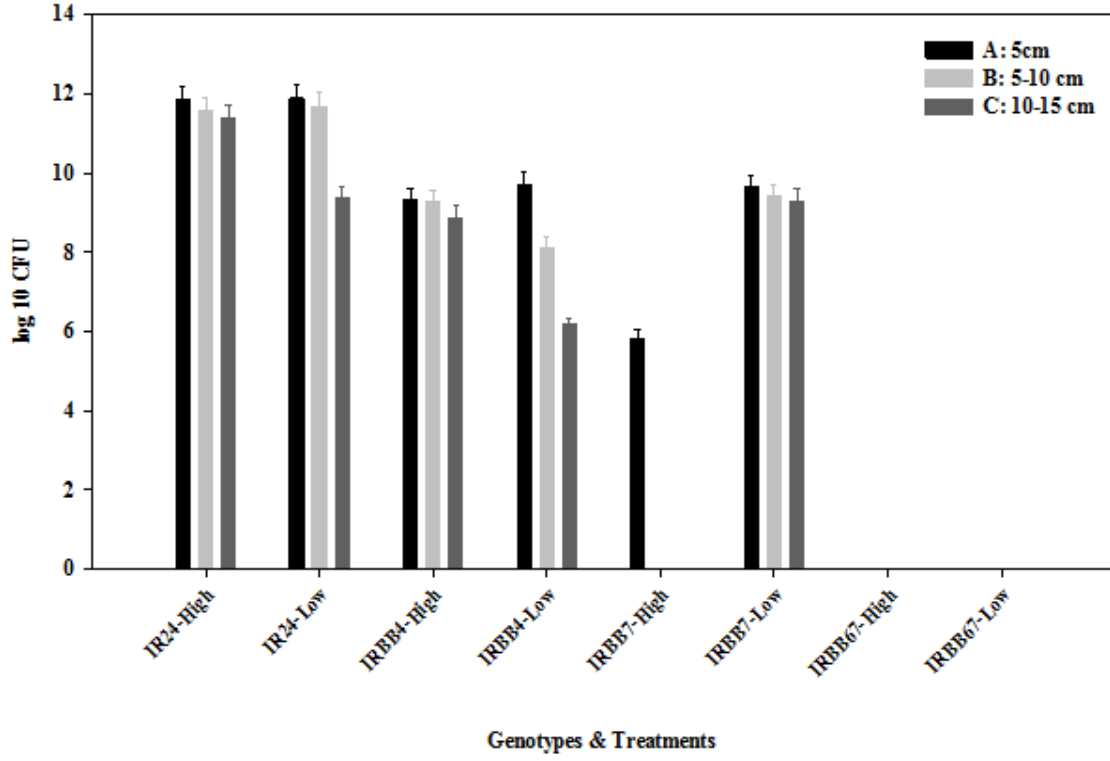
Figure 1C
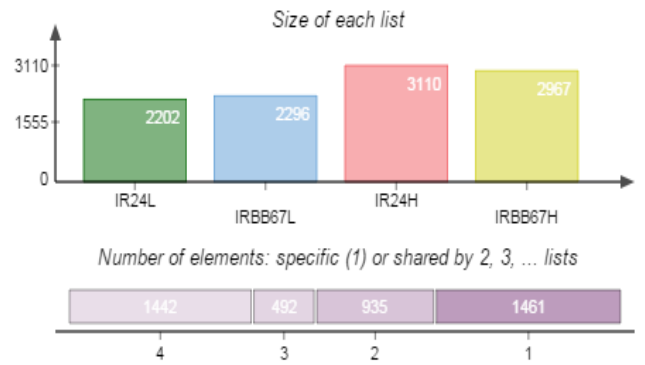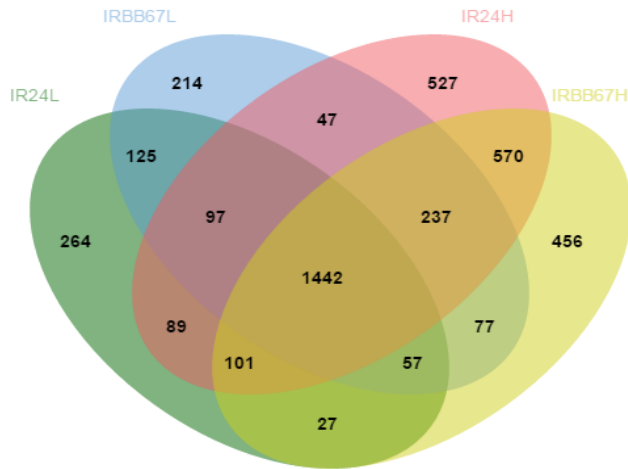
Fig. 2A
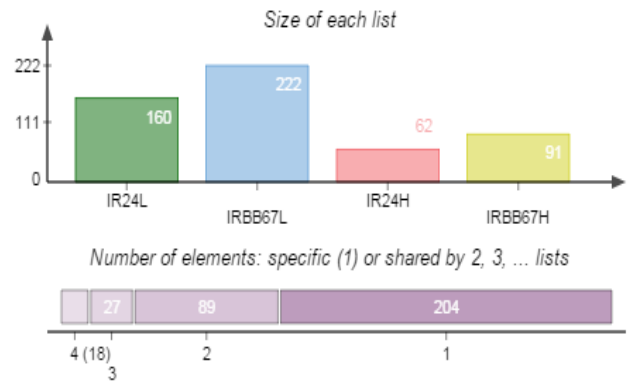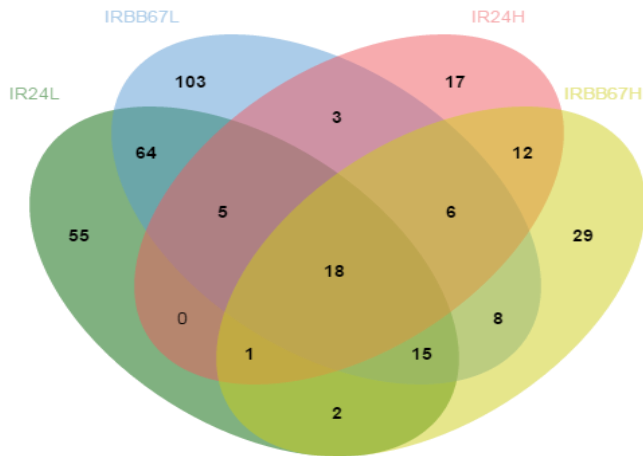
Fig. 2B

Fig. 2C

Figure 2

163
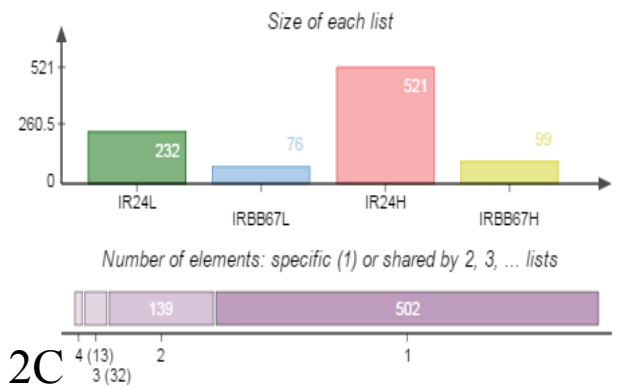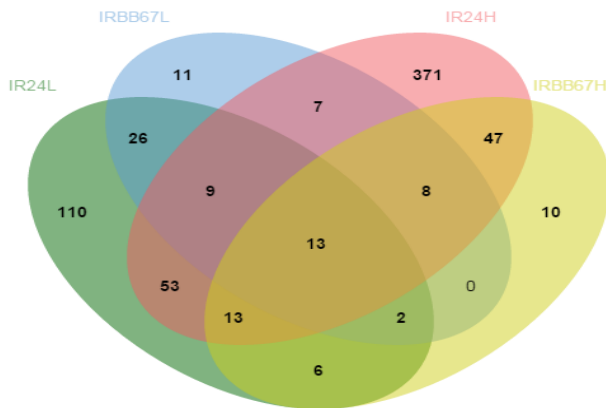
Ontology	IR24H_3hpiup	IR24H_72hpiup	IR24H_120hpiup	IR24L_3hpiup	IR24L_72hpiup	IR24L_120hpiup	IRBB67H-3hpiup	IRBB67H_72hpiup	IRBB67H_120hpiup	IRBB67L_3hpiup	IRBB67L_72hpiup	IRBB67L_120hpiup	IR24H_3hpidown	IR24H_72hpidown	IR24H_120hpidown	IR24L_3hpidown	IR24L_72hpidown	IR24L_120hpidown	IRBB67H-3hpidown	IRBB67H_72hpidown	IRBB67H_120hpidown	IRBB67L_3hpidown	IRBB67L_72hpidown	IRBB67L_120hpidown
amino acid metabolism																								
amino acid metabolism.degradation																								
amino acid metabolism.degradation.branched chain group																								
amino acid metabolism.degradation.branched-chain group.shared																								
amino acid metabolism.misc																								
amino acid metabolism.synthesis																								
amino acid metabolism.synthesis.aromatic aa																								
amino acid metabolism.synthesis.aromatic aa.chorismate																								
amino acid metabolism.synthesis.aromatic aa.chorismate.shikimate kinase																								
amino acid metabolism.synthesis.aromatic aa.tryptophan																								
amino acid metabolism.synthesis.aromatic aa.tryptophan.anthranilate synthase																								
amino acid metabolism.synthesis.aromatic aa.tryptophan.tryptophan synthase																								
amino acid metabolism.synthesis.aspartate family																								
amino acid metabolism.synthesis.aspartate family.methionine																								
amino acid metabolism.synthesis.central amino acid metabolism																								
amino acid metabolism.synthesis.central amino acid metabolism.GABA																								
amino acid metabolism.synthesis.central amino acid metabolism.GABA.Glutamate decarboxylase																								
amino acid metabolism.synthesis.serine-glycine-cysteine group.cysteine																								
Biodegradation of Xenobiotics																								
C1-metabolism																								
cell																								
cell wall																								

cell wall.cell wall proteins.AGPs

cell wall.cell wall proteins.AGPs.AGP

cell wall.cellulose synthesis.COBRA

cell wall.degradation

cell wall.degradation.cellulases and beta -1,4-glucanases

cell wall.degradation.pectate lyases and polygalacturonases

cell wall.hemicellulose synthesis.glucuronoxylan

cell wall.modification

cell wall.precursor synthesis.sugar kinases

cell wall.precursor synthesis.sugar kinases.galacturonic acid kinase

cell.cycle

cell.cycle.peptidylprolyl isomerase

cell.division

cell.organisation

cell.vesicle transport

Co-factor and vitamine metabolism.thiamine.hydroxymethylpyrimidine kinase

development

development.late embryogenesis abundant

development.unspecified

DNA

DNA.repair

DNA.synthesis/chromatin structure

gluconeogenese/ glyoxylate cycle

gluconeogenese/ glyoxylate cycle.isocitrate lyase

gluconeogenese/ glyoxylate cycle.malate synthase

gluconeogenesis.Malate DH

hormone metabolism

hormone metabolism.abscisic acid

hormone metabolism.abscisic acid.induced-regulated-responsive-activated

hormone metabolism.ethylene

hormone metabolism.ethylene.induced-regulated-responsive-activated

hormone metabolism.ethylene.signal transduction

hormone metabolism.ethylene.synthesis-degradation

hormone metabolism.gibberelin

hormone metabolism.gibberelin.synthesis-degradation

hormone metabolism.gibberelin.synthesis-degradation.GA20 oxidase

hormone metabolism.gibberelin.synthesis-

degradation.GA3 oxidase

hormone metabolism.jasmonate

hormone metabolism.jasmonate.synthesis-degradation

hormone metabolism.jasmonate.synthesis-degradation.12-Oxo-PDA-reductase

hormone metabolism.jasmonate.synthesis-degradation.allene oxidase synthase

hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase

hormone metabolism.salicylic acid

hormone metabolism.salicylic acid.synthesis-degradation

lipid metabolism."exotics" (steroids, squalene etc)

lipid metabolism."exotics" (steroids, squalene etc).UDP-glucose:sterol glucosyltransferase

lipid metabolism.FA desaturation

lipid metabolism.FA desaturation.omega 3 desaturase

lipid metabolism.FA synthesis and FA elongation

lipid metabolism.FA synthesis and FA elongation.acyl coa ligase

lipid metabolism.FA synthesis and FA elongation.long chain fatty acid CoA ligase

lipid metabolism.lipid degradation

lipid metabolism.lipid degradation.lipases

lipid metabolism.lipid degradation.lipases.triacylglycerol lipase

lipid metabolism.lipid degradation.lysophospholipases

lipid metabolism.lipid degradation.lysophospholipases.phosphoinositide phospholipase C

major CHO metabolism

major CHO metabolism.degradation

major CHO metabolism.degradation.starch

major CHO metabolism.degradation.sucrose

major CHO metabolism.degradation.sucrose.invertases

major CHO metabolism.degradation.sucrose.invertases.vacuolar

major CHO metabolism.degradation.sucrose.Susy

major CHO metabolism.synthesis

major CHO metabolism.synthesis.starch

major CHO metabolism.synthesis.starch.AGPase

metal handling

metal handling.binding, chelation and storage

minor CHO metabolism

minor CHO metabolism.others

minor CHO metabolism.raffinose family

minor CHO metabolism.raffinose family.galactinol synthases

minor CHO metabolism.raffinose family.galactinol synthases.putative

minor CHO metabolism.raffinose family.raffinose synthases

minor CHO metabolism.raffinose family.raffinose synthases.putative

minor CHO metabolism.trehalose

misc

misc.alcohol dehydrogenases

misc.alcohol dehydrogenases

misc.aminotransferases

misc.aminotransferases.aminotransferase class IV family protein

misc.beta 1,3 glucan hydrolases

misc.beta 1,3 glucan hydrolases.glucan endo-1,3-beta-glucosidase

misc.cytochrome P450

misc.dynamin

misc.GCN5-related N-acetyltransferase

misc.GDSL-motif lipase

misc.gluco-, galacto- and mannosidases

misc.glutathione S transferases

misc.invertase/pectin methylesterase inhibitor family protein

misc.nitrilases, *nitrile lyases, berberine bridge enzymes, reticuline oxidases, troponine reductases

misc.O-methyl transferases

misc.oxidases - copper, flavone etc.

misc.peroxidases

misc.plastocyanin-like

misc.protease inhibitor/seed storage/lipid transfer protein (LTP) family protein

misc.short chain dehydrogenase/reductase (SDR)

misc.UDP glucosyl and glucoronyl transferases

mitochondrial electron transport / ATP synthesis

mitochondrial electron transport / ATP synthesis.cytochrome c reductase

mitochondrial electron transport / ATP synthesis.F1-

ATPase

mitochondrial electron transport / ATP synthesis.NADH-DH.type II

mitochondrial electron transport / ATP synthesis.NADH-DH.type II.external

mitochondrial electron transport / ATP synthesis.NADH-DH.type II.internal matrix

mitochondrial electron transport / ATP synthesis.uncoupling protein

N-metabolism.nitrate metabolism

N-metabolism.nitrate metabolism.NR

nucleotide metabolism.degradation

nucleotide metabolism.degradation.purine

nucleotide metabolism.phosphotransfer and pyrophosphatases

nucleotide metabolism.phosphotransfer and pyrophosphatases.uridylate kinase

nucleotide metabolism.salvage

polyamine metabolism.synthesis.SAM decarboxylase

protein

protein.aa activation

protein.aa activation.cysteine-tRNA ligase

protein.degradation

protein.degradation.AAA type

protein.degradation.aspartate protease

protein.degradation.cysteine protease

protein.degradation.subtilases

protein.degradation.ubiquitin

protein.degradation.ubiquitin.E3

protein.degradation.ubiquitin.E3.BTB/POZ Cullin3

protein.degradation.ubiquitin.E3.BTB/POZ Cullin3.BTB/POZ

protein.degradation.ubiquitin.E3.RING

protein.degradation.ubiquitin.E3.SCF

protein.degradation.ubiquitin.E3.SCF.cullin

protein.degradation.ubiquitin.E3.SCF.FBOX

protein.degradation.ubiquitin.ubiquitin protease

protein.postranslational modification

protein.postranslational modification.kinase

protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VII

protein.synthesis

protein.synthesis.ribosomal protein

protein.synthesis.ribosomal protein.prokaryotic.unknown organellar																			
protein.synthesis.ribosomal protein.prokaryotic.unknown organellar.50S subunit																			
protein.synthesis.ribosomal protein.prokaryotic.unknown organellar.50S subunit.L28																			
protein.targeting																			
protein.targeting.secretory pathway																			
protein.targeting.secretory pathway.unspecified																			
protein.targeting.unknown																			
PS																			
PS.calvin cycle																			
PS.calvin cycle.aldolase																			
PS.calvin cycle.FBPase																			
PS.calvin cycle.GAP																			
PS.calvin cycle.phosphoglycerate kinase																			
PS.lightreaction																			
PS.lightreaction.photosystem I																			
PS.lightreaction.photosystem I.PSI polypeptide subunits																			
PS.photorespiration																			
PS.photorespiration.phosphoglycolate phosphatase																			
redox																			
redox.ascorbate and glutathione.ascorbate																			
redox.ascorbate and glutathione.ascorbate.GDP-L-galactose-hexose-1-phosphate guanyltransferase																			
redox.thioredoxin																			
RNA																			
RNA.processing.ribonucleases																			
RNA.processing.RNA helicase																			
RNA.processing.splicing																			
RNA.regulation of transcription																			
RNA.regulation of transcription.AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family																			
RNA.regulation of transcription.bHLH,Basic Helix-Loop-Helix family																			
RNA.regulation of transcription.C2C2(Zn) CO-like, Constans-like zinc finger family																			
RNA.regulation of transcription.C2H2 zinc finger family																			
RNA.regulation of transcription.HSF,Heat-shock transcription factor family																			
RNA.regulation of transcription.JUMONJI family																			

