Identification of Blast-Resistant Varieties from Landrace, Improved and Wild Species of Rice

Abdu Salih[1], Tanee Sreewongchai[2,*], Prapa Sripichitt[2] and Nonglak Parinthawong[3]

ABSTRACT

An experiment was conducted to identify rice varieties resistant to blast disease. Samples of 311 genetically diversified varieties/accessions comprised of landrace (263), improved (43) and wild (5) rice varieties/accessions were provided by the National Rice Gene Bank of Thailand. The screening for blast at the seedling stage was done using 29 diversified blast isolates. The blast isolates were collected from seven provinces (Phitsanulok, Ubon Ratchathani, Khon Kean, Chiang Rai, Nong Khai, Chaiyaphum and Udon Thani) of Thailand. The results indicated a total of 35 varieties/accessions (25 landrace, 9 improved and 1 wild) were resistant to all tested blast isolates. The 25 resistant landrace varieties were collections from Northern (10), Northeastern (9) and Southern (6) Thailand. Moreover four of the resistant landrace varieties (GS23107, GS19769, GS20874 and GS23774) were highly resistant with no symptom of the disease. From this study, it can be suggested that the resistant landraces from the Southern, Northern and Northeastern regions could be used as sources of resistant varieties in designing future breeding programs aimed at developing disease-resistant genotypes.
Keywords: blast disease, identification, landrace, resistance, rice

INTRODUCTION

Rice (*Oryza sativa*) is the world's second most important cereal and is the staple food for over one third of the world's people (John and Sleeper, 1995). It is recognized as an important strategic food security crop and as a crucial element in the staple food economies of sub Saharan Africa (Kaung and Allan, 1985). It is the leading cereal crop of Southeast Asia and is the only major food crop that can be grown in the vast areas of standing water in tropical and subtropical areas (Pennisi, 2010). In Thailand, it is also one of the major staple foods and an income generating commodity contributing to the gross domestic product. The production and productivity of rice is constrained by factors that vary with different agro-ecology, zones and/or regions. Different diseases, insect pests and weeds impact the yield of rice worldwide. Sixteen diseases are economically important to rice (Baker *et al.,* 1997) of which rice blast disease is the most important disease in rice-growing countries worldwide. This disease has spread to more than 85 countries (Ou, 1985). It is caused by *Magnaporthe grisea* (anamorph: *Pyricularia grisea*) Sacc and is highly adaptable

[1] Afar Pastoral and Agro-Pastoral Research Institute, Afar, Ethiopia.
[2] Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
[3] Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
* Corresponding author, e-mail: taneesree@yahoo.com

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to different environmental conditions and can be found in irrigated lowlands, rain-fed uplands or deepwater rice fields (Ou, 1985; Latif *et al*., 2011). Under favorable environmental conditions, this disease destroys seedlings in nurseries and crops in the tillering stage. Leaf blast stunts plant height and reduces the number of bearing panicles and the weight of individual grains (Thurston, 1998). It also increases plant respiration and reduces the maximum photosynthetic rate at light saturation and initial light use efficiency (Pinnschmidt *et al*., 1994). The economic loss caused by rice blast is estimated to be USD 5 billion annually. Songsak and Aree (2001) reported that in Thailand, a rice blast disease outbreak in 1992 caused over 1.25 million rai (0.2 million ha) of damage that incurred a loss of over THB 1 billion (approximately THB 30 = USD 1). To curb the effects of this serious disease, a multifaceted response was designed.

The use of host resistance is one of the most economical and effective means of controlling blast disease. Resistance to blast disease is governed by a gene-for-gene relationship between a resistance gene in the host and a virulence gene in the blast pathogen (Kiyosawa, 1972; Silue *et al*., 1992). In Thailand, serious attention has been given to blast disease since 1959 (Ou, 1985). Several crossings have been made to develop resistant cultivars in various regions. However, the resistant varieties obtained were unstable and became susceptible or intermediate within a few years of release. Thus, susceptible old cultivars have gradually been eliminated and more resistant ones are in wider use. A clear picture of the genetic base to blast resistance, in presently cultivated rice varieties, will provide important indications to which approach and strategy could be adopted to strengthen the degree of blast resistance and to improve the durability of resistance in future varieties. The objective of this study was to identify blast-resistant rice varieties from the landrace and improved rice (*Oryza sativa L.)* in Thailand.

MATERIALS AND METHODS

Experimental site and plant materials

The study was conducted in the nursery of the Department of Agronomy, Kasetsart University, Bangkok, Thailand during 2011. A total of 311 rice varieties/accessions, including resistant and susceptible controls, were used to screen for blast disease. All rice materials were provided by the National Rice Genebank of Thailand. These varieties/accessions were grouped into three categories—landrace (263 genotypes), improved genotypes (43 varieties) and wild rice species (5 accessions). Out of the 263 landrace rice varieties, 69, 88, 67 and 39 varieties were collected from Central, Northeastern, Northern and Southern Thailand, respectively (Table 1).

Table 1 Blast resistance or susceptible information of 311 rice varieties/accessions used in this study.

