MATERIALS AND METHODS

Blast isolates

Blast isolates used for parental lines and test varieties screening

Isolates were collected from rice and weed hosts around Savanakhet Province. 50 isolates were identified and grouped via pathotyping for screening of parental lines. 22 representative isolates were selected out of the 50 and 5 selected isolates from Thailand for screening of parental lines and test varieties. (Appendix Table 2, 3).

Isolates used for screening the mapping population

Three isolates from 3 diverse blast groups were selected based on the pathogenicity data to screen the mapping population: LPDR16, LPDR41 and LPDR47 (LPDR stands for Lao Public Democratic Republic)

Plant material

Test varieties for blast screening

The parental lines used were JHN, commercial, non-glutinous rice variety resistant to rice blast disease under natural condition and considered to be highly resistant to a broad spectrum of blast isolates in Thailand and KDML 105, a commercial, non-glutinous aromatic rice variety susceptible to rice blast disease. Thirty commercial varieties including JHN and KDML105 were used to identify compatible host-pathogen interaction among various Lao isolates (Table 1).

Table 1. Rice varieties used to identify compatible host-pathogen interaction among various Lao isolates.

No.	Variety	Pedigree	Origin
1	Thadokkham 1 (TDK1)	IR43069-UBN-507-1-2-2	Laos
2	Thadokkham 9 (TDK9)	IR71510-UBN-TDK-6-1-1-2	Laos
3	Thadokkham 3 (TDK3)	L161-7-3-2-1	Laos
4	Thadokkham 5 (TDK5)	SK12-117-2-3	Laos
5	Thadokkham 6 (TDK6)	IR70824-TDK-5-B-1	Laos
6	Thadokkham 7 (TKD7)	IR68101-TDK-B-B-31-1"SK3-1-2-2	Laos
7	Thadokkham 8 (TDK8)	IR71510-UBN-TDK-6-1-1-2	Laos
8	Thadokkham 10 (TDK10)	BKNLR78015-R-R-PSL-3-1	Laos
9	Thasano 1 (TSN1)	IR46463-CPA-5-2-1-1	Laos
10	Thasano 3 (TSN3)	TDK10027-TSN-B-111-5-1	Laos
11	Phone Ngam 1 (PNG1)	IR43086-UBN-505-2-3-1	Laos
12	Phone Ngam 3 (PNG3)	IR68835-88-1-B-B-3-5-B	Laos
13	Phone Ngam 4 (PNG4)	IR68835-44-B-B-B-2-4-B	Laos
14	Phone Ngam 5 (PNG5)	IR8102-TDK-B-3-33-1	Laos
15	Phone Ngam 6 (PNG6)	IR70849-30-3-B-1-B	Laos
16	BL1	TDK10034-B-51-1-1-B	Laos
17	BL2	TDK10042-B-4-3-B	Laos
18	BL6	TDK10025-B-14-3-B	Laos
19	LNTN1	IR68101-TDK-B-B-28-1	Laos
20	NTN1	IR49766-KKN-52-B-2-3	Laos
21	KK2	TDK10047-B-6-1-2-B	Laos
22	KK11	TDK10042-B-1-1-2-B	Laos
23	KK12	TDK10037-B-9-1-3-B	Laos
24	Lai Daw	INDIGENOUS	Laos
25	CR203		Vietnam
26	Khao Dawk Mali 105 (KMDL1O5)		Thailand
27	RD6		Thailand
28	Jao Hawm Nin (JHN)		Thailand
29	AZUCENA		Philippine
30	IR64		International Rice Research Institute (IRRI)
31	IR62266	IR62266-42-6-2	International Rice Research Institute (IRRI)
32	CT9993	CT9993-5-10-1-M	Center of International Tropical Agriculture (CIAT)

RILs for Mapping

587 F6-RILs derived from a cross between KDML105 and JHN were developed by Rice Gene Discovery Unit, BIOTEC at Kasetsart University, Thailand in 2000 by single seed descent. The F6-RILs progenies of KDML105 and Khaw Hom Nin were used to verify the mapping location by using its complete map. The

availability of tightly linked markers facilitates appropriate breeding method for durable blast resistance.

Genotyping (F6-RILs population) and linkage map

In this study, all genotype data and linkage map were provided by Rice Gene Discovery Unit (BIOTEC), Ministry of Science and Environment. Data from sixteen SSR markers were analyzed for the order of markers by MAPMARKER/QTL developed by Lander *et al.,* Distance between markers was analyzed using Kosambi function. The order of six markers on chromosome1 started from RM5, RM246, RM237, RM319, RM212, and RM204 and the distance between markers are 9.3, 7.6, 12.3, 1.5 and 22.7 cM, respectively. The total distance on chromosome1 was 51 cM while that on chromosome11 was as long as 84.9 cM. There were seven markers mapped on chromosome11 in the following order: RM21, RM206, RM254, AC113249, RM144, RM139 and RM244. Distances between these markers were 22.7, 20.7, 9.4, 13.3, 2.9 and 10.6 cM, correspondingly. The total distance on chromosome 12 appeared to be the shortest among the three chromosomes with distances between markers at 12.7 and 27.4 cM, respectively.

