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Developing Blast Disease Resistance of Jasmine Rice by Phenotypic-Genotypic Simultaneous Selection

Thanakorn Wangsawang[1)], Tanee Sreewongchai[1*)], Prapa Sripichitt[1)] and Fisseha Worede[2)]

[1)] Department of Agronomy, Faculty of Agriculture, Kasetsart University, Thailand
[2)] Ethiopian Institute of Agricultural Research, Fogera Rice Research and Training Center, Bahir Dar, Ethiopia

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*) Corresponding author:
E-mail: taneesree@yahoo.com

ABSTRACT

Breeding for resistant varieties of rice is known to be the most preferable way of controlling blast disease (*Pyricularia oryzae*). Identification and introduction of resistance genes into elite rice lines has become possible by the use of molecular markers. KD2-1 line is an isogenic line of KDML105 carrying four resistance genes on chromosome 2, 3, 8 and 12 from IR64 variety. The objective of this research was to transfer blast disease resistant genes from KD2-1 line into RD15 variety by using phenotypic and genotypic selections by the aid of markers. In this study, the four resistance genes were transferred from KD2-1 rice line into a blast susceptible rice variety, RD15. The study resulted in the breeding of four elite rice lines with four resistance genes by phenotypic and foreground selection. The genome-wide SSR marker analysis of the lines showed more than 86.5% background genome recovery of RD15. Pathogenicity assays of the four selected lines exhibited a resistant reaction to all 13 isolates, with agronomic and yield performance, and cooking and eating quality characteristics similar to that of RD15. The phenotypic-genotypic (foreground and background) simultaneous selection strategy is very useful to introduce multiple resistance genes in rice as it is a fast and economical way for identification of anticipated recombinant lines with desired genes.

INTRODUCTION

Rice (*Oryza sativa* L.) plays a key role in food security, with 90% of its production and consumption coming from the Asian continent (Hasan et al., 2015). The two Thai jasmine rice varieties, Khao Dawk Mali 105 (KDML105) and Rice Department 15 (RD15; an isogenic line of KDML105) are the most popular in Thailand and in many other countries because of their aroma, and good cooking and eating qualities (NBACFS, 2003). These rice varieties have gained wide acceptance and increased demand around the world due to the appreciation of their characteristics. Because of this, the price of Thai jasmine rice is 1.3-2.5 times higher than the other aromatic and non-aromatic rice varieties. Nevertheless, these varieties are sensitive to photoperiod, prone to lodging and susceptible to pests (Sriboonjit & Viboonpong, 2000).

Rice blast is a serious disease damaging rice in Thailand and around the world (Abedi, Babaeiyan, & Moumeni, 2012). It is considered as one of the economically important diseases causing up to 50% rice yield loss (Skamnioti & Gurr, 2009). Sriboonjit & Viboonpong (2000) reported that up to 50% of average yield loss in KDML105 and RD15 rice varieties were due to rice blast disease in disease-prevalent areas of Thailand. Rice blast is estimated to cause production losses accounting to US$ 55 million each year in Southeast and South Asia. Rice blast is usually controlled by the use of fungicides that result in high production costs and environmental pollution (Usman Ghazanfar, Wakil, & Sahi, 2009).

The rice variety IR64 has resistance to a wide range of blast isolates from many countries,

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including Asia, Latin America and Africa (Sharma et al., 2012). In Thailand, it has also shown resistance against numerous isolates of blast and is being used as a resistant parent (Sreewongchai et al., 2010). A major gene linked to the simple sequence repeat (SSR) markers RM179 on chromosomes 12 and a minor gene linked to the SSR markers RM208 on chromosomes 2, were responsible for blast resistance in this cultivar (Sirithunya et al., 2004). Using BC_2F_6 progenies derived from a cross of KDML105 and IR64, Waiyalert, Sreewongchai, Chaisan, & Sripichitt (2015) also reported that two minor blast resistance genes are located on chromosomes 3 and 8 linked to SSR markers RM85 and RM38, respectively.

