Chapter 13

The Application of Genomic Approaches in Studying a Bacterial Blight-Resistant Mutant in Rice

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Additional information is available at the end of the chapter

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Abstract

Rice bacterial blight disease (BBD), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the serious diseases in most rice production regions. In this report, we screened for resistance mutants from the mutation pool of TNG67 variety derived by sodium azide (SA) mutagenesis with phenotype investigation and assisted with fluorescent detection. SA0423 is a mutant of broad range resistance against *Xoo* for many years; the resistance was studied following the concept of central dogma. The inheritance of resistance was characterized, and three QTLs were mapped onto the genome of SA0423 using simple sequence repeat (SSR) markers and R/qtl by genomic approach. In transcriptomic approach, only one differential expression QTLs (eQTLs) were identified; two differentially expressed proteins (pQTLs) were identified and genetically characterized by proteomics after *Xoo* challenged in SA0423 mutant. To improve the bacterial blight resistance, makers are developed from QTLs, eQTLs and pQTLs to pyramid the resistance genes through marker-assisted breeding in our rice breeding programs.

Keywords: rice, bacterial blight disease (BBD), resistance, mutant, genetics, genomics, transcriptomics, proteomics, marker-assisted breeding (MAB)

1. Introduction

Rice is a staple food crop and provides more than one-fifth of the calories to humans [1]. However, rice production is often challenged by bacterial blight disease (BBD), which is one of the most destructive diseases caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This disease

was first found in rice by Japanese farmers in 1884. It was not a serious problem in rice production until the release of high-yielding varieties during the 1960s–1970s [2–4]. Some field observations displayed that this disease can lead up to 50% losses in rice planting areas [3, 5]. In Taiwan, BBD often occurs in the second crop season, and its annual pathogenesis area is usually more than 20,000 hectares, accounting for about 4% of the total rice production area. Because of climate change, this disease has become more and more serious recently [6, 7]. Furthermore, International Rice Research Institute (IRRI) proposed that BBD can cause up to 70% of yield loss when susceptible varieties are grown in the environments suitable for *Xoo* pathogens (http://www.knowledgebank.irri.org/index.php?option=com_zoo&task=item&item id=806&Itemid=606).

The existing prevention of BBD includes field management, fertilizer control, pesticide application and resistance varieties with the major resistance gene (*R* gene) or the pattern recognition receptor gene (*PRR* gene). In field management, appropriate spacing could prevent rice plants from the infection of pathogens. Appropriate nitrogen fertilizer application could prevent rice plants from pathogens' infection [8]. The spray of probenazole or other chemicals might prevent the infection before transplantation, but this treatment could not be applied in tropical regions [8–10]. So far, the use of resistant varieties is considered to be the most effective strategy against this disease. In recent years, there is no specific bactericide which could effectively control BBD, and chemicals application also increases production cost, plant injury and environmental pollution. On the other hand, the evolution of pathogens increases the diversity and the difficulty in the breeding program for durable or broad-spectrum resistance [11–13]. Therefore, breeding the bacterial blight-resistant varieties is urgently required to meet the demand of a safe rice production.

Previous studies demonstrated that climate change has been proposed to affect the microflora of *Xoo* in the field and even change the life cycle and evolution of *Xoo* pathogen. Large-scale and long-term cultivation of *Xa4*-mediated resistant varieties also altered the *Xoo* population. Consequently, resistant varieties carried with only *Xa4* have become susceptible to *Xoo* in Southeast and South Asia [14]. Bacterial blight is one of the serious diseases often occurring in the second crop season (August to November) in Taiwan. Our previous results also displayed that the top 20 cultivars with large-scale cultivation in Taiwan were susceptible to *Xoo* (**Table 1**). Therefore, if the bacterial blight disease is endemic, it will cause serious loss to the rice production. These results indicated again that breeding of the bacterial blight-resistant varieties is urgently required to meet the demand of the Taiwanese rice industry.

The availability of resistant sources is the major limitation in breeding. A series of near isogenic lines (NILs) harboured various *Xa* genes (IRBB NILs) that were developed on the susceptible cultivar, IR24, at the International Rice Research Institute (IRRI) [15]. The IRBB lines, often applied in the domestic resistance breeding, were introduced and inoculated with Taiwan local pathogens to test their responses in our previous work. The results indicated that only the IRBB lines carried *Xa5* or *Xa7* showed moderate resistance while all other IRBB lines carried single *Xa* gene showed susceptibility to the local pathogens (**Figure 1**) [16]. Many of the resistance genes were introduced into the susceptible varieties by marker-assisted selection (MAS) to improve bacterial blight resistance [17, 18]. However, many of these genes lose their

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resistance due to the fast evolution of pathogen [19]. It has been reported that durable or broad-spectrum resistance can prolong the bacterial blight resistance in rice [20, 21]. Actually, broad-spectrum and durable resistance can be accomplished by the introduction of one very resistance gene and pyramiding with two to three other resistance genes [22]. However, large-scale and long-term cultivation of resistant varieties might result in changes of pathogen race in the *Xoo* population and cause the breakdown of resistance [14, 23]. These findings indicate that exploration of new germplasms with novel resistance genes become a crucial subject in breeding resistance variety.

Planting area during 2010–2015		**Variety**	**Response for** *Xoo*	
Order	**Ha**		**XM42**	**XF89-b**
1	484,063	Tai Nan No. 11	7	7
2	142,132	Taikeng No. 8	7	7
3	119,641	Taikeng No. 14	7	9
4	98,404	Taikeng No. 16	7	9
5	47,139	Taikeng No. 9	9	9
6	46,204	Taichung-Hsien No. 10	9	9
7	41,424	Taikeng No. 2	7	9
8	39,374	Kaohsiung 139	9	9
9	39,149	Taikeng No. 11	9	7
10	23,767	Taichung-Hsien No. 1	7	9
11	22,965	Taichung 192	9	7
12	19,357	Taikeng No. 4	7	7
13	19,108	Tail Nung No. 71	5	7
14	13,989	Tail Nung No. 67	9	9
15	13,867	Taikeng No. 5	7	9
16	9341	Tai Tung No. 30	9	7
17	9178	Kaohsiung 145	7	7
18	7318	Taoyuan No. 1	5	7
19	7152	Taikeng-No. 1	7	7
20	5660	Taichung-Hsien No. 17	9	9

Note: The resistance of the top 20 rice cultivars was investigated according to the Kauffman's method [66]. The lesion level can be classified by a scale of five scores, such as 1 (HR), 3 (MR), 5, 7 (MS) and 9 (HS).

Table 1. The resistance investigation of the top 20 rice cultivars grown in Taiwan.