Survey and Collection of blast samples

Isolates were collected from different hosts; rice, and weed in 2004 just before the harvesting time of the dry-crop in Savanaket Province. Six districts, representative of the main rice growing areas in Savanakhet Province, were subject to sampling during field trips at the appropriate time of the year, during leaf blast and neck blast epidemics, in particular from blast samples from areas not covered so far. (Figure 1) For increased efficiency of sampling genetic diversity, at least 20 leaves and/or necks from each location will be collected, if possible, with each sample taken at least 10 meter apart. For each sample, the rice cultivars and plant's part which taken from, the location (field), and the date of the sample collection will be noted. Leaves and/or other parts such as collar, necks, and seed samples would be air-dried and stored in a dessicator until further processing.

Figure 1. Map of Laos. Location of *Pyricularia grisea* population sampled at Savanakh , Central Laos.

KH = Khanthabuly; CH = Champhone; XN = Xonbuly; XT = Xayphuthong; XB = Xaybuly

Evaluation of leaf blast resistance

Twenty-two isolates were used to identify for compatible host-plant interaction using 32 varieties, including 3 resistant cultivars JHN, CT9993 and IR62266. The experimental design was randomized complete block in 2 replications. Rice plants were grown in plastic trays divided into 32 rows with 7 plants each. Each variety was planted in 1 row per replication. JHN and KML105 were used as check varieties.

587 RILs of KDML105/JHN were evaluated for leaf blast resistance in greenhouse under controlled temperature against 3 isolates (LPDR16, LPDR41 and LPDR47). The experimental design was randomized complete block without replications. Rice plants were grown in plastic trays divided into 32 rows of 7 plants each. Each genotype was planted in 1 row and on each plastic tray JHN was planted as resistant and KDML105 as susceptible checks.

Fungal inoculation

To obtain conidiophores for inoculation, pieces of colonized filter paper were taken out of storage and revived in petri dishes containing rice polished agar. Inoculum was prepared following the methods of Mackill and Bonman. Seven days before inoculation, 10 ml of distilled water was placed in previously revived petri dishes. The mycelial mat was scraped to induce more growth and then plates were transferred to an ultra-violet cabinet for 2-3 days to induce sporulation. During inoculum preparation, distilled water was added to the petri dish, the mycelial mat was scraped with a sterilized rubber and the suspension was filtered through two layers of cheesecloth. At 3- leaf stage after seeding, a 100 ml single race spore was counted at a concentration of 5 x10 ^{4} conidia /ml suspension with 0.5 % gelatin and was sprayed on each seeded tray. Inoculated plants were kept inside the dew chamber for 16-18 hrs and then transferred to the green house until scoring time.

Scoring of symptoms

The severity of blast disease was scored seven days after inoculation using lesion score with the observed symptoms as described by Roumen *et al*., The infection 0-2 was considered resistant, 3-4 intermediate and 5-6 as susceptible reactions.