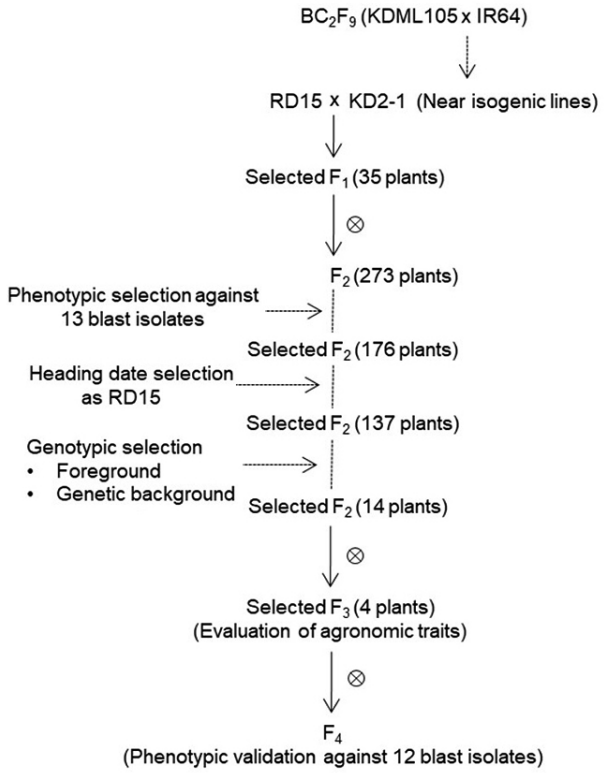

Fig. 1. Schematic representation for the development of blast disease resistance lines using phenotypic -genotypic simultaneous selection.

Breeding of rice varieties with effective and sustained resistance to blast disease is considered as economically feasible and environmental-friendly strategy to manage rice blast (Mackill, 1992). However, the rapid breakdown of varietal resistance to blast has been possible and appeared to be the result of the emergence of a race of virulent pathogens, and it indicates that the genetic diversity for blast resistance within cultivated varieties is narrow (Suh et al., 2013). Therefore, enhancing durable blast resistance in improved rice varieties via the use of genetically diverse blast resistance sources is indispensable. Incorporation of multiple resistance genes with qualitative and quantitative effects need to be incorporated into elite rice varieties to develop more durable blast resistance. The breeding methods currently deployed for identification of blast resistance rice germplasm are based on phenotypic screening (Jayawardana, Jayasekera, Wijesundera, & Dissanayake, 2013). Nevertheless, phenotypic screening is predisposed by environmental conditions, intricate to detect and expensive to practice. Development of blast disease resistant rice lines by classical breeding was hardly successful (Shin, Kim, Park, & Ko, 2011). In addition, identification and transfer of genes with homologous reactions to at least two races through conventional approaches are arduous (Srividhya et al., 2011). This shows the necessity of exploring more efficient strategies for breeding. Identifying blast resistant genes that are tightly linked to molecular markers will save money and time (Joshi, Bimb, Parajuli, & Chaudhary, 2009). These molecular marker tags can be used for direct identification of resistance genes when they are shifted from one varietal background to another (Thippeswamy, Chandramohan, Pravalika, Madhavilatha, & Samreen, 2015). The objective of this research was to transfer blast disease resistant genes from KD2-1 line, an isogenic line of KDML105, into RD15 variety by using phenotypic and genotypic selections by the aid of markers.

MATERIALS AND METHODS

The experiment was conducted from 2015 to 2017 in the green house of Department of Agronomy, Kasetsart University, Thailand.

