OsSWEET11 and *OsSWEET14*: The Possible Key Genes Involved in Bacterial Blight Susceptibility in Rice Cultivar RD47

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ABSTRACT

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the major diseases that impacts rice production in Asia. Rice cultivar RD47 popularly grown in lower Northern Thailand is susceptible to BB disease. The RD47 and IRBB21 plants artificially inoculated with two *Xoo* isolates, Xoo16PT005 and Xoo16PK002, isolated from Phichit and Phisanulok provinces, respectively. The RD47 cultivar was completely susceptible whereas the IRBB21 cultivar carrying the BB resistance gene, *Xa21*, was strongly resistant to both *Xoo* isolates. Lesion lengths occurred on *Xoo*-inoculated leaves indicated that Xoo16PK002 was more virulent than Xoo16PK005. Expression analysis of two BB susceptibility genes, *OsSWEET11* and *OsSWEET14* in the cultivar RD47 inoculated with *Xoo*16PT005 and *Xoo*16PK002 revealed that the expression of both genes was very low or undetectable at 0 hour after *Xoo* inoculation and obviously induced within 48 hours after *Xoo* inoculation. This presumes that the two *Xoo* isolates, Xoo16PT005 and Xoo16PK002 produce the transcription activator-like effectors that are compatible to the effector-binding element of the *OsSWEET11* and *OsSWEET14* promoters leading to activate the transcription. This finding suggests that the *OsSWEET11* and *OsSWEET14* genes may play a major role in the BB susceptibility of rice cultivar RD47.

Key words: bacterial blight, OsSWEET11, OsSWEET14, rice, Xoo, *Xanthomanas*

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INTRODUCTION

Rice (*Oryza sativa*) is one of the most economically important crops in Thailand. Although improvement of yield and seed quality is the first priorities for rice breeding, resistance to insects and diseases is also a major aim of rice breeding. One of the most important diseases in rice growing in lower Northern Thailand is the bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Yield loss caused by the BB disease can reach up to 70% in infected growing areas (http://www.knowledgebank.irri.org, International Rice Research Institute, IRRI). The photoperiod-insensitive rice cultivar RD47 has been one of popular cultivars grown in lower Northern Thailand. It produces high yield and good seed quality. It is rather resistant to brown planthopper (BPH) and the blast disease but susceptible to the BB disease (http://www.brrd.in.th/rkb, Bureau of Rice Research and Development: Rice Department, Thailand).

Xoo pathogenicity depends on a specific class of virulence factors, called transcription activator-like effectors (TALEs), which resemble eukaryotic transcriptional activators (Hutin *et al*., 2015). At the translocation into the plant cell and import in the nucleus, TALEs bind to specific promoter elements, called effector-binding elements (EBEs). This recognition triggers transcription of the targeted gene, whose function often determines the outcome of the interaction. Rice resistance to *Xoo* often relies on executor genes distinct from classical resistance genes, whose transcriptional activation by TALEs triggers immunity, leading to dominant resistance (Zhang *et al*., 2015). TALEs are important pathogenicity determinants in *Xoo* interaction. Several TALEs have been found to be essential virulence factors of *Xoo*, which induce host susceptibility genes and after that promoting pathogen infection and disease development (Yang and White, 2004, Yang *et al*., 2006, Antony *et al*., 2010 and Yu *et al*., 2011).

Xoo TALEs with more substantial effects on virulence uniformly activate members of the *OsSWEET* family genes encoding sucrose transporter proteins to promote disease (Chen *et al*., 2010). Two *SWEET* genes, *OsSWEET11* and *OsSWEET14*, have been identified as targets of four different TALEs from various *Xoo* strains (Chu *et al*., 2006, Yang *et al*., 2006, Antony *et al*., 2010 and Chen *et al*., 2012). The most effective method to control BB is the use of resistant varieties (Khush *et al*., 1989), therefore, the breeding program for BB-susceptible rice varieties through the regulation of *SWEET* gene expression is an alternative approach to improve the BB resistance in rice.

In this study, we determined whether *OsSWEET11* and *OsSWEET14* genes responded to *Xoo* isolated from Phisanulok and Phichit provinces in BB susceptible rice cultivar RD47. This will be helpful to evaluate the direction of molecular breeding to improve rice BB resistance.

Objectives

1. To evaluate the virulence of *Xoo* isolated from Phitsanulok and Phichit provinces on BB susceptible rice cultivar RD47.

2. To characterize the expression of *OsSWEET11* and *OsSWEET14* in rice cultivar RD47 at the early period of *Xoo* infection.

