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Assistance of phenotype-genotype selections for developing blast disease resistance of Thai jasmine rice, RD15

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Abstract. Thai jasmine rice, RD15 variety is well known for its fragrance, cooking and eating qualities. However, it is susceptible to blast disease, a major rice disease caused by the fungus *Pyricularia oryzae*. The aim of this research was to transfer blast disease resistant QTLs from KD4-14 line into RD15 variety by using phenotype and genotype selections by the aid of markers. In this study, the two resistant QTLs were transferred from KD4-14 rice line into a RD15. Our analysis led to the breeding of elite lines with two resistant QTLs for blast disease by phenotype and foreground selections. The background genome recovery of the lines expressed more than 92.4% by using genome wide SSR markers analysis. The pathogenicity assays of the five resistant-QTL-derived lines were validated under greenhouse conditions with 17 blast isolates, prevalent in Thailand. These lines exhibited resistant reaction to all isolates with agronomic and grain quality traits similar to those of the RD15. The strategy of simultaneous phenotype and genotype selections to introduce multiple resistant QTLs is very useful for reducing the cost and time required for the isolation of desirable recombinants with target resistant genes in rice. The resistant-QTL-derived lines have practical breeding value in providing broad spectrum resistance, without a yield penalty, against most of the existing races of blast in Thailand. These lines could have a great impact on the yield stability and sustainability of rice productivity.

1. Introduction

Thai jasmine rice, also called RD15 variety, is a substantial component of one of the most economical crops in Thailand. It is famous for its fragrance, flavor, slender kernel and soft texture [1]. This rice variety has gained wide acceptance and resulted in an increasing demand around the world owing to the appreciation of their characteristics. As a consequence, the price of Hom Mali rice is 1.3-2.5 times higher than that of other aromatic and non-aromatic rice varieties [2]. However, the RD15 has some disadvantages, such as being photoperiod sensitive, prone to severe lodging and being susceptible to



insects and diseases [3]. Among the abiotic and biotic stresses, blast disease is one of the most serious problems for rice production [4].

Rice blast, caused by the fungal pathogen *P. oryzae*, is a serious disease affecting rice in Thailand and around the world [5]. In Thailand, [3] yield losses due to rice blast disease in RD15 rice variety was evaluated and reported up to 50% average yield loss in the disease-infected areas. Rice blast is estimated to cause production losses amounting to US\$ 55 million each year in South and Southeast Asia. Rice blast is controlled usually by the use of fungicides that result in high production costs and environmental pollution [6].

Jaohom Nin (JHN) is a Thai rice variety, which shows a broad-spectrum resistance against rice blast in Thailand [7]. Two Quantitative Trait Loci (QTLs) that are associated with rice blast resistance were mapped on chromosomes 1 and 11 of JHN [8]. The major QTL (*QTL1*) that controls complete resistance was mapped on chromosome 11 which located on the *Pik* locus by the SSR markers RM144 and RM224. The minor QTL (*QTL2*), which confers partial resistance was mapped on chromosome 1 and located on the *Pish* locus by the flanking markers RM319 and RM212 [8]. In Thailand, JHN is used as a rice blast resistant donor in breeding programs. The introgressions of *QTL2* and *QTL1* from JHN via marker-assisted selection (MAS) into susceptible cultivars such as KDML105 [8] and RD6 [9] were successfully accomplished.

To breed rice varieties with more durable blast resistance, multiple resistant genes with qualitative and quantitative traits effect must be incorporated into individual rice varieties. Conventional breeding methods to improve rice cultivars for blast disease resistance have not been very successful [10] due to the dominance and epistasis effects of genes governing disease resistance. Therefore, it is necessary to explore other selection and breeding strategies which are more efficient than those currently used. Identifying blast resistant genes with the tightly-linked molecular markers of the trait which can act as molecular tags, will save money and time [11]. These molecular marker tags can be used for direct identification of resistant genes when they are transferred from one varietal background to another during breeding programs in early segregating generations and at early stages of plant development [12]. The aim of this research was to transfer blast disease resistant QTLs from KD4-14 line into RD15 variety by using phenotype and genotype selections by the aid of markers.

