Identification of SSR Markers Linked to a Bacterial Blight Resistance Gene in Rice Cultivar 'Pin Kaset'

การค้นหาเครื่องหมายโมเลกุล SSR ที่มีความสัมพันธ์กับยีนต้านทานโรคขอบใบแห้งในข้าวพันธุ์ 'ปิ่นเกษตร'

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ABSTRACT

Pin Kaset (PK), a Thai rice cultivar, has a high level of resistance to bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) isolates. In this study, we identified the BB resistant gene in Pin Kaset (PK) using 436 BC_2F_2 plants from a cross between Ba7 and PK. The BC_2F_2 individuals were evaluated with a Xoo isolate TB0304. Based on the phenotypic and genotypic analysis, the SSR marker RM224 showed clearly discriminate banding pattern between resistant and susceptible phenotypes. The result revealed that the BB resistance gene was linked to RM224 on rice chromosome 11. This new gene was tentatively named as *Xa34*(t). Although this identified gene and other BB resistance genes were located in the same region and shared common linked markers on chromosome 11, no evidence has yet been obtained whether they share the same genomic sequence or whether they are tightly linked to each other.

บทคัดย่อ

ข้าวพันธุ์ 'ปิ่นเกษตร' แสดงความต้านทานได้ดีต่อเชื้อโรคขอบใบแห้ง *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) ในประเทศไทย การศึกษานี้ได้ค้นหาตำแหน่งยีนต้านทานโรคขอบใบแห้งในข้าวพันธุ์ปิ่นเกษตร จากประชากรลูกผสมกลับ BC_2F_2 จำนวน 436 ต้น ซึ่งได้จากการผสมข้ามระหว่างข้าวพันธุ์ Ba7 กับพันธุ์ปิ่นเกษตร และทดสอบความต้านทานโรคกับประชากรข้าวโดยใช้เชื้อไอโซเลท TB0304 ผลการตรวจสอบฟีโนไทป์ และจีโนไทป์ พบว่า เครื่องหมายโมเลกุล RM224 บนโครโมโซมคู่ที่ 11 มีความสัมพันธ์อย่างใกล้ชิดกับยีนต้านทาน สามารถแยกสายพันธุ์ต้านทานและอ่อนแอออกจากกันได้อย่างชัดเจน ถึงแม้ว่ายีนต้านทานที่ค้นพบจะวางอยู่บนตำแหน่งเดียวกับยีนต้านทานอื่นที่มีรายงานก่อนหน้านี้ แต่ยังไม่ปรากฏหลักฐานยืนยันว่ายีนเหล่านี้เป็นยีนเดียวกับยีนที่ค้นพบ ดังนั้นจึงเรียกชื่อยีนต้านทานใหม่นี้อย่างไม่เป็นทางการว่า *Xa34*(t)

Key Words : Bacterial blight, SSR marker, *Xa34*(t) gene
คำสำคัญ : โรคขอบใบแห้ง เครื่องหมายโมเลกุลชนิด SSR ยีน *Xa34*(t)

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Introduction

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive diseases in rice-producing areas in Thailand. The disease was prevalent in irrigated and rainfed lowland rice growing ecosystems. The damage of rice crop caused by BB was first reported in Thailand at Pathum Thani province since 1963 (Tabei and Eamchit, 1974). The BB disease can cause yield loss typically ranging from 20-30%. In the severe case, the damage was as high as 50% yield reduction (Ou, 1985). Since the chemical control was not effective, the utilization of resistant varieties have been considered to be the most effective way to control the disease.

The identification and characterization of major BB resistance genes from cultivated rice and wild relatives have been reported and about 30 resistance genes have been mapped on rice chromosomes (Niño-Lui *et al.*, 2006; Wang *et al.*, 2007; Singh *et al.*, 2007). BB resistance genes have been identified in cultivated indica and japonica rice and wild species, *O. longistaminata*, *O. rufipogon*, *O. minuta* and *O. officinalis* (Brar and Khush 1997; Lee *et al.*, 2003). Most of the resistant genes were considered to follow the gene-for-gene theory (Flor, 1971).