Inbred Line Development

KD2-1 line (BC_2F_9), a backcross inbred line (BIL) derived from crossing between KDML105 and IR64 that possesses multiple resistance genes on chromosome 2, 3, 8 and 12, was the male parent and source of blast disease resistant genes. RD15, a blast disease susceptible elite variety with aroma, flavor, slender kernel and soft cooking, was used as the female parent. A cross was made between

RD15 variety and KD2-1 line, and the resulting F_1 progenies were self-pollinated (Fig. 1). The F_2 progenies were inoculated with blast isolates. The F_2 progenies which were blast resistant and had a flowering date as early as RD15 were selected and marker-assisted selection (MAS) was employed to select the plants with resistance alleles. Selection of the F_2 progenies having the top four highest ranking of genetic background as similar as RD15 was carried out. The F_2 progenies were allowed to undergo self-pollination. The validation for blast resistance was performed on F_4 progenies through the inoculation of selected old and new collected blast isolates as described in Table 1.

Table 1. Place of collection of the 12 blast isolates used in this experiment were collected from the infected leaf of KDML 105 rice variety.

Entry	Isolate code	Location collected
1	BAG 2.4	Ubon Ratchathani
2	BAG 4.6	Phitsanulok
3	BAG 4.7	Phitsanulok
4	BAG 8.1	Ubon Ratchathani
5	BAG 20.4	Udon Thani
6	BAG 36.5	Udon Thani
7	BAG 40.2	Chaiyaphum
8	BAG 44.2	Lop Buri
9	BAG 45.3	Roi Et
10	BAG 46.2	Roi Et
11	BAG 48.1	Roi Et
12	PL12	Phitsanulok

Selection in F_2 Progeny

Thirteen blast isolates collected from three regions of Thailand (Table 2) were selected by considering their pathotypic and genetic profiles. Inoculum was performed as described in Roumen, Levy, & Notteghem (1997). About 5 × 104 conidia mL^{-1}, with 0.5 % gelatin was used for inoculation. The selected F2 progenies, parents (KD2-1 and RD15) and check varieties were included in the study; KDML105 and IR64 were applied as negative and positive checks, respectively. The plants were grown in plastic trays (45×30×7 cm) filled with a clay soil. Each treatment includes a single row of 15 plants. Nitrogen fertilizer was applied as described by Roumen, Levy, & Notteghem (1997). About 100 mL of inoculum was sprayed on 2 weeks old plants. After inoculation, the plants were kept under high humidity condition overnight and then transferred to green house. According to Sallaud et al. (2003), disease evaluation was performed at seedling stage seven days after inoculation. The resistant F_2 plants were screened for the flowering date, and plants which were similar in flowering to RD15 were retained.

Table 2. Place of collection of the 13 blast isolates used in this experiment were collected from the infected leaf of KDML 105 rice variety.

Entry	Isolate code	Location collected
1	BAG 1.2	Phitsanulok
2	BAG 2.4	Ubon Ratchathani
3	BAG 3.3	Phitsanulok
4	BAG 4.6	Phitsanulok
5	BAG 4.7	Phitsanulok
6	BAG 20.4	Udon Thani
7	BAG 36.5	Udon Thani
8	BAG 40.2	Chaiyaphum
9	BAG 40.4	Chaiyaphum
10	BAG41.2	Chaiyaphum
11	BAG42.2	Chaiyaphum
12	BAG42.3	Chaiyaphum
13	BAG43.2	Lop Buri

The CTAB method, with little modification, has been employed to extract genomic DNA from frozen leaves of young rice plants (DNA Technology Laboratory, BIOTEC, Bangkok, Thailand). Four gene-specific SSR markers, RM208, RM85, RM38 and RM179, linked to the resistant genes on chromosome 2, 3, 8 and 12, respectively, were used to verify the possessing of the resistant genes in F_2 progenies. Six percent polyacrylamide gel electrophoresis was used to divide PCR products and stained with silver. DNA profiles of each marker were scored in comparison with their parents. SSR markers, 224 in total, distributed over the 12 rice chromosomes were used to screen polymorphism between the two parents. Subsequently, the polymorphic markers were used for further background profiling of the four lines in comparison with RD15.

