AvrBs3-like genes and TAL Effectors Specific to Race Structure in *Xanthomonas axonopodis* pv. *glycines*

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Received: 12 November 2017 Accepted: 13 December 2017

ABSTRACT

The *avrBs3*-like genes designated *avrXg3* and *avrXg2* cloning from representative strains of *Xanthomonas axonopodis* pv. *glycines* (Xag), a causal agent of bacterial pustule disease were harbored by race 1 (new strain SP4); and race 2 (recent strain 12-2) respectively, whereas *avrXg1* (reported by Athinuwat *et al*., 2009) was uniquely detected in race 3 (recent strain KU-P-SW005) that characterized based on their ability to confer virulence toward soybean cultivars. Most of the new strains collected in this study (169 strains) belonged to race 2 (62.72%), the highly virulence strain group. Structural analysis of transcription activator-like effector (TALe) genes determined to particular races was shown that these race strains including race 1 strain SP4, race 2 strain 12-2, and race 3 strain KU-P-SW005 carried TAL effectors of 6 (*tal1a*, *tal1b*, *tal1c*, *tal2a*, *tal2b*, and *tal3*), 6 (same as strain SP4), and 5 (same as strain SP4 but *tal1c* absent) *tale* gene members that guided by 15-23, 15-23, and 15-20 repeat variable diresidues (RVDs) respectively. The individual mutants of each *tal* genes (17-*tal* derivatives) revealed that only *tal2b* in each race strains (XagSP4/*tal2b*, Xag12-2/ *tal2b*, XagKU-P-SW005/*tal2b*) exhibited a positive regulator of similar defense-related phenotype on soybean cultivars of Xag/*avrBs3*-race typing group, suggesting *R* genes mediated specifically recognition of directly *tal2b* induction. Phylogenetic construction and hybridization analysis demonstrated high potential in rapid genotyping of many Xag-race strains using primers and DNA probes designed from either specific *avrBs3* or *tale*-like genes characterized. Genetic mapping and RT-PCR evidence showed that soybean *GmLOB1* resistance genes mediated recognition of Xag12-2/*tal2b*-expressing strain (highly virulent strain) to give disease development on resistance cultivar, Williams82. The *tal2b* and non-TALe type of type III effectors that may establish a co-opted activity targeting host *R* genes other than *GmLOB1* should be experimentally-further analyzed. This is the first description of TALes indicating their translocation through the Xag-T3SS which the work reports two new *avrBs3*-like genes and seventeen *tale*- like genes characterized from soybean-3 pathogenic Xag race strains.

Keywords: Soybean, bacterial pustule, *tal* gene, race-cultivar specific

Thai J. Agric. Sci. (2017) Vol. 50(3−4): 121−145

INTRODUCTION

Xanthomonas axonopodis pv. *glycines* (Xag) causes bacterial pustule on soybean by using effectors secreted from the type III system to block plant defense mechanism. The disease typically occurs on foliage. Symptoms of this disease are including small, pale green spots, which may develop into large necrotic lesions and leading to premature defoliation (Narvel *et al*., 2001). The bacteria are seed borne and can be transmitted to the seedling. Interestingly, the pathogenicity of Xag takes place upon the role of *hrp* system and the delivery of effectors into plant cells (Kim *et al*., 2003). This disease can be severe especially when it infect soybean at 30-40 days old because it's affected to both quality and quantity in soybean production. The prevalence and severity of this disease is varies from year to year because of differences in weather patterns and cultivars of soybean (Prathuangwong *et al*., 1996). The disease causes severe losses when infect the susceptible cultivars, such as local SJ family (Prathuangwong, 1984; Prathuangwong *et al*., 1990; Prathuangwong *et al*., 1996).

The race-cultivar is specific interaction between pathogen and its plant host which described by gene-for-gene concept (Flor, 1971). Gene for gene concept involved resistance (*R*) gene in the host plant and avirulence (*avr*) gene in the pathogen. In several cases of bacteria *avr* genes, an effector effect has been demonstrated and its interference with defense mechanism in plant (Chisholm *et al*., 2006; Mudgett *et al*., 2005; White *et al*., 2000). Many *avr* genes have been shown their contributions to pathogen aggressiveness (Bai *et al*., 2000) and in some case inhibited development of non-specific hypersensitive response in host plant (Chen *et al*., 2000). Diversity of Xag in Thailand can be categorized into 3 races based on their virulence toward specific soybean cultivar. Race 1 is only induced hypersensitive reaction (HR) within 48 hrs in all soybean cultivars. Race 2 caused diseases within 48 hrs on three soybean cultivars including Williams 82, Spencer, and PI520733. On the other hand, race 3 elicited HR on the resistant cultivar (Williams 82) and caused pustule disease on pustule-susceptible cv. Spencer and PI520733. In 2009, Athinuwat *et al.* reported that the *avrBs3*-like genes, *avrXg1,*was identified as race 3 strains KU-P-SW005. Thus, compatible and incompatible reaction on its host and demonstrate multifunction of *avrXg1,* is dependent on pathogen and plant genetic backgrounds.

TAL (transcription activator-like) effectors are transcriptional activators that inject into plant cells by plant pathogenic members of the bacterial genus *Xanthomonas*. TAL effectors are delivered to the nucleus of the host cell during infection via bacteria type III secretions system (Szurek *et al*., 2002). The effector will bind to specific sequences of gene promoters and transcriptionally activate gene expression in host plant (Kay *et al*., 2007). In addition, TAL effectors activated as virulence factors by turning on genes necessary for disease susceptibility (*S* genes), or in a few cases, as avirulence factors by activating a gene that confers resistance (*R* gene) (Antony *et al*., 2010; Boch and Bonas, 2011; Bogdanove *et al*., 2010). Interestingly, TAL effector genes are limited to only members of the genus *Xanthomonas* and *Ralstonia* (Heuer *et al*., 2007). In *Xanthomonas* species, TAL effectors are a significant factor for an ability to infect important crops such as rice, soybean, citrus, pepper, and tomato (Al-Saadi *et al*., 2007; Kay *et al*., 2007; Athinuwat *et al*., 2009; Domingues *et al*., 2010). The first member of TAL effectors that have been identified is *avrBs3*-like genes from *X.campestris* pv. *vesicatoria*, which found to harbor avirulence activity in pepper with the *Bs3* resistance gene. The homologous effector genes *avrXa7* and *avrXa10* were subsequently characterized in *X. oryzae* pv. *oryzae* on the basis of the corresponding avirulence activities. Additional homologs *pthA* and *avrb6* from *X. citri* pv. *citri* and *X. campestris* pv. *malvacearum* were identified as virulence factors. The *avrXa7* was

also demonstrated to the virulence activity (Bai *et al*., 2000). TAL effectors in different strains of *X. oryzae* pv. *oryzae* including PthXo1, PthXo2, and PthXo3, played major roles in the disease process of bacterial blight similar to *AvrXa7* (Yang and White, 2004). Similarity, TAL effectors (PthA4, PthB, PthC, PthA, and PthAw) in different strains of *X. citri* pv. *citri* the causal agent of bacterial canker in citrus, played a role in disease virulence by induction of gene which resulted in enhance bacterial growth and pustule development (Hu *et al*., 2014; Li *et al*., 2014). Difference strains of *X. agardneri* used *AvrBs3* and *AvrHahm1* to induced genes promotes tissue hypertrophy and cell enlargement (Kay *et al*., 2007). Moreover, The induction of TAL20xam668 in X. *axonopodis* pv. *manihotis* strains result in enhances bacterial growth and water soaking symptoms of bacterial blight in cassava (Cohn *et al*., 2014).

Although, major TAL effectors in several pathogens have been report but no specific information on TAL effectors that play major role in disease virulence of *X. axonopodis* pv. *glycines* in soybean. Thus in this study, we were investigated *X. axonopodis* pv. *glycines* by developed specific primer and probe for detection *X. axonopodis* pv. *glycines* in each race. The evidence from this study will provide better understanding in factors that involve in pathogenicity of *X. axonopodis* pv. *glycines*. This information will benefit the breeding programs for bacterial pustule especially effective strategy to protect soybean from this disease in the future.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

Bacterial strains and plasmids used in this study shown in Table 1. Besides *X. axonopodis* pv. *glycines* 12−2 (Prathuangwong, 1983) and KU-P-SW005 (Athinuwat *et al*., 2009) that restored from culture collection, the 169 strains of *X. axonopodis* pv. *glycines* were isolated from infected soybean leaves in Thailand by tissue transplanting method. All strains were tested for pathogenicity on susceptible soybean cultivar SJ.5. Bacterial strains were preserved in Luria-Bertani (LB) broth with sterile 40% glycerol at -80 °C for long-term storage. The *E. coli* strain Top10 was used as host cell. The concentrations of antibiotics amended in the media in this study including kanamycin (50 µg/ml), ampicillin (50 µg/ml), gentamycin (10 µg/ml), spectinomycin (10 µg/ml), and tetracycline (15 µg/ml).

Table 1 Bacterial strains and plasmids used in this study

Bacterial strains or plasmid	Relevant characteristic	Reference or source
X. axonopodis* pv. *Glycines		
SP4	Wildtype, Race 1	This study
12-2	Wildtype, Race 2	Prathuangwong (1984)
KU-P-SW005	Wildtype, Race 3	Athinuwat *et al*. (2009)
SP4*avrXg3*:: Amp^r	*avrXg3*::Amp^r of SP4 by *avrXg3* gene deletion	This study
	avrXg3::Sp^r of SP4 carrying pPZP201	
SP4*avrXg3*:: Sp^r		This study
(pPZP- *avrXg3*)	*tal2b*::Amp^r of SP4 by *tal2b* gene insertion	
SP4*tal2b*::Amp^r	*tal2b*:: Tc^r of SP4 carrying pKEB31	This study

Table 1 (Continue)