Several BB resistance genes have been reported to locate on chromosome 11, i.e. *Xa3*, *Xa4*, *Xa6*, *xa9*, *Xa10*, *Xa21*, *Xa22*, *Xa23*, *Xa26* and *Xa30*. Some of them are multifamily genes and tightly linked together.The *Xa21* from *O. longistaminata* (Khush *et al.*, 1989) was the first resistant gene that has been successfully cloned. The predicted structure of *Xa21* has a cytoplasmic domain containing a serine-threonine kinase, a transmembrane domain, and an extracellular domain with leucine rich repeat (LRRs) receptor kinase like proteins (Song *et al.*, 1995). The *Xa3/Xa26* gene encodes a leucine-rich repeat (LRR) receptor kinase-like protein. The gene belongs to a multigene family consisting of four members (Sun *et al.*, 2004). *Xa3* had the same copy numbers of *Xa26* family members from the rice line Minghui 63 (Xiang *et al.*, 2006). According to the previous reports, *Xa3*, *Xa4*, *Xa6*, *xa9*, *Xa22* and *Xa26* were the same gene (Ogawa *et al.*, 1986; Sun *et al.*, 2004; Xiang *et al.*, 2006). The *Xa10* was identified from rice cultivar Cas 209 (Mew *et al.*, 1982; Yoshimura *et al.*, 1983). It confers race-specific resistance to only a few Philippines races of BB pathogen.

In this study, we attempted to identify SSR markers linked to a BB resistant gene in rice cultivar Pin Kaset. The marker would be useful for the improvement of BB-resistant rice breeding program through marker-assisted selection (MAS).

Materials and methods

Plant materials

Two indica rice cultivars 'Pin Kaset' (PK) and Ba7 were used in this study. Ba7 was used as a male parent and PK was used as a recurrent parent. To develop backcross population, a resistant F_2 plant was crossed with the recurrent parent to generate 98 BC_1F_1 plants. Ten selected BC_1F_1 plants were then crossed with the recurrent parent to generate 122 BC_2F_1 plants. Four lines of BC_2F_1 were self-pollinated to produce the BC_2F_2 population. A total of 436 BC_2F_2 individuals were used to identify the relationship between BB resistant

phenotype and linked DNA markers.

The rice variety IRBB21 carrying *Xa21* was used to compare DNA banding pattern at *Xa21* locus using functional marker PB7-8.

Bioassay for BB resistance

A *Xoo* isolate, TB0304, collected from Chaing Rai province was used for the BB resistance evaluation. This isolate showed an incompatible reaction to PK and a compatible reaction to Ba7. The TB0304 was grown in a peptone sucrose agar medium for 72 h at 28°C. The bacterial cells were suspended in sterile water and adjusted to 10^9 CFU/ml. The suspension was applied to assay for a resistant reaction in the BC_2F_2 population at seedling stage (30 days old plant). The BB inoculation was done in the greenhouse using the leaf-clipping method (Kauffman *et al.*, 1973). Three fully expanded leaves of each plant were inoculated. The resistant reactions were recorded based on the mean of lesion length (LL) of an individual plant. The LL was measured at 12-14 days after inoculation. The disease symptom was classified as resistant when the LL was 0 to 3 cm and as susceptible when it was more than 3 cm.

DNA extraction and SSR analysis

Total genomic DNA from young leaves of the BC_2F_2 individuals and parents were extracted using DNA trap kit (DNA Technology Laboratory). Nineteen BC_2F_2 plants consisted of ten most resistant and nine most susceptible ones were selected based on the LL. Four hundred and fourteen simple sequence repeat (SSR) markers covered whole rice genome were selected and used to identify the location of the resistant gene. These SSR markers were obtained from the public database released by Gramene (http://www.gramene.org/).

The PCR reactions for SSR markers were carried out in a total volume of 10 µl containing 20 ng of genomic DNA, 0.02 µM of each primer, 0.2 mM each of dNTP, 2.5 mM $MgCl_2$, 0.2 unit Taq polymerase and 1X PCR buffer. Amplification was performed for 3 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 60°C, and 1 min at 72°C with a final extension of 5 min at 72°C. The amplification products were separated on 4.5% denaturing acrylamide gel electrophoresis and were detected by silver staining method.

Results

Screening for bacterial blight resistance

Frequency distribution of LL showed skewness toward lower LL based on 436 BC_2F_2 individuals plants inoculated at seedling stage with the *Xoo* isolate, TB0304 (Figure 1). The average LL of PK was 1.5±0.7 cm while Ba7 was 6.7± 1.9 cm. When the cut off of LL for resistance was at 3.0 cm, number of resistance and susceptible plants were 389 and 47, respectively. The ratio of resistance to susceptible in BC_2F_2 was 8R:1S.