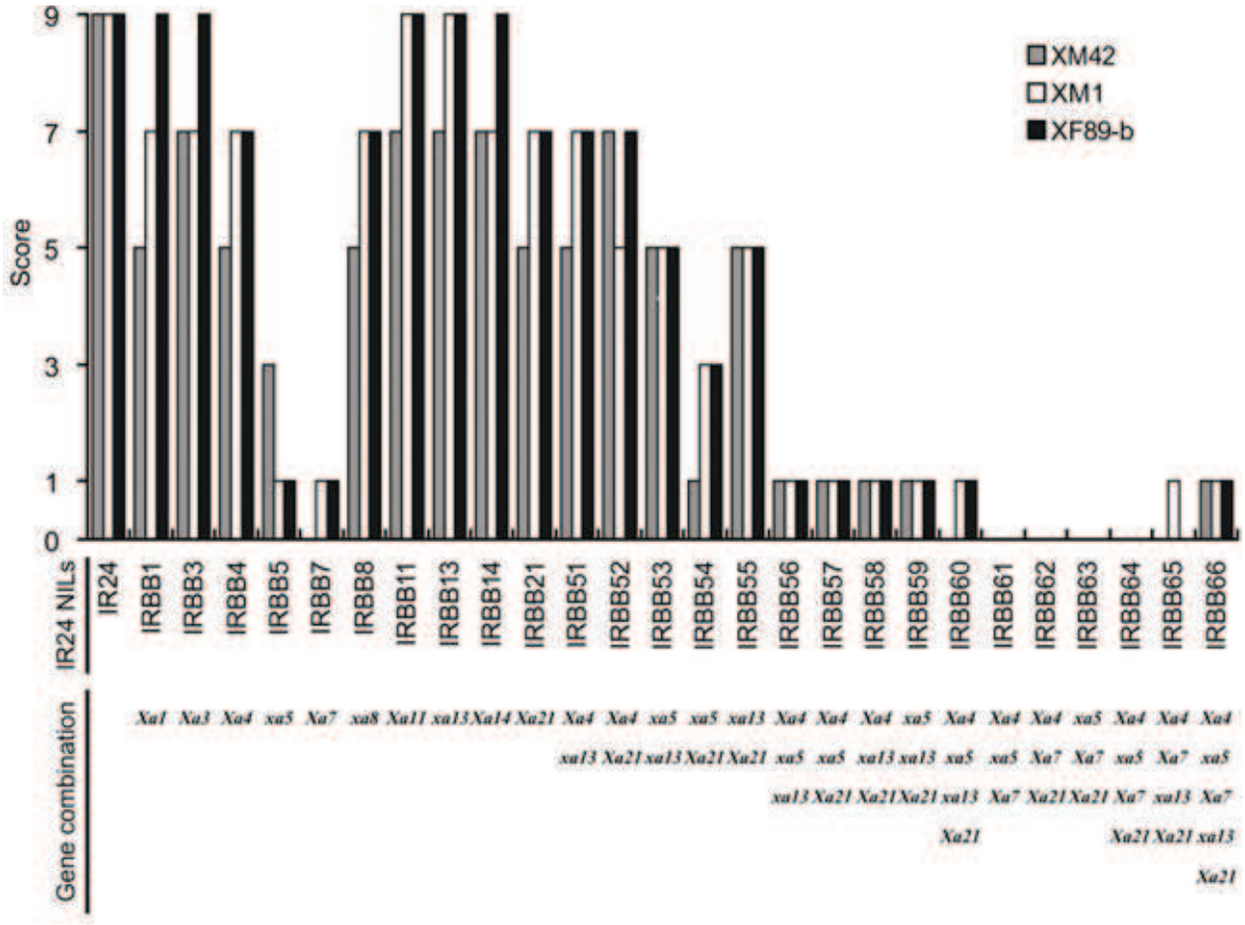

Figure 1. The resistance investigation of IRBB lines against the local pathogens in Taiwan [16].

2. Mutant screening

Sodium azide (NaN_3, SA) induced mutants can be applied to any rice breeding program at any facility, while genetically modified mutants can only be handled in the isolating facilities under the governmental regulation. A TNG67 mutant pool was developed by SA mutagenesis at the Taiwan Agricultural Research Institute (TARI) in our previous breeding program. All the mutants were screened and purified according to their morphological traits by at least 10 generations of self-crossing, selection and purification following pedigree procedures. Over 3000 pure line mutants on the same genetic background of TNG67 variety were maintained in the pool [24]. The genetic diversity of mutant lines in this pool includes disease resistance (blast, bacterial blight and sheath blight) [25], pest resistance (brown planthopper, white backed planthopper and leafroller) [26], herbicide resistance (bentazon, glufosinate and glyphosate) [27] and many agronomic traits; grain quality and morphology diversities seldom found in rice cultivars. These results suggested that the TNG67 mutant pool should have high potential in basic research as well as variety improvement [24].

To improve the bacterial blight resistance for local rice varieties, we attempted to obtain the local resistant germplasms from the selection of TNG67 mutant pool [7]. So far, at least 50 bacterial blight-resistant mutants have been selected from the mutant pool (**Figure 2**). These

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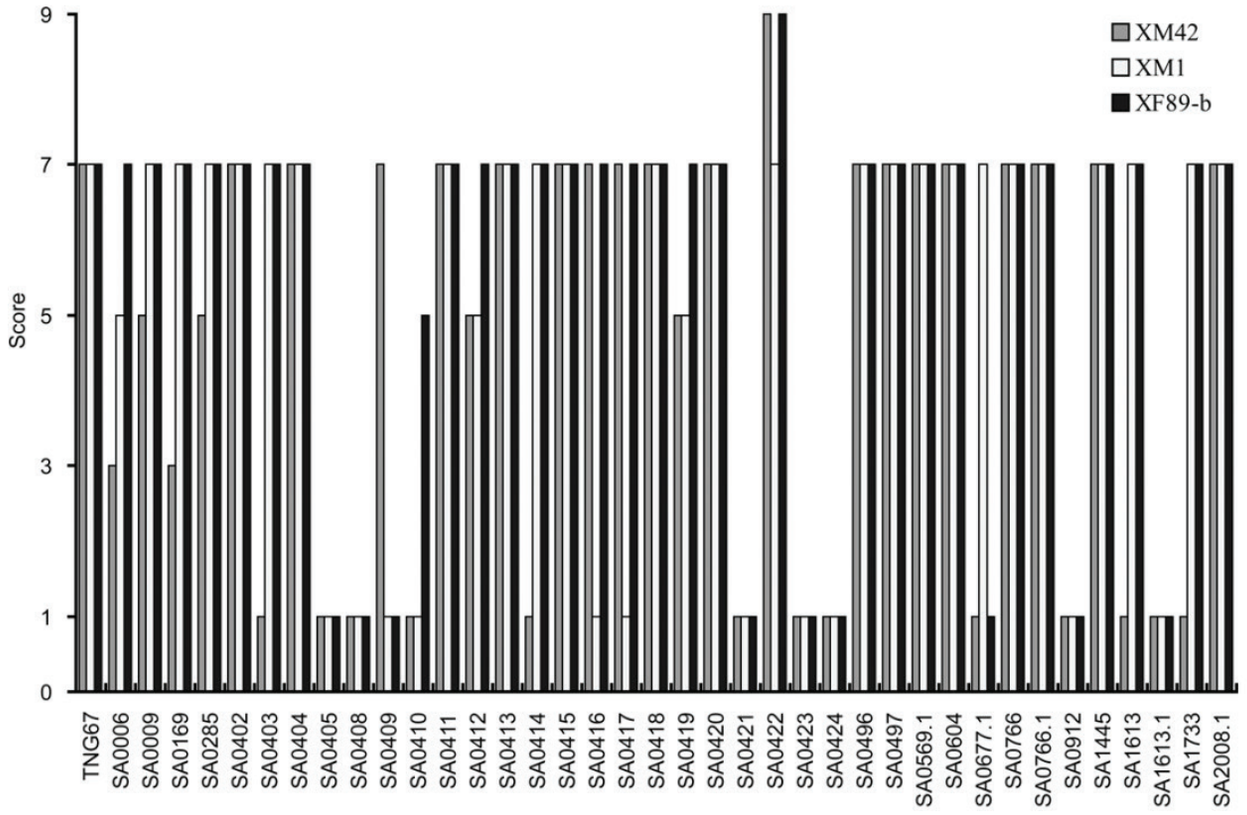