Bacterial strains or plasmid	Relevant characteristic	Reference or source
SP4*tal2b*::Tc^r (pKEB31- *tal2b*)	*avrXg2*::Km^r of 12-2 by *avrXg2* gene deletion	This study
12-2*avrXg2*:: Km^r	*avrXg2*::Gm^r of 12-2 carrying pBBR1MSC-5	This study
12-2*avrXg2*:: Gm^r (pBBR- *avrXg2*)	*tal*2b::Amp^r of 12-2 by *tal2b* gene insertion	This study
12-2*tal2b*::Amp^r	*tal2b*:: Tc^r of 12-2 carrying pKEB31	
12-2 *tal2b*::Tc^r (pKEB31- *tal2b*)	*avrXg2&tal2b*:: Km^r Amp^r of 12-2 by avr*Xg2* and *tal2b* gene deletion	This study
12-2 *avrXg2&tal2b*:: Km^r Amp^r	*avrXg2*::Gm^r of 12-2 carrying pBBR1MSC-5 and *tal2b*:: Tc^r of 12-2	This study
12-2 *avrXg2&tal2b*::Gm^r Tc^r (pBBR-*avrXg2*& pKEB31-*tal2b*)	carrying pKEB31	This study
KU-P-SW005*tal2b* ::Amp^r	*tal*2b::Amp^r of KU-P-SW005 by *tal2b* gene insertion	This study
KU-P-SW005*tal2b*::Tc^r (pKEB31- *tal2b*)	*tal2b*:: Tc^r of KU-P-SW005 carrying pKEB31	This study
		This study
Escherichia coli		
TOP10	F'[lacI^q Tn10(tet^R)] mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 deoR nupG recA1 araD139 Δ(ara-leu)7697 galU galK rpsL(Str^R) endA1 λ$^-$	Invitrogen
Plasmid		
pTok2	ColE1 replicon, suicide plasmid, Tc^r	Kitten and Willis (1996)
pBBR1MCS-5	Broad host range cloning vector, lacZ and Gm^r	Kovach *et al*. (1995)
pPZP201	Broad host range cloning vector, lacZ and Sp^r	Hajdukiewic *et al*. (1994)
pSM7	pBluescriptII-KS(+) (Invitrogen) containing the 4.5 kb *Pst*I fragment of *Tal* gene *aB4.5* interrupted at repeat 9 by EZTN5*Not*IKan-3 transposon, Km^r	Makino (2005)
pKEB31	pDD62 derivative containing Gateway destination vector cassette (Invitrogen) between *Xba*I and *BamH*I sites, Tc^r	Cermak *et al*. (2011)
pKEB31- *tal2b*	pKEB31 containing *tal2b* of 12-2, Tc^r	This study

Phenotypic Race Screening of *X. axonopodis* pv. *glycines*

The 169 new strains of *X. axonopodis* pv. *glycines* 12-2 and KU-P-SW005 were screened followed protocol described in Athinuwat *et al*. (2009). Resistant soybean cultivar, Williams 82, and susceptible cultivars, PI520733, Spencer and SJ.5 were used in this study. A leaf infiltration technique under greenhouse conditions was used to assess phenotypic race on soybean leaves. Briefly, bacterial suspensions at OD_{600} = 0.2, which corresponded to a cell density of $1x10^8$ cfu/ml were infiltrated through a mesophyll using a 1-ml hypodermic syringe without a needle (Athinuwat *et al*., 2009). Infiltrated areas were monitored for tissue collapse and necrosis for 48 hrs after inoculation. The development of HR was scored at 48 hrs after infiltration. The experiment was repeated three times.

Mutation of *avrBs3*-like Genes in *X. axonopodis* pv. *glycines* Race 1 and 2

Cloning and construction

The genomic DNA of *X. axonopodis* pv. *glycines* strain SP4 (race 1) and 12−2 (race 2) were used as DNA template. The primer for amplification of *avrXg3* and *avrXg2* genes (Table 2) in *X. axonopodis* pv. *glycines* were designed from *X. axonopodis* pv. *glycines* 12−2 draft genome (Chatnaparat *et al*., 2012). The targeted single gene mutation of *avrBs3*-like genes named *avrXg3* in *X. axonopodis* pv. *glycines* strain SP4 in race 1 and *avrBs3*-like genes named *avrXg2* in strain 12−2 were accomplished via gene deletion. The flanking fragments of targeted gene were amplified from strains SP4 and 12-2 using primers *avrXg3*-up-F, *avrXg3*-up-R(Amp), *avrXg3*-down-F(Amp), *avrXg3*-down-R, *avrXg2*-up-F, *avrXg2*-up-R(Km), *avrXg2*-down-F(Km) and *avrXg2*-down-R (Table 2). The fragments containing the region upstream and downstream of *avrXg3* and *avrXg2* were ligated into pTok2 (Quick Ligation™ Kit, New England Biolabs), then transformed into *E. coli* Top10 (Invitrogen, CA, USA) by electroporation. Clones were selected on LB agar amended with ampicillin and kanamycin for *avrXg3* and *avrXg2* respectively.

Gene knockout mutagenesis of *avrXg3* and *avrXg2*

The pTok2-*avrXg3* was transformed into *X. axonopodis* pv. *glycines* strains SP4 and pTok2-*avrXg2* into *X. axonopodis* pv. *glycines* strain 12−2 by electroporation. Transformants were selected on LB agar containing antibiotics ampicillin for *avrXg3* mutants and kanamycin for *avrXg2* mutants. Resistant clones were subsequently verified for ampicillin or kanamycin genes insertion by PCR. The mutants were screened for virulence on soybean cv. Williams 82 and Spencer. The *avrXg3* mutant strains of SP4 were confirmed by PCR amplification with internal primers of *avrXg3* (In(*Xg3*)-F: 5' TCAGGCCGATGACAGCTTA3'and In(*Xg3*)-R: 5' TCAGGCCGATGACAGCTTAT3'). The *avrXg2* mutant strain of 12-2 was confirmed by PCR amplification with internal primers of *avrXg2* (In(*Xg2*)-F: 5'GCTTTACTGGCCGCCCGCC3' and In(*Xg2*)-R: 5'TGCCAAGCTGGAGGAA TGA3'). Double mutation of *avrXg2*&*Tal2b* in *X. axonopodis* pv. *glycines* strains 12-2 were also investigated. The pTok2-*avrXg2* was transformed into 12-2 *tal2b*:: Km^r and Amp^r by electroporation. Transformants were selected on NBY agar containing antibiotics kanamycin and ampicillin. Individual colonies of putative mutants were screened for their pathogenicity on soybean cultivars.

Complementary strains of *avr* gene

The primers for amplification of *avrXg3* and *avrXg2* were designed from *X. axonopodis* pv. *glycines* 12-2 draft genome (Chatnaparat *et al*., 2012). The flanking fragments of *avrXg3* and *avrXg2* were amplified from strains SP4 and 12-2 using primer *Xg3*comF-*Xba*I, *Xg3*comR-*Xba*I, *Xg2*comF-*Bam*HI and *Xg2*comR-*Bam*HI, respectively (Table 2). The fragment containing the region of *avrXg3* was ligated into the multiple cloning site vector pPZP201. The *avrXg2* was ligated into the multiple cloning site vector pBBR1MCS-5 with T4 DNA ligase, then

transformed by electroporation into *E. coli* Top10 (Invitrogen, CA, USA) as previously described by Athinuwat *et al*. (2009). Plasmid extraction of *E. coli* were transformed by electroporation into SP4*avrXg3*::Amp^r, 12-2*avrXg2*::Km^r, respectively. The SP4*avrXg3*:: Sp^r (pPZP-*avrXg3*) and 12-2*avrXg2*:: Gm^r (pBBR- *avrXg2*) strains of *avrXg3* and *avrXg2* were selected on LB agar with spectinomycin and LB agar with gentamycin, respectively. Double mutant (12−2*avrXg2*&*tal2b*::Km^{r}Amp^r) was made by synthesized *tal2b* by golden gate TALEN assembly. Gateway® LR clonase™ II enzyme mix was used for cloned into the destination vector pKEB31 (Cermak *et al*., 2011) then, transformed LR reaction into 12-2*avrXg2*:: Gm^r (pBBR- *avrXg2*) resistance clone was selected on LB agar with gentamycin + 15 µg/ml tetracycline.

Table 2 Polymerase chain reaction primer

Primer	Sequence	Description
avrXg3-F	5' ATGGGACTATGCGTTTCAAAGCC 3'	Forward primer for amplification of *avrBs3*-like genes size 1,206 bp named *avrXg3*
avrXg3-R	5' TCATCTCGCCACCGTGACAGG 3'	Reverse primer for amplification of *avrBs3*-like genes size 1,206 bp named *avrXg3*
avrXg2-F	5' ATGCGCTGCCCGCTTGCA 3'	Forward primer for amplification of *avrBs3*-like genes 2,288 bp named *avrXg2*
avrXg2-R	5' TCATTCCTCCAGCTTGGCATC 3'	Reverse primer for amplification of *avrBs3*-like genes size 2,288 bp named *avrXg2*
avrXg3-up-F	5' TCTGCCAGCACGTCGTA 3'	Forward primer for amplification of 1,021 bp of upstream region of *avrXg3* for generate *avrXg3* mutant
avrXg3-up-R	5' GTGCCTCACTGATTAAGCAT GGCCTGATGGGCTGAT 3'	Reverse primer for amplification of 1,021 bp of upstream region of *avrXg3* for generate *avrXg3* mutant
avrXg3-down-F	5' CTTTCACCAGCGTTTCTGGGTG CATAGTCCCATGTCGCTCTC 3'	Forward primer for amplification of 1,010 bp of downstream region of *avrXg3* for generate *avrXg3* mutant
avrXg3-down-R	5' TGCCGTAATGGAAGGC 3'	Reverse primer for amplification of 1,010 bp of downstream region of *avrXg3* for generate *avrXg3* mutant
avrXg2-up-F	5' TCGAGCGTGCGGATCTG 3'	Forward primer for amplification of 1,105 bp of upstream region of *avrXg2*
avrXg2-up-R	5' GAAGCAGCTCCAGCCTACAC AAGCGCATGCCTCGTTTGGA 3'	Reverse primer for amplification of 1,105 bp of upstream region of *avrXg2* for generate *avrXg2* mutant
avrXg2-down-F	5' GGTCGACGGATCCCCGGAA TGAGGAATGATGGCGGC 3'	Forward primer for amplification of 1,056 bp of downstream region of *avrXg2* for generate *avrXg2* mutant

Table 2 (Continue)

Primer	Sequence	Description
avrXg2-down-R	5' ATCCACCGACCGATCTCT 3'	Reverse primer for amplification of 1,056 bp of downstream region of *avrXg2* for generate *avrXg2* mutant
*Xg3*comF-*Xba*I	5' AGTATCTAGA TTCGACCTTGCAAGGCA 3'	Forward primer for amplification of 1,658 bp of *avrXg3* for generate complementation strain of Xag
*Xg3*comR-XbaI	5' AGTATCTAGA AACCAACAACGTTCGCAG 3'	Reverses primer for amplification of 1,658 bp of *avrXg3* for generate complementation strain of Xag
*Xg2*comF-*Bam*HI	5' AGTAGGATCCTCGCCTTC GAACCTGC 3'	Forward primer for amplification of 2,502 bp of *avrXg2* for generate complementation strain of Xag
*Xg2*comR-*Bam*HI	5' AGTAGGATCC CTGTCAGCAACGCATCAT 3'	Reverses primer for amplification of 2,502 bp of *avrXg2* for generate complementation strain of Xag

Mutation *tal* Gene

Detection TAL effectors in *X. axonopodis* pv. *glycines*

High molecular weight genomic DNA of *X. axonopodis* pv. *glycines* strains SP4, 12−2 and KU-P-SW005, the representative strains from races 1, 2 and 3, respectively were prepared for sequencing with Pacific Biosciences single-molecule real-time (SMRT) sequencing technology according to the methods described by Booher *et al*. (2015). Two SMRT cells were sequenced with P4-C2 chemistry, yielding 140x coverage. Reference *tal* gene assemblies were performed and consensus *tal* sequences were extracted using an automated protocol, the PBX toolkit, as described by Booher *et al*. (2015). All three strains that mentioned above also performed with Southern blots analysis. Genomic DNA from each strain was digested with *Sph*I and run on 1% agarose gels. The DNA was transferred to Hybond-N^+ nylon membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The membrane was UV cross-linked to bind the DNA onto the membrane and hybridize with *avrXa7* probe by hybridization as described above.