Type of rice	Susceptible	Intermediate	Resistant	Total
Landrace from Central Thailand	35	34	0	69
Landrace from Northeast Thailand	43	35	10	88
Landrace from Northern Thailand	27	31	9	67
Landrace from Southern Thailand	8	25	6	39
Improved varieties	19	15	9	43
Wild species	2	2	1	5
Total	134	142	35	311

Blast pathogen

Twenty nine diversified blast isolates were collected from seven provinces (Phitsanulok, Ubon Ratchathani, Khon Kean, Chiang Rai, Nong Khai, Chaiyaphum and Udon Thani) of Thailand. These districts are known for the widespread occurrence of rice blast disease and hence the sampling was used to capture the available pathogenic diversity of these hotspot areas. Furthermore, the sampling represented the major rice growing areas of the seven provinces (Uckarach *et al.*, 2011). Samples were collected randomly at 30–40 d after transplanting the rice following disease development at the tillering stage. For each sample, the plant part, locations, and the date of sample collection were recorded. The samples were then taken to the laboratory and stored in desiccators for further processing following the method described by Uckarach *et al.* (2011). The detailed descriptions of the samples are indicated in Table 2.

Table 2 Rice blast fungus isolates used in the study.

Isolate code	Rice variety isolated	Organ infected	Location collected
Bag 1.1	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 1.3	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 1.4	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 2.3	KDML105	Leaf	Mueang, Ubon Ratchathani
Bag 2.4	KDML105	Leaf	Mueang, Ubon Ratchathani
Bag 3.3	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 3.5	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 4.4	KDML105	Neck	Mueang, Phitsanulok
Bag 5.3	1034N.110	Neck	Mueang, Khon Kaen
Bag 5.4	1034N.110	Neck	Mueang, Khon Kaen
Bag 6.1	1030N.8	Neck	Mueang, Khon Kaen
Bag 7.1	KDML105	Leaf Collar	Mueang, Phitsanulok
Bag 7.2	KDML105	Leaf Collar	Mueang, Phitsanulok
Bag 8.2	KDML105	Leaf	Mueang, UbonRatchathani
Bag 8.5	KDML105	Leaf	Mueang, UbonRatchathani
Bag 9.2	KDML105	Neck	Mueang, Chiang Rai
Bag 9.5	KDML105	Neck	Mueang, Chiang Rai
Bag 9.6	KDML105	Neck	Mueang, Chiang Rai
Bag 11.2	RD6	Leaf	Tha Bo, Nong Khai
Bag 12.4	RD6	Leaf	Phon Phisai, Nong Khai
Bag 14.3	KDML105	Leaf	Si Chiang Mai, Nong Khai
Bag 15.1	KDML105	Leaf	Mueang, Udon Thani
Bag 16.1	RD6	Leaf	Phen, Udon Thani
Bag 17.2	KDML105	Leaf	Chatturat, Chaiyaphum
Bag 19.2	KDML105	Leaf	Kut Chap, Udon Thani
Bag 24.1	KDML105	Leaf	Mueang, Chaiyaphum
Bag 24.2	KDML105	Leaf	Mueang, Chaiyaphum
Bag 28.2	RD6	Leaf	Bueng Kan, Nong Khai
Bag 31.1	RD6	Leaf	Ban Dung, Udon Thani

Rice planting

Seeds of all collected rice varieties/accessions were sown on moist tissue paper for about 5–7 d and then the seedlings were transferred to trays containing soil. Each variety/accession was prepared in three replications. Urea and NPK fertilizers were applied twice—that is, as recommended and one day before inoculation. The resistant (IR64) and susceptible (KDML105) varieties were used as standard check varieties in all experiments.

Inoculation and evaluation of disease resistance

Each of the blast isolates was cultured on rice flour agar medium (2.0% rice flour, 0.2% yeast extract and 2.0% agar) and incubated at 25 °C under 12 hours per day of fluorescent light conditions for 8–10 d. Fungal colonies were scraped out of the surface for further sporulation and incubation under the same conditions of culture for 1–2 d. After conidia formation, the conidia were harvested using sterile distilled water. The inocula were adjusted to a concentration of 5×10^4 conidia.mL^{-1} using sterilized distilled water with 0.5% gelatin.

The prepared conidial suspensions of the fungus were sprayed or inoculated at the fourth leaf stage on each rice seedling in the plastic tray. Then, inoculated seedlings were incubated at 25–28 °C and relative humidity of 98–100% in a chamber for about 12–16 hr to create a conducive environment for the penetration of the conidia and for disease development. Seedlings were maintained in the nursery for an additional 7 d. Seven days after inoculation, data for leaf blast symptoms and disease severity were recorded as described by Sirithunya *et al*. (2001) and their disease severity reaction score ranging from 0 (resistant) to 6 (susceptible) was recorded.

Data analysis

The data were classified into two groups based on the reaction of the rice varieties—resistant (R = 0, 1, 2 and 3) and susceptible (S = 4, 5 and 6). A modified resistance index (RI) formula was used to assess the resistance index (Sirithunya *et al*., 2001). The RI was expressed as RI = S/T, where S is the number of isolates showing a resistance reaction and T is the total number of isolates used for screening. Thus, the RI value can range from 0 (susceptible) to 1 (resistant).