Agronomic Performance and Grain Quality Evaluation

The agronomic traits of the lines were evaluated in field trial of Department of Agronomy, Kasetsart University, Bangkok, Thailand. The selected four lines, KD2-1, RD15 and the check varieties were cultivated in a two-row plot. Each row contained 5 plants at a planting density of 20×20 cm. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Standard fertilizer, N-P_2O_5-K_2O, was applied at the rate of 16-16-16 kg per rai on

each experimental plot. Pesticides were sprayed to protect the plants from insect and diseases. Information on agronomic and morphological traits was collected by following the standard evaluation system for rice (SES). Average of three plants per plot were used to compute days of 50% flowering, plant height, flag leaf length, panicle length, number of tillers per plant, number of panicles per plant, number of grains per panicle (the total number of spikelets on the panicle), number of filled grains per panicle (the total number of filled spikelets from the panicle), 100-grain weight (the average weight of 100 fully filled paddy grains, in grams, of each plant), total grain weight per plant (the average weight, in grams, of fully filled paddy grains of each plant) and harvest index per plant.

Statistical Data Analysis

Analysis of variance was performed by using STAR 2.0.1 software. The Duncan's multiple range test (DMRT) was applied to compare significant trait means.

RESULTS AND DISCUSSIONS

Development of Blast Resistant Lines

A total of 35 F_1 progenies were produced from the crosses of RD15 and KD2-1. About 273 F_2 progenies were obtained from the self-pollination of F_1 plants. The 273 progenies were tested against the 13 blast isolates and 176 progenies were found to be resistant. Among these resistant plants, 137 plants were similar in heading time with RD15. Foreground selection of 137 plants using the four SSR markers linked to the genes responsible for blast resistance on chromosome 2, 3, 8 and 12 resulted in identifying 14 plants. The selection of resistant F_2 plants (14 out of 273) was based on the dual-selection procedure of blast-resistance such as phenotyping by inoculation and selection for the flowering date as early as RD15; and blast-resistance genotyping by foreground selection using gene-specific DNA markers and genetic background profiling.

Most indica rice varieties exhibit a higher degree of susceptibility to blast diseases. It is, therefore necessary to develop new durable blast-resistant rice varieties to minimize the loss caused by this disease. Conventional breeding using phenotypic selection with blast inoculation has a weakness as it is difficult to be sure whether the resistant genes are transferred into the elitelinesor 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 (Abedi, Babaeiyan, & Moumeni, 2012). 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 (Hayashi, Yoshida, & Ashikawa, 2006). These resistant genes have facilitated the MAS in rice breeding programs. However, breeding work using both phenotypic and genotypic markers is more plausible and rapid.

Evaluation of Blast Resistance and Flowering Time

Blast resistance evaluation of the 273 F_2 plants, compared to negative and positive check varieties, showed that 176, 41 and 56 plants were found resistant, intermediate and susceptible, respectively. The 176 blast disease resistant plants against 13 blast isolates, without showing any symptoms of blast disease (0-2 score) were evaluated for early flowering. Therefore,137 plants out of 176 examined plants reached flowering as early as RD15.

The cumulative effect of the four resistance genes (on chromosomes 2, 3, 8 and 12) on the lines with RD15 genetic background showed very high resistance to the 13 blast isolates, signifying that the combined action of the genes was more effective to control blast than plants without a resistance gene. Quantitative complementation of numerous resistant genes having an additive effect on all level of resistance was reported (Suh et al., 2011).

Marker Assisted Selection in F_2

DNA was extracted from leaf samples of individual F_2 plants. A total of 137 plants were screened by RM179 marker and subsequently screened with RM208, RM85 and RM38 markers. Consequently, fourteen desirable plants possessing the targeted resistant genes on chromosomes 2, 3, 8 and 12 with KD2-1 background were identified. Stepwise screening of markers was followed. The stepwise method is important in terms of cost and time as it reduces the number of individual plants to be screened (Sreewongchai et al., 2010).