Gene knockout mutagenesis of *tal* gene

A library of 17 *tal* genes knockout strain of *X. axonopodis* pv. *glycines* strains SP4, 12−2 and KU-P-SW005 were generated by transformation with the suicide (non-replicative) plasmid pSM7. The pSM7 plasmid from *E. coli* Top 10 was extracted by using E.Z.N.A.® Plasmid DNA Mini Kit I. *X. axonopodis* pv. *glycines* strains SP4, 12−2, and KU-P-SW005 TAL effectors gene were mutagenesis by transform in to SP4, 12−2, and KU-P-SW005 chemically competent cells, respectively with the suicide (non-replicative) plasmid pSM7. Resistant clones were confirmed by PCR amplification with primer Am-F: 5'ATGCTTAATCAGTGAGGCAC3' and Am-R:

5' CACCCAGAAACGCTGGTGAAAG 3' then, screened for their ability to cause the disease on soybean cultivars.

Complementary strains of *tal* gene

Synthesized of *tal2b* construct of *X. axonopodis* pv. *glycines* strains SP4, 12-2, and KU-P-SW005 by golden gate TALEN assembly. Gateway® LR clonase™ II enzyme mix was used for cloned into the destination vector pKEB31 (Cermak *et al*., 2011). Transformed LR reaction into *X. axonopodis* pv. *glycines* strains SP4, 12-2, and KU-P-SW005 by electroporation. GYE medium supplemented with ampicillin 50 µg/ml and tetracycline 15 µg/ml were used to selected resistant clone. Resistant clones were screened for their ability to cause the disease on soybean cultivars.

Characterization of Mutants

The *avrXg2* and *avrXg3* mutants

Virulence of *X. axonopodis* pv. *glycines* strains SP4, 12−2, SP4*avrXg3*::Amp^r, 12−2*avrXg2*::Km^r, 12−2*avrXg2*&*tal2b*:: Km^r Amp^r, SP4*avrXg3*:: Sp^r (pPZP-*avrXg3*), 12−2*avrXg2*:: Gm^r (pBBR- *avrXg2*) and 12−2 *avrXg2*&*tal2b*::Gm^{r}Tc^r (pBBR-*avrXg2* & pKEB31-*tal2b*) were assessed on soybean cv. Williams 82 and Spencer (Kaewnum *et al*., 2006). Briefly, aqueous cell suspensions of *X. axonopodis* pv. *glycines* (OD_{600} = 0.2, 1 x 10^8 cfu/ml) supplemented with 5 g/l 600 mesh carborundum, were sprayed on leaves of plants maintained in a greenhouse. At 7−10 days after inoculation disease severity was scored by recording the presence or absence of pustules in nine 1 cm^2 sections per leaves, as described by Prathuangwong *et al*. (1990). The experiment was done three times.

The *tal* mutants and complemented strains

The virulence of *X. axonopodis* pv. *glycines* strains SP4, 12−2, and KU-P-SW005, SP4*tal2b*::Amp^r, 12−2*tal2b*::Amp^r, and KU-P-SW005*tal2b*::Amp^r, SP4*tal2b*::Tc^r (pKEB31- *tal2b*), 12−2 *tal2b*::Tc^r (pKEB31- *tal2b*), and KU-P-SW005 *tal2b*::Tc^r (pKEB31- *tal2b*) were tested on 21-days-old of soybean plants cv. Williams 82 and Spencer. Briefly, aqueous cell suspensions *X. axonopodis* pv. *glycines* strains SP4, 12−2, and KU-P-SW005, SP4*tal2b*::Amp^r, 12−2*tal2b*::Amp^r, and KU-P-SW005*tal2b*::Amp^r, SP4*tal2b*::Tc^r (pKEB31-*tal2b*), 12−2 *tal2b*::Tc^r (pKEB31- *tal2b*), and KU-P-SW005 *tal2b*::Tc^r (pKEB31- *tal2b*), OD_{600} = 0.2, 1 × 10^8 cfu/ml, supplemented with 5 g/l 600 mesh carborundum, were sprayed on leaves with same methods described above.

PCR and Hybridization for Race-strain Detection

Primer pair, 2bR1-F/2bR1-R, was used to detect race 1 of *X. axonopodis* pv. *glycines*. These primers were designed from *tal2b* of *X. axonopodis* pv. *glycines* in race 1 compared of *tal2b* of *X. axonopodis* pv. *glycines* in race 2 after analyzed with ClustalW2: Multiple Sequence Alignment. Two primer pairs including *avrXg2*-F/*avrXg2*-R (designed from *avrXg2*) and 2bR2-F/2bR2-R were used for detection of race 2. As for race 3 we also used primer pair (AVR-F/AVR-R) designed from Athinuwat *et al*. (2009). The primer pair, *tal1c*-F /*tal1c*-R was used for separate *X. axonopodis* pv. *glycines* race 3 from race 1 and race 3. Finally, primer *tal*2a-F/*tal*2a-R was used for universal primer to detection *X. axonopodis* pv. *glycines* (Table 3).

Table 3 Polymerase chain reaction primers designed for *X. axonopodis* pv. *glycines*

Primer	Sequence	Description
2bR1-F 2bR1-R	5'-TCGCCAGCAATAGTGGTG-3' 5'-GGGCTTCGAGTTCGGTG-3'	Primer for detection *X. axonopodis* pv. *glycines* in race 1 designed form *tal2b*, the PCR product size is 1,089 bp
avrXg2-F *avrXg2*-R	5'-ATGCCACGCGCTTTACTGG-3' 5'-TCATTCCTCCAGCTTGGCATC-3'	Primer for detection *X. axonopodis* pv. *glycines* in race 2 designed form *avrXg2*, the PCR product size is 2,193 bp
2bR2-F 2bR2-R	5'- CTGAGGCAATAGCTCCATCAA-3' 5'-GTTCCCGAACAGCGCG-3'	Primer for detection *X. axonopodis* pv. *glycines* in race 1 designed form *tal2b*, the PCR product size is 231 bp
AVR-F AVR-R	5′-AATATTGGCGGCAAGCAGGC-3′ 5′ -CGCCTGCTTGCCACCAATATT-3′	Primer for detection *X. axonopodis* pv. *glycines* in race 3 designed form *avrXgI*, the PCR product size is 863 bp
TAL1c-F TAL1c-R	5′-TCACTGAGGCAATAGCTCCATC-3′ 5′-ATGCGGCCTCGGAAGCTAT-3′	Primer for separated *X. axonopodis* pv. *glycines* in race 3 out of race 1 and race 2, the PCR product size is 4,077 bp
TAL2a-F TAL2a-R	5'-TCACTGAGGCAATAGCTCCATC-3' 5'- ATGCGGACTCGGAAGACGTA-3'	Universal primer for detection *X. axonopodis* pv. *glycines* designed form *tal2a*, the PCR product size is 3,261 bp

Phylogenetic analysis

Amino acid sequences of TAL effectors were retrieved from GenBank upon BlastP analysis, using *tal2b* of *X. axonopodis* pv. *glycines* as a model. Proteins were chosen for representing the diversity of lineage and strain origin, especially among the new strains collected in this study (169 strains) of *X. axonopodis* pv. *glycines* and 2 strains of *X. axonopodis* pv. *glycines* 12−2 (Prathuangwong, 1983) and KU-P-SW005 (Athinuwat *et al*., 2009). N- and C-terminal regions were concatenated and aligned using ClustalW2. Sequence alignments and further processed manually to eliminate gaps due to indels. A neighbor-joining tree was constructed using PhyML with program Sea View version 4 and bootstrap analyses were based on 1000 replicates.

Southern blot hybridization with *avrXg3* and *avrXg2 probe*

X. axonopodis pv. *glycines* strain SP4, 12−2 and KU-P-SW005 were used for representative strain in race 1, 2 and 3, respectively. *X. campestris* pv. *campestris* S345 was used for negative control. DNA form SP4, 12−2, and KU-P-SW005 were used to performed Southern blots analysis as described above and then hybridize with *avrXg3* and *avrXg2* probe, respectively.

Prediction and qRT-PCR Analyses

Predicted the targets of *tal2b* in soybean

The effector-binding element (EBE) of *tal2b* of *X. axonopodis* pv. *glycines* strains SP4, 12−2, and KU-P-SW005 were predicted by using the TALE-NT 2.0 Target Finder tool (Doyle *et al.*, 2012), based on the RVD binding region. Prediction s were carried out using the soybean promoterome, defined as the 5' UTR (if annotated) plus 1000 base pairs upstream of the transcriptional start site of each transcript. The output for a gene includes all unique EBE predicted in the promoter of any transcript of that gene. Promoter was recovered from the *Glycine max* (cv. Williams 82) reference genome downloaded from the NCBI of soybean genome annotation (assembly *Glycine max* v.2.0).

Gene expression analysis using qRT-PCR

The soybean leaves were inoculated with *X. axonopodis* pv. *glycines* 12−2 and 12-2*tal2b*::Amp^r. The leaves were collected for total RNA extraction at 24 hrs after inoculation. RNA was extracted using Direct-zol™ RNA MiniPrep kit and protocol following manufacturing structure. RNA quantity and quality were measured using a nanodrop spectrophotometer. One microgram of RNA from each inoculation with *X. axonopodis* pv. *glycines* as indicated were treat with DNaseI (Invitrogen) followed by cDNA synthesis using the iScript Select cDNA synthesis kit (Bio-Red). cDNA derived from 25 ng of total RNA was use for each real-time PCR with gene specific primers. Primer *LOB1*-F: 5' AAAAGTTCGCAAACGTCCAC 3' and *LOB1*-R: 5' AGCATCAAGCTCCTTTTGGA 3' were designed from *Glycine max* cultivar Williams 82 chromosome 5, Glycine_max_ v2.0, whole genome shotgun sequence (NCBI Reference Sequence: NC_016092.2) that amplification of 206 bp. Primer *SWEET* -F: 5' CAGCAACACCTCCAGAACAA 3' and *SWEET* -R: 5' AGCATCAAGCTCCTTTTGGA 3' were designed from *Glycine max* cultivar Williams 82 chromosome 11, Glycine_max_ v2.0, whole genome shotgun sequence (NCBI Reference Sequence: NC_016098.2) that amplification of 206 bp. Melting cure analysis was used to verify amplification of a specific product. The *LOB* and *SWEET* gene were non-detectable in negative control.