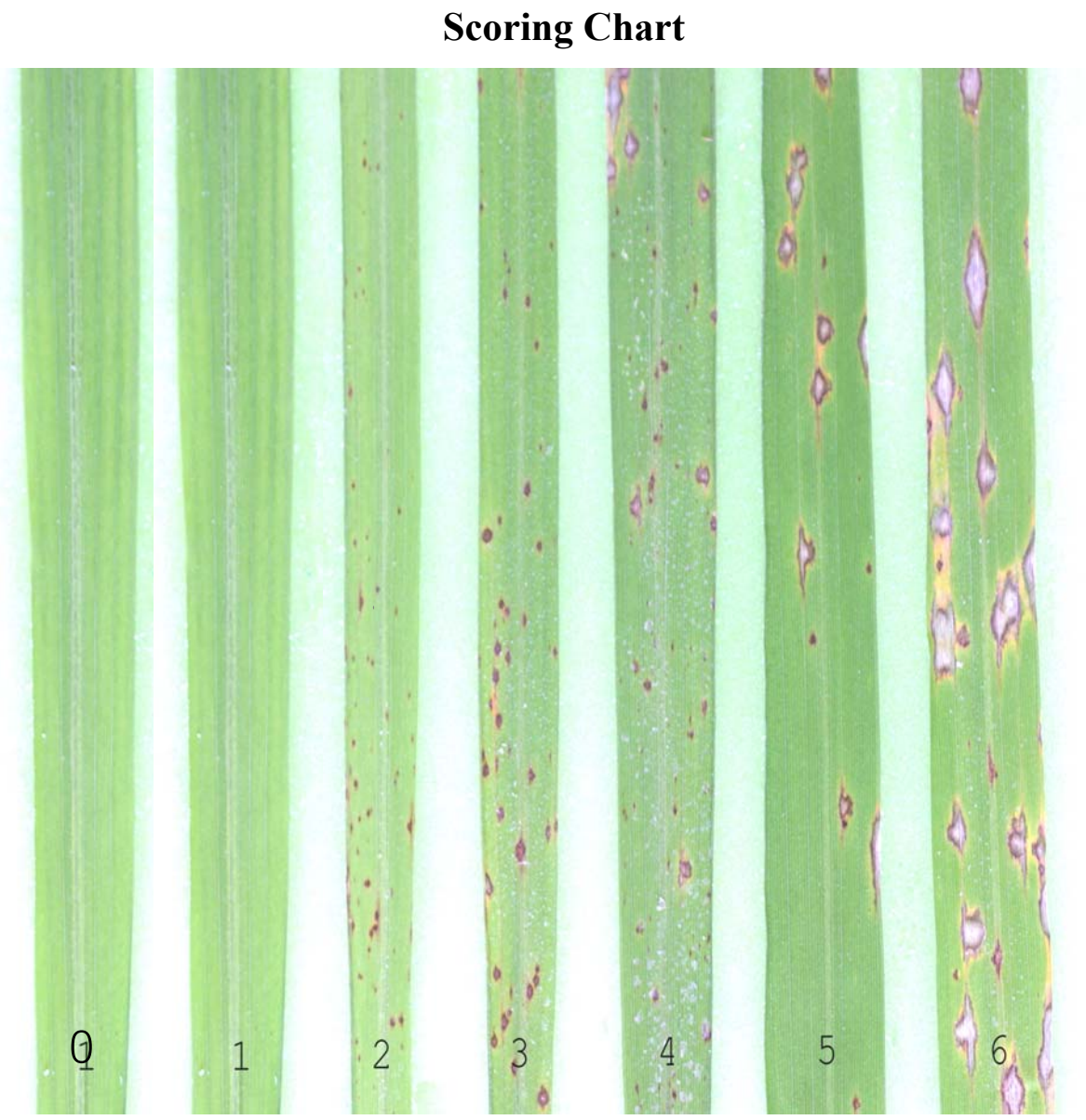

Figure 2. The disease severity index of blast disease with lesion 0-6 type.

Table 2 The description of severity index of blast disease with the seven (0-6) lesion type categories.

Severity index	Description
0	no evidence of infection
1	brown, pinpoint smaller than 0.5 mm., without sporulation
2	brown, pinpoint smaller than 0.5-1mm., without sporulation
3	small eyespots about 1-3 mm with gray center, lesion capable of sporulation
4	small eyespots about 3 mm or more with gray center and dark margin, lesion capable of sporulation
5	susceptible sporulating type, coalescence lesion without dark margin
6	susceptible sporulating type, lesion without dark margin

Note: The data were classified into 2 groups, resistant (R = 0, 1, 2, 3) and susceptible (S = 4, 5, 6) reactions.

Resistance index (RI) formula modified from Ahn (Sirithunya *et al*., 2002) was used to assess the resistance index.

RI = S/T

S = Number of isolates giving resistance reaction

T = Total number of isolates used for screening

The RI value ranged from 0 to 1 where RI equals to 0 or 1 indicated that the rice cultivar or line was considered as susceptible or resistant to all isolates, respectively.

Statistical and QTL Analyses

The data on disease severity score of 32 parental lines screened against 22 blast isolates were analyzed using the average of 2 replications. The resistance index (RI) was analyzed using the mean and most of the data that analyzed phylogenic of blast isolates used NTSYS-pc Version 2.0. For pathotypic data matrix, pathotypic similarities among the samples based on the CANBERRA coefficients were calculated using the Simlnt module of NTSYS-pc. Cluster analysis was performed on similarity matrices with the SAHN module of NTSYS-pc using UPGMA algorithm. All dendrogams were generated from the Graphics and Tree plot module of NTSYS-pc.

The data on disease severity index of 587 RILs were analyzed to get standard deviation, and resistance index using the mean. The data were classified into 2 groups, resistant and susceptible. Chi square test was used to detect the ratio of pathotypic distribution of this population.

QTL analyses were mapped by means of interval mapping (SIM) and simplified composite interval mapping (cSIM) procedures of nQTL, software for interval mapping. The phenotypic data from each of the 3 isolates were analyzed separately. For the nQTL analysis, each data set was analyzed with 1000 permutations at a 5 cM walking speed and type I error of 5%. For cSIM, four background markers with approximately even spacing were specified, with a maximum of three background markers per linkage group. STATGRAPHIC 2.1 software was used as a tool to reconfirm the number, location and effect of the QTLs and to determine the phenotypic variance explained (PVE) or R^2 by QTLs. Two loci interactions of QTLs were determined using regression analysis and ANOVA.

RESEARCH LOCATION AND PERIOD OF STUDY

1. Location

Rice Gene Discovery Unit, Kasetsart University, Kamphaengsaen Campus Greenhouse – Leaf blast screening on RILs population

2. Period of study

June 2003 – March 2006