RNA.regulation of transcription.NAC domain transcription factor family

RNA.regulation of transcription.PHOR1

RNA.regulation of transcription.putative transcription regulator

RNA.regulation of transcription.unclassified

RNA.regulation of transcription.WRKY domain transcription factor family

RNA.transcription

secondary metabolism

secondary metabolism.flavonoids

secondary metabolism.flavonoids.chalcones

secondary metabolism.flavonoids.dihydroflavonols

secondary metabolism.flavonoids.flavonols

secondary metabolism.isoprenoids

secondary metabolism.isoprenoids.non-mevalonate pathway

secondary metabolism.isoprenoids.non-mevalonate pathway.DXS

secondary metabolism.isoprenoids.non-mevalonate pathway.geranylgeranyl pyrophosphate synthase

secondary metabolism.isoprenoids.terpenoids

secondary metabolism.N misc

secondary metabolism.N misc.alkaloid-like

secondary metabolism.phenylpropanoids

secondary metabolism.phenylpropanoids.lignin biosynthesis

secondary metabolism.phenylpropanoids.lignin biosynthesis.CAD

secondary metabolism.phenylpropanoids.lignin biosynthesis.PAL

secondary metabolism.simple phenols

signalling

signalling.calcium

signalling.G-proteins

signalling.light

signalling.phosphinositides

signalling.receptor kinases

signalling.receptor kinases.leucine rich repeat VIII-1

signalling.receptor kinases.leucine rich repeat VIII-2

signalling.receptor kinases.misc

signalling.receptor kinases.proline extensin like

signalling.receptor kinases.S-locus glycoprotein like

Figure 3

		Number of DEGs	Time Points_HT						Number of DEGs	Time Points_HT+Xoo					
			IR24-3hpi	IR24-72hpi	IR24-120hpi	IRBB67-3hpi	IRBB67-72hpi	IRBB67-120hpi		IR24-3hpi	IR24-72hpi	IR24-120hpi	IRBB67-3hpi	IRBB67-72hpi	IRBB67-120hpi
GO_acc	Description														
	Cellular Compartment														
GO:0043226	organelle	81							0						
GO:0043229	intracellular organelle	81							0						
GO:0005622	intracellular	101							0						
GO:0043227	membrane-bounded organelle	79							0						
GO:0043231	intracellular membrane-bounded organelle	79							0						
GO:0005634	nucleus	33							17						
GO:0005773	vacuole	15							12						
GO:0005618	cell wall	12							11						
GO:0030312	external encapsulating structure	12							12						
GO:0009536	plastid	19							10						
GO:0005886	plasma membrane	0							15						
GO:0016020	membrane	0							26						
GO:0005576	extracellular region	0							10						
GO:0005737	cytoplasm	0							35						
GO:0005623	cell	0							91						
GO:0044444	cytoplasmic part	0							29						
GO:0005829	cytosol	0							14						
	Molecular Function														
GO:0005515	protein binding	61							0						
GO:0016772	transferase activity, transferring phosphorus-containing groups	40							0						
GO:0016301	kinase activity	40							0						
GO:0016740	transferase activity	0							23						
GO:0003677	DNA binding	0							11						
GO:0030528	transcription regulator activity	0							11						
GO:0003700	transcription factor activity	0							11						
GO:0003676	nucleic acid binding	0							12						
GO:0003824	catalytic activity	0							74						
GO:0016787	hydrolase activity	0							20						
GO:0005488	binding	0							57						
	Biological Process														
GO:0007165	signal transduction	28							0						
GO:0050789	regulation of biological process	28							0						
GO:0065007	biological regulation	30							0						
GO:0050794	regulation of cellular process	28							0						
GO:0009058	biosynthetic process	64							0						
GO:0008152	metabolic process	182							0						
GO:0016043	cellular component organization	18							0						
GO:0009719	response to endogenous stimulus	44							0						
GO:0009628	response to abiotic stimulus	40							0						
GO:0005975	carbohydrate metabolic process	12							0						
GO:0050896	response to stimulus	96							0						
GO:0006629	lipid metabolic process	17							13						
GO:0009607	response to biotic stimulus	44							13						
GO:0006807	nitrogen compound metabolic process	0							15						
GO:0006139	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	0							13						

Figure 4

GO	Description	Number of DEGs	H–3hpi	H–72hpi	H–120hpi
	Cell Compartment				
GO:0043227	membrane-bounded organelle	28			
GO:0043231	intracellular membrane-bounded organelle	28			
GO:0043226	organelle	29			
GO:0043229	intracellular organelle	29			
GO:0005622	intracellular	32			
GO:0044424	intracellular part	32			
	Molecular Funtion				
GO:0003824	catalytic activity	66			
GO:0016740	transferase activity	36			
GO:0016772	transferase activity, transferring phosphorus-containing groups	25			
GO:0016301	kinase activity	25			
	Biological Process				
GO:0008152	metabolic process	73			
GO:0044260	cellular macromolecule metabolic process	24			
GO:0009987	cellular process	67			
GO:0043170	macromolecule metabolic process	28			
GO:0044267	cellular protein metabolic process	23			
GO:0006464	protein modification process	23			
GO:0043412	macromolecule modification	23			
GO:0019538	protein metabolic process	27			
GO:0050896	response to stimulus	37			
GO:0044238	primary metabolic process	46			
GO:0009058	biosynthetic process	16			

Figure 5

Validation of RNA-Seq data by qRT–PCR
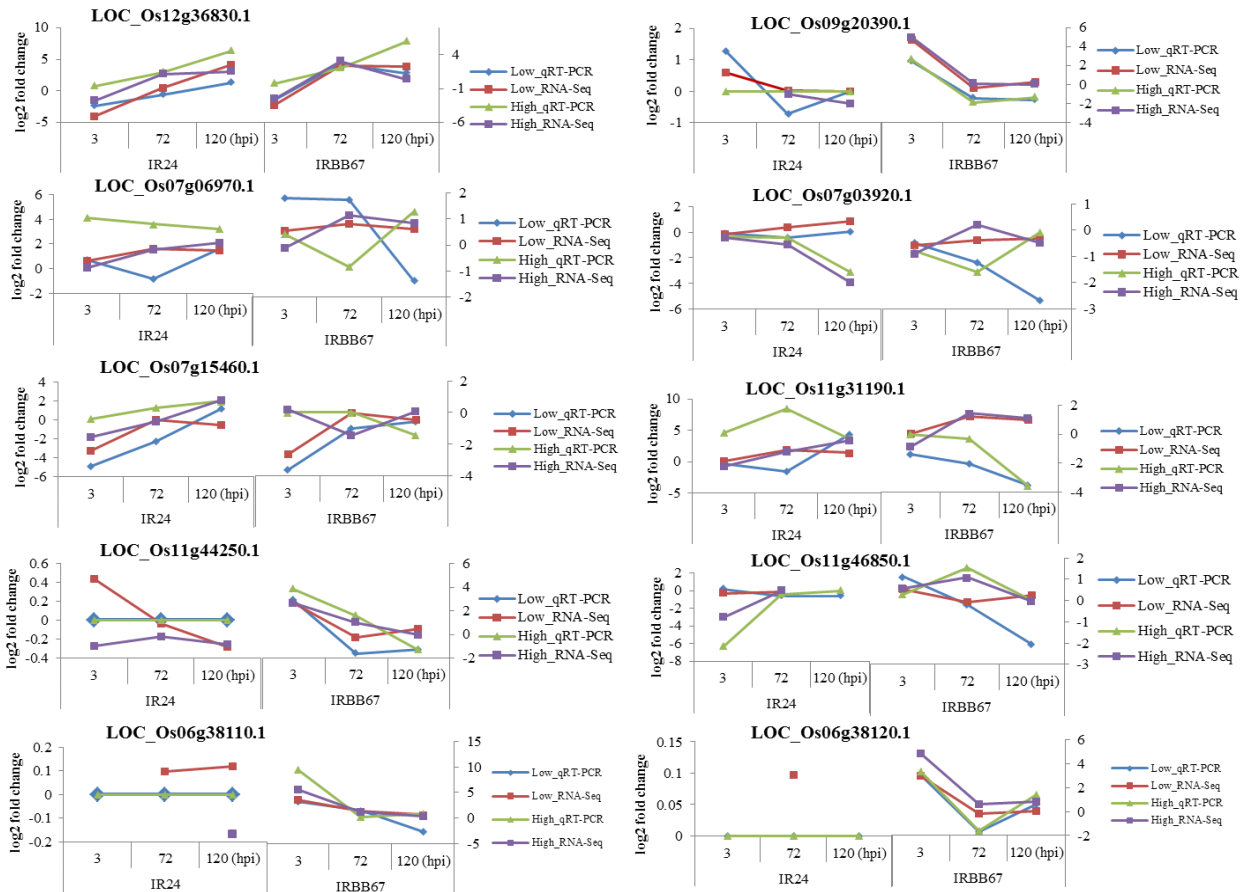
Figure 6
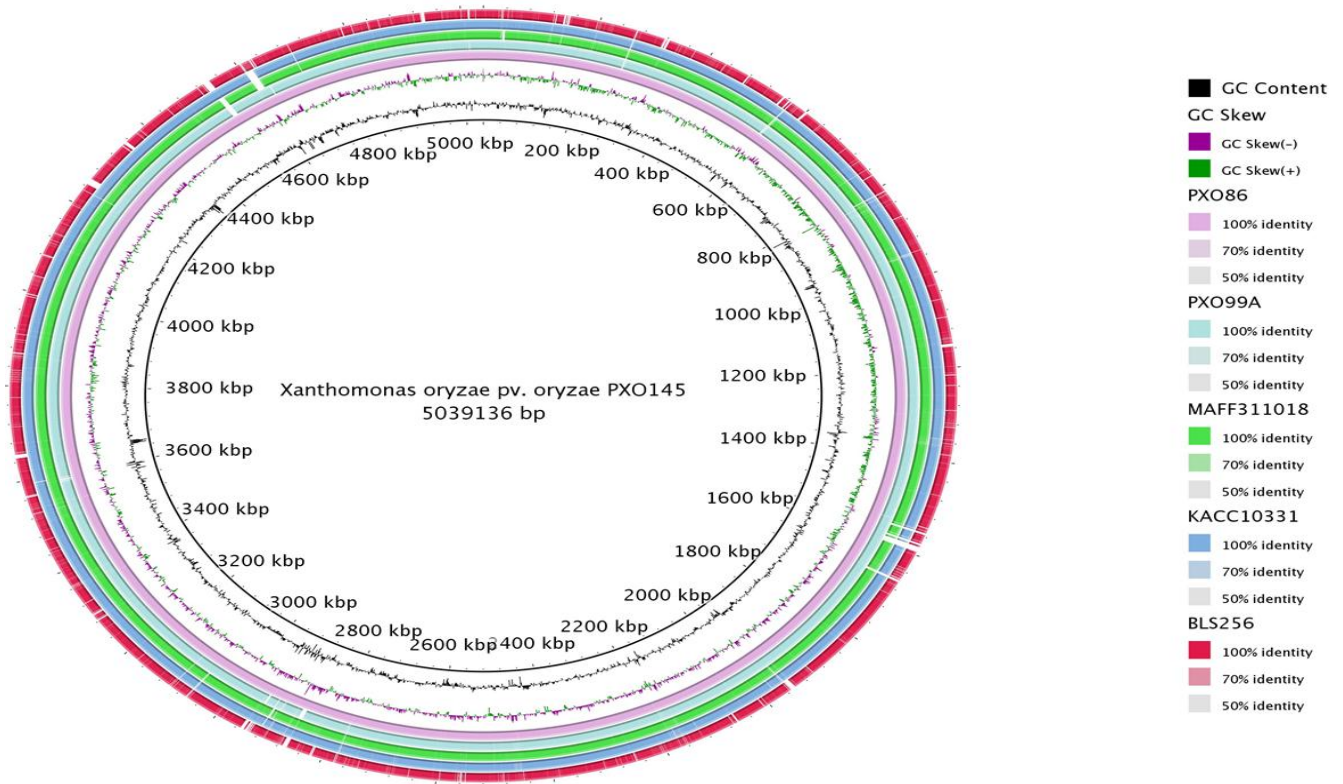
Figure 7
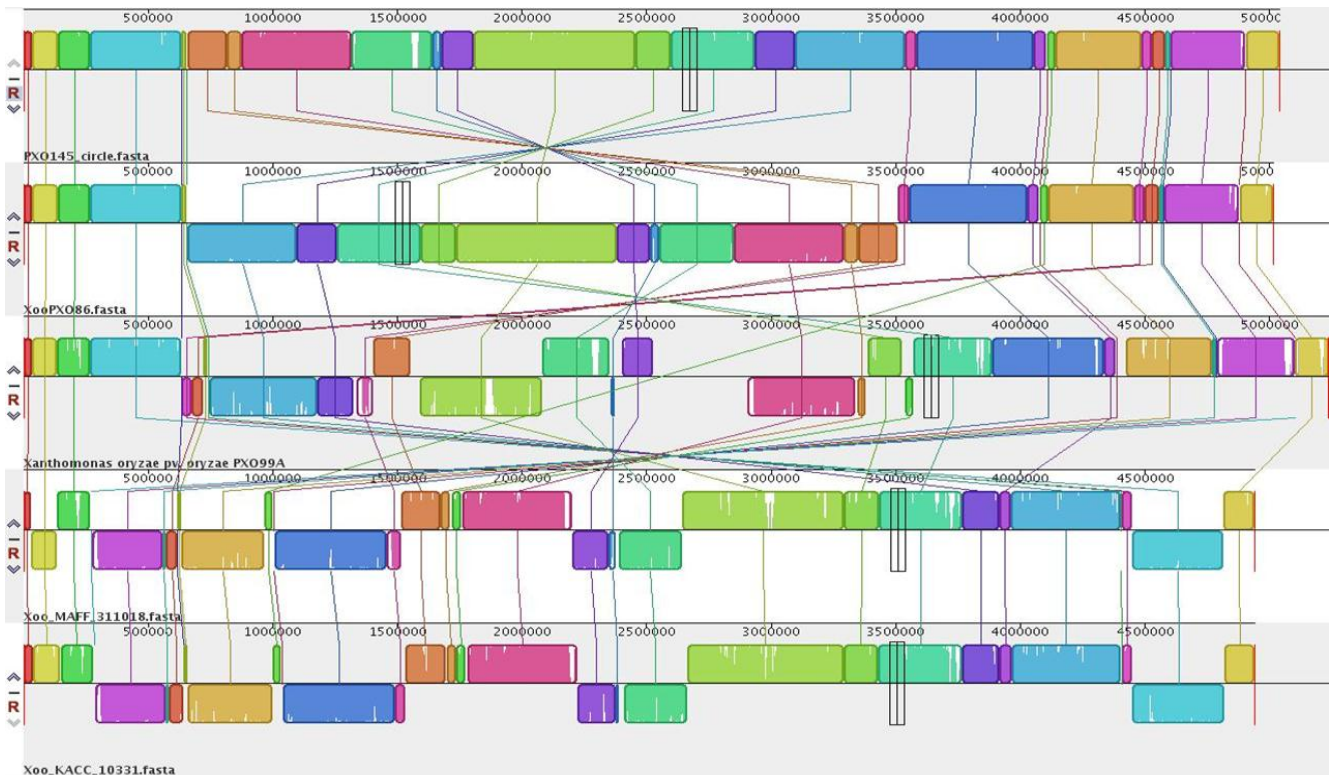
Figure 8

List of supplementary

Supplementary Table 1: qRT-PCR primers for RNA-Seq data confirmation

S/N	Gene	Description	F or R	Sequence (5' 3')	Remarks
1	LOC_Os11g44250	protein kinase, putative, expressed	F	GCGTTATAGCAGGCACTCTAA	
			R	CCCTCTTGCTCACATTCTTCT	
2	LOC_Os11g46850	wall-associated kinase, putative	F	TCGACTGCAACCATAGCTTTAC	
			R	GCTGGATTCCGTGGTGTTAG	
3	LOC_Os07g03920	lectin-like receptor kinase 7, putative	F	GTGAGAAGAAGGCTGAGGTATG	
			R	CAGTGCCCAAGAGATGACTATT	
4	LOC_Os07g06970	HEN1, putative, expressed	F	CAGTACAGTTGGATCGCTTTCT	
			R	CACCACCAAGGAAGCAGTATAG	
5	LOC_Os06g38120	Low affinity cation transporter	F	TTCCTCGCCTTCTCATCTTTC	
			R	GTATTGTCAGCACCGGTAGAA	
6	LOC_Os12g36830	pathogenesis-related Bet v I family protein, putative, expressed	F	CAACGCAGCTCACATTATCAAG	
			R	CGAGCTCATACTCCACGTTTAT	
7	LOC_Os09g20390	OsTTP6	F	AACAAGGGAGTCCTCTTCCAG	Kretzschmar et al., 2015
			R	CTTGAACGCGTCCTCGTC	
8	LOC_Os06g38110	Expressed protein	F	CGCCGTTCTAATGGACTACTT	
			R	AAGGTTTGCGCGGATAGAG	
9	LO_Os07g15460	metal transporter Nramp6, putative, expressed	F	ATGGGGGTGACGAAGGCGGA	
			R	ATTTCCAGGATCGAGGTAA	
10	LOC_Os11g31190	OsSweet14	F	CCTAGGCAACATCATCTCCT	
			R	CGATGTAGATGGTCTCGATG	
11	Actin		F	TCCATCTTGGCATCTCTCAG	
			R	GTACCCTCATCAGGCATCTG	

F: Forward sequence, R: Reverse sequence. Primers were designed using qPCR Assay Design tool of Integrated DNA Technology (IDT, http://sg.idtdna.com/site).

Supplementary Table 2: Mapping results of IR24 and IRBB67 RNA sequencing reads at 3, 72 and 120 hours post-inoculation (hpi) with *Xoo* strain PXO145 and water inoculation under two temperature regimes (low and high)

	Samples			Biological replication I			Biological replication II		
	Temperature treatment	Inoculation treatment	Time Points	Total reads	Total mapped reads	Percentage of mapped reads (%)	Total reads	Total mapped reads	Percentage of mapped reads (%)
IR24	Low	Mock	3hpi	40,674,840	39,746,811	97.72	45,901,604	44,948,818	97.92
IR24	Low	Mock	72hpi	43,936,016	42,991,838	97.85	40,369,096	39,554,307	97.98
IR24	Low	Mock	120hpi	41,557,685	40,785,941	98.14	35,775,914	34,862,571	97.45
IR24	High	Mock	3hpi	38,816,251	38,023,319	97.96	42,282,183	41,385,534	97.88
IR24	High	Mock	72hpi	42,608,972	41,630,584	97.7	25,243,662	24,644,259	97.63
IR24	High	Mock	120hpi	37,366,180	36,536,207	97.78	33,906,037	33,209,507	97.95
IR24	Low	*Xoo*	3hpi	36,569,942	36,056,833	98.6	34,521,855	33,927,051	98.28
IR24	Low	*Xoo*	72hpi	38,381,083	37,500,798	97.71	33,215,355	32,452,033	97.7
IR24	Low	*Xoo*	120hpi	35,997,261	35,273,959	97.99	33,420,582	32,638,429	97.66
IR24	High	*Xoo*	3hpi	49,697,688	48,549,597	97.69	35,330,289	34,660,833	98.11
IR24	High	*Xoo*	72hpi	34,472,139	33,821,975	98.11	37,533,718	36,416,101	97.02
IR24	High	*Xoo*	120hpi	29,264,471	28,384,794	96.99	34,483,333	33,778,667	97.96
IRBB67	Low	Mock	3hpi	39,041,035	38,174,907	97.78	47,598,258	46,647,930	98
IRBB67	Low	Mock	72hpi	44,081,764	43,241,647	98.09	41,233,115	40,359,389	97.88
IRBB67	Low	Mock	120hpi	41,579,556	40,605,006	97.66	30,738,084	30,064,125	97.81
IRBB67	High	Mock	3hpi	44,707,371	43,700,382	97.75	42,754,098	41,869,626	97.93
IRBB67	High	Mock	72hpi	34,623,159	33,908,453	97.94	82,171,104	80,174,653	97.57
IRBB67	High	Mock	120hpi	34,143,236	33,458,910	98	33,053,013	32,324,261	97.8
IRBB67	Low	*Xoo*	3hpi	39,473,668	38,738,159	98.14	30,236,187	29,710,824	98.26
IRBB67	Low	*Xoo*	72hpi	34,082,657	33,333,817	97.8	40,133,169	39,031,327	97.25
IRBB67	Low	*Xoo*	120hpi	35,648,982	34,912,253	97.93	31,983,452	31,327,119	97.95
IRBB67	High	*Xoo*	3hpi	32,953,631	32,346,294	98.16	29,323,127	28,745,107	98.03
IRBB67	High	*Xoo*	72hpi	36,696,325	35,951,404	97.97	34,852,161	34,101,327	97.85
IRBB67	High	*Xoo*	120hpi	25,638,636	24,991,603	97.48	28,561,396	27,864,949	97.56

Suplementary Table 3: 332 DEGs expressed in high and low temperature comparison after mock inoculation in IR24 and IRBB67