2. Materials and Methods

2.1. Plant materials and population development

KD4-14 line (BC₂F₈), a backcross inbred line (BIL) derived from the crossing between KDML105 and JHN and possessing multiple resistant QTLs on chromosome 1 and 11, was used as the male parent. RD15, a susceptible variety for blast disease was used as the female parent. A cross was made between RD15 and KD4-14 and the resulting F₁ progenies were self-pollinated (Figure 1). The F₂ progenies were inoculated with blast isolates. The F₂ progenies which were blast resistance and had a flowering date as early as RD15 were selected and marker-assisted selection was employed to select the plants with resistant alleles. Selection of the F₂ progenies having the top five highest ranking of genetic background as similar as RD15 was carried out. The F₂ progenies were allowed to undergo self-pollination. The validation for blast resistance was performed on F₄ progenies through the inoculation with blast isolates as described in Table 1. The experiment was conducted from 2014 to 2016 at Department of Agronomy, Kasetsart University, Thailand.

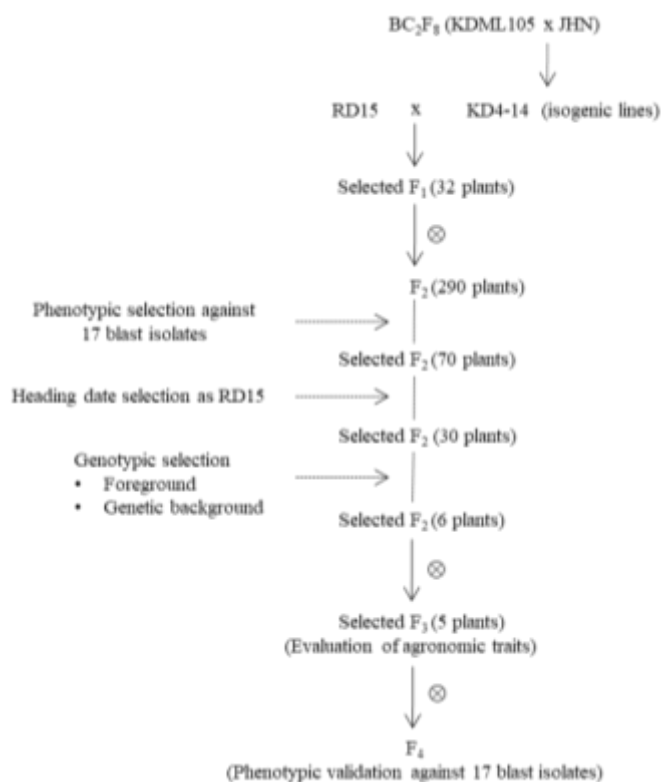


Figure 1. The scheme of applying phenotype -genotype simultaneous selection to improve blast resistance in RD15 rice variety

Table 1. Place of collection, variety and plant parts from which the blast was isolated to use in this experiment.

Entry	Isolate code	Location collected	Rice variety isolated	Organ infected
1	BAG 2.4	Ubon Ratchathani	KDML105	Leaf
2	BAG 4.6	Phitsanulok	KDML105	Leaf
3	BAG 4.7	Phitsanulok	KDML105	Leaf
4	BAG 8.1	Ubon Ratchathani	KDML105	Neck
5	BAG 20.4	Udon Thani	KDML105	Leaf
6	BAG 36.5	Udon Thani	KDML105	Leaf
7	BAG 40.2	Chaiyaphum	KDML105	Leaf
8	BAG 44.2	Lop Buri	KDML105	Leaf
9	BAG 45.3	Roi Et	KDML105	Leaf
10	BAG 46.1	Roi Et	KDML105	Leaf
11	BAG 46.2	Roi Et	KDML105	Leaf
12	BAG 48.1	Roi Et	KDML105	Leaf
13	BAG 49.2	Ubon Ratchathani	KDML105	Neck
14	BAG 49.3	Ubon Ratchathani	KDML105	Leaf
15	BAG 51.1	Chaiyaphum	KDML105	Leaf
16	BAG 52.1	Khon Kaen	KDML105	Leaf
17	BAG 52.2	Khon Kaen	KDML105	Leaf

