การตรวจสอบเครื่องหมายโมเลกุลสำหรับคัดเลือกความต้านทานเพลี้ยกระโดดสีน้ำตาลและโรคไหม้ในพันธุ์ข้าวเหนียวปรับปรุง

Validation of Brown Planthopper and Blast Resistance Markers in Improved Aromatic Glutinous Rice

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ABSTRACT

Bph32 brown planthopper (BPH) resistance gene was incorporated into Hom Xebangfai 4 (HXBF4) together with maintaining two of QTLs BL resistance qBL1 and qBL11. This breeding was processed by a single cross with the BPH and BL resistant glutinous line, RGD13117-115-52-B. Marker-assisted selection was done in F_2 population. In F_3, the 11 selected lines were evaluated for two BPH resistance from Singburi (SBR) and Ayutthaya (AYY) by a modified standard seedbox screening method. The results showed that 10 lines that detected *Bph32 Bph3* and *TPS* had high resistance against both BPH populations. However, the other 1 line carrying *Bph3* and *TPS* had high resistance to AYY but moderately resistant to SBR. After that, the 16 F_4 lines derived from the 1 line of F_3 were evaluated with 7 mixed Thai BL isolates in a greenhouse condition. The results showed that all of the 16 F_4 lines carrying both of qBL1 and qBL11 had resistance against 7 mixed BL isolates and they were more effective rather than that one BL QTL. Therefore, the phenotyping in this study also strongly suggested that the genotyping with high-throughput markers of BPH and BL were very accurate and trustable. Thus, *Bph32*, two QTLs of qBL1 and qBL11 should be recommended for rice breeding programs against BPH and BL in Thailand and Lao PDR.

Keywords: Glutinous rice, Brown planthopper resistance, Blast resistance, Marker-assisted selection

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บทคัดย่อ

ยีนที่ต้านทานเพลี้ยกระโดดสีน้ำตาล (*Bph32*) ถูกถ่ายทอดสู่พันธุ์ข้าวเหนียว Hom Xebangfai 4 (HXBF4) พร้อมกับคงตำแหน่ง QTL ที่ต้านทานต่อโรคไหม้ qBL1 และ qBL11 โดยการผสมข้ามกับสายพันธุ์ RGD13117-115-52-B ซึ่งมียีนดังกล่าว จากนั้นจึงใช้เครื่องหมายโมเลกุลช่วยในการคัดเลือกในประชากรชั่วที่ 2 โดยในชั่วที่ 3 นำสายพันธุ์ที่ถูกคัดเลือกจำนวน 11 สายพันธุ์ มาประเมินความต้านทานต่อเพลี้ยกระโดดสีน้ำตาลประชากรสิงห์บุรี (SBR) และอยุธยา (AYY) ด้วยวิธี modified standard seedbox screening method ผลการทดลองพบว่า สายพันธุ์ที่ถูกคัดเลือก 10 สายพันธุ์ ที่มียีน *Bph32, Bph3* และ *TPS* มีความต้านทานต่อเพลี้ยกระโดดสีน้ำตาลทั้งสองกลุ่มประชากรในระดับที่สูง อย่างไรก็ตาม อีก 1 สายพันธุ์ ที่มีเฉพาะ *Bph3* และ *TPS* มีความต้านทานต่อ AYY ในระดับสูง แต่ต้านทานต่อ SBR ในระดับปานกลาง จากนั้นนำสายพันธุ์ชั่วที่ 4 จำนวน 16 สายพันธุ์ ที่คัดเลือกจาก 1 สายพันธุ์ของชั่วที่ 3 ที่มี qBL1 และ qBL11 มาประเมินความต้านทานต่อโรคไหม้จากเชื้อผสม 7 กลุ่ม พบว่า สายพันธุ์ดังกล่าวมีระดับความต้านทานที่สูงกว่าพันธุ์ที่มี QTL ของโรคไหม้เพียง 1 ตำแหน่ง ดังนั้น การประเมินฟีโนไทป์ของความต้านทานต่อเพลี้ยกระโดดสีน้ำตาลและโรคไหม้จากการใช้เครื่องหมายในการตรวจสอบครั้งละมากๆ (high-throughput) ช่วยในการคัดเลือกมีความแม่นยำและเชื่อถือได้ในระดับสูง จึงสรุปได้ว่า ยีน *Bph32* และ 2 BL QTLs, qBL1 และ qBL11 สามารถใช้เป็นเครื่องหมายในการคัดเลือกในโปรแกรมการปรับปรุงพันธุ์พืชให้ต้านทานเพลี้ยกระโดดสีน้ำตาลและโรคไหม้ในประเทศไทยและลาวได้

คำสำคัญ: ข้าวเหนียว ความต้านทานเพลี้ยกระโดดสีน้ำตาล ความต้านทานโรคใบไหม้ เครื่องหมายช่วยในการคัดเลือก