RESULTS AND DISCUSSION

Phenotypic Screening and Phylogenetic Analysis of *X. axonopodis* pv. *glycines*

The diversity of *X. axonopodis* pv. *glycines* can be categorized into at least 3 races based on phenotypic expression in resistant and susceptible soybean cultivars (Athinuwat *et al.*, 2009). In this study, 169 strains of *X. axonopodis* pv. *glycines* were isolated from infected soybean growing area in four provinces at the Northern part of Thailand including Chiang Mai, Chiang Rai, Lamphuan and Lampang. Thirty strains shown sign of HR in all soybean cultivar (Williams 82, PI520733, Spencer and SJ.5), thus these 30 strains were grouping into race 1 (17.54% of total strains). While, infiltrated leave with 107 strains visualized expressed as necrotic with a surrounding yellow halo on all four soybean cultivars. Therefore, these 107 strains (62.57% of total strains) were group into race 2. On the other hand, 34 strains (19.89% of total strains) which grouping into race 3 induced HR on the resistant cultivar (Williams 82) and caused pustule disease on susceptible cv. PI520733 and Spencer (Table 4).

Table 4 Race-cultivar specificity of 171 strains of *Xanthomonas axonopodis* pv. *glycines* on different soybean cultivars

***X. axonopodis* pv. *glycines* Race**	**Reaction with soybean cultivars[1/]**				**Total**
	Willium82	**PI520733**	**Spencer**	**SJ.5**	
Race1	I	I	I	I	30
Race2	C	C	C	C	107
Race3	I	C	C	C	34
Total					171

[1/] I=Incompatible reaction which results in disease resistance expression by the plant and avirulence expression by the pathogen: C= compatible reaction which results in disease susceptibility in the plant and virulence expression by the pathogen

We used primers that design from this study to construct the phylogenetic tree with new strains of *X. axonopodis* pv. *glycines* that collected in this study (169 strains) and strains of *X. axonopodis* pv. *glycines* 12−2 (Prathuangwong, 1984) and KU-P-SW005 (Athinuwat *et al*., 2009). The primer is specific to *tal2b* gene which is first to identified in this study. Figure 1, shown that primer *tal2b* separated all strains into two main groups including group 1 and group 2 and distinguished with similarity coefficient 0.55 (Figure 1). Group 1, supported by bootstrap values of 99% was weakly strains and separate into two-small subgroup distinguished with similarity coefficient 0.84. The population of *X. axonopodis* pv. g*lycines* in group 1 contains 30 isolates. Group 2, also supported by bootstrap values of 99% and separate into two major group distinguished with similarity coefficient 0.62. The 2A is *X. axonopodis* pv. g*lycines* includes 34 strains, and distinguished with similarity coefficient 0.76. Whereas 2B is *X. axonopodis* pv. g*lycines* includes 107 strains, and separate into small sub-group with similarity coefficient 0.76 (Figure 1). In summary, three groups were separated based on partial sequence of *tal2b* gene. In the same time, the results from phenotypic screening also separated them into three groups as describe above. Together we can conclude that 30 strains in group 1, 107 strains in group 2A and 34 strains in group 2B were categorized into race 1, race 2 and race 3, respectively. These data suggested that the primer designed from *tal2b* gene was appropriate primer to grouping race of *X. axonopodis* pv. g*lycines*.

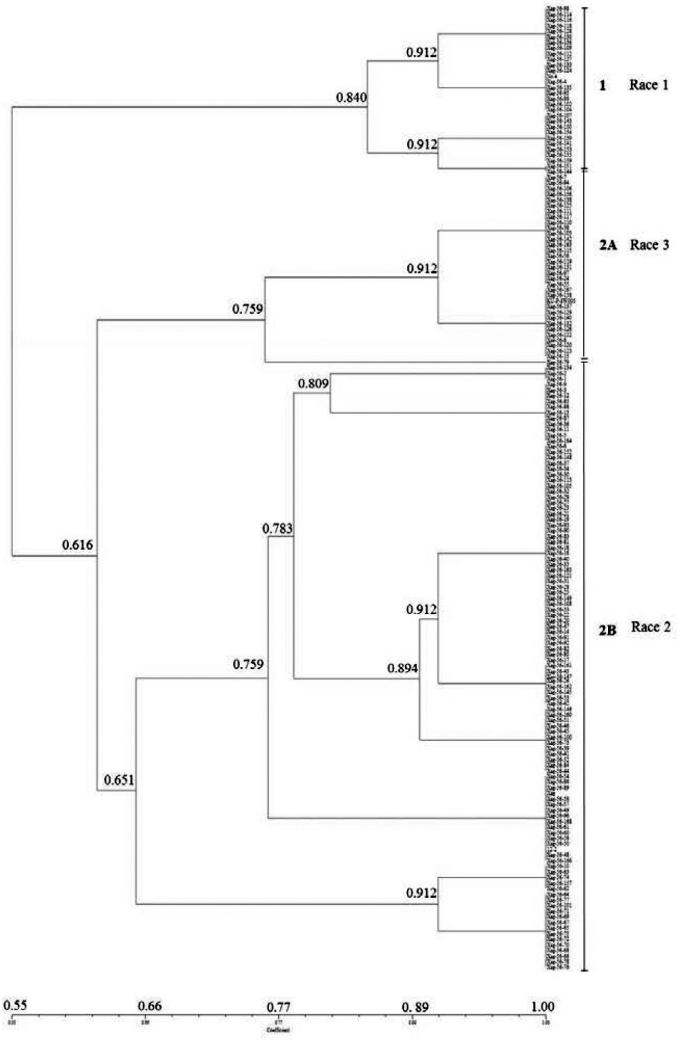

Figure 1 Dendrogram of DNA fingerprinting of 171 strains of *X. axonopodis* pv. *glycines* were identified by repetitive sequence-based (rep-PCR) with *tal2b* primer using PhyML with program Sea View version 4 for analyzed

Result from phenotypic screening and later confirmed with phylogenic tree of all new 169 strains, revealed the genetic diversity of *X. axonopodis* pv. *glycines* race in soybean growing area at the Northern part of Thailand. As mentioned at three strains found in this area, this knowledge will assist the selection of appropriate soybean cultivar for growing in each area.

Detection TAL Effectors of *X. axonopodis* pv. *glycines* in Each Race

X. axonopodis pv. *glycines* causes bacterial pustule on soybean by using effectors secreted by the type III system to block plant defense. One class of T3S effectors, transcription activator-like effectors (TAL effectors), are major pathogenicity factors in many *Xanthomonas*

species. TAL effector generated from *avr* gene because sometime *avr* gene is TAL effector sometime is not depending on nucleotide sequence (Zhang *et al*., 2011). Although, draft genome of *X. axonopodis* pv. *glycines* 12−2 draft genome were previous construct by on short reads sequence (Chatnaparat *et al*., 2012) but the assembly did not capture any *tal* genes. We therefore generated a complete genome assembly to identify all TAL effectors of this strain again. We take advantage from advances of genome sequencing technologies, especially long- molecular technologies such as PacBio sequencing, and better engineering of assembly algrorithms facilitate genome assembly (Korea and Phillippy, 2015). In this study, complete genome sequences were generated for *X. axonopodis* pv. *glycines* strain SP4, 12−2, and KU-P-SW005 which representative race 1, 2, and 3, respectively. The final assembly of the 12−2 genome, consisting of a 5,398,926 bp chromosome, a 26,618 bp large plasmid named pXAG27 (12−2), and a 1,804 bp small plasmid named pXAG2 (12−2) were submitted to NCBI's Genbank. The 12−2 genome (Acession numbers: CP015972), large plasmid pXAG27 (Acession numbers: CP015973) and small plasmid pXAG2 (Acession numbers: CP015974), BioSample: SAMN05179543, BioProject: PRJNA323439.

Named of TAL effector depend on effector binding element (EBEs) in specific host gene promoters. Binding specificity is dictated by a variable number of central, 33−35 amino acid repeats. In each repeat, a pair of variable residue at positions 12 and 13 (called the repeat variable diresidue (RVD)) preferentially associates with a different nucleotide to define the length of the EBE (Boch *et al*., 2009). With this modular protein-DNA recognition mechanism, the pathogen can active multiple susceptibility (*S*) gene in the host by deploying different TAL effectors. From the genome assembly, Six TAL effector genes including *tal1a*, *tal1b*, *tal1c*, *tal1a*, *tal2b* and *tal3* were identified in *X. axonopodis* pv. *glycines* 12−2. The genes range in repeat number of 15 to 23 RVD. Each gene has a unique arrangement of RVD. Similarity, six TAL effector genes including *tal1a*, *tal1b*, *tal1c*, *tal1a*, *tal2b* and *tal3* were identified from *X. axonopodis* pv. *glycines* strain SP4 which the representative of race 1. Interestingly, the genes range in repeat number of 15 to 23 RVD similar to representative strain of race 2 (*X. axonopodis* pv. *glycines* 12−2). On the other hand, in *X. axonopodis* pv. *glycines* strain KU-P-SW005 which is representative in race 3, five TAL effector genes including *tal1a*, *tal1b*, *tal1a*, *tal2b* and *tal3* were identified and the genes range in repeat number of 15 to 20 RVD (Figure 2). As we know, *avrXgI* was identified from strain KU-P-SW005 (Athinuwat *et al*., 2009) but when we try to blast an *avrXgI* with *tal* genes (*tal1a*, *tal1b*, *tal1a*, *tal2b* and *tal3*), its doesn't match because the evolution of *avrXgI* was quickly and depend on host also. Southern blot analysis revealed six candidate TAL effector genes of *X. axonopodis* pv. *glycines* strain SP4, six candidate TAL effector genes of *X. axonopodis* pv. *glycines* 12-2 and, five candidate TAL effector genes of *X. axonopodis* pv. *glycines* strain KU-P-SW005 based on fragment that hybridize with the *avrXa7* probe (universal probe for detection TAL effector of *Xanthomonas* species). The sizes of *Sph*I digestion fragment ranged from 2 kb to 3 kb (Figure 3). The DNA sequencing of the repeat regions of the six, six and five TAL effectors genes showed that the numbers of repeats in the repetitive regions of the respective TAL effectors ranged from 15 to 23 RVD (Figure 3).