Figure 2. The screening of resistance mutants from TNG67 mutation pool by inoculation of local pathogens in Taiwan.

mutants might carry various genotypes of resistance and participate in the resistant pathway. Among them, SA0423 and SA0424 showed stable resistances against various *Xoo* pathogens for many years. The genetic analysis displayed that these two mutants might carry multiple resistant genes to confer broad-spectrum resistance and show different resistant phenotypes (data shown in the following section).

3. Genetic and mapping of resistant genes

At present, planting resistant varieties is accepted as the most efficient, reliable and economic strategy against bacterial blight. It has been proposed that the durable and broad spectrum resistance of plants was usually governed by multiple genes or quantitative trait loci (QTLs) [28]. Therefore, the discovery of novel resistance genes against *Xoo* is very important in the breeding program for disease resistance. So far, 42 resistance loci (*Xa*) for BBD have been identified and characterized [refer to: http://www.nig.ac.jp/labs/PlantGen/english/oryzabase-e/; http://www.gramene.org/; http://www. ricedata.cn/and previous reviews] [29–31]. Most of these genes were found to be controlled by dominance [32], but 14 of them, such as *xa5, xa8, xa13, xa15, xa19, xa20, xa24, xa25, xa26b, xa28, xa31, xa32, xa33* and *xa34*, were found to be regulated in a recessive manner [33, 34]. These genes distribute among 9 chromosomes of rice genome, and 16 of them are clustered on chromosome 4 (*Xa1, Xa2, Xa14, Xa31*(*t*) and *Xa38*) and chromosome 11 (*Xa3/26, Xa4, Xa10, Xa21, Xa22, Xa23, Xa30*(*t*), *xa32*(t), *Xa35*(t), *Xa36*(t) and *Xa40*), respectively. At present, *Xa1, Xa3/Xa26, xa5, Xa10, xa13, Xa21, Xa23, Xa25, Xa27* and *Xa40* have been cloned

and characterized to encode six types of proteins, i.e. NBS-LRR, receptor kinase like protein, ER membrane protein, Os8N3 protein, MtN3/saliva family member and WAK3, indicating the existence of multiple mechanisms of bacterial blight resistance in rice [35–47].

Near isogenic lines (NILs) with various *Xa* genes on the background of IR24, a very susceptible cultivar, named IRBB NILs were applied as the donor parents [15]. Besides, molecular markers linked with *Xa* genes in IRBB NILs were developed through comparative mapping strategy for improving the BBD resistance of commercial cultivars [17, 18]. However, it has been reported that the plant resistance genes may breakdown due to the fast evolution of pathogen isolates [19]. Many studies suggested that large-scale and long-term cultivation of resistant varieties may result in changes of pathogen race in *Xoo* population and caused the breakdown of resistance [14, 23]. These findings indicated that exploration of new resistance genes has become an important subject for breeding resistance variety.

Among the previously selected resistant mutants, SA0423 shows a stable resistance to Taiwan local pathogens for years. Hence, their genetic properties and BBD resistance genes were characterized in our team. Except for the bacterial blight resistance, SA0423 also has thinner leaf blades, shorter plants, more erect plant type and less tiller number than its mutagenesis parent, TNG67 (**Figure 3**). A strong and stable Taiwanese epidemic pathogen, *Xoo* XF89b, has been used for genetic analysis and mapping the bacterial blight-resistance genes. Taichung Native 1 (TN1), a very susceptible *indica* rice cultivar, was used as the recipient parent. The cross TN1/SA0423 was made to generate F_1 and F_2 materials for genetic analysis and mapping of resistant genes. After pathogen infection, the lesion lengths of TN1, SA0423 and TN1/SA0423 F_1 were 17.2 ± 1.1, 1.2 ± 0.7 and 3.4 ± 0.9 cm, respectively, indicating that

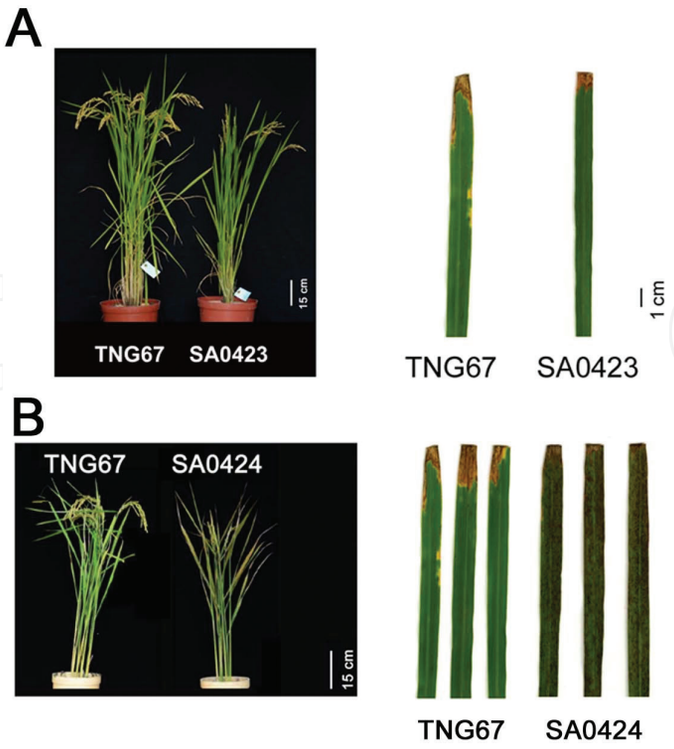

Figure 3. Morphology of TNG67, SA0423 and their disease responses at 28 days after inoculation (DAI) with Taiwanese *Xanthomonas oryzae* pv. *oryzae* XF89b.