Introduction

Brown planthopper (*Nilaparvata lugens* Stål) is the most destructive insect pest for rice production in Southeast Asia (Wu *et al.*, 2018) including Lao PDR (Inthapanya *et al.*, 2011). Planting resistant cultivars is the most ecological friendly strategy to reduce production loss from the insect. Over 30 BPH resistance genes have been identified in cultivated and wild species of *Oryza* (Prahalada *et al.*, 2017; Brar *et al.*, 2009). Only five *Bph* genes, *Bph14, Bph26, Bph3, Bph29*, and *Bph32* have been successfully cloned (Ren *et al.*, 2016). The Sri Lankan rice cultivar Rathu Heenati was found strong and broad-spectrum resistance against BPH (Lakshminarayana and Khush, 1977). Besides, Ikeda and Kaneda (1981) reported that Rathu Heenati was resistant to all four known biotypes of brown planthopper (BPH). The *Bph32*, also previously known as *Bph3* on chromosome 6 was identified by Jairin *et al.* (2007). It was incorporated into KDML105 background and the new breeding lines showed broad resistance to BPH populations in Thailand (Jairin *et al.*, 2009). *Bph3* on chromosome 4 was identified and cloned by Liu *et al.* (2015). This locus contains a cluster of three genes encoding plasma membrane-localized lectin receptor kinases which are *OsLecRK1, OsLecRK2* and *OsLecRK3*. Lectin receptor kinase genes function together to confer broad-spectrum and durable insect resistance. Terpene synthase gene (*TPS*) on chromosome 4 was identified and found to be induced by BPH feeding (Wintai *et al.*, 2013). *TPS* may involve in antixenosis BPH resistance mechanism.

Blast disease caused by *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) is one of the most devastating diseases for rice-growing countries worldwide (Nalley *et al.*, 2016; Asibi *et al.*, 2019). The disease caused about 10 to 20% yield loss in regular seasons and as high as 100% yield loss in years with BL epidemics (Dean *et al.*, 2005). It is also the most serious disease reducing yield substantially in the rain-fed lowland in Laos PDR (Teng and Revilla, 1996; Gnanamanickam, 2009). Over 100 resistance genes and 350 quantitative trait loci (QTLs) have been identified in *Oryza sativa* L. Only 25 BL resistance genes or *Pi* genes have been successfully cloned and applied in breeding programs (Ashkani *et al.*, 2015). Many allelic genes have been reported such as *Pish/Pi35* on chromosome 1 and *Pikh/Pikm/Pik/Pikp/Pi1* on chromosome 11 conferring broad-spectrum resistance to rice BL (Hua *et al.*, 2012; Takahashi *et al.*, 2010). Two QTLs for broad-spectrum resistance, qBL1 and qBL11 were also identified in Thai cultivar Jao Hom Nin (JHN) (Noenplab *et al.*, 2006). These QTLs confer high resistance against BL isolates from Thailand and Lao PDR (Wongsaprom *et al.*, 2010; Korinsak *et al.*, 2011). The tightly linked markers RM212- RM319 and RM224 - RM144 were developed for the selection of the QTLs (Noenplab *et al.*, 2006). These linked markers were successfully applied in rice breeding programs in Thailand and Lao PDR (Manivong *et al.*, 2014; Khanthong *et al.*, 2018; Srichant. *et al.*, 2019).

Hom Xebangfai 4 is an aromatic glutinous rice. It was officially released in Lao PDR in 2017 for blast resistance (qBL1, qBL11), submergence tolerance (*Sub1C*), aroma (*badh2)* and brown planthopper resistance (*Bph3*, *TPS*). It performs well in many aspects but the level of brown planthopper resistance was moderate due to the lacking of *Bph32*. A breeding program for the incorporation of *Bph32* into HXBF4 was initiated in 2017. Desirable traits in this project include blast resistance, submergence tolerance and brown planthopper resistance. A total of seven high-throughput markers were used in the genotyping of F_2 populations. The objective of this study was to validate the BPH resistance in F_3 families and the BL resistance in F_4 families.

Materials and Methods

Plant materials

HXBF4 carrying *Sub1C, badh2,* qBL11 and qBL1 was used as a female parent. RGD13117-115-52-B line (RGD13117), an aromatic glutinous introgression line carrying *Bph32, Bph3, TPS, Sub1C, badh2,* and qBL11 developed by Rice Gene Discovery Unit (RGDU), National Center for Genetic Engineering and Biotechnology (BIOTEC), Kasetsart University Kamphaeng Saen Campus, Thailand, was used as a male parent. *Bph32* was introgressed from the male parent to the female parent (HXBF4) using marker-assisted selection (MAS). The MAS was done in F_2 generation at the segregation loci of *Bph32* and qBL1 including other loci of *Bph3, TPS, Sub1C, badh2,* qBL11. F_3 families derived from the selected F_2 individual plants carrying positive homozygous alleles of *Bph32* were evaluated for BPH resistance. F_4 families derived from the selected F_3 individual plant carrying positive homozygous alleles of qBL1 and qBL11 were evaluated for BL resistance (Figure 1).

Rice growth conditions

This experiment was conducted from September 2017 to December 2020 at RGDU, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The rice plants were seeded in a field nursery. After 30 days, the rice seedlings were transplanted into the paddy field. The F_2, F_3, and F_4 selected plants were grown in 1 row/line of 2.5 x 2.5 m with a spacing of 25 x 25 cm. The management practices were performed following conventional high-yield cultivation approaches.