Race	TALE name	Repeat	Repeat Variable Di-residdue (RVD)
1	*tal1a*	19	NI-HD-NS-NS-NI-NS-NS-HD-NS-NI-NG-NI-NS-HD-NS-HD-NI-NG-NG
	tal1b	17	NI-HD-NS-NI-HD-NS-NS-NS-NS-HD-HD-HD-NG-NS-HD-HD-NG
	tal1c	23	NI-HD-NS-NS-NI-NS-NS-HD-NS-NS-NS-HD-HD-HD-NG-NI-NS-HD-HD-NG-HD-HD-NG
	tal2a	18	NI-NS-HD-NI-HD-NG-HD-NG-HD-NI-NG-NS-HD-NS-HD-NI-NG-NG
	tal2b	15	NI-NI-NG-HD-NG-NG-NI-NI-NI-HD-NS-HD-NI-NI-NG
	tal3	20	NI-NS-HD-NG-HD-NG-NG-HD-NI-NG-NI-NI-HD-NI-HD-NI-HD-NI-NG-NG (A)
2	*tal1a*	19	NI-HD-NS-NS-NI-NS-NS-HD-NS-NI-NG-NI-NS-HD-NS-HD-NI-NG-NG
	tal1b	17	NI-HD-NS-NI-HD-NS-NS-NS-NS-HD-HD-HD-NG-NS-HD-HD-NG
	tal1c	23	NI-HD-NS-NS-NI-NS-NS-HD-NS-NS-NS-HD-HD-HD-NG-NI-NS-HD-HD-NG-HD-HD-NG
	tal2a	18	NI-NS-HD-NI-HD-NG-HD-NG-HD-NI-NG-NS-HD-NS-HD-NI-NG-NG
	tal2b	15	NI-NI-NG-HD-NG-NG-HD-NI-NS-HD-NS-HD-NI-NG-NG (B)
	tal3	20	NI-NS-HD-NG-HD-NG-NG-HD-NI-NG-NI-NI-HD-NI-HD-NI-HD-NI-NG-NG
3	*tal1a*	19	NI-NS-HD-NG-HD-NG-NG-HD-NI-NG-NI-NI-HD-NI-HD-NI-HD-NI-NG-NG
	tal1b	16	NI-HD-NS-NI-HD-NS-NS-NS-NS-HD-HD-HD-NG-HD-HD-NG
	tal2a	18	NI-NS-HD-NI-HD-NG-HD-NG-HD-NI-NG-NS-HD-NI-HD-NI-NG-NG
	tal2b	15	NI-NI-NG-HD-NG-NG-HD-NI-NI-HD-NS-HD-NS-NG-NG (C)
	tal3	20	NI-NS-HD-NG-HD-NG-NG-HD-NI-NG-NI-NI-HD-NI-HD-NI-HD-NI-NG-NG

Figure 2 Repeat variable diresidue (RVD) sequence of TAL effectors of *X. axonopodis* pv. *glycines* strains SP4 (A), 12-2 (B), and KU-P- SW005 (C)

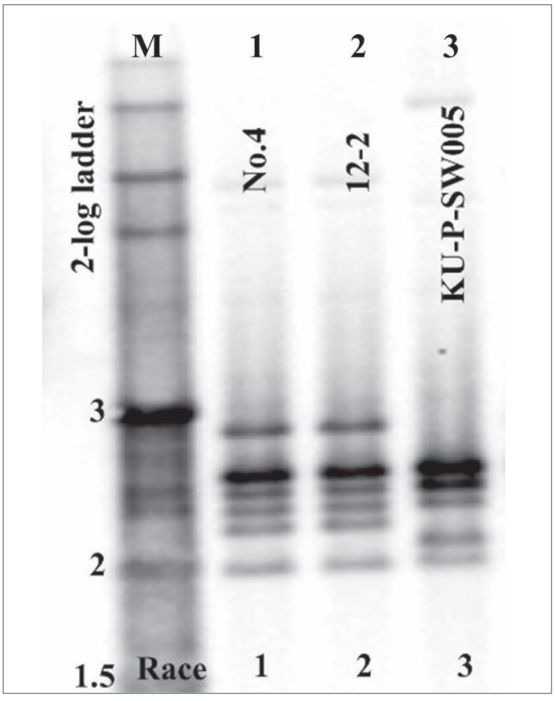

Figure 3 Southern blot results of TAL effector genes of *X. axonopodis* pv. *glycines* strains. Lane M = marker, lane 1 = SP4, lane 2 = 12−2 and lane 3 = KU-P-SW005. Hybridization probe is the *Sph*I segment of TAL effector with *avrXa7* probe

Our results demonstrated that the PacBio data was sufficient to achieve a high-accuracy assembly of the relatively repetitive Xanthomonas genome (Booher *et al*., 2015). Intact sequences of TAL effector genes can be used to predict the potential targets in host genomes, thereby facilitating the identification of possible resistant or susceptible genes (Boch *et al*., 2009; Moscou and Bogdanove, 2009). However, given a relatively high sequencing cost, it is still not realistic to sequence every bacterial strain with PacBio to achieve a finished assembly. The improvement of these long-read sequencing technologies should dramatically reduce the sequencing cost per bacteria strain in the near future. The ability to sequence and completely assemble a large number of independent strains would greatly accelerate genome comparison and the identification of bacterial virulent factors.

Characterization of *avrXg2*, *avrXg3*, *tal* Mutants and Complemented Strains

From the pathogenicity assay of 17 individual of mutants *tal* genes, one out of 17 genes which is *tal2b* showed high possibility to be a major virulent factor (data not show). Even though, *tal2b* gene is present in all 3 races of *X. axonopodis* pv. *glycines* but the nucleotide sequences were difference in each race. Interestingly, *tal2b* mutant strain reduced pustule formation and less population size when compared with wildtype. In addition to the avirulence function, a number of *avr* genes have been shown to enhance the virulence of pathogens on plants lacking the corresponding *R* genes (Abramovitch *et al*., 2003; Chen *et al*., 2014). Mutation of these *avr* genes reduces the fitness of the pathogens on susceptible hosts by affecting pathogen growth and symptom development. Based on these discoveries, it was postulated that *avr* gene were adopted by pathogens as virulence factors but were targeted later by *R* gene during plant-pathogen evolution (Chen *et al*., 2000).

X. axonopodis pv. *glycines* strain SP4, the representative of race 1 induced HR on soybean cv. Williams 82 and cv. Spencer. At 8 day after inoculation, the SP4*avrXg3*::Amp^r caused disease severity of 8.78% on soybean cv. Spencer. On the other hand, mutation of *tal2b* of strain SP4 (SP4*tal2b*::Amp^r) caused disease severity with 3.2% and 10.1 at 8 day after inoculation on soybean cv. Williams 82 and Spencer, respectively. As expected, the complemented strains of *avrXg3* (SP4*avrXg3*::Sp^r(pPZP-*avrXg3*) and *tal2b* (SP4*tal2b*::Tc^r (pKEB31-*tal2b*) were restored HR on soybean cv. Williams82 and Spencer similar to the wildtype (Table 5). In race 2, the representative of race 2, *X. axonopodis* pv. *glycines* strain 12–2, caused disease severity of 50.27 and 98.75% at 8 days

after inoculation on soybean cv. Williams 82 and cv. Spencer, respectively. Interestingly, the mutant strain (12−2*avrXg2*::Km^r) caused HR on soybean cv. Williams82 and disease severity of 5.49% on soybean cv. Spencer at 8 day after inoculation. While, the mutant strain of *tal2b* (12-2*tal2b*::Amp^r) of strain 12−2 was caused disease severity with 16.93 and 50.21% on soybean cv. Williams 82 and Spencer, respectively. In addition, double mutant strain (12−2*avrXg2&tal2b*:: Km^r Amp^r) was induced HR on soybean cv. Williams82 and cv. Spencer. Meanwhile, the complemented strains of12−2*avrXg2*::Gm^r(pBBR-*avrXg2*), 12-2*tal2b*::Tc^r (pKEB31- *tal2b*) and 12−2*avrXg2&tal2b*::Gm^{r}Tc^r (pBBR-*avrXg2*&pKEB31-*tal2b*) were showed the pathogenicity phenotype similar with wildtype. As for race 3, the *X. axonopodis* pv. *glycines* strain KU-P-SW005 which is representative of race 3 was induced HR on the soybean cv. Williams82 and caused pustule disease on soybean cv. Spencer. On the other hand, the mutant strain of *tal2b* of strain KU-P-SW005 (KU-P-SW005*tal2b*::Amp^r) was caused disease with 10.42 and 40.85% on soybean cv. Williams82 and Spencer, respectively. The complemented strain (KU-P-SW005*tal2b*::Tc^r (pKEB31-*tal2b*) was showed the pathogenicity phenotype similar with wildtype (Table 5). Also, the virulence of mutation of the *avrXg3* gene from *X. axonopodis* pv. *glycines* strain SP4, on soybean cv. Spencer was not different when compared with wildtype.

Table 5 Lesion size and disease severity of wildtype, mutansts, double mutant and complementary strains on soybean cultivars Williams82 and Spencer[1/]

Strain[2/]	Lesion mean diam (mm) [2/]		Disease severity (%)[3/]	
	Williams82	Spencer	Williams82	Spencer
SP4	HR	HR	HR	HR
SP4*avrXg3*::Amp^r	HR	0.31^{de}	HR	8.78^{d}
SP4*avrXg3*:: Sp^r (pPZP- *avrXg3*)	HR	HR	HR	HR
SP4*tal2b*::Amp^r	0.14^{d}	0.29^{e}	3.2^{c}	10.1^{d}
SP4*tal2b*::Tc^r (pKEB31- *tal2b*)	HR	HR	HR	HR
12-2	0.61^{a}	0.98^{a}	50.27^{a}	98.75^{a}
12-2*avrXg2*:: Km^r	HR	0.28^{e}	HR	5.49^{d}
12-2*avrXg2*::Gm^r (pBBR-*avrXg2*)	0.45^{b}	0.91^{a}	48.91^{a}	97.84^{a}
12-2*tal2b*::Amp^r	0.32^{c}	0.64^{c}	16.93^{b}	50.21^{b}
12-2 *tal2b*::Tc^r (pKEB31- *tal2b*)	0.57^{a}	0.93^{a}	49.63^{a}	97.48^{a}
12-2*avrXg2&tal2b*:: Km^r Amp^r	HR	HR	HR	HR
12-2*avrXg2&tal2b*::Gm^{r}Tc^r (pBBR-*avrXg2*&pKEB31-*tal2b*)	0.52^{ab}	0.97^{a}	49.17^{a}	98.12^{a}
KU-P-SW005	HR	0.44^{d}	HR	28.64^{c}
KU-P-SW005 *tal2b*::Amp^r	0.29^{c}	0.73^{b}	10.42^{b}	40.85^{b}
KU-P-SW005*tal2b*::Tc^r (pKEB31- *tal2b*)	HR	0.38^{d}	HR	27.23^{c}