Genetic Background Profiling Based on SSR Markers

The presence of substituted chromosome segments in the selected 14 F_2 plants was confirmed

by background analysis. Genome-wide molecular markers were used for the analysis. The background analysis was carried out by 224 SSR markers. The polymorphism of markers between RD15 and KD2-1 was 29.2%. Each line contains an SSR marker-defined genetic background of the female, RD15. Fourlines from F_2 progenies were selected based on the highest genetic background similar to RD15. The average genetic background percentage in R-11, R-13, R-38 and R-87 were 91.3%, 88.5%, 89.4% and 86.5%, respectively (Table 3). In the study, line R-11 inherited the highest size of the genetic background from RD15. Based on the dual-selection (phenotypic and genotypic selection), four F_2 lines with homozygous introduced genes at all four target loci were derived from KD2-1. These four lines showed a high level of resistance to the blast isolates and had approximately the expected background genome recovery of 88.9%.

Four F_2 lines were largely homozygous for the MAS-based target loci with agronomic traits similar to RD15, with a high level of resistance to blast disease. The background genotype recovery varied from 86.5% to 91.3%. Four lines showed high chromosome segments recovery and had similar phenotype with RD15. Theoretically, with one time of self-pollination, the average background genotype recovery should be 50.0%, 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. (2009) reported that recurrent parent background recovery was 82% while studying of stripe rust resistance in wheat in BC_4F_7 progenies. In the present work, phenotypic and genotypic selections were simultaneously employed for a higher degree of background recovery of the female parent. Introgression of resistant genes without maintaining the yield level and the grain quality would not reward, since the developed varieties may not be adopted by the farmers. The four R-gene-derived lines developed in this study without compromising the yield level and grain quality would be of major importance for rice farming areas of high level blast-infestation.

Table 3. Genetic background profiling of the four rice lines performed using polymorphic simple sequence repeat markers between RD15 and KD2-1.

Chr. No. [x]	No. of markers	PM (%) of F/M[y]	Genetic background profiling (%)			
			R-11	R-13	R-38	R-87
1	20	25.0	80.0	80.0	90.0	70.0
2	19	42.1	87.5	100.0	87.5	87.5
3	19	52.6	100.0	100.0	88.9	88.9
4	20	55.0	100.0	100.0	83.3	100.0
5	20	20.0	100.0	66.7	100.0	100.0
6	20	25.0	66.7	66.7	83.3	66.7
7	19	26.3	100.0	100.0	100.0	75.0
8	20	10.0	100.0	100.0	100.0	100.0
9	14	14.3	50.0	0.0	100.0	100.0
10	14	14.3	100.0	50.0	50.0	50.0
11	20	40.0	92.9	100.0	100.0	100.0
12	19	26.3	83.3	66.7	83.3	83.3
Average (Total)	224	29.2	91.3	88.5	89.4	86.5

Remarks: [x] = Chromosome number; [y] = Polymorphism between RD15 (F; female) and KD2-1 (M; male)

Table 4. Major important agronomic traits of the developed lines.

Varieties/Lines	DTF[z]	PH (cm)	FLL (cm)	PL (cm)	T/P	HI
RD15	69.0^{bcd}	143.5^{a}	54.4^{a}	32.3^{a}	6.3^{bc}	0.30
KD2-1	67.5^{cd}	141.3^{a}	54.7^{a}	29.2^{ab}	5.7^{c}	0.27
R-11	66.3^{d}	144.8^{a}	46.0^{a}	30.3^{ab}	6.4^{bc}	0.28
R-13	69.8^{bc}	153.5^{a}	55.0^{a}	33.3^{a}	7.4^{b}	0.27
R-38	71.4^{b}	143.2^{a}	46.6^{a}	28.6^{ab}	6.1^{c}	0.29
R-87	69.0^{bcd}	147.9^{a}	51.9^{a}	32.1^{a}	5.8^{c}	0.28
IR64	69.2^{bcd}	85.6^{b}	32.0^{b}	24.6^{b}	12.2^{a}	0.40
KDML105	92.2^{a}	143.3^{a}	53.9^{a}	32.6^{a}	6.2^{bc}	0.30
F-test	**	**	*	**	**	ns
C.V. (%)	1.7	4.6	14.5	7.97	6.8	14.3

Remarks: DTF[z] =days up to 50% flowering; PH =plant height (cm); FLL =flag leaf length (cm); PL =panicle length (cm); T/P =number of tillers per plant; HI =harvest index; ns =non-significant; *, ** = significance at 0.05 and 0.01 probability levels, respectively. Means within each column of each agronomic traits followed by the same letter are not significantly different according to DMRT.