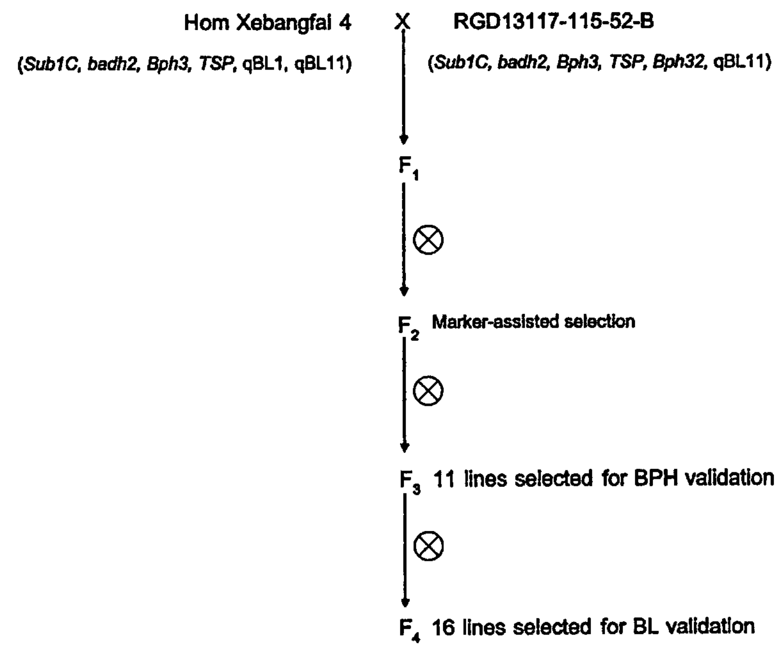

Figure 1 Development of aromatic glutinous rice lines with BPH and BL by using marker-assisted selection

Genotyping

High-throughput genotyping was conducted in F_2 population by using SNP markers developed by RGDU, BIOTEC, Thailand. DNA from the leaf sample was extracted by using DNA trapping method (DNA technology laboratory) (Nubankoh *et al.*, 2020). Polymerase Chain Reaction (PCR) was conducted following the KASP genotyping protocol (LGC Ltd). Allele discrimination was read by using the QuantStudio™ 12K Flex machine (Applied Biosystems™).

Table 1 The SNP markers used for detected target traits

Traits	Gene/QTL	Chro	Markers	Reference
Brown planthopper resistance	*Bph3*	4	*sLecRK3Bph32*	Liu *et al.*, (2015)
	Terpene synthase	4	*OsSTPS2*	Wintai *et al.*, (2013)
	Bph32	6	*Bph32*	Jairin *et al.*, (2007): Ren *et al.*, (2016)
Blast resistance	*qBL1*	1	*TBGI055578*	Wongsaprom *et al.*, (2010)
	qBL11	11	*TBGI454717*	Wongsaprom *et al.*, (2010)
Submergence tolerance	*Sub1C*	9	*Sub1A_SNP1*	Siangliw *et al.*, (2003)
Aromatic	*badh2*	8	*Aroma*	Wanchana *et al.*, (2005)

Validation for brown planthopper resistance

Insect materials: BPH used in this experiment were collected from a rice field in Singburi (SBR) and Ayutthaya (AYY) in 2013. The two populations were reared in 20x30x30 cm^3 40-mesh nylon cages in a controlled room at 26-28 °C with 15 hour-light/9 hour-dark in 50-60% relative humidity. Taichung Native 1 (TN1) seedlings were used as feed and habitat for the insect. New seedlings were replenished every four days. Mass rearing was done for insect multiplication in a greenhouse.

BPH infestation and scoring: Eleven F_3 lines were evaluated for BPH resistance with modified standard seedbox screening described by Saxena (1989) and this method has been used in many experiments (Jairin *et al.*, 2005; 2006; 2009). Germinating seeds of each line were planted in a row (10 seeds/row). Rathu Heenati (resistant check), TN1 (susceptible check), HXBF4 (female parent) and RGD13117-115-52-B (male parent) were also included as checks. At 25 days old, a mix of 2^{nd}- and 3^{rd}-instar BPH nymphs were released at the rate of 25-30 insects/plants. The infestation was recorded as damage scores (DS) according to the Standard Evaluation System (SES) for Rice (IRRI, 2013). The DS was recorded when all susceptible check plants (TN1) died at nine days after infestation (DAI). The DS was recorded again at 17 DAI for durability of the resistance. This experiment was conducted in a completely randomized design with three replications. A score under 3.0 is considered resistance (R). Scores from 3.1 to 5.0 are considered moderately resistant (MR). Scores from 5.1 to 7.0 are considered moderately susceptible (MS). Score from 7.1 to 9.0 are considered susceptible (S).

Validation for blast resistance

Disease materials: Forty-nine blast isolates used in this study were grouped into 7 mixed BL isolates. These blast isolates were collected from different regions in Thailand by RGDU. The collected isolates were classified by genetic diversity cluster analysis by using

Amplified Fragment Length Polymorphism (Rice Gene Discovery, Thailand, unpublished).

BL inoculation and scoring: The 16 F_4 lines were evaluated for BL resistance under greenhouse conditions. Sariceltik (susceptible check), KDML105, and RD6 (moderate resistance check), Jao Hom Nin (resistant check), HXBF4 (female parent) and RGD13117-115-52-B (male parent) were also included in the experiment. Four germinating seeds of each line were planted in plastic trays with 6 x 12 holes. The experiment was conducted in a completely randomized design with three replications. The seedlings were maintained in a greenhouse under high humidity for 21 days old. The preparation of rice plants and the inoculation were followed the protocol described by Korinsak *et al.* (2011), and the lesion score (LS) was recorded at 7 days after inoculation (DAI) on a 0 to 6 scale. Plants exhibiting reactions that scored 0-2 were considered resistant (R), 3-4 as moderately resistant (MR) and 5-6 as susceptible (S).