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the BBD resistance of SA0423 is partial dominance (**Figure 4**). The lesion lengths of the TN1/SA0423 F_2 population showed a continuous distribution (**Figure 5**) and indicated that the disease resistance of SA0423 is controlled by multiple genes or quantitative trait loci (QTLs).

A linkage map covering 12 chromosomes with an average distance of 11.2 cM was constructed and applied to map the resistance of SA0423 using 361 TN1/SA0423 F_2 individuals [48]. QTL analysis was performed using the R program language platform (version 3.1.0; http://www.r-progect.org/) with an add-on package, qtl [46, 47]. Three QTLs are detected on chromosomes 11, 8 and 6 and account for 21.1, 11 and 9.6% of the observed phenotypic variance, respectively (**Table 2** and **Figure 6**). Three QTLs are localized to 6, 7 and 14 cM intervals, respectively; they contribute to approximately 47% of the total phenotypic variation (resistance) and no epistatic effect could be detected among them [48].

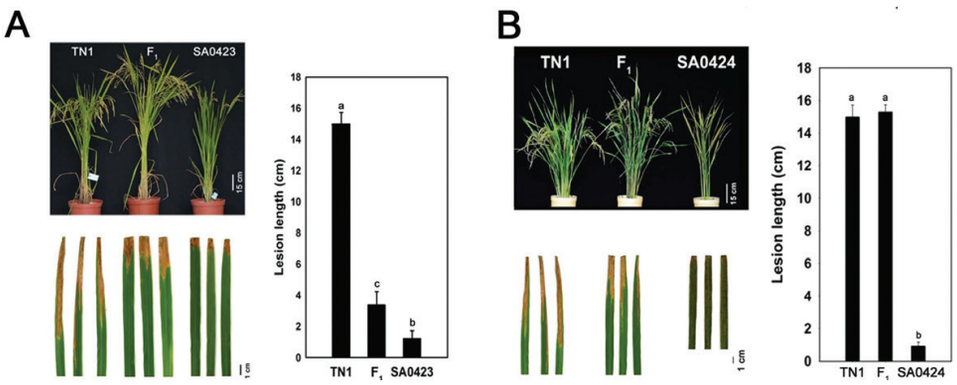

Figure 4. Morphology of TN1, SA0423 and their F_1 individual, and the disease response at 28 days after inoculation (DAI) against Taiwanese *Xanthomonas oryzae* pv. *oryzae* XF89b (A, upper panel). The lower panel of (B) shows the morphology of leaf lesion at 28 DAI; left panel shows the leaf lesion (cm) investigated at 28 DAI. Error bar is the standard error of mean (n = 3). Means with the same letter are not significantly different at 5% level by LSD test.

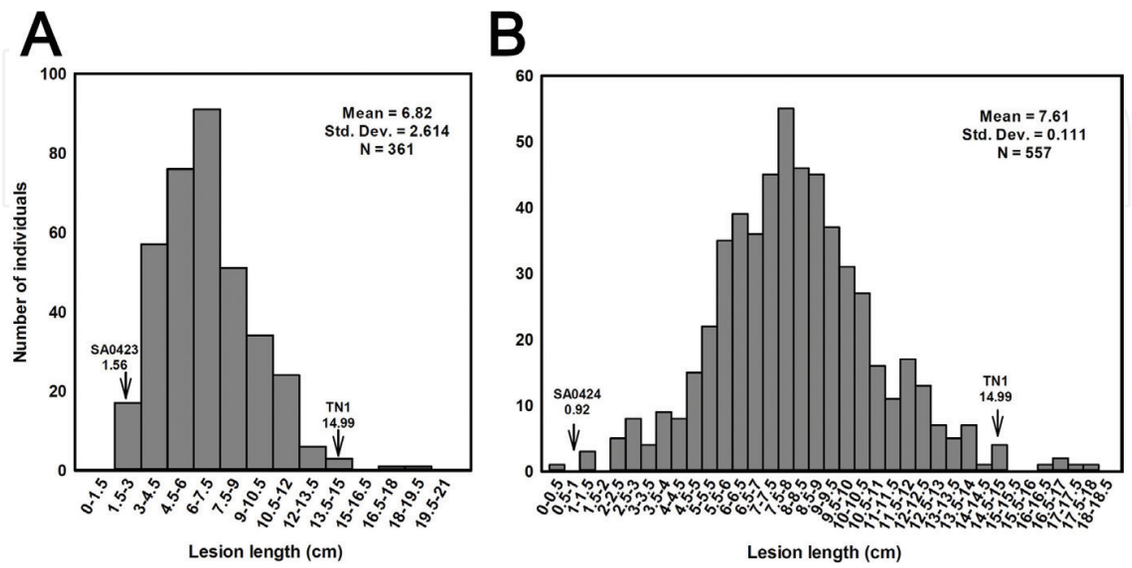

Figure 5. Distribution of lesion length (cm) after inoculation with Taiwanese *Xanthomonas oryzae* pv. *oryzae* XF89b in an F_2 population from the cross, TN1/SA0423.

QTL	Chr.	QTL (Confidence interval) (cM)	LOD	Phenotyping variance (%)	Additive effect	Dominance effect
qBBR11.1 (Q1)	11	124 (121–127)	26.60	21.10	–1.64	–0.44
qBBR08.1 (Q2)	8	39 (34–41)	15.04	11.04	–1.20	–0.82
qBBR06.1 (Q3)	6	120 (111–125)	13.20	9.58	–1.13	0.79

Note: QTLs are labelled according to the principles of previous publications [67, 68].

Table 2. Putative QTLs were identified from the F_2 population of TN1/SA0423.