Gene	Description	IR24-3hpi	IR24-72hpi	IR24-120hpi	IRBB67-3hpi	IRBB67-72hpi	IRBB67-120hpi
LOC_Os01g04280.1	calmodulin binding protein, putative, expressed	-1.93	-0.67	-0.22	-2.86	0.39	-0.07
LOC_Os01g04330.1	OsCML16 - Calmodulin-related calcium sensor protein, expressed	-1.36	-0.62	-0.22	-2.15	-0.03	0.21
LOC_Os01g06590.3	zinc finger, C3HC4 type domain containing protein, expressed	-2.15	0.36	-0.52	-0.58	-0.51	0.12
LOC_Os01g07120.1	AP2 domain containing protein, expressed	-0.38	0.14	-0.26	-2.09	0.20	-0.16
LOC_Os01g09080.1	WRKY DNA-binding domain containing protein, expressed	-0.71	-0.43	-0.07	-2.14	-0.10	-0.01
LOC_Os01g09220.1	transposon protein, putative, CACTA, En/Spm sub-class, expressed	-1.02	-0.38	-0.71	-2.83	0.17	-0.37
LOC_Os01g12490.1	flavin monooxygenase, putative, expressed	2.21	0.85	0.17	2.28	-0.24	0.12
LOC_Os01g20206.1	methyltransferase, putative	-2.40	-0.78	-0.35	-3.76	-0.06	-0.30
LOC_Os01g27340.1	glutathione S-transferase, putative, expressed	-2.31	-0.73	-0.08	-2.88	0.02	-0.09
LOC_Os01g28450.1	SCP-like extracellular protein, expressed	-0.80	-2.24	0.56	-1.21	0.64	0.01
LOC_Os01g28790.1	PRAS-rich protein, putative, expressed	-1.43	-0.18	-0.19	-2.28	0.34	-0.12
LOC_Os01g29280.1	expressed protein	-1.30	-0.74	0.11	-3.30	-0.10	0.01
LOC_Os01g29330.1	expressed protein	-1.93	-1.53	0.04	-3.04	-0.09	0.27
LOC_Os01g31370.1	glycosyltransferase, putative, expressed	-2.39	-0.30	-0.32	-2.81	0.13	-0.15
LOC_Os01g37810.1	expressed protein	-1.21	0.22	-0.21	-2.09	-0.13	-0.03
LOC_Os01g39330.1	helix-loop-helix DNA-binding domain containing protein, expressed	-1.83	-0.45	-0.03	-2.27	-0.13	-0.06
LOC_Os01g40260.1	OsWRKY77 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.29	-1.02	0.44	-2.04	0.33	0.16
LOC_Os01g42370.1	pleiotropic drug resistance protein, putative, expressed	-1.42	-0.85	-0.05	-2.21	0.21	-0.07
LOC_Os01g42380.1	pleiotropic drug resistance protein, putative, expressed	-2.11	-0.71	-0.02	-2.58	0.08	0.05
LOC_Os01g42410.1	pleiotropic drug resistance protein, putative, expressed	-2.03	-0.45	-0.34	-2.17	-0.16	-0.24
LOC_Os01g44120.1	expressed protein	-1.09	-0.04	-0.26	-2.04	-0.12	-0.04
LOC_Os01g45110.1	anthocyanin 3-O-beta-glucosyltransferase, putative, expressed	-1.11	0.15	0.04	-2.45	-0.22	-0.14
LOC_Os01g47580.1	lipid phosphatase protein, putative, expressed	-1.93	-0.36	-0.10	-3.14	-0.20	0.10
LOC_Os01g50100.1	ABC transporter, ATP-binding protein, putative, expressed	-2.56	-1.29	0.19	-3.96	-0.24	-0.02

LOC_Os01g50170.1	eukaryotic aspartyl protease domain containing protein, expressed	-1.43	-1.10	0.22	-2.00	0.29	-0.21
LOC_Os01g50420.1	STE_MEKK_ste11_MAP3K.7 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed	-0.66	-0.55	-0.28	-3.11	-0.64	-0.14
LOC_Os01g51670.1	expressed protein	-1.82	-0.46	-0.06	-3.59	0.21	-0.07
LOC_Os01g51690.1	OsWRKY59 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.40	-0.51	-0.05	-2.97	-0.22	-0.04
LOC_Os01g52730.1	DUF584 domain containing protein, putative, expressed	-1.51	-0.27	-0.34	-2.45	-0.42	-0.35
LOC_Os01g53920.1	receptor-like protein kinase 5 precursor, putative, expressed	-2.08	-0.67	-0.35	-2.97	0.17	-0.30
LOC_Os01g56240.1	OsSAUR2 - Auxin-responsive SAUR gene family member, expressed	-0.98	-0.26	-0.03	-2.41	0.10	-0.03
LOC_Os01g56690.1	helix-loop-helix DNA-binding domain containing protein, expressed	-0.86	-0.63	-0.54	-4.07	-0.25	-0.09
LOC_Os01g60600.1	WRKY DNA-binding domain containing protein, expressed	-2.62	-0.07	-0.02	-3.09	-0.60	-0.17
LOC_Os01g61080.1	OsWRKY24 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.21	-0.67	0.12	-2.21	0.17	-0.16
LOC_Os01g61510.1	ammonium transporter protein, putative, expressed	-2.00	-0.95	-0.16	-2.46	0.14	-0.22
LOC_Os01g61990.1	ankyrin repeat-containing protein, putative, expressed	-1.81	-0.54	-0.34	-2.46	-0.12	-0.04
LOC_Os01g62430.2	C2 domain containing protein, putative, expressed	-1.85	-0.73	-0.31	-2.70	0.31	-0.21
LOC_Os01g62430.3	C2 domain containing protein, putative, expressed	-2.40	-1.52	0.09	-3.82	0.24	0.13
LOC_Os01g62670.1	avr9/Cf-9 rapidly elicited protein, putative, expressed	-1.56	-0.07	-0.57	-2.09	0.30	-0.40
LOC_Os01g64440.1	expressed protein	-1.76	-0.84	0.25	-2.02	-0.08	0.09
LOC_Os01g64470.1	harpin-induced protein 1 domain containing protein, expressed	-1.67	-0.58	-0.22	-2.42	0.21	-0.05
LOC_Os01g64670.2	soluble inorganic pyrophosphatase, putative, expressed	-1.39	-2.14	0.31	-0.14	0.70	-0.09
LOC_Os01g66860.1	serine/threonine protein kinase, putative, expressed	-2.49	-0.44	0.20	-2.89	0.04	-0.05
LOC_Os01g66860.2	serine/threonine protein kinase, putative, expressed	-2.27	-0.74	0.25	-2.73	0.32	-0.14
LOC_Os01g66860.3	serine/threonine protein kinase, putative, expressed	-2.26	-0.64	0.31	-3.42	-0.10	-0.03
LOC_Os01g67810.1	transposon protein, putative, unclassified, expressed	-2.24	-0.14	-0.23	-2.58	-0.02	0.03
LOC_Os01g67820.1	exo70 exocyst complex subunit domain containing protein, expressed	-2.04	-0.24	-0.51	-2.53	-0.05	-0.08
LOC_Os01g68060.1	copine, putative, expressed	-1.74	-0.23	-0.29	-2.23	-0.18	-0.01
LOC_Os01g70820.1	lumenal PsbP, putative, expressed	-1.62	-0.25	0.12	-2.34	-0.27	0.39
LOC_Os01g71340.1	glycosyl hydrolases family 17, putative, expressed	-0.87	-2.01	0.16	-0.34	-0.26	0.00
LOC_Os01g71690.2	recA protein, expressed	-0.99	-0.88	0.26	-3.23	0.82	-0.27
LOC_Os01g71760.1	amino acid permease family protein, putative	-1.96	-1.13	0.46	-2.44	0.37	0.12
LOC_Os01g71890.1	transposon protein, putative, unclassified	-2.03	-1.01	-0.14	-0.67	0.36	-0.24
LOC_Os01g72080.1	calmodulin-like protein 1, putative, expressed	-1.52	-0.49	-0.11	-2.88	-0.08	-0.07

LOC_Os01g72530.1	OsCML31 - Calmodulin-related calcium sensor protein, expressed	-1.47	0.02	-0.38	-2.62	-0.33	0.07
LOC_Os01g72810.1	secreted glycoprotein, putative, expressed	-2.49	-0.77	0.06	-2.45	0.50	-0.23
LOC_Os01g74370.1	domain of unknown function DUF966 domain containing protein, expressed	-1.79	-0.85	-0.06	-2.81	0.21	0.00
LOC_Os02g01590.1	glycosyl hydrolases, putative, expressed	0.53	0.32	-0.04	2.06	-0.62	-0.52
LOC_Os02g02780.1	protein kinase family protein, putative, expressed	-1.64	-0.71	-0.18	-2.11	0.21	-0.27
LOC_Os02g03020.1	EF hand family protein, putative, expressed	-1.20	-0.40	0.17	-2.49	-0.22	0.19
LOC_Os02g03400.1	microtubule associated protein, putative, expressed	-1.16	-1.00	-0.05	-2.45	0.18	0.01
LOC_Os02g04130.1	DUF1645 domain containing protein, putative, expressed	-1.24	-0.25	-0.22	-2.42	0.06	-0.41
LOC_Os02g04630.1	sodium/calcium exchanger protein, putative, expressed	-1.44	-1.06	-0.17	-2.17	0.76	-0.08
LOC_Os02g04750.2	cycloartenol synthase, putative, expressed	1.07	0.28	0.06	2.76	-0.26	0.01
LOC_Os02g04750.3	cycloartenol synthase, putative, expressed	1.83	0.85	-0.13	2.66	0.46	0.04
LOC_Os02g04760.1	cycloartenol synthase, putative	1.50	0.32	-0.06	2.20	0.07	0.04
LOC_Os02g06090.1	phytosulfokine receptor precursor, putative, expressed	-1.96	-0.68	0.09	-3.35	0.16	-0.05
LOC_Os02g06930.2	protein kinase, putative, expressed	-1.72	-0.52	-0.78	-2.01	-0.16	-0.24
LOC_Os02g11070.1	3-ketoacyl-CoA synthase, putative, expressed	-2.47	-0.93	0.06	-2.71	-0.46	-0.15
LOC_Os02g11070.2	3-ketoacyl-CoA synthase, putative, expressed	-1.57	-0.10	-0.09	-2.72	0.32	-0.10
LOC_Os02g11859.1	expressed protein	-1.94	-0.91	-0.43	-2.73	-0.02	0.02
LOC_Os02g14440.1	peroxidase precursor, putative, expressed	-1.54	-0.83	-0.05	-2.62	-0.07	-0.15
LOC_Os02g21040.1	aspartic proteinase nepenthesin precursor, putative, expressed	-1.57	-0.29	-0.36	-2.25	0.19	-0.27
LOC_Os02g22160.1	DNA binding protein, putative, expressed	-2.54	-1.44	-0.13	-3.71	0.10	-0.23
LOC_Os02g26430.1	OsWRKY42 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.55	-0.25	-0.09	-2.05	0.04	0.16
LOC_Os02g26670.1	F-box/Kelch-repeat protein, putative, expressed	-1.08	0.02	0.16	-2.05	-0.28	-0.15
LOC_Os02g27310.1	TKL_IRAK_DUF26-lc.6 - DUF26 kinases have homology to DUF26 containing loci, expressed	-1.69	-0.90	0.32	-2.14	0.16	0.06
LOC_Os02g33680.1	U-box domain containing protein, expressed	-1.44	0.07	0.06	-2.03	-0.01	-0.48
LOC_Os02g35329.1	RING-H2 finger protein ATL3F, putative, expressed	-1.70	-0.37	0.27	-3.02	-0.26	-0.11
LOC_Os02g36530.1	hypothetical protein	-1.71	-0.19	-0.08	-2.47	-0.02	0.04
LOC_Os02g36740.7	zinc finger, C3HC4 type, putative, expressed	0.12	-0.30	0.21	-2.13	0.38	-0.01
LOC_Os02g41510.1	MYB family transcription factor, putative, expressed	-1.37	-0.71	0.05	-2.56	0.01	-0.02
LOC_Os02g41670.1	phenylalanine ammonia-lyase, putative, expressed	-0.87	-1.03	-0.11	-3.47	-0.26	-0.23
LOC_Os02g43790.1	ethylene-responsive transcription factor, putative, expressed	-1.70	-0.76	0.02	-3.13	-0.21	-0.09

LOC_Os02g45450.1	dehydration-responsive element-binding protein, putative, expressed	-1.51	-0.41	-0.18	-2.49	-0.42	-0.03
LOC_Os02g45780.1	zinc finger, C3HC4 type domain containing protein, expressed	-1.71	-0.34	-0.21	-2.71	0.08	-0.23
LOC_Os02g46910.1	glycosyl hydrolases family 16, putative, expressed	-1.65	-0.29	-0.10	-2.12	-0.29	-0.13
LOC_Os02g48320.2	DNA binding protein, putative, expressed	-2.06	-0.49	-0.29	-2.44	-0.15	-0.25
LOC_Os02g48570.2	peptide transporter PTR2, putative, expressed	-0.29	-0.34	-0.07	-2.10	0.55	0.14
LOC_Os02g50110.1	hypothetical protein	-1.41	-0.14	-0.46	-2.01	-0.30	0.03
LOC_Os02g50600.1	glycosyl transferase 8 domain containing protein, putative, expressed	-1.64	-0.29	-0.35	-2.68	0.12	-0.14
LOC_Os02g52040.1	phosphate-induced protein 1 conserved region domain containing protein, expressed	-2.47	-0.10	0.01	-2.63	-0.07	-0.06
LOC_Os02g52170.1	expressed protein	-2.30	-0.57	0.05	-3.45	0.24	0.09
LOC_Os02g53670.1	MYB family transcription factor, putative, expressed	1.37	0.34	-0.09	2.11	0.00	-0.25
LOC_Os02g53700.3	DENN domain containing protein, expressed	-0.88	0.19	-0.12	2.17	0.49	-0.12
LOC_Os02g53750.2	tyrosine protein kinase domain containing protein, putative, expressed	-1.38	-0.02	-0.22	-2.01	0.02	-0.24
LOC_Os02g55970.1	ANTH, putative, expressed	-1.78	-0.47	0.03	-2.20	0.06	-0.11
LOC_Os02g56930.1	expressed protein	-1.48	-0.09		-2.91	-0.05	0.04
LOC_Os03g01740.1	expressed protein	-1.58	-0.47	-0.34	-2.04	-0.05	-0.07
LOC_Os03g03370.2	fatty acid hydroxylase, putative, expressed	-2.66	-0.30	0.19	-1.69	-0.16	0.17
LOC_Os03g03790.1	AMP-binding domain containing protein, expressed	-1.78	-0.43	0.80	-3.07	-0.55	0.17
LOC_Os03g05334.1	expressed protein	-2.09	-0.16	-0.69	0.49	-0.08	0.18
LOC_Os03g05920.1	expressed protein	-2.16	0.03	0.02	-1.66	0.06	-0.49
LOC_Os03g08310.1	ZIM domain containing protein, putative, expressed	-2.14	-0.74	-0.13	-2.69	0.10	-0.12
LOC_Os03g08320.1	ZIM domain containing protein, putative, expressed	-1.91	-0.52	-0.13	-2.41	0.38	-0.12
LOC_Os03g08410.1	flavin-containing monooxygenase family protein, putative, expressed	-0.42	-0.08	0.18	-4.95	0.07	0.01
LOC_Os03g08900.1	MATE efflux family protein, putative, expressed	-1.03	0.25	0.06	-2.23	-0.70	-0.36
LOC_Os03g08940.1	conserved hypothetical protein	-1.40	-0.15	0.16	-1.99	0.05	-0.19
LOC_Os03g09170.1	ethylene-responsive transcription factor, putative, expressed	-1.50	-0.33	-0.24	-2.63	-0.68	0.02
LOC_Os03g09900.1	membrane protein, putative, expressed	-0.84	-0.50	0.17	-2.17	0.01	-0.11
LOC_Os03g10300.1	haemolysin-III, putative, expressed	-1.21	-0.18	-0.13	-2.07	-0.05	-0.18
LOC_Os03g10640.1	calcium-transporting ATPase, plasma membrane-type, putative, expressed	-1.15	-0.10	-0.46	-2.41	0.27	0.15
LOC_Os03g12890.5	aminotransferase domain containing protein, putative, expressed	-2.57	-0.69	-0.34	-1.00	0.06	-0.54
LOC_Os03g13740.1	immediate-early fungal elicitor protein CMPG1, putative, expressed	-1.89	-0.37	-0.37	-2.73	0.02	-0.04
LOC_Os03g15780.2	anthranilate synthase component I-1, chloroplast precursor, putative, expressed	-2.01	-0.47	0.05	-1.98	0.23	-0.15

LOC_Os03g15780.4	anthranilate synthase component I-1, chloroplast precursor, putative, expressed	-2.46	-0.55	-0.43	-2.02	0.12	-0.10
LOC_Os03g17700.1	CGMC_MAPKCGMC_2_ERK.2 - CGMC includes CDA, MAPK, GSK3, and CLKC kinases, expressed	-1.72	-0.61	-0.18	-2.08	-0.17	-0.17
LOC_Os03g18910.1	COBRA-like protein 7 precursor, putative, expressed	-2.13	-0.11	0.00	-2.97	-0.13	-0.14
LOC_Os03g19070.1	long cell-linked locus protein, putative, expressed	-2.51	-0.84	-0.37	-3.53	0.16	0.02
LOC_Os03g20330.1	VQ domain containing protein, putative, expressed	-2.02	-1.18	-0.05	-2.25	0.56	0.07
LOC_Os03g20380.4	CAMK_KIN1/SNF1/Nim1_like.2 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-1.23	-0.90	0.41	-2.30	0.05	0.64
LOC_Os03g20380.5	CAMK_KIN1/SNF1/Nim1_like.2 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-0.74	-0.90	0.28	-2.09	0.13	0.42
LOC_Os03g20380.8	CAMK_KIN1/SNF1/Nim1_like.2 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-1.34	-0.96	0.32	-2.01	-0.33	0.70
LOC_Os03g24100.1	expressed protein	-1.67	-0.96	0.12	-2.33	0.10	0.09
LOC_Os03g32230.1	ZOS3-12 - C2H2 zinc finger protein, expressed	-1.69	-0.95	-0.11	-3.93	-0.42	-0.16
LOC_Os03g33520.1	exo70 exocyst complex subunit, putative	-1.19	-0.35	-0.01	-2.52	0.36	-0.13
LOC_Os03g44810.3	expressed protein	-1.00	0.15	0.00	-2.35	-0.38	0.09
LOC_Os03g45210.1	2-aminoethanethiol dioxygenase, putative, expressed	1.05	0.30	-0.16	2.09	-0.50	-0.65
LOC_Os03g45960.1	thaumatin, putative, expressed	-2.11	-1.46	-0.31	-1.40	0.33	-0.66
LOC_Os03g46200.1	acetyltransferase, GNAT family, putative, expressed	-1.05	-0.79	0.21	-2.07	-0.38	0.06
LOC_Os03g46884.1	transposon protein, putative, CACTA, En/Spm sub-class	-1.83	-0.70	0.35	-3.02	0.52	0.02
LOC_Os03g47280.1	VQ domain containing protein, putative, expressed	-1.51	-0.92	-0.34	-2.27	-0.12	-0.13
LOC_Os03g49380.1	lipoxygenase, putative, expressed	-1.86	-0.87	-0.81	-2.42	-0.09	-0.35
LOC_Os03g51350.1	expressed protein	-0.43	-0.86	0.11	-2.15	0.83	-0.39
LOC_Os03g53340.5	HSF-type DNA-binding domain containing protein, expressed	-1.36	-0.75	-0.31	-2.23	-0.03	-0.17
LOC_Os03g55430.1	expressed protein	-1.11	-0.67	-0.27	-2.26	0.23	-0.26
LOC_Os03g56250.1	LRR receptor-like protein kinase, putative, expressed	-1.50	-0.51	-0.18	-2.02	0.05	-0.13
LOC_Os03g56820.2	fatty acid hydroxylase, putative, expressed	-2.16	-0.28	0.01	-0.64	-0.05	0.10
LOC_Os03g57310.1	syntaxin, putative, expressed	-1.47	-0.38	-0.01	-2.08	-0.06	-0.14
LOC_Os03g57640.1	gibberellin receptor GID1L2, putative, expressed	-1.67	-0.73	-0.22	-2.22	-0.15	-0.14
LOC_Os03g57880.1	glucan endo-1,3-beta-glucosidase precursor, putative, expressed	-0.90	-0.47	-0.38	-2.15	-0.02	-0.46
LOC_Os03g60570.1	ZOS3-22 - C2H2 zinc finger protein, expressed	-1.65	-0.39	-0.25	-3.20	0.10	-0.06
LOC_Os03g61360.1	hydrolase, alpha/beta fold family domain containing protein, expressed	-1.32	-0.11	-0.36	-2.11	-0.14	-0.14
LOC_Os03g61490.1	expressed protein	-0.68	-0.22	-0.16	-3.68	0.20	-0.20