Results

Genotyping in F_2 population

Marker-assisted selection was done in 240 F_2 individual plants. Step-wise genotyping 60 lines for BPH (*Bph32*) and 10 lines for BL resistance (qBL1) were selected respectively to reduce the sample size. Genotyping at other loci including *Bph3, TPS*, qBL11, *Sub1C* and *badh2* was also confirmed of existence in progeny lines. Ten F_2 individual plants with three homozygous positive alleles for BPH resistance loci and two BL resistance loci were selected (Table 2). The RGD17020-MS373 with a negative allele for *Bph32* was also maintained to compare between progeny lines. Eleven F_3 families were obtained from self-fertilization of the selected F_2. All 11 families were evaluated for brown planthopper resistance.

Table 2 Genotypes of 11 F_2 selected lines compared with their parents, resistant and susceptible checks for BPH and BL

Line	SNP on BPH resistance locus			Blast resistance genes	
	Bph3	*TPS*	*Bph32*	qBL1	qBL11
Rathu Heenati (R check)[1/1]	G/G	T/T	G/G	C/C	C/C
TN1 (S check)[1/2]	C/C	C/C	C/C	C/C	T/T
Jao Hom Nin (R check)[2/1]	C/C	C/C	C/C	T/T	T/T
Sariceltik (S check)[2/2]	C/C	C/C	C/C	C/C	C/C
HXBF4 (female parent)	G/G	T/T	C/C	T/T	T/T
RGD13117-115-52-B (male parent)	G/G	T/T	G/G	C/C	T/T
RGD17020-MS30	G/G	T/T	G/G	T/T	T/T
RGD17020-MS36	G/G	T/T	G/G	T/T	T/T
RGD17020-MS42	G/G	T/T	G/G	T/T	T/T
RGD17020-MS45	G/G	T/T	G/G	T/T	T/T
RGD17020-MS87	G/G	T/T	G/G	T/T	T/T
RGD17020-MS94	G/G	T/T	G/G	T/T	T/T
RGD17020-MS143	G/G	T/T	G/G	T/T	T/T
RGD17020-MS166	G/G	T/T	G/G	T/T	T/T
RGD17020-MS194	G/G	T/T	G/G	T/T	T/T
RGD17020-MS338	G/G	T/T	G/G	T/T	T/T
RGD17020-MS373	G/G	T/T	C/C	T/T	T/T

[1/1] = Positive for BPH validation

[1/2] = Negative for BPH validation

[2/1] = Positive for BL validation

[2/2] = Negative for BL validation

BPH resistance in F_3 generation

The experiment on BPH evaluation in F_3 generation was well controlled which the resistant check showed the lowest DS and the susceptible check showed the highest DS from the infestation by both BPH populations. Different BPH populations were similar to the pattern of infestation. However, SBR population made more damage than that of the AYY in all selected plants and checks. The levels of damage were also obviously observed and shown in Fig. 2 and Fig. 3. According to the SES, ten of F_3 lines containing all three homozygous *Bph* resistance loci were scored under 3.0 at 9 DAI. They performed as well as the resistant check and the male parent. This suggests that the three *Bph* loci conferred high levels of resistance to both BPH populations. On the other hand, RGD17020-MS373 with a negative allele of *Bph32* was resistant to AYY, but it had moderate resistance against SBR at 9 DAI.

For durability, DS from AYY population was still lower than 3.0 even at 17 DAI for ten F_3 lines. Besides, their damage from SBR population was still lower than 5.0 which was moderately resistant at 17 DAI. RGD17020-MS373 with a negative allele of *Bph32* was susceptible to SBR population at 17 DAI. However, it was resistant to AYY population with a DS of 3.7 at 17 DAI. The damage trend of RGD17020-MS373 was the same as the female parent.

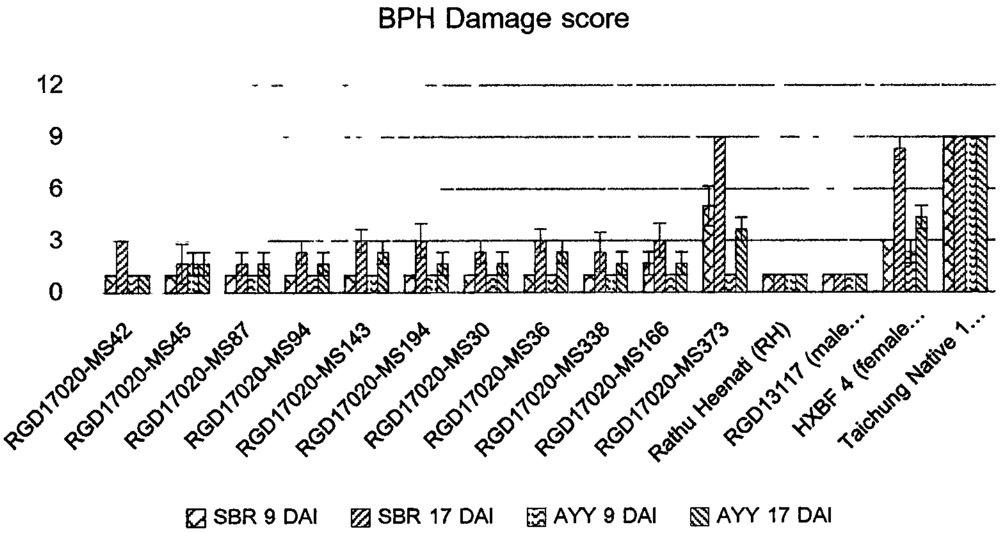

Figure 2 Average damage scores of 11 F_3 lines after infested with Singburi and Ayutthaya BPH populations at 9 DAI and 17 DAI. Scoring 0-3 = resistant (R), 3-4 = moderately resistant (MR), 5-7 = moderately susceptible (MS), and 7-9 =susceptible (S)