Detection of BB resistance gene using SSR markers

Of 114 SSR markers, 62 showed very clear polymorphisms between PK and Ba7. These markers were used to identify 19 BC_2F_2 individual plants (10 resistant and 9 susceptible) and their parents. A SSR marker RM224 located on the long arm of rice chromosome 11 showed distinguishable banding patterns between resistant and susceptible plants as shown in Figure 2.

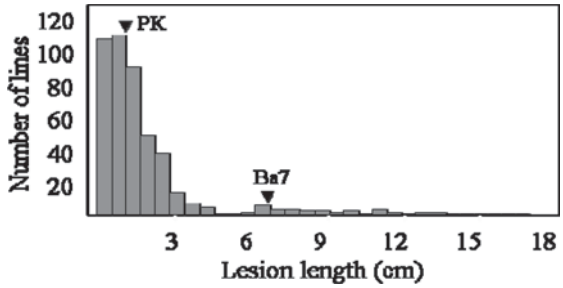

Figure 1 Frequency distribution of LL of BC_2F_2 population after inoculation with the TB0304 isolate. The average LL of PK and Ba7 were 1.5±0.7cm and 6.7±1.9 cm, respectively.

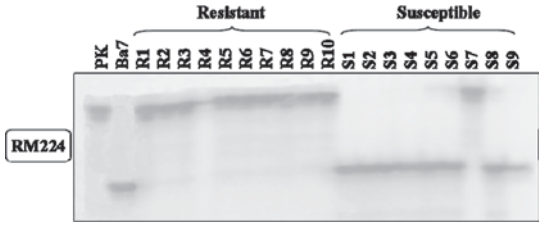

Figure 2 The SSR marker RM224 linked to BB resistant gene. The marker was identified in resistant and susceptible individual plants of the BC_2F_2 population.

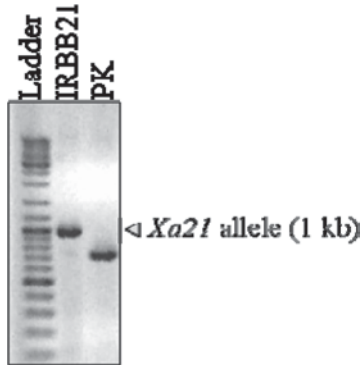

Figure 3 The difference of DNA fragments between rice cultivars IRBB21 and PK at the *Xa21* locus.

DNA banding pattern of IRBB21 and PK using PB7-8

The functional marker PB7-8 was used to detect *Xa21* gene in IRBB21 (carrying *Xa21*) and PK. The result showed that the amplified fragments of IRBB21 and PK were different. IRBB21 carried 1 kb allele of *Xa21*, whereas PK carried only 0.7 kb allele (Figure 3).

Discussion

The BB resistant gene identified in rice cultivar PK was located on chromosome 11. According to the previous studies, there are five BB resistance genes, *Xa21*, *Xa10*, *Xa23*, *Xa3* and *Xa26*, which have been reported to locate in this region. In the present experiment, the BB resistance gene in PK was different from *Xa21* in IRBB21. The results confirmed that a BB resistant gene in PK was not *Xa21*.

The resistance gene *Xa10* was identified from rice cultivar Cas 209 (Mew *et al.*, 1982; Yoshimura *et al.*, 1983). The *Xa10* locus was initially mapped between the RAPD marker O072000 and RFLP marker CDO365 (Yoshimura *et al.*, 1995). Corresponding to the genome position, *Xa23* and *Xa10* were located at approximately 21.40 and 21.66 Mb, respectively while the BB resistant gene in PK was located at around 29.49 Mb on the rice chromosome 11. The experimental observation suggested that BB resistant gene in PK might be different from *Xa23* and *Xa10* genes.

The *Xa3* and *Xa26* were concluded as the same gene (Sun *et al.*, 2004; Xiang *et al.*, 2006). Its locus was mapped between flanking markers RM224 and Y6855RA (Yang *et al.*, 2003).

Although, *Xa3/Xa26* and the resistance gene in PK shared common linked markers but no evidence has obtained yet whether they share the same genomic sequence or they are different loci and tightly linked to each other. The answers of these questions could be provided in the near future when the BB resistant genes have been cloned. Consequently, the BB resistant gene in PK was tentatively designated as *Xa34*(t).

Conclusions

The BB resistant gene in rice cultivar PK was linked with the SSR marker RM224 on the rice chromosome 11. This new gene was tentatively designated as *Xa34*(t). The closely linked marker found in this study will be useful for improvement of BB resistance through MAS in rice breeding programs.

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