MATERIALS AND METHODS

Plant and *Xoo* materials

Rice (*Oryza sativa* L. ssp. *indica*) cultivars RD47 (carrying null-*Xa21*) and IRBB21 (carrying *Xa21*) derived from Phitsanulok Rice Research Center, Thailand were planted in small pots containing 720 g clay soil/pot. The experimental rice plants were grown in the greenhouse under natural light and temperature. Two *Xoo* isolates, Xoo16PT005 (isolated from rice paddy field in Phichit province, Thailand) and Xoo16PK002 (isolated from rice paddy field in Phitsanulok province, Thailand) were used for the inoculation test and the *OsSWEET* expression analysis. The BB infected leaves were cleaned with soap and washed with sterile distilled water. Under the laminar flow condition, they were surface sterilized with 10% Clorox for 10 min, and rinsed three times with sterile distilled water. After drying on tissue papers, leaves cut in pieces (approximately 1-2 cm long) were deposited on the NA medium (beef extract 3 g/L, peptone 5 g/L and agar 15 g/L) and incubated at 28°C for 72 h. The yellowish *Xoo* bacteria developing around and exudate oozing from the leaf pieces were re-streaked on the new NA medium to isolate single colonies. To confirm that the isolated bacteria were *Xoo*, PCR tests using the TXT primers (Sakthivel *et al*., 2001) and Xoo4009 primers (Lang *et al*., 2010) were performed.

***Xanthomonas oryzae* pv. *oryzae* (*Xoo*) inoculum and inoculation**

The *Xoo* inoculum was prepared by suspending bacterial colonies in sterilized distilled water and adjusted a dilution to OD_{600} = 0.2 then inoculated to 60-day old experimental plants using the clipping method (Kauffman, 1973). The experimental rice plants were grown in the greenhouse under natural light and temperature. The Xoo16PT005 and Xoo16PK002 isolates were inoculated to five leaves of each plant of the RD47 and IRBB21cultivars. Five replicates of each cultivar were carried out. *Xoo*-free inoculation was used as a control. The disease response was evaluated 7, 14 and 21 days after inoculation by measuring the lesion lengths of inoculated leaves, and scored for BB resistance according to the IRRI standard evaluation system as follows: resistance (R, lesion length 0-5 cm), moderate resistance (MR, lesion length >5-10 cm), moderately susceptible (MS, lesion length>10-15 cm), susceptible (S, lesion length>15 cm) (http://www .knowledgebank.irri.org/decision-tools/rice-doctor/rice-doctor-fact- sheets/item/bacterial-blight, International Rice Research Institute Knowledge Bank).

Expression analysis of *OsSWEET11* and *OsSWEET14*

1. RNA isolation and RT-PCR

Leaves of 21-day old RD47 plants were inoculated with the two *Xoo* isolates, *Xoo*16PT005 and *Xoo*16PK002. Inoculated leaf segments from five plants were bulk and ground into fine powder using liquid nitrogen. The inoculated samples were collected at 0 and 48 hours post inoculation (HPI) and frozen in liquid nitrogen immediately. Total RNA was isolated using the FavorPrep™ Plant Total RNA Mini kit (Favorgen, Taiwan) following the manufacturer's recommendation. For RT-PCR analysis, the first strand cDNA was synthesized from 500 ng total RNA using first-strand cDNA synthesis kit (Thermo Fisher Scientific Inc. Waltham, MA) following the manufacturer's recommendation. Then PCR was carried out using GoTaq® Green Master Mix (Promega, USA). Five microliters of cDNA and 0.5 μM each primer were used in 20 μl of PCR reaction. Primers used for detection of *OsSWEET11* transcripts were 5´-GTCAAGTTCCTCGGCAGCG-3´ and 5'-GCAGAACCACGCGACGGC-3´ (GenBank no. XM_015792937). Primers used for detection of *OsSWEET14* transcripts were 5´-GGCGACCGCCGCATCGTGGTT-3´ and 5´-GCCCAGCACGTTGGGAAGAGCG-3´ (Valerie *et al*., 2012). The *endothelial differentiation factor* (*edf*) gene was used as a reference gene. Amplification of *edf* was performed using primers based on Wang *et al*. (2016). The reaction mixture was amplified for denaturation step of 5 min at 94°C followed by 35 cycles of 10s at 94°C, 30s at 60°C and 40 s at 72°C, and ending with a final elongation step of 5 min at 72°C.