Figure 3 Symptoms of 25 days old F_3 seedlings infested with Singburi BPH population 9 DAI compared with resistant check (RH), susceptible check (TN1), HXBF4 (female parent) and RGD13117 (male parent)

BL resistance in F_4 generation

The 16 F_4 lines derived from a single F_3 plant were subjected to BL screening with their parents by using 7 mixed BL isolates groups. The three susceptible checks, (Sariceltik, RD6 and KDML105) and a resistant check (JHN) were included as controlled varieties. The result showed that Sariceltik was susceptible to all of BL isolates, while RD6 and KDML105 were susceptible (Fig 4 and 5). JHN was resistant to all of the mixed isolates. The male parent carrying only qBL11 was resistant to the mixed 2, 3, 4, 5, and 6 and moderately resistant to the mixed 1 and 7. While female parent carrying both qBL1 and qBL11 showed resistance to all of the 7 mixed BL isolates. In the same way, the 16 F_4 tested lines carrying these two BL QTLs were resistant to all of the 7 mixed BL isolates as well as the HXBF4.

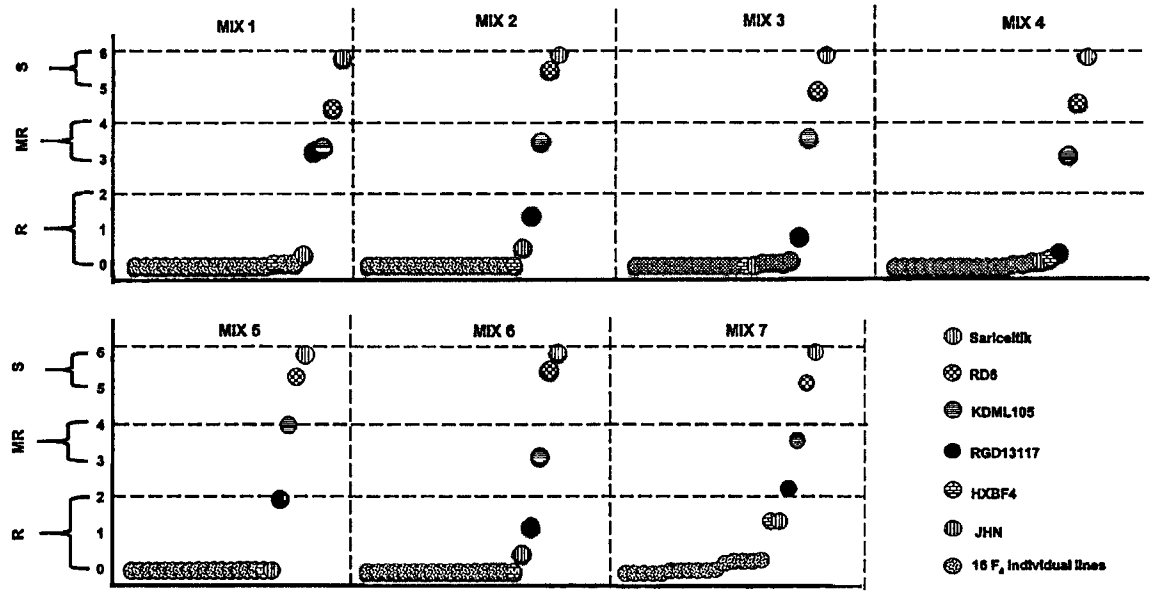

Figure 4 Average damage scores of 16 F_4 lines carrying qBL1, qBL11 inoculated with 7 mixed BL isolates compared with Sariceltik and RD6 (susceptible checks), HXBF4 (female parent) and RGD13117 (male parent). 0-2 = resistance (R), 3-4 = moderately resistant (MR), and 5-6 = susceptible (S)