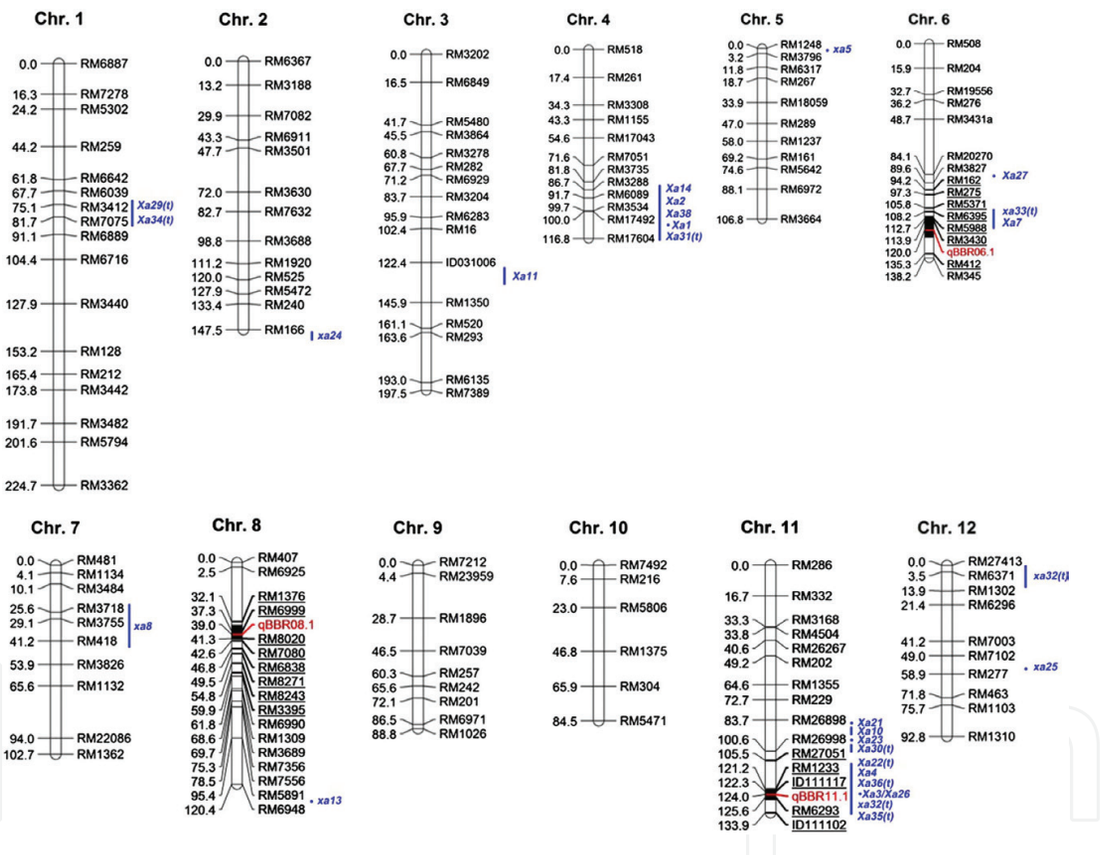

Figure 6. The linkage mapping of SSR/InDEL markers and SA0423 resistance QTLs in the F_2 population of TN1/SA0423. The markers and genetic distances (cM) are labelled to the right and left of the chromosome, respectively. The QTLs are coloured with red, and other published genes and QTLs associated with BBR are labelled as blue dots and lines, respectively.

4. Transcriptomic studies

According to QTL analysis, all the three identified QTLs contribute to 47% of the resistance indicating that other resistance genes may exist in SA0423 [48]. Therefore, the transcriptomes of TNG67 and SA0423 were determined by microarray technologies to explore the bacterial-resistant genes in SA0423.

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For a precise and non-destructive investigation in the infection process of bacterial blight pathogen after inoculation, a *Xanthomonas fluorescent* expression plasmid, pRBBZsGFP, was constructed with a strong fluorescent gene *ZsGFP* and the pBBR1MCS vector for simultaneous detection of bacterial blight pathogen infection and the gene expression [49]. Pathogens infection with $XF89b_{ZsGFP}$ was conducted on the dark-treated albino seedlings of TNG67 rice variety; the multiplication and colonization of $XF89b_{ZsGFP}$ could be detected in 0.5 hour after inoculation, and the maximum fluorescence was observed on the same leaf in 1 hour after inoculation (**Figure 7**). However, the fluorescence was reduced in the following time course indicating that the multiplication and colonization of $XF89b_{ZsGFP}$ might be suppressed by the endogenous immune system of rice. At 7 DAI (days after inoculation), the stronger fluorescence was observed again on the same leaf and extended continuously to the leaf base, suggesting that the rice immune system was broken down by the $XF89b_{ZsGFP}$.

After the infection of *Xoo* XF89b, RNA samples prepared from the leaves of TNG67 and SA0423 collected at 0, 0.5, 1, 2 and 6 hours, respectively, were applied in the transcriptomic analysis with Agilent Oligo Microarray (60K, custom-made, Agilent Technologies) [50]. The results demonstrated that 2727, 3585 and 18,432 differentially displayed transcripts were identified in SA0423, TNG67 and in both, respectively. Among them, 58 genes involved in SA0423 resistance were further conducted by bioinformatics strategies [refer to: http://www.nig.ac.jp/labs/PlantGen/english/oryzabase-e/; http://www.gramene.org/; http://www.ricedata.cn/and previous reviews] [29–31] as well as "plant-pathogen interaction" pathway (http://www.genome.jp/kegg), and clustered with BioLayout Express^{3D} [51]. By confirming with real-time RT-PCR, 17 resistance gene candidates (**Table 3**) were selected for bioinformatics analysis, they have been proposed to be involved in plant-pathogen interaction pathway, biosynthetic pathway of plant hormones, autophagy and signal transduction prior to the induction of plant immune system [52].

To confirm the function of the identified genes from transcriptomic analysis, the SSR markers flanking in 5 cM region of these genes were retrieved from GRAME web site, screened for the polymorphic markers between TN1 (the susceptible parent) and SA0423 (the resistant parent), and then genotyping was performed in the F_2 population [53]. Simultaneously, the disease lesion of F_2 individuals was investigated to represent the resistance phenotype after the inoculation of *Xoo* XF89b. The linkage between genotype and phenotype was conducted using R/qtl software by the single marker regression model. The results displayed that only RM6838 adjacent to *Ankyrin* showed a significantly high LOD (6.86) (**Table 4**) indicting that *Ankyrin* (LOC_Os08g15840) has a high potential to be involved in the resistance of SA0423. The bioinformatics analysis shows that this Ankyrin protein shares 76% similarity with the *Arabidopsis* RING type ligase, XBAT32, of an XB3 family. In *Arabidopsis*, Ankyrin has been proposed to negatively regulate 1-aminocyclopropane-1-carboxylate synthase (ACS), a key enzyme involved in the ethylene biosynthesis pathway, and then compromised immune system [54]. The real-time RT-PCR displayed that the expression level of *Ankyrin* in SA0423 was lower than that of TNG67, and higher expression levels of *OsACS1* and *OsACS3* were found in the BBD resistance mutant, SA0423 (**Figure 8**). These findings showed that ethylene metabolism may involve in the disease resistance of SA0423. A total of 15 mutations in the coding region resulting two mutation residues, Ser280Pro and Thr381Ala, were discovered in the *Ankyrin* of SA0423 through cloning and sequencing (data not shown). At the same time, the transgenic rice plants with less expression of *ankyrin* showed a significant