LOC_Os04g03796.3	OsSub37 - Putative Subtilisin homologue, expressed	-0.23	-2.48	0.25	-1.16	0.25	0.18
LOC_Os04g12960.1	UDP-glucoronosyl/UDP-glucosyl transferase, putative, expressed	-1.75	-0.97	-0.04	-2.15	0.28	-0.15
LOC_Os04g15580.1	serine/threonine-protein kinase receptor precursor, putative, expressed	-1.35	-0.59	0.07	-2.07	-0.11	0.07
LOC_Os04g15920.1	dehydrogenase, putative, expressed	-1.85	-0.31	0.55	-3.20	-0.52	0.40
LOC_Os04g22470.1	SHR5-receptor-like kinase, putative	-1.41	-1.13	-0.08	-2.02	-0.04	-0.04
LOC_Os04g25900.1	go35 NBS-LRR, putative, expressed	-2.53	-0.59	-0.57	0.31	-0.53	0.25
LOC_Os04g32480.1	zinc-finger protein, putative, expressed	-1.70	-0.93	-0.17	-2.72	-0.23	0.13
LOC_Os04g32920.2	potassium transporter, putative, expressed	-1.49	-0.53	0.00	-3.34	-0.59	0.32
LOC_Os04g33640.1	glycosyl hydrolases family 17, putative, expressed	-2.40	-0.06	0.06	-3.01	0.10	-0.13
LOC_Os04g33640.2	glycosyl hydrolases family 17, putative, expressed	-2.34	0.28	-0.01	-3.31	-0.48	0.30
LOC_Os04g33820.1	OsFBX132 - F-box domain containing protein, expressed	-1.27	-0.52	-0.18	-2.62	0.16	-0.20
LOC_Os04g37490.1	oxidoreductase, aldo/keto reductase family protein, putative, expressed	-0.97	-0.64	0.09	-2.57	-0.01	0.10
LOC_Os04g39350.1	heavy metal associated domain containing protein, expressed	-1.71	-1.07	-0.45	-2.38	-0.07	-0.01
LOC_Os04g40310.2	dehydrogenase, putative, expressed	-0.22	-1.00	0.90	-2.26	0.60	-0.28
LOC_Os04g41960.1	NADP-dependent oxidoreductase, putative, expressed	-1.84	-0.63	-0.23	-2.47	0.27	-0.21
LOC_Os04g43440.1	NB-ARC/LRR disease resistance protein, putative, expressed	-3.23	-0.60	-0.46	-3.99	-0.04	-0.04
LOC_Os04g43680.1	MYB family transcription factor, putative, expressed	-1.54	-0.95	-0.20	-2.48	-0.27	-0.09
LOC_Os04g46830.1	LTPL122 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.69	0.00	-0.05	-2.42		
LOC_Os04g46970.1	glucosyltransferase, putative, expressed	-1.93	-1.05	0.02	-3.27	0.34	-0.06
LOC_Os04g48850.1	aminotransferase, classes I and II, domain containing protein, expressed	-0.85	-0.67	-0.68	-3.80	-0.68	-0.18
LOC_Os04g49510.2	CAMK_CAMK_like.27 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-0.98	-0.07	-0.14	-2.17	-0.04	-0.06
LOC_Os04g51460.1	glycosyl hydrolases family 16, putative, expressed	-2.46	-0.43	-0.32	-4.01	-0.10	-0.18
LOC_Os04g52750.1	expressed protein	-1.83	-0.88	-0.24	-3.02	0.11	0.07
LOC_Os04g55100.1	expressed protein	-1.51	-0.55	0.13	-2.29	0.38	-0.25
LOC_Os04g56110.3	protein kinase, putative, expressed	-1.16	-0.29	0.19	-2.06	0.25	-0.20
LOC_Os04g57810.3	GA18008-PA, putative, expressed	-1.69	-0.50	0.30	-2.85	-0.16	0.18
LOC_Os04g58090.1	harpin-induced protein 1 domain containing protein, expressed	-1.89	-0.27	-0.02	-2.89	0.37	-0.54
LOC_Os04g58220.1	transporter family protein, putative	-2.17	-0.42	0.09	-1.40	-0.10	-0.10
LOC_Os04g58810.1	CAF1 family ribonuclease containing protein, putative, expressed	-0.92	-0.99	-0.10	-1.99	-0.30	-0.03
LOC_Os04g58920.1	U-box domain-containing protein, putative, expressed	-1.15	-0.42	0.12	-2.70	0.05	0.00

LOC_Os05g02140.1	clathrin assembly protein, putative, expressed	-1.35	-0.39	0.22	-2.15	-0.02	-0.10
LOC_Os05g07940.2	glyoxalase family protein, putative, expressed	-1.13	-0.21	-0.41	-2.21	0.07	-0.62
LOC_Os05g08830.1	expressed protein	-0.14	-1.03	-0.05	-2.29	-0.05	-0.04
LOC_Os05g08860.1	expressed protein	-2.13	-0.76	-0.01	-3.41	0.68	-0.06
LOC_Os05g08890.1	transposable element protein, putative, containing Pfam profile: PF03108, MuDR	-1.86	-0.35	-0.05	-3.31	0.03	0.03
LOC_Os05g08900.1	expressed protein	-2.32	-0.66	0.09	-3.39	0.44	-0.03
LOC_Os05g08910.1	expressed protein	-1.48	-0.41	-0.20	-2.17	0.17	0.00
LOC_Os05g10840.2	calmodulin-binding protein, putative, expressed	-0.39	0.55	-0.15	-2.35	-0.14	0.02
LOC_Os05g21180.3	phosphatidic acid phosphatase-related, putative, expressed	-2.40	-0.21	-0.14	-2.17	-0.09	0.13
LOC_Os05g21180.4	phosphatidic acid phosphatase-related, putative, expressed	-1.61	-0.82	-0.16	-2.68	-0.08	-0.30
LOC_Os05g24770.1	reticulon domain containing protein, putative, expressed	-2.02	-0.18	-0.24	-2.31	0.23	0.09
LOC_Os05g24780.1	OsCML21 - Calmodulin-related calcium sensor protein, expressed	-1.70	-0.12	-0.09	-2.36	0.26	0.18
LOC_Os05g27730.1	OsWRKY53 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.25	-0.44	0.10	-2.07	0.14	-0.02
LOC_Os05g30500.1	expressed protein	-1.83	-0.14	-0.32	-2.39	-0.86	0.02
LOC_Os05g33400.1	basic 7S globulin precursor, putative, expressed	-1.91	-0.47	0.00	-2.39	0.35	-0.29
LOC_Os05g35290.1	phenylalanine ammonia-lyase, putative, expressed	-1.65	-0.20	0.09	-2.41	-0.73	-0.23
LOC_Os05g36260.1	soluble inorganic pyrophosphatase, putative, expressed	-2.35	-0.40	-0.09	-2.17	-0.24	0.25
LOC_Os05g39720.1	OsWRKY70 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-0.83	-0.36	0.14	-3.33	-0.32	0.12
LOC_Os05g41370.1	TKL_IRAK_DUF26-la.1 - DUF26 kinases have homology to DUF26 containing loci, expressed	-2.03	-0.43	-0.26	-2.32	0.28	-0.06
LOC_Os05g41610.1	glycosyl hydrolases family 17, putative, expressed	-1.49	-0.13	0.13	-2.06	-0.02	0.00
LOC_Os05g45100.1	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed	-1.61	-0.22	-0.10	-2.84	-0.11	-0.15
LOC_Os05g46020.1	OsWRKY7 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.77	-0.19	0.02	-2.76	0.05	-0.16
LOC_Os05g46750.1	STE_MEKK_ste11_MAP3K.18 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed	-1.61	0.02	-0.45	-2.06	-0.69	0.29
LOC_Os05g46760.1	STE_MEKK_ste11_MAP3K.19 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed	-1.67	-0.16	-0.29	-2.47	0.09	0.11
LOC_Os05g46790.1	expressed protein	-0.76	-1.01	0.13	-3.69	0.62	-0.39
LOC_Os05g50100.1	expressed protein	-1.92	-0.53	0.04	-2.54	-0.11	0.09
LOC_Os05g50570.1	OsSCP29 - Putative Serine Carboxypeptidase homologue, expressed	-0.91	-0.69	0.11	-2.37	-0.23	-0.06
LOC_Os05g50770.1	C4-dicarboxylate transporter/malic acid transport protein, expressed	-2.35	-0.19	0.01	-3.62	0.03	-0.03
LOC_Os06g03810.1	expressed protein	-1.00	-1.06	0.00	-2.42	0.45	0.02

LOC_Os06g04220.1	expressed protein	-2.08	-1.24	-0.14	-2.93	-0.02	0.05
LOC_Os06g04240.1	expressed protein	-1.45	-0.53	-0.60	-2.11	-0.07	-0.08
LOC_Os06g09310.1	zinc finger, C3HC4 type domain containing protein, expressed	-1.26	-0.94	-0.65	-3.24	-0.02	-0.16
LOC_Os06g09370.2	PTF1, putative, expressed	-1.49	0.27	-0.02	-2.07	-0.73	0.64
LOC_Os06g09370.3	PTF1, putative, expressed	-2.22	-0.55	-0.36	-2.42	0.00	-0.41
LOC_Os06g09980.1	expressed protein	-1.46	-0.59	0.24	-2.27	0.30	0.33
LOC_Os06g10210.1	expressed protein	-1.40	0.62	-0.39	-2.52	0.21	0.03
LOC_Os06g10210.2	expressed protein	-2.04	-0.36	-0.40	-2.10	-0.50	0.24
LOC_Os06g10210.3	expressed protein	-2.00	-0.56	-0.21	-1.88	-0.06	-0.59
LOC_Os06g13180.1	metalloendoproteinase 1 precursor, putative, expressed	-1.12	-0.31	-0.08	-2.15	0.24	-0.23
LOC_Os06g13940.1	expressed protein	-1.84	-0.15	-0.31	-2.75	-0.28	-0.35
LOC_Os06g14370.1	caleosin related protein, putative, expressed	-2.23	-0.31	-0.74	-0.75	0.08	-0.08
LOC_Os06g14450.2	exo70 exocyst complex subunit family protein, putative, expressed	-1.77	-0.21	0.14	-2.24	-0.15	0.05
LOC_Os06g20900.1	expressed protein	-1.05	-0.59	0.05	-2.32	-0.47	0.08
LOC_Os06g23350.1	late embryogenesis abundant protein D-34, putative, expressed	-0.17	-1.08	0.18	-2.29	0.70	-0.13
LOC_Os06g28050.3	expressed protein	-2.24	-0.35	-0.01	-1.95	0.01	0.09
LOC_Os06g33970.1	VQ domain containing protein, putative, expressed	-1.69	-1.07	0.09	-2.85	0.14	0.13
LOC_Os06g37300.1	cytochrome P450, putative, expressed	-1.49	-2.19	0.28	-0.75	-0.05	-0.28
LOC_Os06g43080.1	expressed protein	-1.76	-0.91	-0.04	-3.28	-0.18	-0.28
LOC_Os06g44010.1	OsWRKY28 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-1.28	-1.30	0.15	-2.17	0.11	-0.02
LOC_Os06g46950.1	EF hand family protein, putative, expressed	-1.54	-0.64	-0.26	-2.46	0.05	0.07
LOC_Os06g48160.1	glycosyl hydrolases family 16, putative, expressed	-2.21	-0.07	-0.19	-3.50	-1.17	-0.03
LOC_Os07g03020.1	hypothetical protein	-1.33	-0.76	0.19	-2.16	0.17	-0.07
LOC_Os07g03710.1	SCP-like extracellular protein, expressed	-0.72	-2.49	0.58	-1.71	0.48	0.18
LOC_Os07g04560.1	no apical meristem protein, putative	-2.40	-0.85	-0.02	-3.50	0.48	-0.53
LOC_Os07g04820.1	protein kinase, putative, expressed	-1.02	0.15	-0.25	-2.50	0.35	-0.17
LOC_Os07g05940.1	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed	-0.53	-0.31	-0.06	-2.30	-0.60	-0.12
LOC_Os07g09420.1	ATPase, putative, expressed	-1.56	-0.46	0.05	-2.27	-0.02	-0.14
LOC_Os07g33280.1	expressed protein	-0.78	-0.55	-0.38	-2.21	-0.24	0.16
LOC_Os07g34260.1	chalcone and stilbene synthases, putative, expressed	-0.76	-1.23	-0.08	-2.81	0.40	-0.18
LOC_Os07g34280.1	CXE carboxylesterase, putative, expressed	-0.64	-1.30	-0.18	-3.27	0.04	-0.09

LOC_Os07g34520.2	isocitrate lyase, putative, expressed	2.38	0.04	-0.03	0.60	-0.46	-0.92
LOC_Os07g34520.3	isocitrate lyase, putative, expressed	2.10	0.06	-0.08	0.63	-0.33	-0.79
LOC_Os07g35280.1	TKL_IRAK_DUF26-lc.1 - DUF26 kinases have homology to DUF26 containing loci, expressed	-1.09	-0.36	0.18	-2.43	0.17	0.05
LOC_Os07g35290.1	TKL_IRAK_DUF26-lc.10 - DUF26 kinases have homology to DUF26 containing loci, expressed	-1.18	-0.20	-0.14	-2.01	-0.31	0.04
LOC_Os07g35330.1	TKL_IRAK_DUF26-lc.13 - DUF26 kinases have homology to DUF26 containing loci, expressed	-1.64	-0.77	-0.18	-2.70	-0.25	-0.11
LOC_Os07g36560.1	transferase family protein, putative, expressed	-1.48	-0.40		-2.67	0.11	-0.06
LOC_Os07g36570.1	KI domain interacting kinase 1, putative, expressed	-1.24	-0.56	0.03	-2.14	-0.04	-0.11
LOC_Os07g37400.1	OsFBX257 - F-box domain containing protein, expressed	-2.14	-0.67	-0.30	-2.22	-0.31	0.15
LOC_Os07g37730.1	NADH-ubiquinone oxidoreductase, mitochondrial precursor, putative, expressed	-0.93	-0.54	-0.23	-4.34	-0.21	-0.17
LOC_Os07g37920.1	no apical meristem protein, putative, expressed	-1.25	0.30	-0.29	-2.04	0.25	-0.38
LOC_Os07g40240.1	GASR9 - Gibberellin-regulated GASA/GAST/Snakin family protein precursor, expressed	-0.55	-0.71	0.31	-2.46	0.12	-0.10
LOC_Os07g42940.2	CAMK_CAMK_like.7 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-1.73	-0.35	-0.01	-2.01	-0.71	0.81
LOC_Os07g42940.7	CAMK_CAMK_like.7 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-1.36	-0.57	-0.22	-2.01	-0.04	-0.36
LOC_Os07g42940.8	CAMK_CAMK_like.7 - CAMK includes calcium/calmodulin depedent protein kinases, expressed	-1.93	-0.36	-0.07	-2.35	-0.18	0.29
LOC_Os07g43160.1	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	-1.27	-0.22	0.06	-2.14	0.13	-0.10
LOC_Os07g43800.1	EF hand family protein, putative, expressed	-1.40	-0.90	0.23	-2.43	0.34	-0.01
LOC_Os07g44140.1	cytochrome P450 72A1, putative, expressed	-1.41	0.20	0.00	-2.00	0.17	-0.15
LOC_Os07g46920.1	sex determination protein tasselseed-2, putative, expressed	-2.24	-0.93	0.41	-1.51	-0.19	0.25
LOC_Os08g04340.1	plastocyanin-like domain containing protein, putative, expressed	-1.74	-1.23	0.05	-3.31	0.10	-0.03
LOC_Os08g04350.1	plastocyanin-like domain containing protein, putative, expressed	-0.73	-0.88	-0.01	-4.02	0.34	-0.39
LOC_Os08g04360.1	plastocyanin-like domain containing protein, putative, expressed	-1.35	-0.10		-2.85	0.02	-0.04
LOC_Os08g04370.1	plastocyanin-like domain containing protein, putative, expressed	-1.69	-0.48	0.09	-3.86	-0.11	-0.01
LOC_Os08g04500.2	terpene synthase, putative, expressed	1.63	2.01	-0.04	1.16	-0.50	-0.05
LOC_Os08g04630.1	external NADH-ubiquinone oxidoreductase 1, mitochondrial precursor, putative, expressed	-1.40	-1.08	-0.37	-2.30	0.27	-0.43
LOC_Os08g07100.1	terpene synthase, putative, expressed	-0.89	-0.92	-0.01	-3.76	0.30	-0.14
LOC_Os08g07620.1	hypothetical protein	-0.58	-0.18	-0.02	-2.69	0.17	0.08
LOC_Os08g10500.1	hypothetical protein	-1.55	-0.20	-0.10	-2.40	0.15	-0.24

LOC_Os08g13570.1	exo70 exocyst complex subunit family protein, putative, expressed	-1.69	-0.39	0.10	-2.10	0.33	0.06
LOC_Os08g27170.1	calmodulin binding protein, putative	-1.66	-0.73	0.42	-2.83	-0.05	0.14
LOC_Os08g28710.1	receptor protein kinase CRINKLY4 precursor, putative, expressed	-2.16	-0.90	0.03	-2.60	0.06	0.03
LOC_Os08g29570.1	pleiotropic drug resistance protein 3, putative, expressed	-1.94	-0.83	0.23	-2.05	-0.25	0.29
LOC_Os08g30020.3	membrane protein, putative, expressed	2.17	0.65	0.05	0.10	0.47	-0.54
LOC_Os08g31850.1	expressed protein	-2.04	-0.19	-0.26	-2.39	0.18	-0.30
LOC_Os08g31860.1	expressed protein	-2.16	-0.35	-0.11	-1.86	0.25	-0.34
LOC_Os08g32750.1	bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor, putative, expressed	-2.41	-1.40	-0.10	-3.14	0.50	0.03
LOC_Os08g32780.1	bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor, putative	-2.39	-1.05	-0.11	-3.66	0.57	-0.02
LOC_Os08g34790.1	AMP-binding domain containing protein, expressed	-2.10	-0.47	-0.05	-2.21	-0.58	-0.34
LOC_Os08g39850.1	lipoxygenase, chloroplast precursor, putative, expressed	-2.20	-1.58	0.21	-2.28	0.18	0.05
LOC_Os08g39850.2	lipoxygenase, chloroplast precursor, putative, expressed	-0.76	-1.71	0.23	-3.51	0.22	0.11
LOC_Os08g39850.4	lipoxygenase, chloroplast precursor, putative, expressed	-2.19	-1.49	0.37	-2.68	0.12	0.23
LOC_Os08g40270.1	lectin-like protein kinase, putative	-2.36	-0.94	-0.32	-3.66	0.06	-0.36
LOC_Os08g40530.1	calcium-transporting ATPase 9, plasma membrane-type, putative, expressed	-1.39	-0.58	-0.20	-2.02	-0.06	-0.06
LOC_Os09g00999.1	conserved hypothetical protein	-2.09	-0.09	0.84	-0.35	-0.60	0.56
LOC_Os09g20090.1	L-ascorbate oxidase precursor, putative, expressed	-1.63	-0.84	-0.44	-2.73	-0.05	-0.29
LOC_Os09g27010.1	tyrosine protein kinase domain containing protein, putative, expressed	-1.36	-0.54	-0.39	-2.03	-0.58	-0.05
LOC_Os09g28160.1	phosphate carrier protein, mitochondrial precursor, putative, expressed	-1.72	-0.30	-0.62	-2.33	-0.24	-0.09
LOC_Os09g29510.1	OsWAK80 - OsWAK receptor-like protein kinase, expressed	-0.78	-0.85	-0.02	-2.05	-0.15	0.03
LOC_Os09g31031.2	ubiquitin family protein, putative, expressed	-0.34	-0.37	0.13	0.23	-2.06	0.26
LOC_Os09g34160.1	resistance protein, putative, expressed	-1.90	-0.55	-0.38	-2.18	0.18	-0.03
LOC_Os09g34230.1	UDP-glucoronosyl/UDP-glucosyl transferase, putative, expressed	-1.46	-0.16	-0.14	-2.40	-0.23	-0.06
LOC_Os09g34250.1	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed	-1.69	-0.23	-0.32	-2.39	-0.06	-0.15
LOC_Os09g34330.1	helix-loop-helix DNA-binding domain containing protein	-1.30	-0.34	0.20	-3.28	-0.11	0.41
LOC_Os09g35780.1	BAP2, putative, expressed	-1.33	-0.44	0.04	-2.44	-0.12	-0.03
LOC_Os09g36500.1	zinc finger, C3HC4 type domain containing protein, expressed	-1.59	-0.03	-0.21	-2.17	0.24	-0.09
LOC_Os09g37080.1	expressed protein	-1.52	-0.72	-0.38	-2.27	-0.07	-0.16
LOC_Os09g38800.1	OsWAK88 - OsWAK pseudogene	-1.88	-0.71	0.10	-4.11	-0.08	0.02
LOC_Os09g38840.1	OsWAK90 - OsWAK receptor-like protein kinase, expressed	-0.66	-0.98	0.05	-4.95	-0.05	-0.10