2.2. Selection in F_2 progeny

Seventeen blast isolates collected from northern, northeastern and central Thailand were selected. Inoculum preparation was performed by following the method of Roumen et al. [13]. The isolates were re-grown from the stock cultures on rice flour agar medium (2.0% of rice flour, 0.2% of yeast extract and 2.0% of agar). The selected F_2 progenies, the parental and check varieties were included in the study. KDML105 and JHN were introduced as negative and positive check varieties, respectively. The plants were grown in a greenhouse in plastic trays filled with clay soil. An ample nitrogen fertilizer was applied as described by [13]. Inoculations were performed by spraying 100 ml of inoculum on the leaves of 2 week-old plants in the check rows in plastic trays. The inoculated plants were incubated under high humidity conditions overnight before transferring them into a greenhouse. Disease evaluation was performed at seedling stage by following the method of Roumen et al. [13]. The selected F_2 resistant plants were screened for flowering date similar to RD15.

Genomic DNA was extracted from fresh frozen leaves of rice plants using the CTAB method with little modification [14]. Two QTLs-specific flanking markers, RM319 and RM212 as well as RM144 and RM224, linked to the resistant *QTL1* and *QTL11*, respectively, were used to confirm the presence of resistant QTLs in F_2 progenies. The PCR products were separated in 6% polyacrylamide gel electrophoresis and stained with silver. DNA profiles of each marker were scored in comparison with their parents. A total of 225 SSR markers of known chromosomal positions distributed evenly across the 12 chromosomes were used in a genome-wide survey to identify the polymorphic between the parents. The polymorphic SSR markers between the parents were used for the background profiling in the selected five lines compared with the RD15.

2.3. Evaluation of agronomic performance and grain quality

The parent, the check and the selected five lines were planted in a randomized complete block design with three replications and the agronomic traits were evaluated in the rice experimental field of the Department of Agronomy, Kasetsart University, Bangkok, Thailand. The amount of standard fertilizer application in the experimental field was $N-P_2O_5-K_2O = 16-16-16$ kg/rai. Commercial pesticides were applied for the protection of plant materials. For each line, three plants in the rows were used to determine days to 50% flowering, plant height, flag leaf length, panicle length, number of tillers per plant, number of panicles per plant, number of grains per panicle, number of filled grains per panicle, 100-grain weight, total grain weight per plant and harvest index.

The analysis of variance was performed and Duncan's multiple range test (DMRT) was used for multiple mean comparisons using the STAR 2.0.1 software.

3. Results and Discussion

3.1. Development of blast resistant lines

A total of 32 F_1 progenies were produced from the cross of RD15 and KD4-14. About 290 F_2 progenies were obtained from the self-pollination of F_1 plants. The 290 F_2 progenies were tested against the 17 blast isolates showed that 70, 146 and 74 plants were found resistant, intermediate and susceptible, respectively. The 70 blast disease resistant plants against all of blast isolates, without showing any symptoms of blast disease (0-2 score) were evaluated for early flowering. Therefore, 30 plants out of 70 examined plants reached flowering as early as RD15. DNA was extracted from leaf samples of individual F_2 plants. A total of 30 plants were screened by RM319 and RM212 markers and subsequently screened with RM144 and RM224 markers. Consequently, six desirable plants possessing the targeted resistant QTLs on chromosomes 1 and 11 with KD4-14 background were identified. The selection of resistant F_2 plants (6 out of 290) was based on the dual-selection procedure of blast-resistance such as phenotype by inoculation and selection for the flowering date as early as RD15; and blast-resistant genotype by foreground selection using gene-specific DNA markers and genetic background profiling.