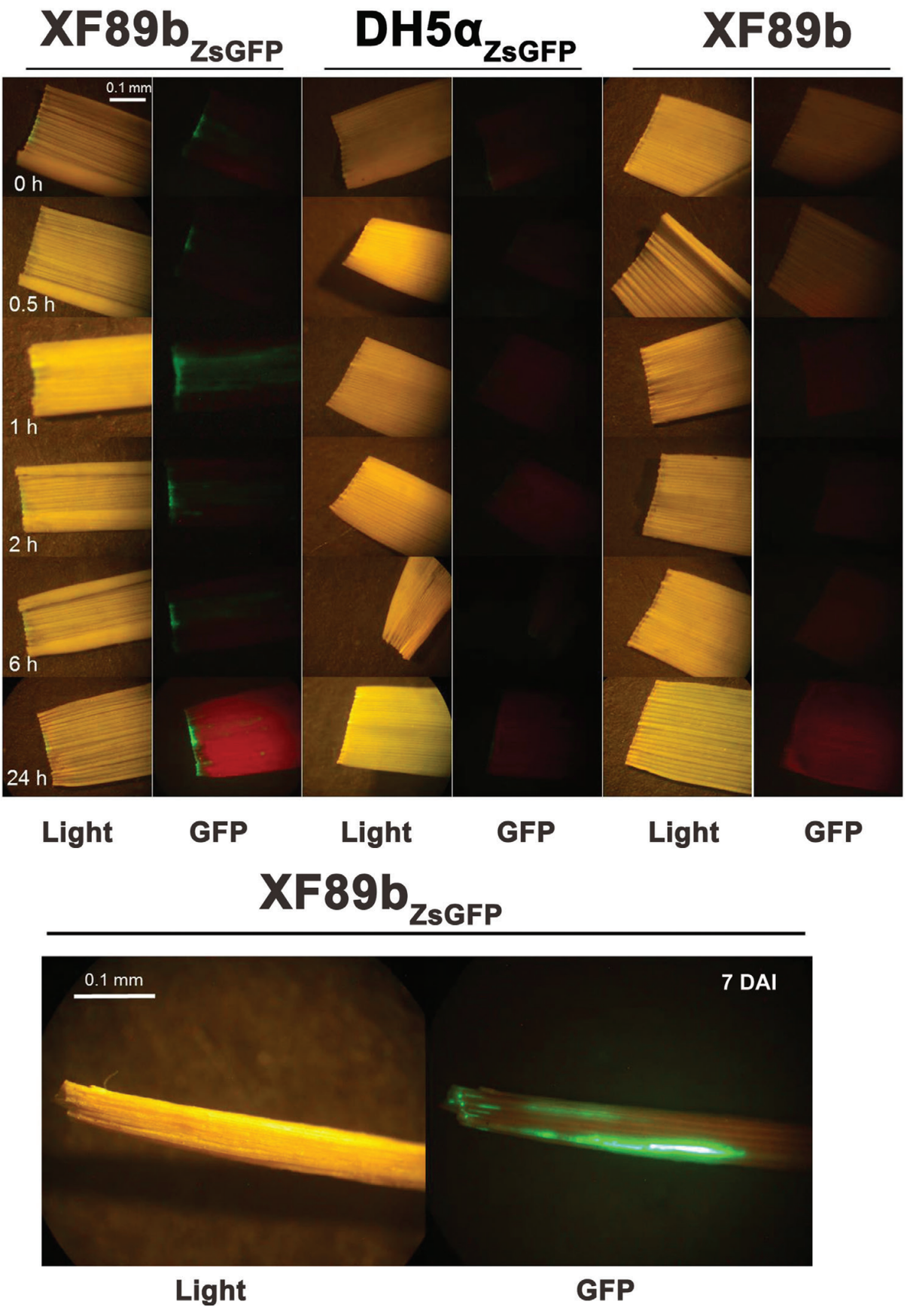

Figure 7. Visualization of *X. oryzae* pv. *oryzae* and *E. coli* expressing *GFP* in the dark-treated albino TNG67 seedlings.

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Gene name	Gene ontology	
*Ankyrin*Ankyrin repeat-rich protein	BP	Cellular process, biosynthetic process, protein modification process, post-embryonic development, anatomical structure morphogenesis, response to endogenous stimulus
	MF	Binding, protein binding, catalytic activity,
*ATG1*ATG1	BP	Cellular process, cellular component organization, protein modification process
	CC	Plasma membrane
	MF	Molecular function
*CaM_Chr.1-1*OsCam1-3-Calmodulin	BP	Biological process, response to abiotic stimulus, post-embryonic development, signal transduction
	MF	Binding, protein binding
*CaM Chr.1-2*OsCam3-Calmodulin	BP	Biological process, response to abiotic stimulus, post-embryonic development, signal transduction
	MF	Binding, protein binding
*CaM_Chr.2*EF hand family protein	BP	Protein modification process, biosynthetic process
	CC	Cytoplasm
	MF	Binding
*CaM_Chr.5*OsCam2-Calmodulin	BP	Signal transduction
	CC	Plasma membrane
	MF	Signal transducer activity, binding, protein binding
*CMPG*Immediate-early fungal elicitor protein CMPG1	BP	Protein modification process, biological process
	CC	Intracellular
	MF	Catalytic activity, binding
*DUF26*Domain of Unknownfunction 26-lc	BP	Protein modification process, cellular process, metabolic process
	CC	Plasma membrane
	MF	kinase activity, protein binding, cellular process,
*FMO*Flavin-containing monooxygenase family protein	BP	Cell death, signal transduction, metabolic process, response to biotic stimulus, cellular process, response to stress
	CC	Endoplasmic reticulum, membrane, cell
	MF	Nucleotide binding, catalytic activity, binding
*JOM*JasmonateO-methyltransferase	BP	Multicellular organismal development, cellular process, metabolic process
	CC	Cellular component
	MF	Binding, protein binding, transferase activity
*PxMP*Peroxisomal membraneprotein	BP	Biological process
	CC	Peroxisome, membrane
	MF	Molecular function

Gene name	Gene ontology	
*SAM*SAM dependent carboxyl methyltransferase	BP	Biological process, cellular process, metabolic process
	CC	Cellular component
	MF	Transferase activity
*SNARE*SNARE associated Golgiprotein	CC	Cytosol
*Ubi*Ubiquitin family protein	MF	Molecular function
*Xa2*OsSAUR21—Auxin-responsive SAUR gene family member	BP	Response to endogenous stimulus
	MF	Molecular function
*Xa25*Nodulin MtN3 family protein	BP	Biological process, cellular process, transport
	CC	Plasma membrane, membrane, cell
	MF	Transporter activity
*xa5*Transcription initiation factor IIA gamma chain	BP	Biosynthetic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
	CC	Nucleoplasm

Note: BP, biological process; CC, cellular component; MF, molecular function.

Table 3. The resistance gene candidates identified from transcriptomic analysis in a bacterial blight-resistant mutant, SA0423.

Gene	Chromosome	Position (cM)	Marker	LOD[z]
CaM_Chr.1-1	1	50.8	RM6039	0.8941
CaM Chr.1-2	1	50.9	RM572	1.7618
CaM_Chr.2	2	25.3	RM6378	0.0886
CMPG	2	131	RM13938	0.5618
Xa2	4	107.4	RM17492	1.2685
JOM	4	120.3	RM17604	0.5006
xa5	5	3	RM17741	0.2725
CaM_Chr.5	5	104.7	RM6972	0.5717
FMO	6	19.1	RM19556	0.2034
SAM	6	33.5	RM276	0.1920
DUF26	7	73.2	RM3826	0.1547
SNARE	7	116.1	RM1362a	0.2325
Ankyrin	8	42.9	RM6838	6.8579
ATG	10	73.7	RM5471a	0.0479
Ubi	10	99.8	RM147	0.2880
Xa25	12	57.9	RM28157	0.4657
PxMP	12	69.6	RM519	0.2940

Note: [z] LOD, $\log_{10}$ of odds.