[1/] Numbers followed by different letters show significance at P ≤ 0.05

[2/] Lesion mean diameter with 0.5 cm showed the necrotic symptom after 48 hrs infiltrated

[3/] Disease severity was evaluated as described in text. For each strain at least three leaves, collected from the top, middle and basal portion of three plants, were evaluated

Based on information from this study we concluded that function of *avrXg3* and *avrXg2* should be depended on genetic background of soybean cultivar. Because the *avrXg3* that present in *X. axonopodis* pv. *glysines* strain SP4 (race 1) when using the TALE-NT 2.0 Target Finder tool (Doyle *et al*., 2012) predicted the target of *R* gene in plant. The gene interaction between resistance gene of soybean cv. Williams82 and *avrXg3* in race 1 resulted in express hypersensitive response (HR) in these soybean cultivars. Meanwhile, the X. *axonopodis* pv. *glycines* 12−2, the representative of race 2 (present *avrXg2*) caused disease in all of soybean cultivars. Because the *avrXg3* function in induced susceptible (*S*) gene such as *LOB* and *SWEET* gene of soybean cv. Williams82. Therefore, the *X. axonopodis* pv. *glycines* in race 2 result in disease severity in all cultivars of soybean in this study. For race 3, *avrXg1* was identified from strains KU-P-SW005 that conferred compatible and incompatible reaction on its host and demonstrates multifunction of *avrXg1* is dependent on pathogen and plant genetic backgrounds (Athinuwat *et al*., 2009).

PCR and Hybridization for Race-strain Detection

Primer for detection of *X. axonopodis* pv. *glycines* in race 1 and race 2 were designed by analyzed nucleotides sequences of strain SP4 and 12−2, respectively. With NCBI's BLAST tool and analyzed with ClustalW2: Multiple Sequence Alignment and design specific primer, six primer pairs were designed for *avrXg2, avrXg3* and *tal* gene in all races as described in Table 3. Two primer pairs, 2bR1 and 2bR2, were highly specific to *tal* gene in race 1and race 2, respectively. As shown in Figure 4, primer 2bR1 only amplified with sample in race 1; similarity, primer 2bR2, was only amplified in race 2 sample. Primer *tal1c* was amplified with sample in race 1 and race 3, but not race 3 (Figure 4E). This data suggested that this primer pairs is appropriate to use to identify race 3 out from the other two races. On the other hand, two primer pairs, *avrXg2* and AVR which designed based on sequence of *avrXag* gene, were able to identified race 2 and race 3, respectively (Figure 4C, 4D). In addition, primer TAL2a was consider as universal primer for *Xag,* as shown in amplication of sample from all races (Figure 4F).

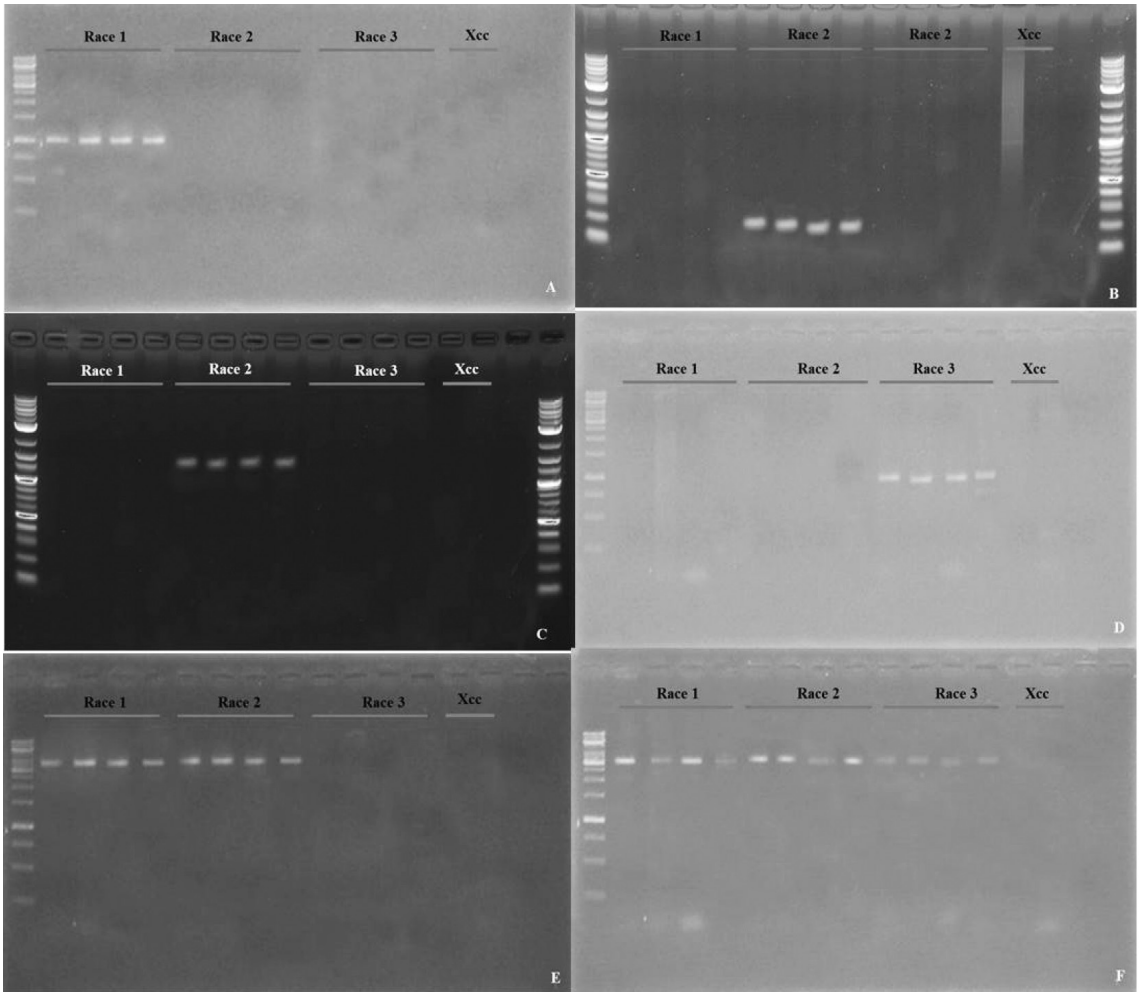

Figure 4 The specific band of *X. axonopodis* pv. *glycines* (Xag) race 1 designed from *tal2b* of race 1 (A), specific band of race 2 designed from *tal2b* of race 2 (B), *avrXg2* (C), specific band of race 3 designed from *avrXg1* (D), specific band1designed from *tal1C* for separate race 3 out of race 1 and race 2 (E) and universal band of Xag (F) designed from *tal2a* separated by electrophoresis on a 1.0% agarose gel. Lane M = 1 kb DNA Ladder, lanes 1−4 = Xag race 1, lanes 5−8 = Xag race 2, lanes 9−12 = Xag race 3, lane 13 = X. *campestris* pv. *campestris* S345 and lane 14 = X. *campestris* pv. *campestris* 2015

Southern blot analysis revealed that *avrXg3* probe was specific for identified *X. axonopodis* pv. *glycines* in race 1. As shown in Figure 5, only strain SP4 show the band size 1,180 bp on the membrane. While, *X. axonopodis* pv. *glycines* strain 12−2 shown band size 2,028 bp on the membrane after *avrXg2* probe. On the other hand, no band present in sample from strain KU-P-SW005 whether uses *avrXg3* or *avrXg2* as a probe. This data suggested that the *avrXg3* and *avrXg2* probe were highly specific probe for *X. axonopodis* pv. *glycines* in race 1 and race 2, respectively (Figure. 5).

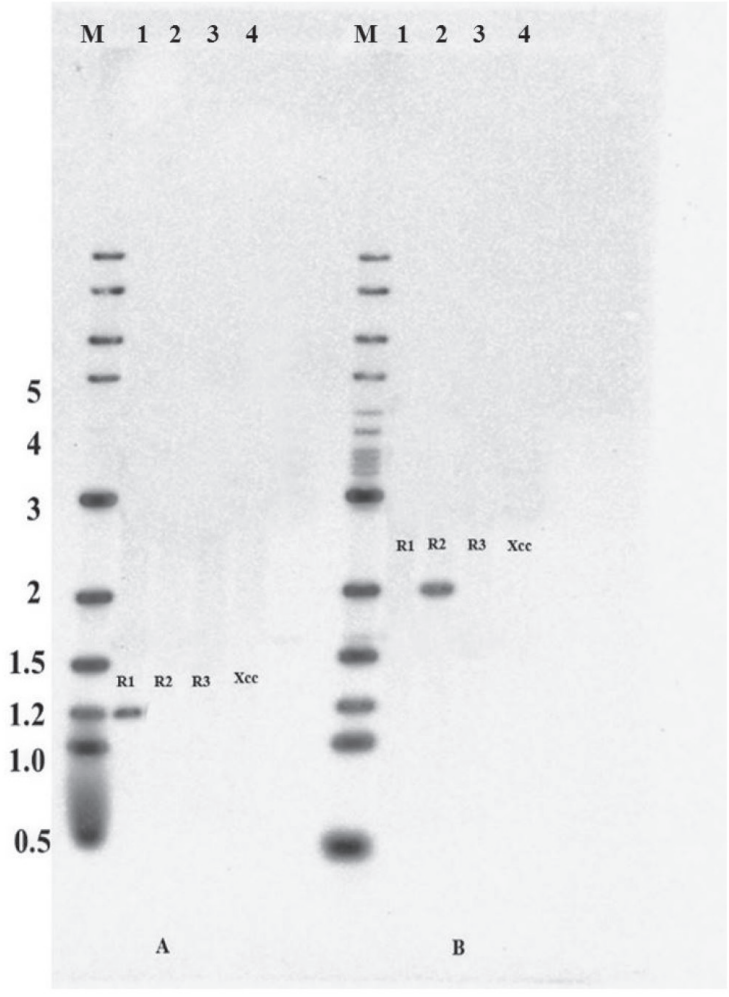

Figure 5 Southern blot results of *avr* genes of *X. axonopodis* pv. *glycines* strain SP4, 12−2 and KU-P-SW005 from Race 1, 2 and 3 respectively and X. *campestris* pv. *campestris* S345. Lane M = 1 kb DNA Ladder, lanes1 = SP4, lane 2 = 12−2, lane 9−3 = KU-P-SW005 and lane 4 = X. *campestris* pv. *campestris* S345. Genomic DNA was digested with *Sph*I and hybridization using *avrX*g3 (A) and *avrX*g 2 (B) as a probe