Table 5. Major important yield component of the developed lines.

Varieties/Lines	P/P[z]	S/P	G/P	GW (g)	W/P (g)
RD15	4.7^{c}	116.0^{b}	101.9^{b}	3.4^{a}	2.7
KD2-1	5.4^{c}	107.4^{b}	94.7^{b}	3.2^{a}	2.7
R-11	5.7^{bc}	111.3^{b}	101.6^{b}	3.3^{a}	2.6
R-13	6.8^{b}	109.0^{b}	91.6^{b}	3.3^{a}	2.4
R-38	5.1^{c}	107.6^{b}	102.9^{b}	3.7^{a}	2.1
R-87	5.1^{c}	110.7^{b}	99.9^{b}	3.3^{a}	2.6
IR64	11.0^{a}	150.5^{a}	132.2^{a}	2.1^{b}	2.3
KDML105	4.6^{c}	105.1^{b}	89.9^{b}	3.5^{a}	1.9
F-test	**	**	**	**	ns
C.V. (%)	8.9	10.4	10.7	11.5	20.8

Remarks: P/P[z] =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; **= significance at 0.01 probability levels. Means within each column of each yield component followed by the same letter are not significantly different according to DMRT.

Agronomic Performance and Quality Characteristics of Lines

The performance evaluation in the field showed non-significant differences between the selected four lines and RD15 for most of the traits, including yield component as well as cooking and eating characteristics (Table 4 and Table 5). Days to 50% flowering of four lines ranged from 66.3 to 71.4 with a mean of 69.1 days, which was similar to RD15 (69 days). The 100-grain weight ranged from 3.3 g for R-11, R-13 and R-87 lines to 3.7 g for R-38 line, while that of RD15 was 3.4 g. The range of total grain weight per plant for the four lines was between 2.1 g for R-38 line and 2.6 g for R-11 and R-87 lines, while the value of RD15 was 2.7 g. As compared to RD15, the four lines did not have much difference in the total grain weight per plant and 100-grain weight. There was no significant difference between RD15 and the four lines for flag leaf length, panicle length, plant height, number of panicles per plant, number of tillers per plant, number of filled grains per panicle, number of grains per panicle, harvest index, aroma, amylose content of milled rice and alkali digestion value. It indicates that the blast resistance-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 (Ye & Smith, 2010). 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 resistance genes.

Validation of Blast Resistance in the Lines by Phenotyping

The validation of blast resistance on four lines of F4 progenies was performed by comparing

them with KD2-1 line, IR64 and RD15 varieties. The plants were inoculated with old blast isolates prevalent at different locations, including newly collected blast isolates. The total of 4 lines that were resistant to blast disease exhibited a very high level of resistance to all the 12 new blast isolates without any symptoms of blast disease.The lines with the combination of the four resistance genes will provide a wider range of resistance to the blast isolates, and will have implication on rice yield stability in the region (Suh et al., 2011).

CONCLUSION AND SUGGESTION

Phenotypic and marker-assisted selection methods performed together are effective for pyramiding of resistant genes. The present study demonstrated that the successful transfer of four blast resistant genes into RD15, a Thai jasmine rice variety, without reduction of grain yield and quality, and without changing the flowering time. The dual-selection methodology of phenotypic and genotypic selection is effective to identify improved breeding lines of rice with four genes governing blast disease resistance. Efforts to transfer these multiple genes of blast resistance to elite or commercial varieties of rice may help to reduce the yield and quality loss caused by the pathogen.

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