2. Experimental design and statistical analysis

Experiments were laid out using a complete randomized design (CRD). Test samples included of five plants per rice cultivar (RD47 and IRBB21) with 5 leaves of each cultivar. Data on average lesion lengths were subject to analysis of variance (ANOVA). Means with a significant difference were subject further to Least Significant (LSD) using IBM SPSS Statistics Version 19 software with 0.05 alpha as an error value.

RESULTS

***Xoo* infection tests on rice cultivars RD47 and IRBB21**

Sixty-day old RD47 and IRBB21 plants artificially inoculated with two *Xoo* isolates, *Xoo*16PT005 and *Xoo*16PK002 were classified into different groups of BB score according to the lesion length (LL) at 21 days after inoculation (Table 1 and Figure 1). The IRBB21 cultivar carrying *Xa21* classified in the BB resistance group showed very slow progress of LL at the rate of 0.04-0.06 cm/day in *Xoo*16PT005 infection and 0.07-0.11 cm/day in *Xoo*16PK002 infection. The RD47 cultivar carrying null-*Xa21* classified in the BB susceptibility group showed approximately 13-29 times and 11-19 times faster LL progress than the IRBB21 cultivar when it was infected with *Xoo*16PT005 and *Xoo*16PK002, respectively.

Table 1 Lesion length (LL) measurement (cm) on leaves of rice cultivars RD47 and IRBB21 inoculated with *Xoo*16PT005 and *Xoo*16PK002 at 7, 14 and 21 days after *Xoo* inoculation (DAI).

Days after inoculation (DAI)	Xoo16PT005		Xoo16PK002	
	IRBB21	RD47	IRBB21	RD47
7	0.29 ± 0.07[a]	3.90±0.33[b]	0.49 ± 0.08[a]	5.79±0.39[b]
14	0.82 ± 0.10[a]	21.81±1.72[b]	1.22 ± 0.13[a]	31.82±2.03[b]
21	1.32 ± 0.13[a]	37.33±2.09[b]	2.42 ± 0.30[a]	44.13±2.96[b]
BB resistance score*	R	S	R	S

Data are presented as the mean ± standard error of lesion lengths of 20 leaves per rice cultivar. Means followed by the same letter are not significantly different according to DMRT at p = 0.05.

* BB resistance was scored at 21 DAI. R = Resistant (lesion length 0-5 cm), S = Susceptible (lesion length >15 cm).

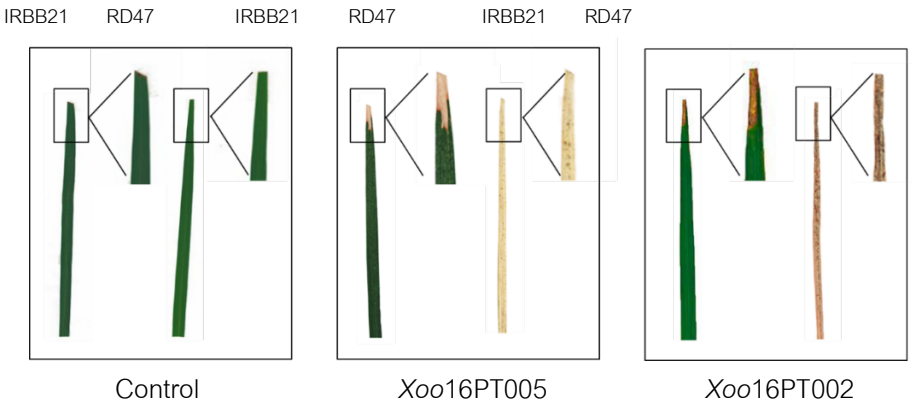

Figure 1 The BB disease severity evaluated on *Xoo*-inoculated leaves of rice cultivars RD47 and IRBB21 inoculated with *Xoo*16PT005 and *Xoo*16PK002 at 21 days.

Expression analysis of *OsSWEET11* and *OsSWEET14* in rice cultivar RD47 inoculated with Xoo16PT005 and Xoo16PK002.

The 21-day-old RD47 seedlings inoculated with Xoo16PT005 and Xoo16PK002 showed very low and undetectable levels of *OsSWEET11* and *OsSWEET14* transcripts at 0 HPI (Figure 2). The induction of *OsSWEET11* and *OsSWEET14* expression was obviously detected at 48 HPI (Figure 2).