Most indica rice varieties exhibit a higher degree of susceptibility to blast disease. It is, therefore necessary to develop new durable blast-resistant rice varieties to minimize the loss caused by this

disease. Conventional breeding using phenotype selection with blast inoculation has a weakness as it is difficult to be sure whether the resistant genes are transferred into the elite lines or not. As a result, tagging of molecular markers to a specific resistant gene in a variety and the use of that variety in marker-assisted selection has increased the precision of introgressions [15]. Few blast resistant genes have been characterized, and the markers derived from resistant genes have been developed to accommodate their combination into elite breeding lines [16]. These resistant genes have facilitated the MAS in rice breeding programs. However, breeding work using both phenotype and genotype markers is more plausible and rapid.

3.2. Genetic background profiling based on SSR markers

The presence of substituted chromosome segments in the selected 6 F₂ plants was confirmed by background analysis. Genome-wide molecular markers were used for the analysis. The background analysis was carried out by 225 SSR markers. The polymorphism of markers between RD15 and KD4-14 was 5.78%. Each line contains an SSR marker-defined genetic background of the female, RD15. Five lines from F₂ progenies were selected based on the highest genetic background similar to RD15. The average genetic background percentage in R-44, R-47, R-48, R-55 and R-56 were 92.4, 92.7, 92.8, 93.1 and 92.4, respectively (Table 2). Based on the dual-selection (phenotype and genotype selections), five F₂ lines with homozygous introduced QTLs at all two target loci were derived from KD4-14. These five lines showed a high level of resistance to the blast isolates.

Theoretically, with one time of self-pollination, the average background genotype recovery should be 50%, and that background recovery rate is less than that of the selected lines in this study, because MAS was used to select population in each generation. On the contrary, without marker-assisted background selection, Randhawa et al. [17] reported that recurrent parent background recovery was 82% while studying of stripe rust resistance in wheat in BC₄F₇ progenies. In the present work, phenotype and genotype selections were simultaneously employed for a higher degree of background recovery of the female parent.

Table 2. Simple sequence repeat markers with polymorphism between the RD15 and the KD4-14 and genetic background profiling in four lines of rice.

Chr. No. ^x	No. of markers	PM (%) of F/M ^y	Genetic background profiling (%)				
			R-44	R-47	R-48	R-55	R-56
1	20	5.00	100.0	91.3	91.3	91.3	91.3
2	20	25.00	91.3	95.0	95.0	93.8	93.8
3	20	35.00	91.3	91.3	93.0	93.0	93.0
4	20	5.00	91.3	95.7	91.3	95.7	95.7
5	20	15.00	91.3	95.7	100.0	100.0	91.3
6	20	0.00	91.3	91.3	91.3	91.3	91.3
7	19	0.00	91.3	91.3	91.3	91.3	91.3
8	20	5.00	91.3	91.3	91.3	91.3	91.3
9	14	0.00	93.5	93.5	93.5	93.5	93.5
10	14	0.00	93.5	93.5	93.5	93.5	93.5
11	19	5.26	91.3	91.3	91.3	91.3	91.3
12	19	5.26	91.3	91.3	91.3	91.3	91.3
Average (Total)	225	5.78	92.4	92.7	92.8	93.1	92.4

Note: ^x Chromosome number, ^y Polymorphism between RD15 (F: female) and KD4-14 (M: male).