Table 4. Linkage analysis between the resistance gene candidates and the resistance trait of SA0423 by R/qtl.

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resistance against *Xoo* XF89b isolate. Therefore, Ankyrin is considered to be one of the expression quantitative trait loci (eQTLs) involved in the bacterial blight resistance of SA0423.

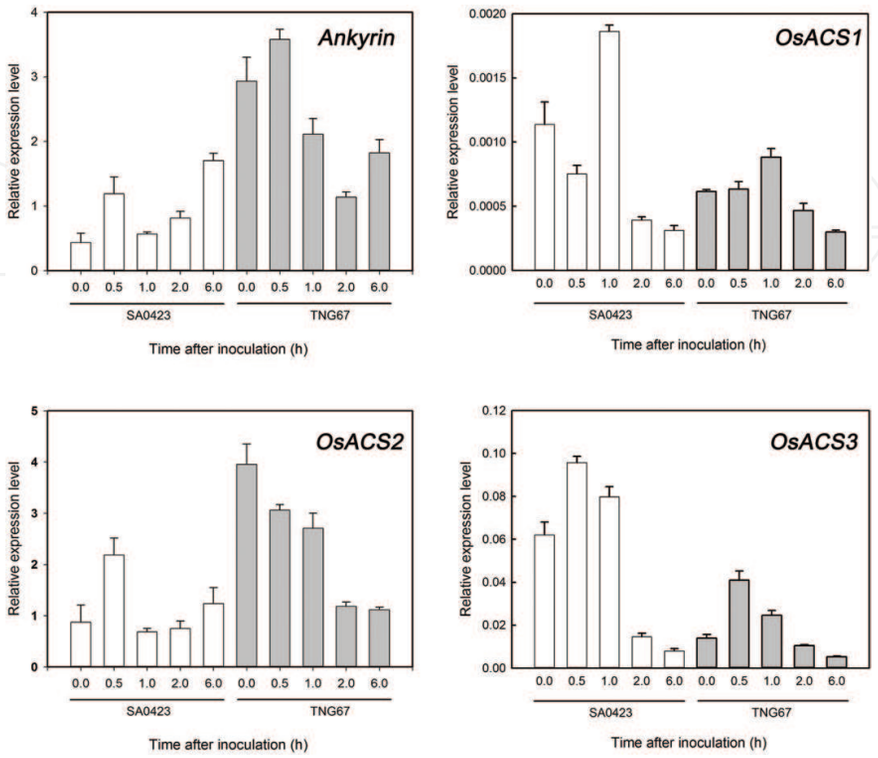

Figure 8. Quantitative analysis of mRNA expression of *Ankyrin* and *OsACS* homologs in TNG67 and SA0423 after inoculated with *Xoo* XF89b by using real-time RT-PCR.

5. Proteomics study

Proteomics technology provides a direct investigation of proteins which may participate in rice disease resistance. In previous studies, plasma membrane (PM) proteomic analysis of the genetically modified rice suspension cells with *Xa21* demonstrated that PM-associated ATPase, phosphatase, hypersensitive-induced response protein, prohibitin, zinc finger/C2 domain protein, universal stress protein and heat shock protein might be involved in the early immune response against compatible and incompatible *Xoos* [55]. A proteomic analysis of Java 14 seedling revealed that 20 differentially displayed proteins were responded to bacterial inoculation and categorized into energy, metabolism and defence pathways [56]. These proteomic studies were conducted at 0, 12, 24 even 72 hours after inoculation [55, 56] whereas considering the rapidity of defence observed in other plant-pathogen interactions [57] and the short life cycle of *Xoo*, it is expected that *Xoo* might induce rice reprogramming immediately after pathogen infection.

A comparative proteomics analysis was conducted to characterize the proteomic profiling in leaves of TN1 (as a susceptible control), TNG67 and SA0423 after the infection of *Xoo* XF89b at 0, 6, 48 and 72 hours after pathogen inoculation (**Figure 9**). There were 60, 38 and 96 differentially displayed protein spots identified only in SA0423, TNG67 and TN1, respectively, by the separation of two-dimensional gel electrophoresis (2-DE). Finally, a total of 150 disease resistance-related proteins were identified from these protein spots through the ESI-Q-TOF mass

spectrometry (MS) analyses. Ten resistance protein candidates (**Table 5**) were then determined by bioinformatics approach including annotation of metabolic pathway, comparative mapping analysis with published resistance loci [refer to: http://www.nig.ac.jp/labs/PlantGen/english/oryzabase-e/; http://www.gramene.org/; http://www.ricedata.cn/and previous reviews] [29–31] as well as 'plant-pathogen interaction' pathway (http://www.genome.jp/kegg), and clustered with BioLayout Express^{3D} [51]. These candidates were proposed to be involved in ascorbate, glyoxylate and glutathione, and oxidative phosphorylation metabolisms.

The candidate genes identified from proteomics approach were genetically confirmed as previously described, the SSR markers flanking in 5 cM region of them were retrieved from GRAMENE web site, and screened for polymorphism TN1 (the susceptible parent) and SA0423 (the resistant parent). Genotyping analysis was performed in 94 TN1/SA0423 F_2 individuals using the polymorphic markers. The lesion of these F_2 individuals was investigated after the inoculation of *Xanthomonas oryzae* pv. *oryzae* XF89b as the resistance phenotype. The linkage between genotyping and resistance was analysed by MapDisto according to Lorieux's protocol [60]. The result displayed that only RM5970 adjacent to the putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (BIPM) and RM14099 adjacent to aspartate aminotransferase (AST) showed significant association with the SA0423 resistance (**Table 5**). BIPM has been proposed to have some important roles in glycolysis, stomatal movement, vegetative growth and pollen production in *Arabidopsis* [61], but it was usually found to be differentially expressed under abiotic or biotic stress [62–64]. AST was found to be up-regulated in rice *spotted leaf 5* (*spl5*) mutant that showed spontaneous HR-like lesions on its leaves, and a broadly enhanced resistance against rice blast and bacterial blight pathogens [65]. Based on these findings, BIPM and AST are found to have high potential to participate in the resistance mechanism of SA0423. These results provide novel insights into the molecular mechanisms of rice response to *Xoo* infection and discovery of new resistance genes as the basis for application in molecular breeding. Therefore, both BIPM and AST are considered to be the proteomic quantitative trait loci (pQTLs) for the bacterial blight resistance in SA0423.