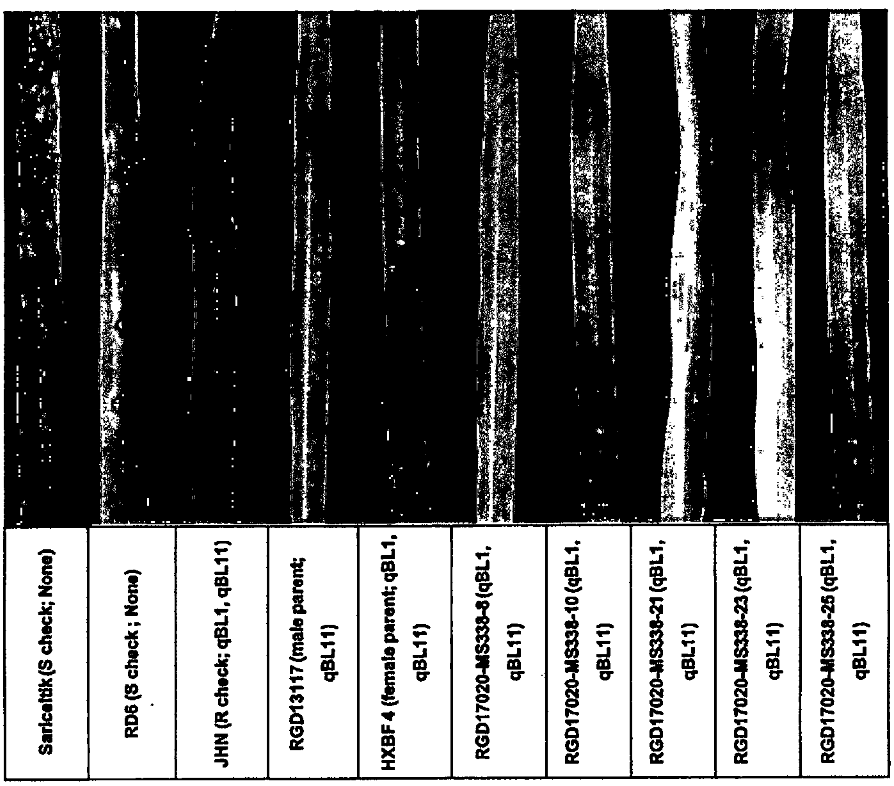

Figure 5: Symptoms of rice leaves on 21 day-old F_4 seedlings inoculated MIX 1 compared with Sariceltik and RD6 (susceptible checks), HXBF4 (female parent) and RGD13117 (male parent)

Discussion

DS scores of BPH in the F_3 lines with three BPH resistance genes of *Bph32, Bph3* and *TPS* were lower than those of the line without *Bph32* and the susceptible check-in both SES and durability tests. This suggested that more resistance genes of *Bph* gave better BPH resistance than a single gene. The results solidified the gene pyramiding concept that had been proved by other researchers in insect and disease resistance. Hu *et al.* (2010) reported that Shanyou 63 improved hybrid rice carrying *Bph14* and *Bph15* had higher resistance than that of *Bph14* or *Bph15* alone. Besides, the pyramiding of *Bph6* and *Bph9* in LuoYang 69 improved hybrid rice was found to show stronger resistance than that of a single gene (Wang *et al.*, 2017). Moreover, two *Bph* genes *Bph3* and *Bph32* were effective for BPH resistance. The *Bph3* that was identified in Rathu Heenati was found that it had resistance to four BPH biotypes (Pathak and Heinrichs, 1982). The *Bph3* has also been used and widely recommended in rice breeding programs for over 30 years (Jena *et al.*, 2015). *Bph32* still an unknown domain-containing protein, but the results in the experiment of Ren *et al.* (2016) were found that *Bph32* was highly expressed of resistance in leaf sheath, and might inhibit feeding in BPH. Besides, it was confirmed that *Bph32* was very valuable for rice defense against BPH (Ren *et al.*, 2016; Jairin *et al.*, 2007).

In this study, all 10 F_3 lines carrying *Bph32, Bph3* and *TPS* were resistant against two SBR and AYY populations at 9 DAI and 17 DAI. RGD17020-MS373 carrying *Bph3* and *TPS* was resistant against AYY but moderately resistant against SBR at 9 DAI and susceptible at 17 DAI. This suggests that the F_3 plants that carrying all three *Bph* genes especially, the lines carrying *Bph32* in a combination were expressed more resistance levels than those of the progenies carrying only two genes.

The 16 F_4 lines derived from one single F_3 plant were screened for BL resistance. The results showed that the 16 F_4 lines carrying two BL QTLs, qBL1 and qBL11 exhibited a high level of resistance to 7 mixed BL isolates as well as their parent 'HXBF4'. The results were resisted to BL similar to the reported of Manivong *et al.* (2014) by reporting that, the improved lines in the F_5 population from three-way crossed by F_5 lines carrying two BL QTLs, qBL1 and qBL11 were resisted against all 15 Lao PDR BL isolates and all 42 Thai BL isolates. This indicated that the two BL QTL used in this study were board-spectrum resistance to BL and numerous studies have also reported the success of BL resistant gene introgression by using these two BL QTLs. Sreewongchai *et al.* (2010) reported that, the improved lines in F_4 population between IR64 and Jao Hom Nin carrying four BL QTLs on chromosomes 2, 12, 1, and 11 showed resistance against 11 Thai blast isolates while the report of Wongsaprom *et al.* (2010) to qBL1 and qBL11 in BC_4F_2 of improved lines between IR64 and Jao Hom Nin showed resistance against 8 Thai BL isolates. Besides, the development of aromatic glutinous rice of Khanthong *et al.* (2018) was reported that the F_5 new improved lines carrying qBL1 and qBL11 gave a high level of blast resistance against 4 blast isolates from rice production areas in Thailand, and not only that, the reported of Ruengphayak *et al.* (2015), Khanthong *et al.* (2018) and Srichant *et al.* (2019) were give a similar resistance by using two of these BL QTLs "qBL1 and qBL11".