Prediction of *tal2b* Target in Soybean and qRT-PCR Analyses

Candidate targets of *tal2b* of *X. axonopodis* pv. *glycines* 12−2 were investigated by using the TALE-NT 2.0 Target Finder tool. We found that two candidate plant susceptibility (*S*) gene of *tal2b* in *X. axonopodis* pv. *glycines* 12−2 were identified. One candidate gene encodes from sugars will eventually be exported transporters (*GmSWEET1*) on chromosome 1 and 11 induced of Glyma 01G186700.1, Glyma11G117100.1 and Glyma11G186700.3 and the other one encodes of lateral organ boundaries (*GmLOB1*) domain family protein (TACcAaAgAAaCCTACaT)

on chromosome 5 of Glyma 05G040500.1, Glyma05G040500.2 and Glyma05G040500.2 were express disease on soybean all of cultivar test within 48 h (Table 6) these genes they might have a role in pustule formation. Interestingly, The *tal2b* in each race from different strains recognized different effector binding elements (EBEs) on host. The *tal2b* effector from *X. axonopodis* pv. *glycines* strain SP4 (race 1) in targeting the EBEs of enhanced disease resistance gene: *GmPR2* (TcCcACtAcACCCTACCc) was induced hypersensitive response (HR) on soybean cv. Williams82, Spencer and PI520733 within 48 hrs. The *tal2b* effector from *X. axonopodis* pv. *glycines* strain 12−2 (race 2) in targeting the EBEs of lateral organ boundaries (*GmLOB1*) domain family protein (TACcAaAgAAaCCTACaT) and *GmSWEET*: sugars will eventually be exported transporters (TACAAaAcAAgCaTACCT) were express disease on soybean cv. Williams82, Spencer, PI520733 and SJ.5 within 48 hrs. The *tal2b* effector from *X. axonopodis* pv. *glycines* strain KU-P-SW005 (race 3) in targeting the EBEs of Pathogenesis-related thaumatin superfamily protein gene: *GmPR9* (TAaAAaAAAACaCTgCCT) was express HR on resistant cultivar (Williams82) of soybean and lateral organ boundaries (*GmLOB1*) domain family protein (TACcAaAgAAaCCTACaT) was express disease on susceptible (Spencer, PI520733 and SJ.5) soybean cultivars. (Figure 6)

Table 6 Candidate targets of *tal2b* of *X. axonopodis* pv. *glycines* in each race were predicted by using the TALE-NT 2.0 Target Finder tool

Race	TAL effector	Candidate gene	Transcript	EBE	Annotation
1	*tal2b*	Chromosome 9	Glyma 09G212100.1	TcCcACtAcACCCTACCc	*GmPR2*
		Chromosome 9	Glyma 09G212100.2	TcCcACtAcACCCTACCc	*GmPR2*
2	*tal2b*	Chromosome 5	Glyma 05G040500.1	TACcAaAgAAaCCTACaT	*GmLOB1*
		Chromosome 5	Glyma 05G040500.2	TACcAaAgAAaCCTACaT	*GmLOB1*
		Chromosome 5	Glyma 05G040500.3	TACcAaAgAAaCCTACaT	*GmLOB1*
		Chromosome 1	Glyma 01G186700.1	TACAAaAcAAgCaTACCT	*GmSWEET1*
		Chromosome 11	Glyma 11G117100.1	TACAAaAcAAgCaTACCT	*GmSWEET1*
		Chromosome 11	Glyma 11G186700.3	TACAAaAcAAgCaTACCT	*GmSWEET1*
3	*tal2b*	Chromosome 12	Glyma 12G064300.1	TAaAAaAAAACaCTgCCT	*GmPR9*
		Chromosome 1	Glyma 17G086300.1	TACcAaAgAAaCCTACaT	*GmLOB1*
		Chromosome 5	Glyma 05G040500.1	TACcAaAgAAaCCTACaT	*GmLOB1*

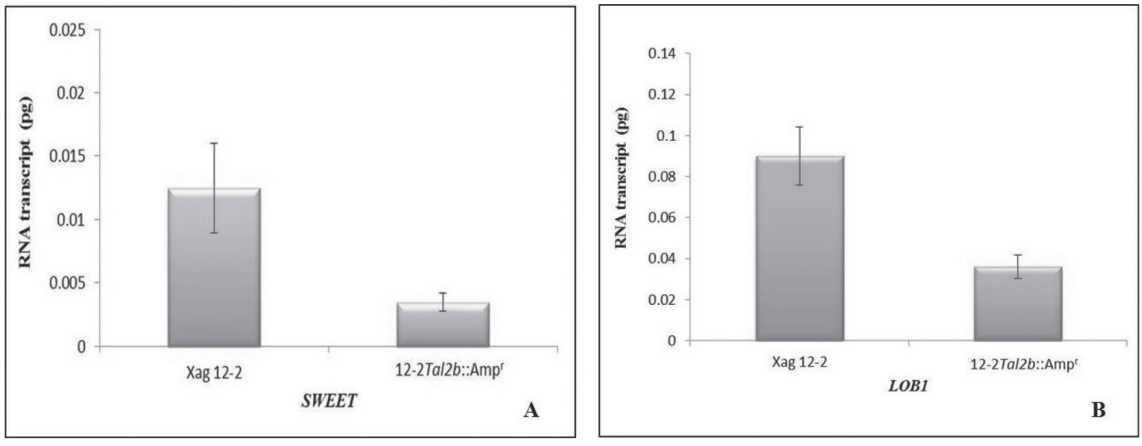

Figure 6 Transcriptional levels of *SWEET* and *LOB1* gene were determined by quantitative RT-PCR: *SWEET* (A) and *LOB1* (B)

Previous reports have indicated that functional region can be located in the same or different regions of the crystal structure formed a b-barrel structure and showed high similarity to the pathogenesis-related (PR) thaumatin superfamily protein thaumatin-like xylanase inhibitor (TL-XI) an elicitor. The region between amino acids 35 and 53 of HapG , identified in *X.axonopodis* pv. *glycines*, induced HR and exhibited elicitor activity (Kim *et al*., 2003). The TAL effectors are a large family of closely related type III effector proteins, which transcriptionally activate host gene expression by directly interacting with promoter elements of host genes and have a varying degree of contribution in bacterial virulence, proliferation and other disease symptomatology (Boch and Bonas, 2011; White and Yang, 2009). So far, TAL effectors with virulence contribution have been detected in strains of *X. oryzae* pv. *oryzae*, *X. citri* pv. *citri*, *X. campestris* pv. *vesicatoria*, *X. campestris* pv. *malvacearum* and *X. oryzae* pv. *oryzicola* (Antony *et al*., 2010; Cernadas *et al*., 2014; Hu *et al*., 2014; Kay *et al*., 2007; Li *et al*., 2014; Wichmann and Bergelson, 2004; Zhou *et al*., 2015). TAL effectors are commonly present in various Xanthomonas strain and contribute to virulence. *avrBs3*-like genes, one of the earliest characterized TAL effector members from *X. campestris* pv. *vesicatoria* was shown to serve as an apparent virulence factor when *S* gene *upa20* is induced in the host (Kay *et al*., 2007). For the citrus bacterial canker causal strains, many of them carry more than one TAL effector gene. However, only one gene in each strain is known to be the major virulence effector (Al-Saadi *et al*., 2007). Whether TAL effectors play roles as virulence factors in other *Xanthomonas*-associated disease is unknown. An understanding of the mechanism of TAL effector mediated pathogenesis on hosts will be gained by exploring more pathosystem. Thus, further work is still need to confirm the two candidate *S* genes as targets of *tal2b* of *X. axonopodis* pv. *glycines* 12−2. An understanding of the mechanisms of TAL effector mediated pathogenesis on hosts will be gained by exploring more pathosystems.

The reverse transcriptase-PCR of *SWEET* gene (sugars will eventually be exported transporters) and *LOB1 gene* (lateral organ boundaries domain family protein production) were used to measure effect of *Tal2b* on gene expression. We found that the level of RNA transcript of wildtype (Xag 12-2) of these gene higher than *tal2b* mutant

strain (12−2*tal2b*::Amp^r) on soybean leaf. Therefore, the influence of *SWEET* and *LOB1* were associated with a reduction in the rate of transcription level of *SWEET* and *LOB1* in soybean leaf when inoculation with mutant strain. Besides, the period or cycle was directly related to RNA concentration (data not shown). The soybean leaf that inoculated with mutant strains (12−2*tal2b*::Amp^r) was expression *SWEET* and *LOB* less and slower than wildtype. The result suggested that *tal2b* of Xag12−2 interaction with *SWEET* and *LOB* during infected soybean plant.

CONCLUSION

The 169 strains of *X. axonopodis* pv. *glycines* can be categorized into 3 races , and the representative strains of each race were selected as strain SP4 for race 1, strain 12−2 for race 2, and strain KU-P-SW005 for race 3. In this work, most of the recently isolated of *X. axonopodis* pv. *glycines* strains belonged to race 2, the highly virulent strain group. The *tal* genes from the representative strains of each race were identified and characterized by PacBio genome sequencing and Southern blot hybridization. Race 1 (strain SP4) and race 2 (strain 12−2) carry 6 *tal* genes, named *tal1a*, *tal1b*, *tal1c*, *tal2a*, *tal2b* and *tal3*, respectively, according to their positions in the certain genome. Race 3 (strain KU-P-SW005) carries 5 *tal* genes, almost same as the formers except for *tal1c*. *R* gene isolation has traditionally been accomplished by positional cloning, a labor intensive and time-consuming strategy that depends on parental lines with dense marker maps and robust phenotypic distinction for the trait of interest. Since, TAL effectors act by direct induction of their target promoters, gene activation is a molecular signature for the point of interaction between TAL effector and host. The genetic analyses of TAL effectors in *X. axonopodi*s pv. *glycines* will provide better understating into the pathogenicity mechanism of this pathogen. As expected each race carries TAL effectors and one TAL effector that found to play major role in virulence of pathogen is *tal2b*. Due to the specificity of *tal2b* gene in all three races of *X. axonopodi*s pv. *glycines,* the primers designed from this gene was able to identified race of this pathogen as shown in phylogenetic tree construct from this primer. Thus, race identification of this pathogen can be done by PCR with TAL2b primer and no need for phenotypic screening. More importantly, the *tal2b* also associated with the virulence and avirulence factor in race 1 and race 2, respectively. As in race 1, *tal2b* link to *R* gene which result in non-pathogenetic of this race. On the other hand, *tal2b* in race 2 which is the most virulence strain was found linked to *S* gene including *SWEET* and *LOB1* gene. Beside TAL effectors, *avr* gene also function in virulence level of *X. axonopodi*s pv. *glycines,* as shown in HR that presence in soybean cv. Williams82 and cv. Spencer after induced from double mutant strain (12−2*avrXg2&tal2b*:: Km^r Amp^r).