LOC_Os09g38850.1	OsWAK91 - OsWAK receptor-like protein kinase, expressed	-2.04	-0.74	-0.17	-3.33	0.05	0.02
LOC_Os09g39620.1	protein kinase family protein, putative, expressed	-2.20	-0.51	-0.03	-1.71	-0.13	0.33
LOC_Os10g04380.1	conserved hypothetical protein	-1.40	-0.08	-0.11	-2.45	0.17	-0.10
LOC_Os10g25310.1	SPX domain containing protein, putative, expressed	-2.95	-0.42	-0.79	0.35	-0.33	0.18
LOC_Os10g28240.1	calcium-transporting ATPase, plasma membrane-type, putative, expressed	-1.61	-0.27	-0.29	-2.51	0.30	0.07
LOC_Os10g28680.1	DUF581 domain containing protein, expressed	0.95	0.79	0.03	2.05	-0.48	-0.13
LOC_Os10g35460.1	COBRA, putative, expressed	-0.41	-0.54	-0.46	-2.49	-0.58	0.02
LOC_Os10g35950.1	transferase family protein, putative, expressed	-1.71	-0.38	0.53	-2.84	-0.13	0.09
LOC_Os10g36360.1	expressed protein	-1.56	-0.14	-0.09	-2.44	0.00	-0.09
LOC_Os10g37570.1	OsFBDUF49 - F-box and DUF domain containing protein, expressed	-1.38	-0.34	0.18	-2.62	0.06	-0.01
LOC_Os10g39140.2	flavonol synthase/flavanone 3-hydroxylase, putative, expressed	-1.68	-1.08	0.09	-2.28	-0.06	-0.02
LOC_Os10g39680.1	CHIT14 - Chitinase family protein precursor, expressed	-1.28	-1.39	-0.02	-2.02	-0.04	-0.07
LOC_Os10g39700.1	CHIT15 - Chitinase family protein precursor, putative, expressed	-1.60	-0.86	-0.24	-2.56	-0.07	-0.28
LOC_Os10g40480.1	LTPL143 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.78	-0.09	0.00	-3.21	-0.30	-0.08
LOC_Os10g41330.2	AP2 domain containing protein, expressed	-1.50	-0.73	-0.86	-3.17	0.09	0.04
LOC_Os10g42020.2	RALFL29 - Rapid ALkalinization Factor RALF family protein precursor, expressed	-2.38	-1.71	1.14	-0.28	0.03	0.53
LOC_Os10g42040.1	expressed protein	-1.77	-2.56	0.57	-1.62	0.00	0.11
LOC_Os10g42690.1	jmjC domain containing protein, expressed	-1.67	-0.58	-0.21	-2.06	-0.17	-0.07
LOC_Os10g43060.1	expressed protein	-1.45	-0.70	-0.16	-2.08	-0.10	0.05
LOC_Os11g04560.1	calmodulin-like protein 1, putative, expressed	-0.98	-0.41	-0.17	-2.11	0.08	0.19
LOC_Os11g08100.1	eukaryotic aspartyl protease domain containing protein, expressed	-1.72	-0.64	-0.37	-2.15	0.20	0.01
LOC_Os11g09010.1	lipase, putative, expressed	-2.23	-0.22	-0.25	-2.35	0.68	0.11
LOC_Os11g10470.1	expressed protein	-1.85	-0.38	-0.43	-2.55	-0.72	-0.04
LOC_Os11g19340.1	lipase, putative, expressed	-1.74	-0.43	0.01	-3.79	-0.02	-0.04
LOC_Os11g31540.1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	-2.22	-0.89	0.45	-0.57	0.53	-0.47
LOC_Os11g35330.1	LYK, putative, expressed	-1.07	-0.41	-0.01	-2.10	-0.03	0.15
LOC_Os11g37230.2	zinc finger, C3HC4 type domain containing protein, expressed	-1.20	-0.26	-0.18	-2.10	0.46	-0.12
LOC_Os11g44600.1	calmodulin binding protein, putative, expressed	-1.87	-0.24	-0.11	-3.51	-0.43	-0.03
LOC_Os11g46860.1	wall-associated receptor kinase-like 4 precursor, putative, expressed	2.05	-0.55	0.26	-0.58	0.43	0.41
LOC_Os12g04360.1	calmodulin-like protein 1, putative, expressed	-1.29	-0.78	-0.30	-2.04	0.05	0.15

LOC_Os12g08700.1	expressed protein	-1.45	-0.58	-0.02	-2.02	0.00	0.02
LOC_Os12g08850.1	expressed protein	-1.70	-0.45	0.36	-3.25	-0.01	0.02
LOC_Os12g25660.1	cytochrome P450, putative, expressed	-1.71	-0.70	0.24	-4.15	-0.12	-0.04
LOC_Os12g28550.1	ATPase, AAA family domain containing protein, expressed	-1.33	-0.03	0.20	-3.08	-0.15	0.05
LOC_Os12g32610.1	expressed protein	-2.33	-0.52	0.03	-3.22	0.24	0.00
LOC_Os12g36110.1	calmodulin binding protein, putative, expressed	-2.47	-0.57	-0.01	-2.77	0.05	-0.07
LOC_Os12g36880.1	pathogenesis-related Bet v I family protein, putative, expressed	-0.77	-2.18	0.75	-0.26	0.16	0.11
LOC_Os12g38760.2	nucleotide pyrophosphatase/phosphodiesterase, putative, expressed	-1.25	-0.74	0.26	-2.21	-0.14	-0.11
LOC_Os12g39310.1	cytochrome P450, putative, expressed	-1.63	-0.49	-0.02	-2.62	-0.18	-0.11
LOC_Os12g41110.1	OsCML5 - Calmodulin-related calcium sensor protein, expressed	-1.65	-0.19	-0.12	-2.31	0.19	-0.05
LOC_Os12g43410.1	thaumatin, putative	-0.94	-2.04	0.44	-0.49	0.20	0.05

Supplementary Table 4: 156 DEGs expressed in high and low temperature comparison after *Xoo* inoculation in IR24 and IRBB67

Gene	Description	IR24-3hpi	IR24-72hpi	IR24-120hpi	IRBB67-3hpi	IRBB67-72hpi	IRBB67-120hpi
LOC_Os01g01430.1	No apical meristem protein, putative, expressed	0.09	0.37	2.32	0.02	0.25	-0.05
LOC_Os01g01840.1	helix-loop-helix DNA-binding domain containing protein, expressed	0.21	0.85	0.88	-0.16	2.68	2.22
LOC_Os01g11620.1	GDSL-like lipase/acylhydrolase, putative, expressed	-0.77	-1.16	-1.33	-1.11	-2.48	-0.60
LOC_Os01g27630.1	glutathione S-transferase, putative, expressed	0.06	-1.14	-1.01	-0.17	-2.70	-0.41
LOC_Os01g28450.1	SCP-like extracellular protein, expressed	-0.25	-1.44	-1.41	-1.36	-2.51	-0.94
LOC_Os01g32460.1	expressed protein	-0.22	0.86	0.44	0.49	2.42	1.40
LOC_Os01g39020.1	HSF-type DNA-binding domain containing protein, expressed	0.17	0.79	2.07	0.32	1.34	0.78
LOC_Os01g50400.1	STE_MEKK_ste11_MAP3K.5 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed	0.54	-0.23	2.37	0.15	0.92	0.98
LOC_Os01g50910.1	late embryogenesis abundant protein, group 3, putative, expressed	0.51	1.67	2.57	-0.64	2.75	0.52
LOC_Os01g50910.2	late embryogenesis abundant protein, group 3, putative, expressed	-0.22	0.24	2.02	-0.60	1.38	0.07
LOC_Os01g54030.1	NADP-dependent malic enzyme, putative, expressed	0.38	0.77	2.12	-0.48	2.17	0.90
LOC_Os01g55510.1	dynein light chain type 1 domain containing protein, expressed	-0.80	0.60	2.03	-1.01	0.75	0.22
LOC_Os01g59780.1	AP2 domain containing protein, expressed	0.14	0.71	2.17	-0.90	1.39	0.64
LOC_Os01g65060.1	S-domain receptor-like protein kinase, putative	0.44	-0.96	-1.78	0.19	-2.07	-1.07
LOC_Os01g68650.1	plant-specific domain TIGR01615 family protein, expressed	-0.05	0.63	2.06	-0.29	1.15	-0.11
LOC_Os01g70850.1	esterase, putative, expressed	0.05	0.14	-0.28	-0.59	-2.48	-1.29
LOC_Os01g70850.2	esterase, putative, expressed	-0.27	0.70	-2.16	0.14	-1.50	-0.43
LOC_Os01g72270.1	cytochrome P450, putative, expressed	0.18	0.27	2.04	-0.23	0.59	0.34
LOC_Os02g01590.1	glycosyl hydrolases, putative, expressed	0.56	1.69	0.23	1.27	2.11	0.54
LOC_Os02g02210.1	aminotransferase, putative, expressed	0.05	0.29	2.17	0.08	2.16	0.71
LOC_Os02g04750.3	cycloartenol synthase, putative, expressed	0.59	2.00	0.54	0.73	0.61	0.74
LOC_Os02g06670.1	retrotransposon protein, putative, unclassified, expressed	0.23	0.39	2.09	-0.13	1.10	0.29
LOC_Os02g07170.1	MYB family transcription factor, putative, expressed	-0.04	-0.05	2.17	0.00	-0.05	-0.06
LOC_Os02g08440.2	OsWRKY71 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-0.24	-0.92	2.28	-0.46	0.08	0.49
LOC_Os02g17620.1	isochorismatase family protein, putative, expressed	-0.28	-1.67	-1.71	-0.04	-2.19	-1.12
LOC_Os02g26720.1	Inositol 1, 3, 4-trisphosphate 5/6-kinase, putative, expressed	0.10	0.05	2.16	-0.76	1.17	1.05
LOC_Os02g43330.1	homeobox associated leucine zipper, putative, expressed	1.25	0.82	2.75	0.77	1.43	1.11
LOC_Os02g45490.3	expressed protein	-0.20	-0.56	-0.17	0.43	-1.99	-0.01
LOC_Os02g46560.1	helix-loop-helix DNA-binding protein, putative, expressed	0.01	-0.70	-0.16	-0.34	-2.08	-0.40

LOC_Os02g47780.1	hydrolase, alpha/beta fold family domain containing protein, expressed	-0.21	1.13	-0.56	0.08	2.09	0.09
LOC_Os03g02050.1	LTPL151 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	0.21	1.19	2.51	0.18	1.15	0.67
LOC_Os03g02190.1	protein kinase domain containing protein, expressed	-0.23	-1.54	-1.74	-0.08	-2.00	-0.26
LOC_Os03g04080.1	expressed protein	0.20	1.60	3.08	0.11	2.06	0.50
LOC_Os03g06360.1	late embryogenesis abundant protein D-34, putative, expressed	0.03	0.70	1.38	0.05	2.03	0.78
LOC_Os03g06360.2	late embryogenesis abundant protein D-34, putative, expressed	0.49	0.60	2.01	-0.48	1.62	0.44
LOC_Os03g12510.1	non-symbiotic hemoglobin 2, putative, expressed	0.15	-0.82	2.44	-0.73	-0.07	0.05
LOC_Os03g14010.2	glycosyl hydrolase family 10 protein, putative, expressed	-0.03	-0.89	-0.03	-0.16	-2.22	0.05
LOC_Os03g14654.1	LTPL108 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.25	-1.98	-0.90	-1.07	-2.39	-0.28
LOC_Os03g17350.1	white-brown complex homolog protein, putative, expressed	-0.14	1.53	-0.15	-0.24	2.13	0.00
LOC_Os03g28330.2	sucrose synthase, putative, expressed	-0.46	-1.58	-0.75	-1.01	-2.41	-0.45
LOC_Os03g28330.3	sucrose synthase, putative, expressed	-0.58	-1.05	-0.63	-0.27	-2.12	-0.72
LOC_Os03g28330.4	sucrose synthase, putative, expressed	-0.65	-1.11	-0.09	-0.31	-2.13	-1.83
LOC_Os03g28330.5	sucrose synthase, putative, expressed	-0.81	-1.06	-0.86	-0.36	-2.36	-0.92
LOC_Os03g48710.3	expressed protein	0.39	-0.10	2.05	0.16	0.56	0.81
LOC_Os03g48770.1	Cupin domain containing protein, expressed		-1.00	0.43	-0.07	-2.00	-0.08
LOC_Os03g54750.1	COBRA-like 3 protein precursor, putative, expressed	-0.53	-1.39	-0.98	-0.19	-2.18	-0.52
LOC_Os03g55090.1	alpha-glucan phosphorylast isozyme, putative, expressed	0.30	-1.20	-1.35	-0.20	-2.43	-0.26
LOC_Os03g57460.1	fasciclin domain containing protein, expressed	-0.61	-1.61	-1.31	-0.56	-2.19	-0.58
LOC_Os03g57980.1	LTPL99 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-0.06	1.15	-0.09	-0.08	2.05	-0.13
LOC_Os03g58290.1	indole-3-glycerol phosphate lyase, chloroplast precursor, putative, expressed	-0.62	2.10	0.03	-0.92	0.50	1.79
LOC_Os03g61150.1	expressed protein	0.44	0.81	1.95	0.04	2.25	1.08
LOC_Os03g61150.3	expressed protein	0.33	1.63	2.13	-0.02	0.75	0.14
LOC_Os03g63390.1	plastocyanin-like domain containing protein, putative, expressed	-0.41	-1.23	-0.30	0.23	-2.09	-0.24
LOC_Os04g03210.1	receptor kinase, putative	-0.06	-1.48	-0.60	-0.06	-2.27	-1.52
LOC_Os04g27670.1	terpene synthase family, metal binding domain containing protein, expressed	0.41	0.46	2.29	0.02	1.13	0.02
LOC_Os04g39880.3	Os4bglu12 - beta-glucosidase, exo-beta-glucanase, expressed	-0.78	-1.42	-1.21	-0.63	-2.17	-0.56
LOC_Os04g40630.1	BTBZ4 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with TAZ zinc finger and Calmodulin-binding domains, expressed	-1.28	-1.16	0.51	0.40	-2.20	-0.09
LOC_Os04g40630.4	BTBZ4 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with TAZ zinc finger and Calmodulin-binding domains, expressed	0.21	-0.14	-0.13	0.76	-2.04	-0.08
LOC_Os04g44500.1	GEM, putative, expressed	0.09	1.37	2.38	0.17	1.87	1.44
LOC_Os04g48850.1	aminotransferase, classes I and II, domain containing protein, expressed	-0.21	-1.09	2.23	-0.75	-0.66	0.34
LOC_Os05g01140.1	methyltransferase, putative, expressed	-0.27	0.69	0.33	-0.85	1.88	2.00
LOC_Os05g03130.1	OsRCI2-7 - Putative low temperature and salt responsive protein, expressed	0.18	0.55	2.03	-0.12	1.65	0.40
LOC_Os05g04380.1	peroxidase precursor, putative, expressed	-0.83	-2.26	-0.47	-0.65	-2.66	-1.04
LOC_Os05g20100.1	glycerol-3-phosphate acyltransferase, putative, expressed	-0.26	1.35	-0.08	-0.26	2.42	0.31