Conclusion

The validation in the F_3 lines with three *Bph* genes of *Bph3, TPS* and *Bph32* by screening both BPH populations was confirmed that these *Bph* genes were very effective against BPH. The pyramiding of all three *Bph* genes in a combination was shown more effectiveness than two genes in a combination (*Bph3* and *TPS*). It also give durability resistance to rice seedlings at 17 DAI. The result of two BL QTLs, qBL1 and qBL11 were shown broad-spectrum resistance to all of 7 mixed BL isolates more than one BL QTL. Thus, it is also strongly suggested that these BPH and BL QTL can be used as the donor parents in other breeding programs. Besides, the genotyping with high-throughput markers for BPH and BL is very accurate and trustworthy. Therefore, *Bph32*, qBL1 and qBL11 are recommended to use in the breeding program against BPH and BL in Thailand and Lao PDR. However, more validation should be conducted by using other BPH populations and BL isolates to ensure broad-spectrum resistance of these loci.

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References

Ashkani, S., Rafii, M. Y., Shabanimofrad, M., Miah, G., Sahebi, M., Azizi, P., Tanweer, F. A., Akhtar, M. S., and Nasehi, A. 2015. Molecular breeding strategy and challenges towards improvement of blast disease resistance in rice crop. *Frontiers in Plant Science*, *6*(886), 1-14.

Asibi, A., Chai, Q., and Coulter, J. 2019. Rice blast: a disease with implications for global food security. *Agronomy*, *9*(451), 1-14.

Brar, D. S., Virk, P. S., Jena, K. K., and Khush, G. S. 2009. Breeding for resistance to planthoppers in rice. In *Plant Hoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia*, K.L. Heong and B. Hardy, eds. pp 401-409. Los Banõs: International Rice Research Institute.

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., and Birren, B. W. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature, 434*(7036), 980-986.

Gnanamanickam, S.S. 2009. *Biological Control of Rice Diseases*. New York: Springer Science and Business Media.

Hu, J., Li, X., Wu, C., Yang, C., Hua, H., Gao, G., and He, Y. 2010. Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. *Molecular Breeding, 29*(1), 61-69.

Hua, L., Wu, J., Chen, C., Wu, W., He, X., Lin, F., and Wang, L. 2012. The isolation of Pi1, an allele at the Pik locus which confers broad-spectrum resistance to rice blast. *Theoretical and Applied Genetics*, *125*(5), 1047-1055.

Inthapanya, S., Preston, T. R., and Leng, R. A. 2011. Ensiled brewers' grains increased feed intake, digestibility and N retention in cattle fed ensiled cassava root, urea and rice straw with fresh cassava foliage or water spinach as main source of protein. *Livestock Research for Rural Development*, *28*(2), 1-5.

IRRI. 2013. *Standard Evaluation System (SES) for Rice*. Los banõs: International Rice Research Institute.

Ikeda, R. and Kaneda C. 1981. Genetic analysis of resistance to brown planthopper, *Nilaparvata lugens* (Stal), in rice. *Japanese Journal of Breeding*, *31*(3), 279–285.

Jairin, J. , Toojinda, T., Tragoonrung, S., Tayapat, S., and Vanavichit, A. 2005. Multiple genes determining brown planthopper (*Nilaparvata lugens* Stal) resistance in backcross introgressed lines of Thai Jasmine Rice 'KDML105'. *Science Asia, 31*, 129-135.

Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A., and Toojinda, T. 2006. Mapping of a broad- spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. *Molecular Breeding, 19*(1), 35-44.

Jairin, J. , Teangdeerith, S. , Leelagud, P. , Phengrat, K. , Vanavichit, A., and Toojinda,T.. 2007. Physical mapping of *Bph3*, a brown planthopper resistance locus in rice. *Maejo International Journal of Science and Technology, 1*(2), 166-177.

Jairin, J., Teangdeerith, S., Leelagud, P., Kothcharerk, J., Sansen, K., Yi, M., and Toojinda, T. 2009. Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker- assisted selection. *Field Crops Research, 110*(3), 263-271.

Jena M., Panda R., Sahu R., Mukherjee A. K., and Dhua, U. 2015. Evaluation of rice genotypes for rice brown planthopper resistance through phenotypic reaction and genotypic analysis. *Crop Protection*. *78*, 119–126.

Khanthong, S., Kate-Ngam, S., and Riabroy, K., T. 2018. Development of aromatic glutinous rice for rainfed lowland areas by marker-assisted selection. *Chiang Mai Journal of Science*, *45*(6), 2312-2321.

Korinsak, S., Sirithunya, P., Meakwatanakarn, P., Sarkarung, S., Vanavichit, A., and Toojinda, T. 2011. Changing allele frequencies associated with specific resistance genes to leaf blast in backcross introgression lines of Khao Dawk Mali 105 developed from a conventional selection program. *Field Crops Research, 122*(1), 32–39.

Lakshminarayana, A., and Khush, G. S. 1977. New genes for resistance to the brown planthopper in rice. *Crop Science*, *17*, 96-100.

Liu, Y., Wu, H., Chen, H., Liu, Y., He, J., Kang, H., and Wan, J. 2015. A gene cluster encoding lectin receptor kinases confers broad- spectrum and durable insect resistance in rice. *Nature Biotechnology, 33*(3), 301-305.

Manivong, P., Korinsak, S., Korinsak, S., Siangliw, J.L., Vanavichit, A., and Toojinda, T. 2014. Marker-assisted selection to improve submergence tolerance, blast resistance and strong fragrance in glutinous rice. *Thai Journal of Genetic, 7*(2), 110–122.