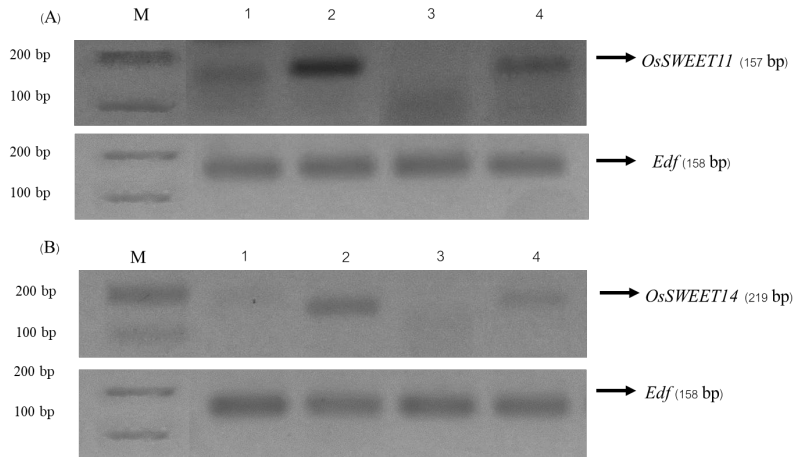

Figure 2 The RT-PCR products of *OsSWEET11* and *OsSWEET14*. (A) The expression of *OsSWEET11* in *Xoo*16PT005 and *Xoo*16PK002-infected RD47 plants. (B) The expression of *OsSWEET14* in *Xoo*16PT005 and *Xoo*16PK002-infected RD47 plants. Lanes M, 50 bp Marker (Hyper Ladder™ 50bp, Bioline); Lanes 1 and 2, RD47 at 0 and 48 hours after inoculation with *Xoo*16PT005, respectively; Lanes 3 and 4, RD47 at 0 and 48 hours after inoculation with *Xoo*16PK002, respectively. *Edf* is a reference gene.

DISCUSSION

In this research, we determined whether the potential BB susceptibility genes, *OsSWEET11* and *OsSWEET14* were involved in the BB susceptibility caused by the *Xoo* isolates *Xoo*16PT005 and *Xoo*16PK002 in rice cultivar RD47. The *Xoo* inoculation test showed that the RD47 plants was completely susceptible to both *Xoo* isolates. The IRBB21 plants carrying the BB resistance gene, *Xa21*, used as the BB resistant control was strongly resistant. Lesion lengths measured on leaves infected with both *Xoo* implied that Xoo16PK002 was more virulent than Xoo16PT005. However, these *Xoo* isolates hardly invaded the BB resistant IRBB21 cultivar. The *Xa21*, a broad-spectrum BB resistance gene, could be a key gene for BB resistance. Sagun *et al*. (2019) reported that *Xa21* enhanced the BB resistance in the RD47 cultivar but the BB resistance level was classified into the moderate resistance. Other factors in the genetic background of the RD47 cultivar either the BB susceptibility genes or the co-factors of the BB resistance genes may involve in the response of the RD47 plants to *Xoo* attack. Expression analysis of the BB susceptibility genes, *OsSWEET11* and *OsSWEET14* in the RD47 plants revealed that both Xoo16PT005 and Xoo16PK002 induced their expression within 48 h after inoculation. Although many reports detected the expression of *OsSWEET11* and *OsSWEET14* at 48 h after *Xoo* inoculation, the expression of *OsSWEET11* and *OsSWEET14* was induced as early as 24 h after *Xoo* inoculation in BB susceptible rice cultivar IR24 (Zaka *et al*., 2018). The response of *OsSWEET11* and *OsSWEET14* in the RD47 cultivar presumes that Xoo16PT005 and Xoo16PK002 produced the specific TALEs, which are compatible to the EBEs located in both promoters of *OsSWEET11* and *OsSWEET14*

leading to activate the transcription of both genes. Therefore, the *OsSWEET11* and *OsSWEET14* may play a major role in the BB susceptibility of the RD47 cultivar. The EBEs of both genes would be the targets of genome editing for mutation to prevent the recognition of the TALEs from Xoo16PT005 and Xoo16PK002 causing no induction of the *OsSWEET11* and *OsSWEET14* expression. Mutations of the *OsSWEET14* EBE sequences for AvrXa7 and Tal5 TALEs led to BB resistance in BB susceptible rice cultivar Kitaake (Blanvillain-Baufume *et al*., 2017). This suggests that the EBE sequence editing is the alternative approach from the use of the *Xa21* gene to improve the BB resistance in rice cultivar RD47.

CONCLUSION

Rice cultivar RD47 was completely susceptible to the *Xoo* isolates Xoo16PT005 and Xoo16PK002, which were derived from Phichit and Phitsanulok provinces, respectively. These two *Xoo* isolates could induce the expression of *OsSWEET11* and *OsSWEET14* genes within 48 HPI. They could be key genes involved in BB susceptibility in rice cultivar RD47.

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