LOC_Os05g24660.1	IPP transferase, putative, expressed	-0.35	-1.76	-0.85	-0.93	-2.18	-0.92
LOC_Os05g32110.2	COBRA, putative, expressed	-0.29	-1.38	-1.32	0.57	-2.07	-0.27
LOC_Os05g38290.2	protein phosphatase 2C, putative, expressed	0.53	1.07	2.15	0.26	1.28	0.60
LOC_Os05g39320.1	thiamine pyrophosphate enzyme, C-terminal TPP binding domain containing protein, expressed	-0.18	-1.24	-0.72	0.08	-2.01	-0.44
LOC_Os05g45370.1	cell cycle control protein, putative, expressed	0.55	-1.63	-1.52	0.05	-3.48	-0.19
LOC_Os05g48890.1	fasciclin domain containing protein, expressed	-0.27	-0.68	-0.74	-0.15	-2.26	-0.30
LOC_Os05g48900.1	fasciclin domain containing protein, expressed	-0.56	-1.44	-1.85	0.05	-2.47	-0.98
LOC_Os05g49730.1	protein phosphatase 2C, putative, expressed	0.64	1.00	1.87	0.26	2.24	0.89
LOC_Os05g50260.1	polygalacturonase, putative, expressed	-0.05	0.96	1.14	0.11	2.07	0.67
LOC_Os06g03670.1	dehydration-responsive element-binding protein, putative, expressed	0.36	0.43	2.31	0.13	0.78	1.03
LOC_Os06g14670.1	ODORANT1, putative, expressed	-0.19	0.58	2.09	-0.18	0.93	0.74
LOC_Os06g16350.1	peroxidase precursor, putative, expressed	-0.19	1.77	-0.21	-0.27	2.38	0.13
LOC_Os06g17070.1	retrotransposon protein, putative, Ty1-copia subclass	0.54	-0.43	2.01	0.22	-0.50	-0.07
LOC_Os06g21910.1	late embryogenesis abundant group 1, putative, expressed	0.11	0.83	2.24	-0.28	2.98	0.31
LOC_Os06g27910.1	oleosin, putative, expressed	-0.02	0.58	2.21	0.05	0.70	0.09
LOC_Os06g30370.1	osMFT1 MFT-Like1 homologous to Mother of FT and TFL1 gene; contains Pfam profile PF01161: Phosphatidylethanolamine-binding protein, expressed	0.89	1.08	1.51	0.80	2.21	0.85
LOC_Os06g35520.1	peroxidase precursor, putative, expressed	0.20	0.60	2.66	-0.05	-0.14	0.16
LOC_Os06g36270.1	receptor-like protein kinase 5 precursor, putative, expressed	-1.01	-0.84	0.35	-0.37	-2.03	-0.49
LOC_Os06g36560.1	inositol oxygenase, putative, expressed	-0.37	0.03	2.07	-0.12	0.28	0.35
LOC_Os06g37140.2	retrotransposon protein, putative, Ty3-gypsy subclass, expressed	0.41	-0.32	2.09	0.13	0.13	0.08
LOC_Os06g42560.2	tryptophan synthase beta chain 2, putative, expressed	-0.61	-1.34	-0.57	-0.26	-2.14	-0.84
LOC_Os07g03920.1	lectin-like receptor kinase 7, putative	0.08	-1.27	-2.27	0.28	0.15	-0.13
LOC_Os07g04560.1	no apical meristem protein, putative	0.31	-1.82	-0.83	-0.77	-2.14	-0.78
LOC_Os07g04940.1	uncharacterized PE-PGRS family protein PE_PGRS54 precursor, putative	0.02	1.52	-0.05	-0.11	2.35	-0.14
LOC_Os07g05940.1	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed	0.80	-0.20	3.19	0.30	1.22	1.02
LOC_Os07g34520.2	isocitrate lyase, putative, expressed	0.31	0.13	2.25	0.20	1.84	0.80
LOC_Os07g34520.3	isocitrate lyase, putative, expressed	0.45	0.22	2.56	0.24	2.36	0.29
LOC_Os07g35350.1	glucan endo-1,3-beta-glucosidase precursor, putative, expressed	0.01	-2.54	0.44	0.06	-1.90	-1.29
LOC_Os07g40850.1	retrotransposon protein, putative, unclassified, expressed	-0.06	1.65	-0.05	-0.50	2.16	0.04
LOC_Os07g40870.1	igA FC receptor precursor, putative, expressed	-0.45	1.67	-0.37	-0.11	2.21	-0.04
LOC_Os07g40890.1	igA FC receptor precursor, putative, expressed	-0.29	1.85	-0.07	-0.51	2.53	-0.06
LOC_Os07g41350.1	B12D protein, putative, expressed	-0.77	0.21	2.21	-0.52	1.43	0.07
LOC_Os07g45060.1	uncharacterized GPI-anchored protein At5g19240 precursor, putative, expressed	-0.52	-1.48	-1.16	-0.13	-2.05	-0.85
LOC_Os07g46920.1	sex determination protein tasselseed-2, putative, expressed	-0.33	1.33	-0.67	-0.52	2.12	0.10
LOC_Os07g47210.1	GDSL-like lipase/acylhydrolase, putative, expressed	-0.28	1.20	-0.15	-0.32	2.32	0.14

LOC_Os07g48050.1	peroxidase precursor, putative, expressed	0.29	0.47	-0.35	0.02	-2.13	0.03
LOC_Os08g01370.1	expressed protein	0.18	1.25	2.06	-0.12	2.29	1.02
LOC_Os08g04500.1	terpene synthase, putative, expressed	1.54	2.27	2.03	0.56	2.61	2.14
LOC_Os08g04500.2	terpene synthase, putative, expressed	1.39	2.24	1.55	0.30	2.29	2.00
LOC_Os08g04540.1	decarboxylase, putative, expressed	-0.74	-1.70	-0.61	-1.34	-2.19	-1.17
LOC_Os08g07080.1	terpene synthase, putative, expressed	0.18	0.31	1.17	0.17	3.32	0.43
LOC_Os08g19420.1	O-methyltransferase, putative, expressed	0.49	0.88	2.05	0.22	1.65	0.99
LOC_Os08g23870.1	late embryogenesis abundant group 1, putative, expressed	0.59	0.78	2.00	-0.35	1.79	0.14
LOC_Os08g32750.1	bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor, putative, expressed	-1.28	-2.42	-2.45	-1.56	-1.64	-1.93
LOC_Os08g32780.1	bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor, putative	-0.72	-1.89	-2.47	-0.92	-1.21	-1.77
LOC_Os08g34390.1	retrotransposon protein, putative, unclassified, expressed	0.73	1.05	2.53	-0.02	0.86	0.65
LOC_Os08g35710.1	expressed protein	-0.46	-2.38	-1.71	-0.31	-3.34	-0.67
LOC_Os08g36920.1	AP2 domain containing protein, expressed	-0.66	-0.11	2.30	-0.87	-0.07	0.35
LOC_Os08g37300.1	expressed protein	-0.60	1.81	-0.11	-0.16	2.39	-0.01
LOC_Os08g38270.1	fasciclin domain containing protein, expressed	-0.73	-1.32	-0.48	-0.05	-2.28	-0.13
LOC_Os08g39490.1	expressed protein	0.06	0.17	-2.32	0.20	0.19	-0.72
LOC_Os08g39850.2	lipoxygenase, chloroplast precursor, putative, expressed	-0.89	-1.05	0.11	-2.01	-1.26	-0.61
LOC_Os08g39870.1	Os8bglu28 - beta-glucosidase homologue, similar to Os4bglu12 exoglucanase, expressed	-0.40	-1.59	-1.78	-0.34	-2.34	-0.69
LOC_Os08g42570.1	plant protein of unknown function domain containing protein, expressed	-0.10	-1.11	-1.01	-0.25	-2.10	-1.04
LOC_Os09g03190.1	expressed protein	0.71	0.14	2.41	0.42	0.30	0.38
LOC_Os09g03200.1	hypothetical protein	1.01	-0.04	2.02	0.49	0.32	0.53
LOC_Os09g04339.1	expressed protein	-0.21	-0.05	1.25	0.26	-2.22	-1.01
LOC_Os09g12660.1	glucose-1-phosphate adenylyltransferase large subunit, chloroplast precursor, putative, expressed	-0.03	-1.08	-1.33	-0.26	-2.92	-0.38
LOC_Os09g21120.1	armadillo/beta-catenin repeat family protein, putative, expressed	1.01	0.95	2.41	0.60	1.96	1.06
LOC_Os09g27010.1	tyrosine protein kinase domain containing protein, putative, expressed	0.10	0.12	1.99	-0.30	0.44	0.69
LOC_Os09g31430.1	Os9bglu30 - beta-glucosidase, similar to Os4bglu12 exoglucanase, expressed	-0.85	-0.60	-0.84	-0.77	-2.68	-0.37
LOC_Os09g32570.1	alcohol dehydrogenase GroES-like domain containing protein, expressed	-0.58	-1.34	-0.97	-0.50	-2.29	-0.76
LOC_Os09g36700.1	ribonuclease T2 family domain containing protein, expressed	-0.68	-1.42	-0.34	0.04	-2.09	0.10
LOC_Os09g39410.1	male sterility protein, putative, expressed	-0.11	1.47	-0.28	-0.02	2.21	-0.30
LOC_Os10g11310.3	expressed protein	0.10	0.23	-2.03	0.27	-0.62	-0.39
LOC_Os10g13700.1	phosphoenolpyruvate carboxykinase, putative, expressed	0.23	-0.04	2.37	0.45	1.24	0.70
LOC_Os10g23820.1	transferase family protein, putative, expressed	-0.10	-1.77	-0.21	-0.65	-2.07	-1.03
LOC_Os10g25310.1	SPX domain containing protein, putative, expressed	-0.86	-1.99	-0.11	0.23	-0.72	-0.25
LOC_Os10g36100.1	LTPL157 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	0.02	0.34	3.53	-0.05	0.73	1.01

LOC_Os10g37400.1	DUF538 domain containing protein, putative, expressed	-0.47	1.57	0.24	0.34	2.19	0.06
LOC_Os10g38120.1	cytochrome P450, putative, expressed	0.09	-2.01	-0.96	-0.15	-1.97	-0.67
LOC_Os10g39680.1	CHIT14 - Chitinase family protein precursor, expressed	-0.71	-1.90	-0.80	-1.00	-2.13	-0.98
LOC_Os10g39700.1	CHIT15 - Chitinase family protein precursor, putative, expressed	-0.63	-2.46	-0.62	-0.20	-2.55	-0.29
LOC_Os10g42040.1	expressed protein	0.03	-1.71	-1.35	-0.61	-2.11	-0.54
LOC_Os11g02540.1	OsWRKY50 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-0.06	0.97	1.06	-0.20	2.34	1.96
LOC_Os11g07911.1	expressed protein	0.19	0.68	2.71	-0.65	1.82	0.56
LOC_Os11g14910.1	NADP-dependent oxidoreductase, putative, expressed	0.12	-2.48	-0.99	0.08	-1.33	-0.51
LOC_Os11g26750.1	dehydrin, putative, expressed	0.43	2.21	2.35	-0.28	2.94	0.54
LOC_Os11g26780.1	dehydrin, putative, expressed	0.73	1.14	1.90	0.01	2.60	0.73
LOC_Os11g31090.1	transferase family protein, putative, expressed	-0.71	1.01	-0.32	-0.38	2.03	-0.11
LOC_Os11g42200.1	laccase precursor protein, putative, expressed	0.25	-1.53	-2.13	-0.96	-2.65	-1.70
LOC_Os11g46000.1	von Willebrand factor type A domain containing protein, putative, expressed	-0.13	0.40	0.40	0.07	2.58	1.55
LOC_Os12g02470.1	OsWRKY65 - Superfamily of TFs having WRKY and zinc finger domains, expressed	-0.34	0.10	1.16	-0.78	2.31	2.28
LOC_Os12g14440.1	Jacalin-like lectin domain containing protein, putative, expressed	0.81	0.44	0.74	0.31	1.00	2.04
LOC_Os12g27830.1	dehydrogenase/reductase, putative, expressed	0.09	0.59	2.31	-0.18	2.71	0.91
LOC_Os12g37350.1	lipozygenase protein, putative	-0.04	1.73	0.94	-0.10	2.26	0.74
LOC_Os12g37519.1	retrotransposon protein, putative, unclassified, expressed	0.32	-0.33	2.05	0.13	-0.44	0.23
LOC_Os12g38770.1	nucleotide pyrophosphatase/phosphodiesterase, putative, expressed	-0.22	0.59	2.28	-0.44	1.75	0.52
LOC_Os12g40180.1	expressed protein	-0.18	0.09	2.16	-0.10	0.94	0.50
LOC_Os12g43410.1	thaumatin, putative	-0.19	-2.12	-0.92	-0.07	-3.11	-1.05
LOC_Os12g43440.1	thaumatin, putative, expressed	-0.23	-1.75	-0.34	-1.18	-2.51	-0.98
LOC_Os12g44050.1	purple acid phosphatase precursor, putative, expressed	-0.24	-0.90	-0.07	-0.87	-2.07	-0.48

Supplementary Table 5: 145 DEGs induced in comparison between IRBB67 and IR24 after *Xoo* inoculation across the three time points under low (L) temperature

DEGs ID	Description	*L-3hpi*	*L-72hpi*	*L-120hpi*
LOC_Os02g43860.2	amino acid permease, putative, expressed	-1.17332	-1.11553	-2.41756
LOC_Os06g16420.3	amino acid transporter, putative, expressed	-1.08992	-0.88597	-2.07977
LOC_Os05g45100.1	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed	2.530754	0.130024	-0.12798
LOC_Os10g07970.1	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed	-1.0842	-2.81756	-2.10173
LOC_Os03g08490.1	AP2 domain containing protein, expressed	-3.54605	-2.36232	-3.30238
LOC_Os06g16300.1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	-2.52179	-2.25919	-2.08362
LOC_Os03g10640.1	calcium-transporting ATPase, plasma membrane-type, putative, expressed	2.13141	0.732054	0.955736
LOC_Os11g44340.1	calmodulin binding protein, putative	-4.15576	-2.33324	-1.99956
LOC_Os11g44310.1	calmodulin binding protein, putative, expressed	-5.63959	-3.8695	-3.45226
LOC_Os11g44680.1	calmodulin binding protein, putative, expressed	-2.64773	-1.01934	-1.99154
LOC_Os06g12940.1	conserved hypothetical protein	-2.39245	-1.29127	-0.61583
LOC_Os11g37140.1	conserved hypothetical protein	2.222485	0.533981	0.574019
LOC_Os11g42590.1	conserved hypothetical protein	1.190916	1.155552	2.158446
LOC_Os11g44260.1	conserved hypothetical protein	2.498451	1.132176	1.679368
LOC_Os05g47540.3	CPuORF26 - conserved peptide uORF-containing transcript, expressed	-2.24453	-1.39035	-2.51173
LOC_Os05g47540.4	CPuORF26 - conserved peptide uORF-containing transcript, expressed	-2.04203	-1.68335	-1.6317
LOC_Os05g47540.5	CPuORF26 - conserved peptide uORF-containing transcript, expressed	2.235462	0.410622	1.690458
LOC_Os03g10650.1	cyclin, putative, expressed	2.341483	1.281531	1.227796
LOC_Os02g39010.2	cyclin-dependent kinase G-1, putative, expressed	2.284552	0.903509	0.417002
LOC_Os02g12130.1	cysteine-rich receptor-like protein kinase 35 precursor, putative, expressed	-2.56024	-1.27746	-1.50349
LOC_Os06g15680.1	cytochrome P450 71A6, putative	2.559062	1.105753	1.184552
LOC_Os06g37330.1	cytochrome P450, putative, expressed	2.164737	0.955665	2.038015
LOC_Os11g47120.1	DEFL48 - Defensin and Defensin-like DEFL family, expressed	-1.17089	-2.27281	-1.58909
LOC_Os03g09020.1	dehydrogenase, putative, expressed	2.977628	1.671782	2.500217
LOC_Os05g49440.2	DUF1264 domain containing protein, putative, expressed	2.632096	0.362221	0.345543
LOC_Os11g43790.1	DUF581 domain containing protein, expressed	0.264527	2.516845	1.543991

LOC_Os03g06890.1	DUF593 domain containing protein	2.00471	0.391361	0.023391
LOC_Os10g42220.1	enoyl-CoA hydratase/isomerase family protein, putative	-2.28984	-1.56211	-1.8326
LOC_Os10g42210.1	enoyl-CoA-hydratase, putative, expressed	-2.63269	-1.43331	-1.8848
LOC_Os01g66310.1	expressed protein	-2.24406	-2.13975	-2.3592
LOC_Os02g23939.1	expressed protein	1.086042	1.162446	2.106175
LOC_Os03g04580.1	expressed protein	0.94452	1.078523	2.346658
LOC_Os03g04930.1	expressed protein	4.162021	0.311608	4.24955
LOC_Os03g04930.2	expressed protein	-3.88265	-3.80518	-3.56148
LOC_Os03g06835.1	expressed protein	3.064404	1.732996	2.469337
LOC_Os03g07410.1	expressed protein	2.484993	1.524403	2.168718
LOC_Os03g08030.1	expressed protein	1.999362		
LOC_Os03g54240.1	expressed protein	-2.18971	-2.02877	-3.03288
LOC_Os05g01330.1	expressed protein	-2.99973	-2.46677	-3.02957
LOC_Os05g03320.1	expressed protein	-4.61004	-0.21217	-5.13674
LOC_Os05g03390.1	expressed protein	-1.40697	-0.74556	-2.97492
LOC_Os05g46470.1	expressed protein	-1.62317	-2.09703	-1.89645
LOC_Os05g46630.1	expressed protein	2.566898	1.499949	1.354047
LOC_Os05g48790.2	expressed protein	1.427175	0.261487	2.035762
LOC_Os05g48790.3	expressed protein	2.835146	0.216631	0.234296
LOC_Os06g12455.1	expressed protein	2.251372	1.453547	0.508675
LOC_Os06g16140.1	expressed protein	-2.32102	-2.38307	-1.88089
LOC_Os06g35165.1	expressed protein	-2.7879	-2.62128	-2.62009
LOC_Os06g38110.1	expressed protein	3.423657	1.102293	0.762949
LOC_Os06g38210.1	expressed protein	6.715736	0.079007	6.14724
LOC_Os06g38210.2	expressed protein	5.416072	0.104087	6.250925
LOC_Os06g38594.1	expressed protein	1.803122	1.394789	2.066424
LOC_Os06g38660.1	expressed protein	-1.33495	-2.10052	-1.5514
LOC_Os06g38680.1	expressed protein	-3.18032	-1.92754	-1.90682
LOC_Os06g39110.1	expressed protein	2.262224	1.210601	1.875511
LOC_Os06g39120.1	expressed protein	-3.22079	-4.00758	-3.87525
LOC_Os06g42060.2	expressed protein	4.068029	0.124942	5.246743

LOC_Os07g05510.1	expressed protein	1.968914	1.995229	2.626074
LOC_Os08g14195.1	expressed protein	-1.75316	-1.75143	-2.46756
LOC_Os09g17329.2	expressed protein	0.255656	0.17939	3.234041
LOC_Os09g27135.1	expressed protein	0.920837	1.273145	2.233695
LOC_Os11g09979.1	expressed protein	-2.00287	-0.58205	-1.22798
LOC_Os11g29500.1	expressed protein	-4.12671	-0.26297	-4.94223
LOC_Os11g35540.1	expressed protein	-0.59116	-1.25229	-2.25203
LOC_Os11g42850.1	expressed protein	-2.04296	-2.55564	-2.52905
LOC_Os11g42850.2	expressed protein	-1.33819	-2.32001	-1.9082
LOC_Os11g43390.1	expressed protein	1.726026	1.279666	2.14279
LOC_Os11g43895.1	expressed protein	-2.74069	-2.15327	-3.04909
LOC_Os11g43990.1	expressed protein	-3.23871	-2.03876	-2.2559
LOC_Os11g44330.1	expressed protein	-1.99438	0.303231	0.021902
LOC_Os11g44380.1	expressed protein	-6.57085	-0.24237	-4.97008
LOC_Os11g44800.1	expressed protein	-2.35184	-1.29062	-1.09668
LOC_Os11g47370.1	expressed protein	-4.78496	-0.3985	-4.79287
LOC_Os07g05400.1	ferredoxin--NADP reductase, chloroplast precursor, putative, expressed	5.094868	0.187138	0.317455
LOC_Os05g45860.1	glucan endo-1,3-beta-glucosidase precursor, putative, expressed	2.790977	0.846886	0.703957
LOC_Os03g07270.1	glycine-rich cell wall protein, putative, expressed	3.737792	0.133888	4.844386
LOC_Os11g44950.2	glycosyl hydrolase family 3 protein, putative, expressed	1.122281	1.253246	2.124103
LOC_Os05g46240.1	green ripe-like, putative, expressed	2.941236	1.93459	1.234
LOC_Os05g46240.2	green ripe-like, putative, expressed	3.474341	0.191211	0.248265
LOC_Os05g46240.3	green ripe-like, putative, expressed	3.273121	0.232445	2.036524
LOC_Os05g46240.4	green ripe-like, putative, expressed	2.720919	1.477819	0.726256
LOC_Os02g43100.1	hypothetical protein	-2.14488	-1.90525	-2.464
LOC_Os06g42650.1	hypothetical protein	-4.14257	-1.44043	-2.21171
LOC_Os11g17650.1	hypothetical protein	-2.21211	-0.71031	-1.50275
LOC_Os11g44300.1	hypothetical protein	-2.77956	-1.96442	-1.09603
LOC_Os06g12870.1	leaf senescence related protein, putative, expressed	-2.01029	-0.87989	-1.18378
LOC_Os07g03920.1	lectin-like receptor kinase 7, putative	2.991725	1.551647	2.077128
LOC_Os05g46090.1	Leucine Rich Repeat domain containing protein	-1.19149	-1.58962	-2.09313