Nalley, L., Tsiboe, F., Durand-Morat, A., Shew, A., and Thoma, G. 2016. Economic and environmental impact of rice blast pathogen (*Magnaporthe oryzae*) alleviation in the United States. *PloS One, 11*(12), e0167295.

Noenplab, A. Vanavichit, A. Toojinda, T. Sirithunya, P. Tragoonrung, S. Sriprakhon, S. , and Vongsaprom, C. 2006. QTL mapping for leaf and neck blast resistance in Khao Dawk Mali105 and Jao Hom Nin recombinant inbred lines. *Science Asia, 32*, 133-142.

Nubankoh, P., Wanchana, S., Saensuk, C., Ruanjaichon, V., Cheabu, S., Vanavichit, A., and Arikit, S. 2020. QTL-seq reveals genomic regions associated with spikelet fertility in response to a high temperature in rice (*Oryza sativa* L.). *Plant Cell Reports, 39*(1), 149-162.

Pathak, P., and Heinrichs E. 1982. Selection of biotype populations 2 and 3 of *Nilaparvata lugens* by exposure to resistant rice varieties. *Environmental Entomology. 11*(1), 85–90.

Prahalada, G. D., Shivakumar, N., Lohithaswa, H. C., Sidde Gowda, D. K., Ramkumar, G., Kim, S.-R., Ramachandra, C., Hittalmani, S., Mohapatra, T., and Jena, K. 2017. Identification and fine mapping of a new gene, *BPH3 1* conferring resistance to brown planthopper biotype 4 of India to improve rice, *Oryza sativa* L. *Rice, 10*(1), 41.

Ren, J., Gao, F., Wu, X., Lu, X., Zeng, L., Lv, J., and Ren, G. 2016. *Bph32*, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. *Scientific Reports, 6*, 37645.

Ruengphayak, S., Chaichumpoo, E., Phromphan, S., Kamolsukyunyong, W., Sukhaket, W., Phuvanartnarubal, E., Korinsak, S., Korinsak, S., and Vanavichit, A. 2015. Pseudo-backcrossing design for rapidly pyramiding multiple traits into a preferential rice variety. *Rice, 8*, 7.

Saxena, R.C., 1989. Durable resistance to insect pests of irrigated rice. In *Progress in Irrigated Rice Research: Selected Papers and Abstracts from International Rice Research Conference*, International Rice Research Institute, ed. pp 111-132. Manila: International Rice Research Institute.

Siangliw, M., Toojinda, T., Tragoonrung, S., and Vanavichit, A. 2003. Thai jasmine rice carrying QTLch9 (SubQTL) is submergence tolerant. *Annals of Botany, 91*(2), 255-261.

Sreewongchai T, Toojinda T, Thanintorn N, Kosawang C, Vanavichit A, Tharreau D, Sirithanya, P. 2010. Development of elite indica rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. *Plant Breed, 129,* 176–180.

Srichant, N., Chankaew, S., Monkham, T., Thammabenjapone, P., and Sanitchon, J. 2019. Development of Sakon Nakhon Rice Variety for Blast Resistance through Marker Assisted Backcross Breeding. *Agronomy, 9*, 67.

Takahashi, A., Hayashi, N., Miyao, A., and Hirochika, H. 2010. Unique features of the rice blast resistance Pish locus revealed by large scale retrotransposon-tagging. *BMC Plant Biology, 10*(1), 175.

Teng, PS. and Revilla, IM. 1996. Technical issues using crop-loss data for research prioritization. In *Rice Research in Asia: Progress and Priorities*, R.E. Evenson, R.W. Herdt and M. Hossain, eds. pp 261-275. Wallingford: CAB International in Association with International Rice Research Institute.

Wanchana, S., Kamolsukyunyong, W., Ruengphayak, S., Toojinda, T., Tragoonrung, S., and Vanavichit, A. 2005. A rapid construction of a physical contig across a 4.5 cM region for rice grain aroma facilitates marker enrichment for positional cloning. *Science Asia, 31*(3), 299-306.

Wang, Y., Jiang, W., Liu, H., Zeng, Y., Du, B., Zhu, L., and Chen, R. 2017. Marker-assisted pyramiding of *Bph6* and *Bph9* into elite restorer line 93-11 and development of a functional marker for *Bph9*. *Rice, 10*(1), 51.

Wintai, K. Sukhaket, W. Ruanjaichon, V. Toojinda, T., and Vanavichit, A. 2013. Single-feature polymorphism mapping of isogenic rice lines identifies the influence of terpene synthase on brown planthopper feeding preferences. *Rice, 6*(1), 18.

Wongsaprom, C., Sirithunya, P., Vanavichit, A., Pantuwan, G., Jongdee, B., Sidhiwong, N., Siangliw, J., and Toojinda, T. 2010. Two introgressed quantitative trait loci confer a broad-spectrum resistance to blast disease in the genetic background of the cultivar RD6 a Thai glutinous jasmine rice. *Field Crops Research, 119*, 245-251.

Wu, S. F., Zeng, B., Zheng, C., Mu, X. C., Zhang, Y., Hu, J., Zhang, S., Gao, C. F., and Shen, J. L. 2018. The evolution of insecticide resistance in the brown planthopper (*Nilaparvata lugens* Stål) of China in the period 2012-2016. *Scientific Reports, 8*(1), 4586.

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