3.3. Agronomic performance and quality characteristics of lines

The performance evaluation in the field showed non-significant differences between the selected five lines and RD15 for most of the traits, including yield components as well as cooking and eating characteristics (Table 4 and Table 5). Days to 50% flowering of five lines ranged from 65.7 to 70.2, which was similar to RD15 (68.1 days). There was no significant difference between RD15 and the

five lines for plant height, flag leaf length, panicle length, number of tillers per plant, harvest index, number of panicles per plant, number of grains per panicle, number of filled grains per panicle, 100-grain weight, total grain weight per plant, aroma, amylose content of milled rice and alkali digestion value. It indicates that the blast resistant-genes have no adverse effects on grain quality and agronomic traits of rice. The female parent determines aroma, cooking and eating characteristics of rice. Consequently, the alternative of the female parent plays a decisive part in rice breeding programs to improve quality traits [18]. The agronomic traits and yield of the selected lines in this study are also cognate to RD15, indicating the absence of punishment associated with the resistant QTLs.

Table 3. Performance of principle agronomic traits of five lines, which were selected as the most promising lines.

Varieties/Lines	DTF ^z	PH (cm)	FLL (cm)	PL (cm)	T/P	HI
RD15	68.1 ^{bc}	143.1	53.9	30.7	7.0	0.32
KD4-14	94.7 ^a	141.8	52.8	28.9	6.0	0.29
R-44	65.7 ^c	142.8	47.4	29.7	6.5	0.35
R-47	68.8 ^{bc}	150.8	51.7	31.3	7.1	0.32
R-48	70.2 ^b	142.5	51.2	29.2	6.4	0.28
R-55	68.4 ^{bc}	146.6	51.2	31.0	5.9	0.39
R-56	67.3 ^{bc}	146.3	50.5	30.2	6.4	0.39
KDML105	92.7 ^a	141.4	51.5	31.6	6.3	0.33
F-test	**	ns	ns	ns	ns	ns
C.V. (%)	2.2	3.7	6.3	4.7	8.7	13.0

Note: ^z DTF: days to 50% flowering, PH: plant height (cm), FLL: flag leaf length (cm), PL: panicle length (cm), T/P: number of tiller per plant, HI: harvest index. ns = non-significant and ** = significantly different at $P \leq 0.01$. Means in the same column followed by the same letter are not significantly different by DMRT.

Table 4. Performance of principle yield components of five lines, which were selected as the most promising lines.

Varieties/Lines	P/P ^z	S/P	G/P	GW (g)	W/P (g)
RD15	4.6	113.3	98.5	3.2	2.8
KD4-14	5.1	109.8	93.3	3.3	2.7
R-44	5.4	109.0	98.2	3.4	3.0
R-47	5.8	109.3	94.9	3.3	2.7
R-48	4.9	107.6	96.2	3.5	2.4
R-55	5.4	114.0	101.2	3.3	2.7
R-56	5.6	107.2	99.6	2.9	2.5
KDML105	5.8	108.5	96.6	3.2	2.5
F-test	ns	ns	ns	ns	ns
C.V. (%)	15.2	7.0	8.0	8.8	12.5

Note: ^z P/P: number of panicles per plant, S/P: number of grains per panicle, G/P: number of filled grain per panicle, GW: 100-grain weight (g), W/P: total grain weight per plant (g). ns = non-significant.

3.4. Validation of blast resistance in the lines by phenotype

The validation of blast resistance on five lines of F₄ progenies was performed by comparing them with KD4-14 line, JHN and RD15 varieties. The plants were inoculated with 17 blast isolates. The total 5 lines that were resistant to blast disease exhibited a very high level of resistance to all the blast isolates without any symptoms of blast disease. The lines with the combination of the two resistant QTLs will provide a wider range of resistance to the blast isolates, and will have implication on rice yield stability in the region [12].

4. Conclusion

The blast disease resistant QTLs from KD4-14 lines have successfully transferred into RD15 variety by using phenotype and genotype selections simultaneously with the aid of markers. The new blast resistant lines which were controlled by a major *QTL1* and a minor *QTL* have been developed.

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