LOC_Os11g29110.1	Leucine Rich Repeat family protein, expressed	4.285871	0.419829	0.317032
LOC_Os02g40130.1	leucine-rich, putative, expressed	2.183729	1.194568	1.219134
LOC_Os09g18159.1	light repressible receptor protein kinase, putative, expressed	1.799087	0.314541	4.03369
LOC_Os06g38120.1	low-affinity cation transporter, putative, expressed	4.309904	0.283214	0.378859
LOC_Os03g08900.1	MATE efflux family protein, putative, expressed	-0.45698	-1.88616	-2.11834
LOC_Os07g02570.1	NB-ARC domain containing protein	-2.95131	-2.65558	-1.96395
LOC_Os11g44990.1	NB-ARC domain containing protein, expressed	1.188558	1.536231	2.080183
LOC_Os11g45090.1	NB-ARC domain containing protein, expressed	0.743014	1.244376	2.37905
LOC_Os11g46210.1	NB-ARC domain containing protein, expressed	-3.99819	-3.45074	-3.87747
LOC_Os11g29090.1	NB-ARC/LRR disease resistance protein, putative	3.115347	0.161341	0.113785
LOC_Os01g11670.1	OsSCP2 - Putative Serine Carboxypeptidase homologue, expressed	-2.85125	-0.65175	-0.98628
LOC_Os11g42390.1	OsSCP64 - Putative Serine Carboxypeptidase homologue, expressed	-0.9642	-3.46927	-2.23355
LOC_Os11g47140.1	OsWAK123 - OsWAK receptor-like protein kinase, expressed	3.556176	0.125273	3.823489
LOC_Os02g42810.1	oxidoreductase, short chain dehydrogenase/reductase family domain containing protein, expressed	-2.81477	-2.2403	-2.29416
LOC_Os11g29490.1	plasma membrane ATPase, putative, expressed	-2.34538	-2.19425	-2.35146
LOC_Os05g46360.2	possible lysine decarboxylase domain containing protein, expressed	2.615184	2.28311	1.10909
LOC_Os02g43080.1	PPR repeat domain containing protein, putative	-2.34315	-2.07167	-2.18174
LOC_Os09g18594.1	protein kinase domain containing protein, expressed	-0.83633	-2.16596	-2.92228
LOC_Os05g41950.1	protein kinase, putative, expressed	2.131787	1.158887	1.326898
LOC_Os11g44250.1	protein kinase, putative, expressed	2.531544	1.050811	1.286531
LOC_Os02g38780.2	protein phosphatase 2C containing protein, expressed	2.076739	1.132669	2.578884
LOC_Os06g45080.2	rabGAP/TBC domain-containing protein, putative, expressed	-0.84815	-1.21887	-2.12055
LOC_Os06g12790.1	ras-related protein, putative, expressed	2.539213	1.487975	1.775321
LOC_Os06g12790.2	ras-related protein, putative, expressed	-1.55516	-1.6109	-2.25543
LOC_Os02g40190.1	receptor kinase, putative	2.598084	0.939081	1.490533
LOC_Os11g07170.1	receptor kinase, putative, expressed	-0.5909	-1.41267	-2.5323
LOC_Os02g40180.1	receptor-like protein kinase 5 precursor, putative, expressed	-3.28973	-2.84479	-2.80228
LOC_Os06g38640.1	receptor-like protein kinase precursor, putative	0.913235	1.490023	2.377775
LOC_Os06g38670.1	receptor-like protein kinase precursor, putative	-2.87161	-1.50282	-1.40711
LOC_Os06g38650.1	receptor-like protein kinase precursor, putative, expressed	2.193768	1.388587	3.613617
LOC_Os08g28050.1	retrotransposon protein, putative, Ty1-copia subclass	2.849546	0.70318	0.681723

LOC_Os06g43150.1	retrotransposon protein, putative, unclassified	-4.27869	-3.87947	-4.73366
LOC_Os07g05440.1	retrotransposon protein, putative, unclassified, expressed	-3.22081	-4.13589	-4.25039
LOC_Os07g14514.3	retrotransposon protein, putative, unclassified, expressed	-1.82471	-2.05712	-3.34636
LOC_Os07g14514.6	retrotransposon protein, putative, unclassified, expressed	-1.95544	-3.84402	-3.92694
LOC_Os10g34884.1	RIPER7 - Ripening-related family protein precursor, expressed	-2.86252	-1.11366	-1.96814
LOC_Os09g19500.1	senescence-induced receptor-like serine/threonine-protein kinase precursor, putative	-0.52152	-1.02975	-2.34717
LOC_Os02g43110.1	sodium/calcium exchanger 1 precursor, putative, expressed	-2.65321	-2.5491	-2.7149
LOC_Os03g04500.2	tetratricopeptide repeat domain containing protein, expressed	-2.2444	-0.82701	-1.08489
LOC_Os11g42480.1	transferase family domain containing protein, expressed	1.910609	0.362161	3.522168
LOC_Os05g36230.1	transposon protein, putative, CACTA, En/Spm sub-class	-1.06838	-0.67859	-2.39426
LOC_Os03g61870.1	transposon protein, putative, Mariner sub-class	2.075976	0.490669	0.689371
LOC_Os06g39090.1	transposon protein, putative, unclassified	-3.30187	-2.66019	-2.92295
LOC_Os02g43370.1	transposon protein, putative, unclassified, expressed	0.862154	1.633074	2.590441
LOC_Os05g50990.1	TTL3, putative, expressed	-2.05235	-0.33272	-0.18517
LOC_Os03g06460.1	type I inositol-1,4,5-trisphosphate 5-phosphatase, putative	2.062986	0.12364	3.528923
LOC_Os11g42510.2	tyrosine aminotransferase, putative, expressed	1.864674	2.135048	1.951698
LOC_Os07g46660.1	ubiquitin carboxyl-terminal hydrolase domain containing protein, expressed	1.894504	2.867151	4.049469
LOC_Os05g42040.1	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed	3.459452	2.460186	2.66189
LOC_Os05g42060.1	UDP-glucoronosyl/UDP-glucosyl transferase, putative, expressed	3.290321	1.810991	0.335875
LOC_Os09g20390.1	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	0.135014	0.112583	5.012703
LOC_Os09g20390.2	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	0.144629	0.148214	4.087343
LOC_Os09g20390.3	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	0.137037	0.21946	3.088164
LOC_Os07g37454.1	urate anion exchanger, putative, expressed	-3.05437	0.113914	-0.25094
LOC_Os07g30980.1	uvrD/REP helicase family protein, putative, expressed	-3.48131	-2.95705	-3.80947
LOC_Os11g46850.1	wall-associated kinase, putative	3.000758	1.130686	1.118614
LOC_Os11g46860.1	wall-associated receptor kinase-like 4 precursor, putative, expressed	5.00399	1.938128	3.903878

Suplementary Table 6: 188 DEGs induced in comparison between IRBB67 and IR24 after *Xoo* inoculation under high (H) temperature

DEGs ID	Description	H-3hpi	H-72hpi	H-120hpi
LOC_Os01g11670.1	OsSCP2 - Putative Serine Carboxypeptidase homologue, expressed	-2.65	-1.27	-1.65
LOC_Os01g24820.1	NB-ARC domain containing protein	-0.53	-2.33	-1.92
LOC_Os01g66310.1	expressed protein	-2.28	-1.91	-2.72
LOC_Os01g70850.1	esterase, putative, expressed	-0.04	-1.82	-2.17
LOC_Os02g12130.1	cysteine-rich receptor-like protein kinase 35 precursor, putative, expressed	-3.27	-1.25	-1.53
LOC_Os02g23939.1	expressed protein	1.26	2.11	2.21
LOC_Os02g38780.2	protein phosphatase 2C containing protein, expressed	2.50	2.72	3.14
LOC_Os02g40130.1	leucine-rich, putative, expressed	2.84	1.50	2.04
LOC_Os02g40180.1	receptor-like protein kinase 5 precursor, putative, expressed	-4.35	-3.43	-3.40
LOC_Os02g40190.1	receptor kinase, putative	2.44	3.60	3.69
LOC_Os02g40330.3	retrotransposon protein, putative, Ty3-gypsy subclass, expressed	-1.89	-1.41	-3.03
LOC_Os02g40340.1	expressed protein	-0.45	-0.59	-2.04
LOC_Os02g42810.1	oxidoreductase, short chain dehydrogenase/reductase family domain containing protein, expressed	-3.42	-2.23	-3.06
LOC_Os02g43080.1	PPR repeat domain containing protein, putative	-2.09	-2.50	-2.76
LOC_Os02g43100.1	hypothetical protein	-2.46	-2.18	-2.70
LOC_Os02g43110.1	sodium/calcium exchanger 1 precursor, putative, expressed	-3.09	-2.89	-3.07
LOC_Os02g43860.2	amino acid permease, putative, expressed	-1.09	-1.73	-2.42
LOC_Os02g44155.1	expressed protein	0.78	1.61	2.07
LOC_Os03g02514.2	hydrolase, alpha/beta fold family protein, putative, expressed	-0.13	0.02	-2.07
LOC_Os03g04580.1	expressed protein	0.89	1.99	2.31
LOC_Os03g04930.1	expressed protein	4.66	5.58	5.16
LOC_Os03g04930.2	expressed protein	-4.61	-4.42	-3.35
LOC_Os03g05860.1	expressed protein	-2.91	-0.75	-0.54
LOC_Os03g05920.1	expressed protein	-2.07	-0.43	-0.82
LOC_Os03g06460.1	type I inositol-1,4,5-trisphosphate 5-phosphatase, putative	2.24	3.31	4.40

| LOC_Os03g06835.1	expressed protein	3.17	2.70	2.90
LOC_Os03g06850.5	B3 DNA binding domain containing protein, expressed	0.51	2.04	1.93
LOC_Os03g07270.1	glycine-rich cell wall protein, putative, expressed	3.96	4.86	5.71
LOC_Os03g07410.1	expressed protein	2.05	2.20	3.09
LOC_Os03g07410.2	expressed protein	0.88	2.30	1.50
LOC_Os03g07430.4	protein kinase domain containing protein, expressed	-2.23	-2.09	-1.44
LOC_Os03g08490.1	AP2 domain containing protein, expressed	-2.56	-2.14	-2.86
LOC_Os03g08840.1	zinc finger protein, putative, expressed	-1.99	-2.05	-2.33
LOC_Os03g08900.1	MATE efflux family protein, putative, expressed	-0.85	-1.60	-2.26
LOC_Os03g09020.1	dehydrogenase, putative, expressed	3.16	2.79	3.10
LOC_Os03g10540.1	OsFBX78 - F-box domain containing protein, expressed	-2.02	-1.63	-1.87
LOC_Os03g10650.1	cyclin, putative, expressed	3.46	2.04	2.01
LOC_Os03g38940.1	expressed protein	-0.47	-2.40	-2.33
LOC_Os03g53800.2	periplasmic beta-glucosidase precursor, putative, expressed	-0.68	-0.35	-2.72
LOC_Os03g54240.1	expressed protein	-2.15	-2.78	-3.05
LOC_Os03g55150.2	eukaryotic translation initiation factor 5A, putative, expressed	2.65	0.43	0.84
LOC_Os03g55150.4	eukaryotic translation initiation factor 5A, putative, expressed	2.64	0.44	0.89
LOC_Os03g61500.1	uncharacterized Cys-rich domain containing protein, putative	-0.20	0.01	-2.75
LOC_Os05g01330.1	expressed protein	-2.60	-2.86	-3.15
LOC_Os05g03320.1	expressed protein	-4.74	-5.18	-5.03
LOC_Os05g03390.1	expressed protein	-1.79	-2.06	-2.03
LOC_Os05g03390.3	expressed protein	-1.78	-2.06	-1.36
LOC_Os05g36230.1	transposon protein, putative, CACTA, En/Spm sub-class	-1.71	-1.60	-2.63
LOC_Os05g41240.3	Myb-like DNA-binding domain containing protein, putative, expressed	1.40	2.31	1.65
LOC_Os05g41950.1	protein kinase, putative, expressed	2.00	1.60	1.46
LOC_Os05g42040.1	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed	4.67	3.30	3.26
LOC_Os05g42060.1	UDP-glucoronosyl/UDP-glucosyl transferase, putative, expressed	3.17	3.84	4.30
LOC_Os05g42210.1	serine/threonine-protein kinase receptor precursor, putative, expressed	2.23	1.55	1.23
LOC_Os05g45170.1	glucosyl transferase, putative, expressed	2.14	-0.35	0.65
LOC_Os05g45954.1	AP2 domain containing protein, expressed	1.28	2.59	2.76
LOC_Os05g45980.1	hypothetical protein	2.05	0.40	1.12

LOC_Os05g46090.1	Leucine Rich Repeat domain containing protein	-1.46	-1.82	-2.76
LOC_Os05g46240.1	green ripe-like, putative, expressed	2.23	2.54	3.22
LOC_Os05g46240.2	green ripe-like, putative, expressed	3.11	3.10	3.82
LOC_Os05g46240.3	green ripe-like, putative, expressed	3.41	2.52	3.06
LOC_Os05g46360.2	possible lysine decarboxylase domain containing protein, expressed	3.47	2.63	2.68
LOC_Os05g46470.1	expressed protein	-1.86	-2.45	-1.64
LOC_Os05g46630.1	expressed protein	3.11	2.96	3.34
LOC_Os05g47520.1	hypothetical protein	-2.63	-1.90	-2.27
LOC_Os05g47540.3	CPuORF26 - conserved peptide uORF-containing transcript, expressed	-3.95	-1.55	-1.16
LOC_Os05g47540.5	CPuORF26 - conserved peptide uORF-containing transcript, expressed	2.00	0.98	1.66
LOC_Os05g48790.2	expressed protein	0.65	2.92	2.76
LOC_Os05g48790.3	expressed protein	2.89	4.76	5.09
LOC_Os05g49440.2	DUF1264 domain containing protein, putative, expressed	2.87	1.16	-0.16
LOC_Os05g50390.1	expressed protein	2.51	1.26	2.10
LOC_Os05g50990.1	TTL3, putative, expressed	-2.74	-0.26	-0.02
LOC_Os06g10750.1	integral membrane protein DUF6 containing protein, expressed	0.11	-0.08	-2.01
LOC_Os06g12455.1	expressed protein	2.06	2.32	2.74
LOC_Os06g12460.1	CSLA3 - cellulose synthase-like family A; mannan synthase, expressed	0.84	1.62	2.31
LOC_Os06g12630.2	glutathione S-transferase, N-terminal domain containing protein, expressed	1.40	1.80	3.35
LOC_Os06g12790.1	ras-related protein, putative, expressed	2.67	1.17	1.60
LOC_Os06g12790.2	ras-related protein, putative, expressed	-1.27	-2.42	-2.04
LOC_Os06g12940.1	conserved hypothetical protein	-3.41	-1.13	-1.27
LOC_Os06g15680.1	cytochrome P450 71A6, putative	2.17	2.12	2.43
LOC_Os06g16140.1	expressed protein	-2.22	-2.44	-2.63
LOC_Os06g16300.1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	-2.76	-2.23	-1.46
LOC_Os06g16640.1	carboxyl-terminal peptidase, putative, expressed	-2.84	-1.15	-0.38
LOC_Os06g35165.1	expressed protein	-2.28	-2.82	-2.48
LOC_Os06g37140.1	retrotransposon protein, putative, Ty3-gypsy subclass, expressed	-1.85	-0.78	-2.37
LOC_Os06g37140.2	retrotransposon protein, putative, Ty3-gypsy subclass, expressed	-1.07	-0.66	-2.40
LOC_Os06g37150.1	L-ascorbate oxidase precursor, putative, expressed	0.62	-2.50	-0.42
LOC_Os06g38110.1	expressed protein	3.70	0.88	1.17

| LOC_Os06g38120.1	low-affinity cation transporter, putative, expressed	3.96	0.61	1.21
LOC_Os06g38210.1	expressed protein	7.12	7.13	7.50
LOC_Os06g38210.2	expressed protein	6.33	5.14	6.45
LOC_Os06g38594.1	expressed protein	2.36	2.43	2.65
LOC_Os06g38640.1	receptor-like protein kinase precursor, putative	0.90	1.89	2.44
LOC_Os06g38650.1	receptor-like protein kinase precursor, putative, expressed	2.82	3.88	4.49
LOC_Os06g38660.1	expressed protein	-1.69	-1.15	-3.08
LOC_Os06g38670.1	receptor-like protein kinase precursor, putative	-3.55	-0.98	-2.33
LOC_Os06g38680.1	expressed protein	-3.33	-2.41	-3.12
LOC_Os06g39090.1	transposon protein, putative, unclassified	-3.45	-3.02	-3.25
LOC_Os06g39120.1	expressed protein	-2.63	-5.26	-3.82
LOC_Os06g42060.2	expressed protein	4.40	5.23	5.91
LOC_Os06g42650.1	hypothetical protein	-4.81	-1.72	-3.93
LOC_Os06g43150.1	retrotransposon protein, putative, unclassified	-4.50	-5.11	-5.35
LOC_Os06g45080.2	rabGAP/TBC domain-containing protein, putative, expressed	-1.35	-1.52	-2.32
LOC_Os07g02570.1	NB-ARC domain containing protein	-3.57	-2.05	-2.83
LOC_Os07g03920.1	lectin-like receptor kinase 7, putative	3.28	3.49	5.17
LOC_Os07g03970.1	lectin-like receptor kinase 7, putative	1.54	2.92	3.40
LOC_Os07g05400.1	ferredoxin--NADP reductase, chloroplast precursor, putative, expressed	5.64	6.17	7.11
LOC_Os07g05440.1	retrotransposon protein, putative, unclassified, expressed	-3.48	-4.99	-4.78
LOC_Os07g05510.1	expressed protein	1.92	2.71	2.94
LOC_Os07g14514.1	retrotransposon protein, putative, unclassified, expressed	-0.70	-1.95	-2.38
LOC_Os07g14514.2	retrotransposon protein, putative, unclassified, expressed	-1.15	-1.85	-2.45
LOC_Os07g14514.3	retrotransposon protein, putative, unclassified, expressed	-2.20	-3.80	-4.09
LOC_Os07g14514.6	retrotransposon protein, putative, unclassified, expressed	-2.36	-4.12	-4.29
LOC_Os07g29960.1	cytochrome P450, putative, expressed	0.05	0.93	2.04
LOC_Os07g30240.1	mutS family domain IV containing protein	0.92	2.66	2.92
LOC_Os07g30980.1	uvrD/REP helicase family protein, putative, expressed	-3.98	-3.88	-3.77
LOC_Os07g31870.1	expressed protein	0.57	2.27	2.82
LOC_Os07g37454.1	urate anion exchanger, putative, expressed	-2.25	0.10	0.26
LOC_Os07g38280.2	insulin-degrading enzyme, putative, expressed	0.94	1.61	2.08