ACKNOWLEDGEMENTS

We express the appreciation to the Thailand Research Fund (TRF) for the financial support of this research under the Royal Golden Jubilee Ph.D (RGJ) Program. Partial support from Center for Advanced Studies in Tropical Natural Resources is gratefully acknowledged.

REFERENCES

Abramovitch, R.B., Y.J. Kim, S. Chen, M.B. Dickman and G.B. Martin. 2003. *Pseudomonas* type III effector *AvrPtoB* induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J. 22: 60−69.

Al-Saadi, A., J.D. Reddy, Y.P. Duan, A.M. Brunings, Q. Yuan and D.W. Gabriel. 2007. All five host-range variants of *Xanthomonas citri* carry one *pthA* homolog with 17.5 repeats that determines pathogenicity on citrus, but none determine host-range variation. Mol. Plant Microbe Interact. 20: 934−943.

Antony, G., J. Zhou, S. Huang, T. Li, B. Liu, F. White and B. Yang. 2010. Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-*11N3*. Plant Cell 22: 3864−3876.

Athinuwat, D., S. Prathuangwong and T.J. Burr. 2009. *Xanthomonas axonopodis* pv. *glycines* -soybean cultivar virulence specificity determinate by *avrBs3* homolog, *avrXag1*. Phytopathology 99: 996−1004.

Bai, J., S.H. Choi, G. Ponciano, H. Leung and J.E. Leach. 2000. *Xanthomonas oryzae* pv. *oryzae* avirulence genes contribute differently and specifically to pathogen aggressiveness. Mol. Plant Microbe Interact. 13: 1322−1329.

Boch, J., H. Scholze, S. Schornack, A. Landgraf, S. Hahn, S. Kay, T. Lahaye, A. Nickstadt and U. Bonas. 2009. Breaking the code of DNA-binding specificity of TAL-Type III effectors. Science 326: 1509−1502.

Boch, J. and U. Bonas. 2011. *Xanthomonas* AvrBs3 family-type III effectors: Discovery and function. Annu. Rev. Phytopathol. 48: 419−436.

Bogdanove, A.J., S. Schornack and T. Lahaye. 2010. TAL effectors: Finding plant genes for disease and defense. Curr. Opin. Plant Biol.13: 394−401.

Booher, N.J., S.C.D. Carpenter, R.P. Serbra, L. Wang, S.L. Salzberg, J.E. Leach and A.J. Bogdanove. 2015. Single molecule real-time sequencing of *Xanthomonas oryzae* genomes reveals a dynamic structure and complex TAL (transcription activator-like) effector gene relationships. Microb. Genom. 1(4): e000032.

Cermak, T., E.L. Doyle, M. Christian, L. Wang, Y. Zhang, C. Schmidt, J.A. Baller, N.V. Somia, A.J. Bogdanove and D.F. Voytas. 2011. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. 39(12): e82.

Cernadas, R.A., E.L. Doyle, D.O. Nino-Liu, K.E. Wilkins, T. Bancroft, L. Wang, C.L. Schmidt, R. Caldo, B. Yang, F.F. White, D. Nettleton. R.P. Wise and A.J. Bogdanove. 2014. Code-assisted discovery of TAL effector targets in bacterial leaf streak of rice reveals contrast with bacterial blight and a novel susceptibility gene. PLoS Pathog. 10: e1003972.

Chatnaparat, T., S. Prathuangwong, M. Ionescu and S.E. Lindow. 2012. *XagR*, a *LuxR* homolog, contributes to the virulence of *Xanthomonas axonopodis* pv. *glycines* to soybean. Mol. Plant Microbe Interact. 25: 1104−1117.

Chen, L.Q. 2014. SWEET sugar transporters for phloem transport and pathogen nutrition. New Phytol. 201(4): 1150−1155.

Chen, Z., A.P. Kloek, J. Boch, F. Katagiri and B.N. Kunkel. 2000. The *Pseudomonas syringae avrRpt2* gene product promotes pathogen virulence from inside plant cells**.** Mol. Plant Microbe Interact. 13: 1312−1321.

Chisholm, S.T., G. Coaker, B. Day and B.J. Staskawicz. 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell 124: 803−814.

Cohn, M., R.S. Bart, M. Shybut, D. Dahlbeck, M. Gomez, R. Morbitzer, B.H. Hou, W.B. Frommmer, T. Lahaye and B.J. Staskawics. 2014. *Xanthomonas axonopodis* virulence is promoted by a transcription activator-like effector-mediated induction of a SWEET sugar transporter in cassava. Mol. Plant Microbe Interact. 27: 1186−1198.

Domingues, M.N., T.A. de Souza, R.A. Cernadas, M.L. de Oliveira, C. Docena, C.S. Farah and C.E. Benedetti. 2010. The *Xanthomonas citri* effector protein *PthA* interacts with citrus proteins involved in nuclear transport, protein folding and ubiquitination associated with DNA repair. Mol. Plant Pathol. 11(5): 663−675.

Doyle, E.L., N.J. Booher, D.S. Standage, D.F. Voytas, V.P. Brendel, J.K. VanDyk and A.J. Bogdanove. 2012. TAL Effector-Nucleotide Targeter (TALE-NT) 2.0: Tools for TAL effector design and target prediction**.** Nucleic Acids Res. 40: 117−122.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9: 275−296.

Hajdukiewicz, P., Z. Svab and P. Maliga. 1994. The small, versatile *pPZP* family of *Agrobacterium* binary vectors for plant transformation. Mol. Plant Microbe Interact. 25(6): 989−994.

Heuer, H., Y. Yin, Q. Xue, K. Smalla and J. Guo. 2007. Repeat domain diversity of *avrBs3*-like genes in *Ralstonia solanacearum* strains and association with host preferences in the field. Appl. Environ. Microbiol. 73(13): 4379−4384.

Hu, Y., J. Zhang, H. Jia, D. Sosso, T. Li, W.B. Frommer, B. Yange, F. White, N. Wang and J.B. Jonesa. 2014. Lateralorgan boundaries is a disease susceptibility gene for citrus bacterial canker disease. Proc. Natl. Acad. Sci. U.S.A. 111: 521−529.

Kaewnum, S., S. Prathuangwong and T.J. Burr. 2006. A pectate lyase homolog, *xagP*, in *Xanthomonas axonopodis* pv. *glycines* is associated with hypersensitive response induction on tobacco. Phytopathology 96: 1230−1236.

Kay, S., S. Hahn, E. Marois, G. Hause and U. Bonas. 2007. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. Science 318(5850): 648−651.

Kim J.G., B.K. Park, C.H. Yoo, E. Jeon, J. Oh and I. Hwang. 2003. Characterization of the *Xanthomonas axonopodis* pv. *glycine*s Hrp pathogenicity island. J. Bacteriol. 185(10): 3155−3166.

Kitten, T. and D.K. Willis. 1996. Suppression of a sensor kinase-dependent phenotype in *Pseudomonas syringae* by ribosomal proteins L35 and L20. J. Bacteriol. 178: 1548−1555.

Koren, S. and A.M. Phillippy 2015. One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly. Curr. Opin. Plant Biol. 23: 110−120.

Kovach, E.M., P.H. Elzer, D.S. Hill, G.T. Robertson, M.A. Farris, R.M. Roop and K.M. Peterson. 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene 166(1): 175−176.

Li, Z., L. Zou, G. Ye, L. Xiong, Z. Ji, M. Zakria, N. Hong, G. Wang and G. Chen. 2014. A Potential disease susceptibility gene *CsLOB* of citrus is targeted by a major virulence effector PthA of *Xanthomonas citri* subsp. *citri*. Mol. Plant 7(5): 912−915.

Makino, S. 2005. Molecular Mechanisms of Pathogenesis in Pathovars of the Rice Pathogenic Bacterial Species *Xanthomonas Oryzae*. M.S. thesis, Iowa State University, USA.

Moscou, M.J. and A.J. Bogdanove. 2009. A simple cipher governs DNA recognition by TAL effectors. Science 326(5959): 1501.

Mudgett, M.B. 2005. New insights to the function of phytopathogenic bacterial type III effectors in plants. Annu. Rev. Plant Biol. 56: 509−531.

Narvel, J.M., L.R. Jakkula, D.V. Phillips, T. Wang, S.H. Lee and H.R. Boerma. 2001. Molecular mapping of *Rxp* conditioning reaction to bacterial pustule in soybean. J. Hered. 92(3): 267−270.

Prathuangwong, S. 1983. Effect of different soybean plant ages on susceptibility of *Xanthomonas campestris* pv. *glycines.* J. Thai Phytopathol Soc. 3: 148−153.

Prathuangwong, S. 1984. A simple technique for inoculation of *Xanthomonas campestris* pv. *glycines.* Thai J. Agri. Sci. 17(3): 183−189.

Prathuangwong, S., C. Preecha, N. Thaveechai and S. Taeesombat. 1990. Distribution and severity of soybean bacterial pustule in Thailand. pp.76−80. *In* Proc. of the 3rd International Conference on Plant Protection in the Tropics, Vol. VI. Mar. 20−23, 1990. Malaysia.

Prathuangwong, S., S. Saisangthong, A. Fareaendee and P. Chutawantana. 1996. The incidence and new occurrence of soybean disease at central area of Thailand during 1994-1996. pp. 242−258. *In* Proc. of the 6th Nat. Soybean Res. Con. Chang Mai, Thailand.

Szurek, B., O. Rossier, G. Hause and U. Bonas. 2002. Type III-dependent translocation of the *Xanthomonas AvrBs3* protein into the plant cell. Mol. Microbiol. 46(1): 13−23.

White, F.F. and B. Yang. 2009. Host and pathogen factors controlling the rice-*Xanthomonas oryzae* interaction. Plant Physiol**.** 150(4): 1677−1886.

White, F.F., B. Yang and L.B. Johnson. 2000. Prospects for understanding avirulence gene function. Curr. Opin. Plant Biol. 3(4): 291−298.

Wichmann, G. and J. Bergelson. 2004. Effector genes of *Xanthomonas axonopodis* pv. *vesicatoria* promote transmission and enhance other fitness traits in the field. Genetics 166 (2): 693−706.

Yang, B. and F.F. White. 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(11): 1192−1200.

Zhang, F., L. Cong, S. Lodato, S. Kosuri, G.M. Church and P. Arlottal. 2011. Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. Nat. Biotechnol. 29: 149−153.

Zhou, J., Z. Peng, J. Long, D. Sosso, B. Liu, J.S. Eom, S. Huang, S. Liu, C.C. Vera, W.B. Frommer, F.F. White and B. Yang. 2015. Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. Plant J. 82(4): 632−643.