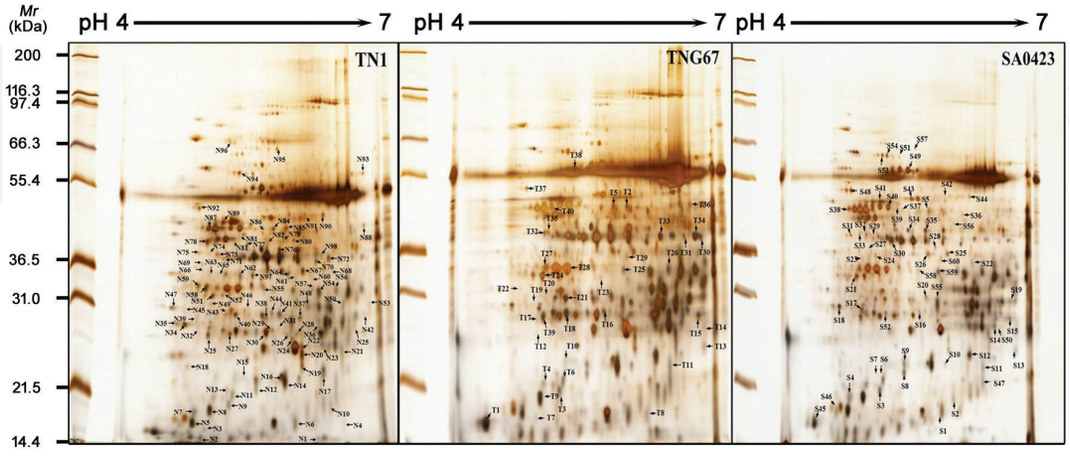

Figure 9. 2-DE image analysis of rice leaf proteome under *Xoo* XF89b infection. Total leaf proteins were extracted and separated by 2-DE then stained with sliver staining according to the previous protocol [58, 59]. An equal amount (200 μg) of the total proteins was loaded on each gel strip. The differentially expressed resistance-related proteins in TN1, TNG67 and SA0423 are marked as N, T and S, respectively.

Gene	Marker	hmzA	hmzB	htz	n	m(hmzA)	m(hmzB)	m(htz)	*R2*	*A*	*D*	*|D/A|*	*F*	*p*	
L-ascorbate peroxidase 1, cytosolic (APX1)	RM7197	30	35	29	94	6.56	8.28	7.57	0.05	0.862	0.15158	0.18	2.5	0.08795	
Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (BIPM)	RM5970	16	28	50	94	8.29	8.841	6.52	0.12	0.276	–2.04831	7.41	6.05	0.00337	* *
Glyceraldehyde-3-phosphate dehydrogenase, putative, expressed (G3PD)	RM14336	23	23	40	86	6.75	7.833	7.4	0.02	0.539	0.10949	0.2	0.77	0.46538	
Aspartate aminotransferase (AST)	RM14099	42	22	14	78	6.61	9.433	7.29	0.14	1.412	–0.73185	0.52	6.34	0.00281	**
2,3-bisphosphoglycerate-independent phosphoglycerate mutase, putative, expressed (BIPME)	RM8084	24	52	18	94	6.74	8.134	6.73	0.05	0.695	–0.70982	1.02	2.34	0.10172	
Triosephosphate isomerase (TRI)	RM24714	16	33	45	94	7.43	8.23	7.01	0.03	0.401	–0.81535	2.03	1.44	0.24307	
30S ribosomal protein S4, chloroplastic (RP30S)	RM5579a	18	36	40	94	6.59	8.479	7.05	0.06	0.943	–0.48336	0.51	3	0.05439	
Fructose-bisphosphate aldolase, chloroplastic (FBPA)	RM26143	10	46	35	91	8.09	7.596	7.31	0.01	–0.247	–0.53336	2.16	0.24	0.78348	
Cysteine synthase (CYS1)	RM520	23	28	43	94	7.8	6.835	7.8	0.02	–0.483	0.47717	0.99	0.91	0.40443	

Table 5. Linkage analysis between the resistance protein candidates and the resistance trait of SA0423 by MapDisto. The "**" was indicated "statistical significance" ($p \leq 0.05$).

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6. Conclusion

Breeding resistance variety is the best strategy to overcome the bacterial blight disease damage in rice and is a very challengeable work. Availability of resistant genotype is the major limitation to the resistance improvement. However, plant disease resistance is a complex trait usually regulated by QTLs, epistatic effect, and influenced by the interactions among pathogen, host and environment.

In this review, a durable resistance mutant, SA0423, was firstly obtained from screening a sodium azide-induced mutation pool on the genetic background of TNG67 rice variety. The genomic approaches and technologies were conducted according to the concept and flow of Central Dogma. In the genomic study, the inheritance and gene corresponding to the BBD resistance of SA0423 was conducted. Linkage maps were constructed, and three QTLs (qBBR06.1, qBBR08.1 and qBBR11.1) for resistance were identified from SA0423. Meanwhile, the linkage markers for each QTL were developed according to the linkage map for marker-assisted breeding.

The transcriptomics and proteomics technologies were applied to identify the expressed genes and proteins corresponding to the pathogen inoculation for BBD resistance on SA0423. The differential displayed genes (or proteins) were annotated by blast with the gene database (NCBI and GRAMENE websites), and then their putative biological functions or the participating pathways were predicted by GO analysis. Besides, they were compared with the published resistance genes in *Xa* locus or putative rice 'plant-pathogen interaction' pathway to confirm the resistance genes or pathway in SA0423. The results demonstrated that 17 candidate genes (eQTLs) and 10 candidate proteins (pQTLs) might be involved in SA0423 resistance mechanism. The association between these candidates and SA0423 resistance was further evaluated by integration of genotyping and phenotyping of TN1/SA0423 F_2 progeny through genetic approach. Both genomic and bioinformatics approaches were integrated to confirm the function and genetic relationship of the candidate genes with BBD resistance. The final results suggested that only one major expression QTLs (eQTLs) [53] and two protein QTLs (pQTLs) (Lin et al., 2017, paper in preparation) are confirmed to confer the resistance of SA0423. It is worth to note that both the eQTLs and pQTLs identified in this study are not identified in the genetic mapping approach, and the products of eQTLs were not found in the protein profiling (pQTLs), and vice versa. These results showed that the genomic approach alone cannot unravel all the genes involved in the disease resistance of SA0423.

Phenomics or phenotype can provide the solid evidence for gene function. Our previous findings were tested through transgenic approach as well as marker-assisted backcrossing (MABC). The transgenic rice plants with less expression of *ankyrin* and *BIPM* showed significant resistance against *Xoo* XF89b isolate, supporting that these two eQTLs are involved in BBD resistance in rice. The identified resistance QTL, qBBR11.1, of SA0423 was introduced and improved the BBD resistance in a very susceptible indica variety, TCS10, through MABC approach. These results demonstrated that the QTLs identified from genomic, transcriptomic and proteomic approaches can be practically applied to improve the BBD resistance in rice breeding program.

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