LOC_Os07g41350.1	B12D protein, putative, expressed	0.51	0.67	-2.02
LOC_Os07g46660.1	ubiquitin carboxyl-terminal hydrolase domain containing protein, expressed	4.63	4.02	4.63
LOC_Os08g04210.1	cysteine-rich repeat secretory protein 55 precursor, putative, expressed		-0.27	-2.37
LOC_Os08g14195.1	expressed protein	-2.28	-1.97	-2.63
LOC_Os08g28050.1	retrotransposon protein, putative, Ty1-copia subclass	2.17	0.56	0.49
LOC_Os08g43120.1	Plant PDR ABC transporter associated domain containing protein, expressed	-0.52	-0.30	-2.00
LOC_Os09g17152.1	OsFBX319 - F-box domain containing protein, expressed	-2.08	-0.56	-1.76
LOC_Os09g17329.2	expressed protein	2.71	3.49	4.10
LOC_Os09g17870.1	cytidylyltransferase domain containing protein, expressed	2.18	2.18	2.12
LOC_Os09g18159.1	light repressible receptor protein kinase, putative, expressed	1.97	4.20	4.61
LOC_Os09g18594.1	protein kinase domain containing protein, expressed	-1.22	-2.40	-1.83
LOC_Os09g19160.1	serine/threonine-protein kinase, putative, expressed	2.00	2.25	2.21
LOC_Os09g19280.1	retrotransposon protein, putative, unclassified	2.70	0.89	1.88
LOC_Os09g19380.1	receptor-like protein kinase precursor, putative, expressed	0.38	1.40	3.51
LOC_Os09g19390.1	senescence-induced receptor-like serine/threonine-protein kinase precursor, putative, expressed	0.53	1.28	3.58
LOC_Os09g19400.1	senescence-induced receptor-like serine/threonine-protein kinase precursor, putative	1.08	1.83	3.38
LOC_Os09g20390.1	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	7.73	4.69	5.70
LOC_Os09g20390.2	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	7.41	4.30	5.26
LOC_Os09g20390.3	uncharacterized glycosyl hydrolase Rv2006/MT2062, putative, expressed	7.70	3.58	4.28
LOC_Os09g27135.1	expressed protein	0.90	1.84	2.30
LOC_Os10g04170.1	hypothetical protein	-1.49	-1.95	-2.21
LOC_Os10g07970.1	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed	-0.96	-1.62	-2.40
LOC_Os10g34884.1	RIPER7 - Ripening-related family protein precursor, expressed	-5.96	-2.73	-2.46
LOC_Os10g36100.1	LTPL157 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-0.04	-0.18	-2.09
LOC_Os10g42210.1	enoyl-CoA-hydratase, putative, expressed	-3.09	-1.91	-2.37
LOC_Os10g42220.1	enoyl-CoA hydratase/isomerase family protein, putative	-3.11	-2.13	-2.56
LOC_Os11g09979.2	expressed protein	-1.74	-1.66	-2.25
LOC_Os11g17650.1	hypothetical protein	-2.47	-1.27	-1.75
LOC_Os11g29090.1	NB-ARC/LRR disease resistance protein, putative	3.58	2.77	3.32
LOC_Os11g29110.1	Leucine Rich Repeat family protein, expressed	4.86	5.17	5.31
LOC_Os11g29490.1	plasma membrane ATPase, putative, expressed	-2.01	-1.12	-2.78

| LOC_Os11g29500.1	expressed protein	-4.64	-4.83	-5.16
LOC_Os11g29790.1	receptor kinase, putative	-2.29	-0.31	-0.23
LOC_Os11g31190.1	nodulin MtN3 family protein, putative, expressed	-0.09	-0.13	-2.36
LOC_Os11g41410.1	expressed protein	-1.45	-2.40	-2.02
LOC_Os11g42390.1	OsSCP64 - Putative Serine Carboxypeptidase homologue, expressed	-1.04	-0.40	-2.60
LOC_Os11g42480.1	transferase family domain containing protein, expressed	2.44	4.37	4.36
LOC_Os11g42510.2	tyrosine aminotransferase, putative, expressed	2.87	1.89	1.80
LOC_Os11g42580.1	Leucine Rich Repeat family protein	1.26	1.83	2.42
LOC_Os11g42590.1	conserved hypothetical protein	1.05	2.04	2.44
LOC_Os11g42720.1	retrotransposon protein, putative, unclassified	0.82	1.82	2.04
LOC_Os11g42850.1	expressed protein	-1.77	-2.94	-2.93
LOC_Os11g42850.2	expressed protein	-1.56	-2.75	-3.05
LOC_Os11g43390.1	expressed protein	2.07	2.41	2.80
LOC_Os11g43790.1	DUF581 domain containing protein, expressed	2.04	3.34	2.37
LOC_Os11g43800.1	PPR repeat domain containing protein, putative	-1.26	-1.53	-2.07
LOC_Os11g43860.2	sodium/calcium exchanger protein, putative, expressed	1.78	2.03	2.13
LOC_Os11g43895.1	expressed protein	-3.05	-3.53	-3.70
LOC_Os11g43990.1	expressed protein	-3.48	-2.23	-3.00
LOC_Os11g44310.1	calmodulin binding protein, putative, expressed	-5.72	-4.10	-3.90
LOC_Os11g44340.1	calmodulin binding protein, putative	-4.62	-1.81	-1.73
LOC_Os11g44380.1	expressed protein	-7.55	-5.29	-5.69
LOC_Os11g44430.1	protein kinase, putative, expressed	-2.97	0.05	0.06
LOC_Os11g44680.1	calmodulin binding protein, putative, expressed	-2.53	-1.79	-1.45
LOC_Os11g44800.1	expressed protein	-2.17	-1.71	-2.14
LOC_Os11g44950.2	glycosyl hydrolase family 3 protein, putative, expressed	1.08	2.03	0.16
LOC_Os11g44990.1	NB-ARC domain containing protein, expressed	1.17	2.42	3.79
LOC_Os11g45090.1	NB-ARC domain containing protein, expressed	0.61	1.62	2.71
LOC_Os11g45840.1	expressed protein	0.93	1.69	2.76
LOC_Os11g46210.1	NB-ARC domain containing protein, expressed	-3.80	-3.96	-4.48
LOC_Os11g46850.1	wall-associated kinase, putative	2.23	1.53	1.34
LOC_Os11g46860.1	wall-associated receptor kinase-like 4 precursor, putative, expressed	4.44	2.91	3.85

LOC_Os11g47120.1	DEFL48 - Defensin and Defensin-like DEFL family, expressed	-0.87	-1.88	-2.06
LOC_Os11g47140.1	OsWAK123 - OsWAK receptor-like protein kinase, expressed	3.71	4.11	3.96
LOC_Os11g47370.1	expressed protein	-5.55	-5.85	-5.98
LOC_Os11g47400.1	hypothetical protein	-1.46	-1.90	-2.03
LOC_Os11g47500.1	glycosyl hydrolase, putative, expressed	0.54	-1.99	-3.69
LOC_Os11g47600.1	glycosyl hydrolase, putative, expressed	-0.09	-0.03	-2.07
LOC_Os11g47910.1	SCARECROW, putative	-1.65	-1.61	-2.24
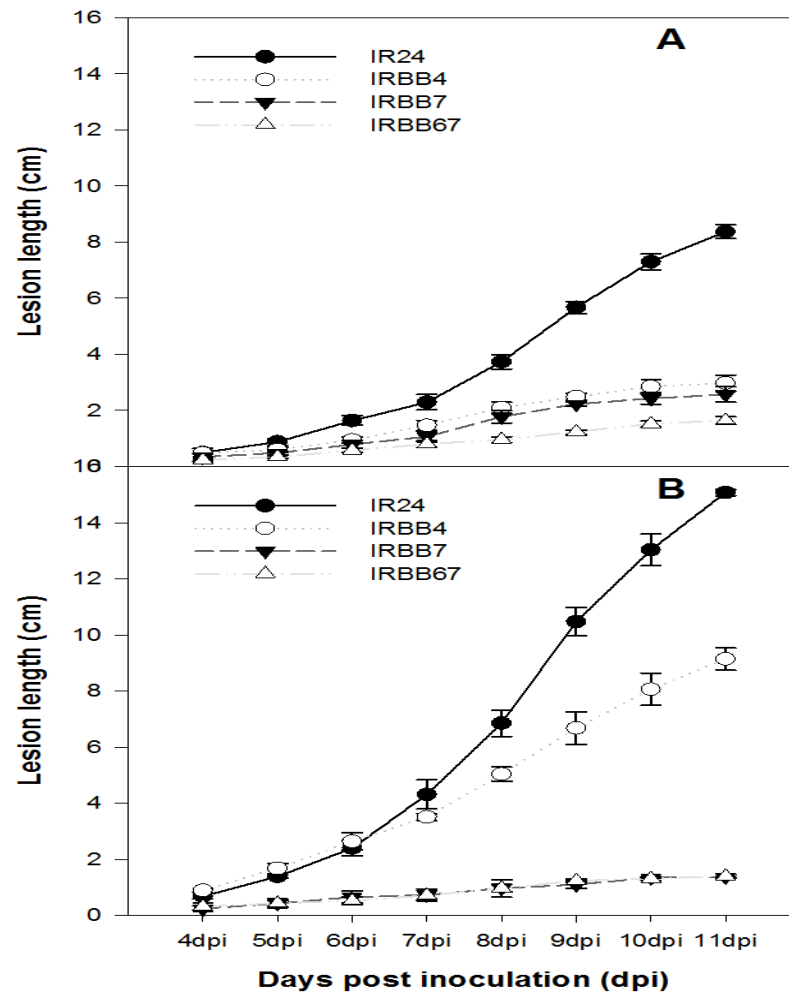
Supplementary Figure 1: A) bacterial blight lesion length progression under low temperature regime; B) bacterial blight lesion length progression under high temperature regimes
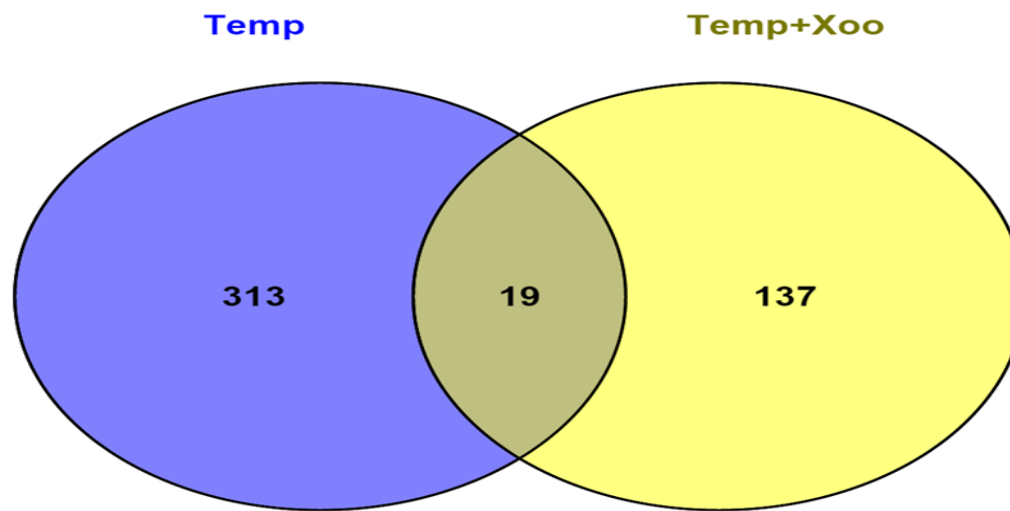
Supplementary Figure 2: Venn diagram showing distribution of temperature and temperature combined with *Xoo* response genes.
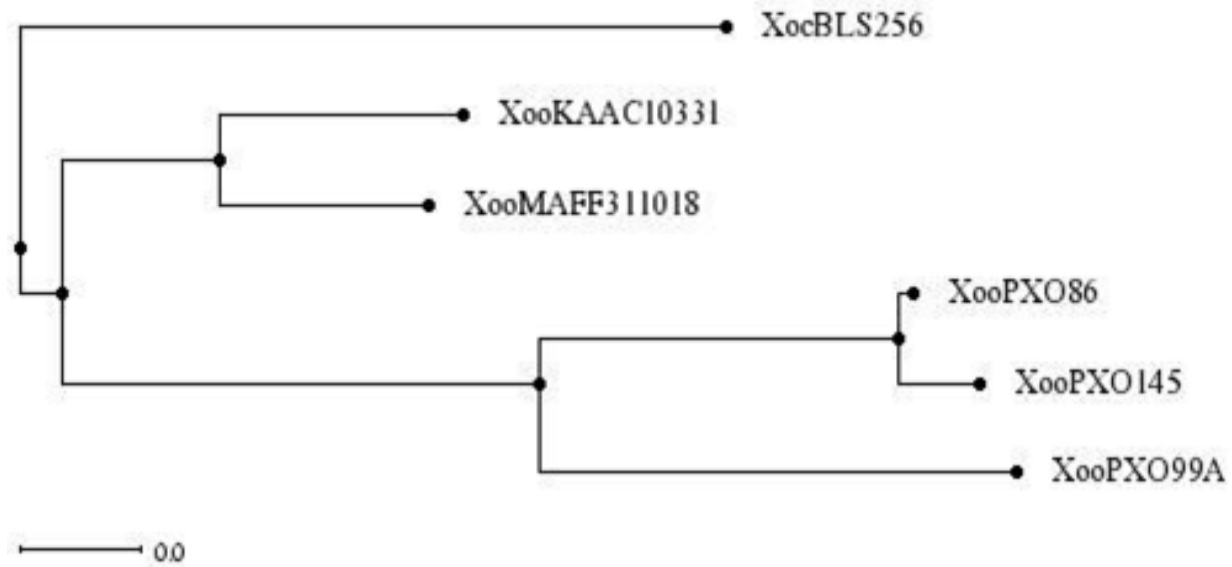
Supplementary Figure 3: Phylorelationship between PXO145 and other *Xoo* and *Xoc* strain BLS256 based on whole genome alignment generated using MAUVE v2.3.1.

Chapter 6: Conclusion and future perspectives

Climate change impact on host-plant resistance to pathogens reveals complex. This study presents a comprehensive analysis of rice response to bacterial blight under high temperature and also under drought stress treatment. The results demonstrate that high temperature or drought stress may have positive or negative effects on rice response to bacterial blight. The outcome of the interaction varied according to the presence or absence of *R* genes which recognize the corresponding avirulence protein of the pathogen, revealing the *Xa4* mediated resistance compromised under abiotic stress, while *Xa7* mediated resistance became more effective. Moreover, the results highlighted that a rice genotype with a single *Xa4 R* gene or *Xa4* combined with drought *qDTY* is affected by drought stress, while genotypes with single *qDTY* are drought tolerant, but are susceptible to bacterial blight. Comparing *qDTY* lines to IRBB NILs and *Xa4+qDTY* lines, drought *qDTY* lines were more susceptible to bacterial blight under drought stress conditions. The phenotypes of IRBB7 and IRBB67 suggest that the *Xa7 R* gene may be involved in both biotic and abiotic stress response pathways.

The use of wild type rice genotypes remains an important approach for new discoveries of resistance or tolerance genes. *Oryza glaberrima* accessions have shown their resistance enhanced under high temperature as shown in this study. Although no *Xa* resistance gene was detected, the *Oryza glaberrima* phenotype suggests that these accessions may possess BB resistance genes which could be different from the known *Xa R* genes. High temperature has shown the complementary effects of rice *Xa4* to *Xa7* in the pyramided line IRBB67 with complete restriction of *Xoo* spread *in planta* beyond the symptomatic area. Time course transcriptome profiles of IRBB67 and IR24 suggest that IRBB67 responds with higher resistance to *Xoo* infection under high temperature than under low temperature. The results from transcriptome profiling also suggest that IRBB67, in response to heat and *Xoo*, maintains homeostasis in cation efflux which supports cell membrane integrity. Additionally, regulation of glucose metabolism by *OsTPP6* gene contributes to coping with heat stress and

allows the host plant to respond efficiently to the invader. IRBB7 with *Xa7 R* gene showed resistance to BB under high temperature, but also under drought stress, suggesting that this genome may be important in breeding for abiotic and biotic stress tolerance, such as BB and high temperature or BB and drought stress tolerance. Prediction of TAL effectors from *Xoo* strain PXO145 provides additional information to understand the rice-*Xoo* pathosystem. The PXO145 genome sequence showed close relatedness to PXO86 in term of whole genome similarity and also TAL effector occurrence. Further study of the PXO145 TAL effectors' target genes would provide insight into the rice-*Xoo* interaction and provide additional information for the use of genome editing in engineering genotypes with broad spectrum resistance.

List of Publications

Gerbert Sylvestre Dossa, Ricardo Oliva, Edgar Maiss, Casiana Vera Cruz, Kerstin Wydra. High temperature enhances the cultivated African rice *Oryza glaberrima* resistance to bacterial blight. *Plant Disease*. http://dx.doi.org/10.1094/PDIS-05-15-0536-RE

Conference Papers

- **Gerbert Sylvestre C. Dossa**, R. Torres, A. Henry, R. Oliva, E. Maiss, C. M. Vera Cruz, K. Wydra. 2015. Rice response to simultaneous stress of bacterial blight and drought: Evidence from two major rice *R* genes mediated resistance to bacterial blight. The 18th International Plant Protection Congress. Berlin, Germany, August 24-27, 2015. **Poster**
- **Gerbert Sylvestre C. Dossa**, I. Quibod, A. Henry, R. Torres, A. Kumar, R. Oliva, E. Maiss, K. Wydra, J. E. Leach, C. M. Vera Cruz. 2015. Effectiveness of rice bacterial blight R gene Xa4 decreased with increase of drought and high temperature stresses. APS Annual Meeting, 2015. Pasadena, California, USA, August 1-5, 2015. **Poster**
- **Gerbert Sylvestre C Dossa**, Casiana M Vera Cruz, A. Henry, R. Torres, A. Kumar, R. Oliva, E. Maiss, K. Wydra. 2014. Would Combination of Drought QTLs and Rice *Xa* Genes Be Suitable to Control Bacterial Blight under Climate Change? Tropentag conference, bridging the gap between increasing knowledge and decreasing resources. Venue: Czech University of Life Sciences Prague, Prague, Czech Republic, September 17 - 19, 2014. **Poster**
- **Gerbert Sylvestre C. Dossa**, Kerstin Wydra, Johnny Balidion, Ricardo Oliva, Edgar Maiss, Casiana M Vera Cruz. 2014. Two Rice Major *Xa* Genes: *Xa4* and *Xa7* Showed Complementary Effects to Combined Stresses of Bacterial Blight and High Temperature, Tropentag conference, Bridging the gap between increasing knowledge and decreasing resources. Venue: Czech University of Life Sciences Prague, Prague, Czech Republic, September 17 - 19, 2014. **Poster**
- **Gerbert Sylvestre C. Dossa**, Casiana M Vera Cruz, Johnny Balidion, Ricardo Oliva, Edgar Maiss and Kerstin Wydra. 2014. Effects of temperature on plant defense response to bacterial blight in rice. XVI International Congress on Molecular Plant-Microbe Interactions (MPMI), 6-10 July 2014, Rhodes (Greece). **Poster**
- **Gerbert Sylvestre C. Dossa**, A. Henry, R. Torres, A. Kumar, R. Oliva, E. Maiss, K. Wydra, and Casiana M Vera Cruz. 2014. Climate changes and rice bacterial blight: an approach to enhance rice tolerance to abiotic and biotic stresses. 13th International Conference on Plant Pathogenic Bacteria (ICPPB), 8-13 June 2014, Shanghai (China). **Oral presentation**

Genelou Atienza-Grande, Eula Oreiro) and the former members Elenita Silab, Isabelita Ona and Flavio Maghirang for their valuable help and support.

I am extremely grateful to Dr. Valerien Pede for his strong support through my whole stay at IRRI. Without his unstinted flow of encouragement and brotherly advices, my stay at IRRI would not have been pleasant. I deeply thank Dr. Finbarr Horgan for his supports by allowing me to use his greenhouse for my experiments and for the conviviality and cheerful atmosphere.

I am extremely thankful to Gertrude, mom, dad, sisters and brothers for their love and support.

CURRICULUM VITAE

Personal information

Name   : Codjo Sylvestre Gerbert Dossa

Date of Birth   : 28.11.1986

Place of Birth   : Totchangni (Benin)

Nationality   : Beninese

Education

2012-2015   : Doctoral degree program in Horticultural Sciences at Leibniz
                          University of Hannover (Germany)

2012-2015   : Affiliated Ph.D Scholar at the International Rice Research Institute, Los
                          Banos (Philippines)

2010-2012   : Master program in Crop Protection at Georg-August University of
                          Göttingen (Germany), graduation with M.Sc.

2008-2009   : Agrar Engineer Diploma program at University of Parakou (Benin),
                          graduation with Dipl. Ing.

2004-2008   : General Agronomy Diploma (B.Sc.), University of Parakou (Benin)

1997-2004   : High School (Benin)

1990-1997   : Primary School (Benin).