RESULTS

Blast disease resistance of rice varieties/accessions

The analysis was based on the Resistance Index (RI) methodology (Sirithunya *et al*., 2001) of disease data analysis to determine the available genetic variation in disease resistance. Accordingly, out of the 311 varieties/accessions compared a total of 35, 142, and 134 cultivars were found resistant, intermediate and susceptible respectively. Among the 35 resistant varieties (25 from landraces, 9 from improved and 1 wild rice) only 9 were highly resistant to blast disease with no symptom of blast disease (0 score) when tested against the 29 individual blast isolates.

The nine highly resistant rice varieties plus IR64 (resistant check) and KDML105 (susceptible check) were tested again against the 29 isolates. The results showed that from the landraces, GS23107, GS19769, GS20874 and GS23774 and from the improved varieties, Nat 1, Suphanburi 1, Suphanburi 60, Suphanburi 90, JHN and IR64, were resistant to all isolates with an RI value of one, while KDML105 (susceptible control) was susceptible to all isolates with an RI value of 0 (detailed descriptions are provided in Table 3). Comparisons among the regions indicated that landrace varieties which were collected from the central region showed no resistance while the highest percentage of those landraces from the southern region showed resistance against all blast isolates. The detailed descriptions of the landrace rice varieties are indicated in Table 4.

Table 3 Name, Resistance level, type of cultivar and origin of nine highly resistant cultivars and one resistant and one susceptible control.

Code/Name	Resistance level	Type of cultivar	Origin	Score	RI value
GS 23107	Resistant	Landrace	Thailand	0	1
GS 19769	Resistant	Landrace	Thailand	0	1
GS 20874	Resistant	Landrace	Thailand	0	1
GS 23774	Resistant	Landrace	Thailand	0	1
Nat 1	Resistant	Improved	Thailand	0	1
Suphan Buri 1	Resistant	Improved	Thailand	0	1
Suphan Buri 60	Resistant	Improved	Thailand	0	1
Suphan Buri 90	Resistant	Improved	Thailand	0	1
JHN	Resistant	Improved	Thailand	0	1
IR64	Resistant	Resistant control	IRRI	0	1
KDML 105	Susceptible	Susceptible control	Thailand	6	0

IRRI = International Rice Research Institute, Los Baños, Laguna, the Philippines.
RI = Resistance index.

Table 4 Local/landrace varieties collected from some regions of Thailand and their response to rice blast fungus.

Region	Susceptible	Intermediate	Resistance	Total
Central	35 (50.72%)	34 (49.28%)	0	69
Northeast	43 (48.85%)	35 (39.8%)	10 (11.35%)	88
Northern	27 (40.3%)	31 (46.27%)	9 (13.43%)	67
Southern	8 (20.52%)	25 (64.10%)	6 (15.38%)	39

The numbers in parentheses show the percentage of the level of disease incidence against blast isolates.

DISCUSSION

The results from this study indicated the genetic diversity of the rice varieties collected from the different regions of Thailand against blast pathogens and their interaction. It was clear that the blast disease caused by *M. oryzae* is one of the destructive diseases of rice and can cause severe damage and yield reduction with favorable environmental conditions and susceptible varieties. This experiment showed differences in the resistance to blast among cultivars collected from different regions in Thailand. These differences are in agreement with the report of Ou (1985) which recorded variability in resistance from region to region or from country to country. The differences could probably be related to the availability of predisposing factors that favor disease development (Babujee and Gnanamanickam, 2000). Artificial inoculation conducted in the greenhouse showed that 80% of the local cultivars tested against blast showed either susceptible or intermediate resistance to the 29 isolates of *P. grisea*. Four (GS23107, GS19769, GS20874 and GS23774) out of the 263 local cultivars were highly resistant to all 29 isolates of *P. grisea*. Similar research was carried out in other rice growing countries; in screening trials in Bangladesh, Mohanta *et al*. (2003) reported that among 28 restored lines and 3 standard checks, 3 were highly resistant, 12 were resistant and 16 were moderately susceptible. Similarly Dissanayake (1995), in Sri Lanka revealed that out of 22 cultivated rice varieties used in his study, only 6 varieties were resistant to blast at two sites, with this difference attributed to parental sources.

A comparison of the regions in the current study showed that all resistant varieties were obtained from the Southern, Northern and Northeastern provinces of Thailand. However, the results from this inoculation study need to be verified with field-based observations. The susceptibility of most of the varieties clearly suggests the need for the development and promotion of blast-resistant varieties.

CONCLUSION AND RECOMENDATION

This study showed that there is variability in resistance among the different rice genotypes evaluated. All resistant genotypes were obtained from Southern, Northern and Northeastern Thailand. These, regions/areas could be used as a potential source of genotypes resistant to blast disease. The inclusion of blast disease resistance as one of the criteria in rice breeding programs and the use of these resistant genotypes will help to develop rice genotypes that are agronomically important and blast-disease resistant.

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