Large scale screening for blast resistance of Thai indigenous lowland rice germplasm

Menson R Kwanwah[1], Tanawat WongSa[2], Tidarat Monkham[2], Sompong Chankaew[2],

Jirawat Sanitchon[2]*

Abstract: Rice blast has become a problematic situation for rice cultivation worldwide. Blast disease is caused by a fungus *Magnaporthe grisea (Pyricularia oryzae)*, essentially diminished yield and value of rice grains. This study was conducted to evaluate for resistant of the rice germplasm against rice blast disease in a natural infection in field condition, in order to give faster screening for rice blast resistance as a forerunner in a breeding system. A total of 347 Thai lowland indigenous rice germplasm with 14 check varieties, were screened on a natural infection in blast incidents hot spots in field conditions. The screening was conducted at two locations that have a history of high incidence of blast disease occurrence and are among the famous blast screening locations in Thailand. A completely randomized design with 3 replications was conducted in field conditions, using standard evaluation system (SES) (IRRI, 2013) 0-9 method to score the disease infection. The finding revealed that two third of indigenous lowland rice accession were classified as blast resistant under natural field infection in both experimental years with score lower than 2.00. Additionally, the top ten elected indigenous lowland rice accession including LLR276, LLR397, LLR054, LLR425, LLR055, LLR057, LLR253, LLR334, LLR363, and LLR258 also shown resistant ability under upland short row condition, that indicated that those accessions providing an emerging alternate sources of blast resistance varieties in Thailand, for future rice breeding programs.

Keywords: *Magnaporthe grisea (Pyricularia oryzae)*, KDML105, Neck blast, Upland short row, Natural infections

---

Recevied May 16, 2019

Accepted August 29, 2019

[1] International Program of Agriculture, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

[2] Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand.

* Co-responding author: e-mail: jirawat@kku.ac.th

Introduction

Rice (*Oryza sativa* L.), serves as a standard diet for over 3 billion people globally, is the second largest cereal cultivation crop next to maize with over 685 million tons recorded (FAO STAT, 2019). The world's cultivated land covered under rice production is about 156 million hectares (FAO STAT, 2019). A significant portion of 90% of rice eating and cultivation happens in Asian countries (Khush, 1997). However, the rice production program had a series of constraints from biotic and abiotic pressure. Blast disease which is caused by fungus *Magnaporthe grisea*, is one of the stress, it is a destructive disease that accounts for worldwide losses of 30-70% in rice yields (Manandhar et al., 1992).

The disease distributes outbreak in tropical, subtropical as well as the temperate territories where rice is cultivated globally (Chadha, 2005). *Magnaporthe grisea* has been observed and announced in the minimum of 85 countries and caused yield losses in the tune of 157 million tons, that can feed roughly, 60 million people (Oerke et al., 1994). Thai aromatic rice varieties, KDML105 and RD6 which occupied 87% of the area under rice cultivation in northeastern Thailand are not exempted from this serious concern about the prevalence of blast pathogen (Aoki and Miyagawa, 2000).

Raised temperature and relative humidity up to 85-89% and excessive nitrogen fertilizer usage support the disease growth (Piotti et al., 2005). The blast pathogen attacks at varying levels of the rice plant. The disease is more visible when attacks take place on the leaf blades, nodes, panicle, and neck (Ou, 1985). Early attacked of the disease often lead to total destructions in seedlings stage (Piotti et al., 2005; Thruston, 1998; Rao, 1994; Shim et al., 2005; Candole et al., 2000; Padmanabhan, 1965). Rice yield losses up to 70-80% were reported in the fields infected by neck blast in a serious situation (Manandhar et al., 1992; Ou, 1985).

The chemical control method in blast is accurate but has graven negative implications with the agricultural ecosystem and also increases the production cost. Therefore, the utilization of genetic resistance varieties is an effective way to control the disease (IRRI, 2006). The complete blast resistance is being linked with major 'breakdown' of varietal resistance (Crill et al., 1981). Such as in Egypt, the Japanese cultivar Reiho possessing the gene *Pita-2* for complete resistance which was released in 1984, unfortunately, lost its resistance after the first year of production, leading to severe epidemic (Reddy and Bonman, 1987). Regrettably, the rapid genetic progression of the fungus often defeats the resistance given to by major genes after a few years of intensive agricultural use (Sharma et al., 2012; Lavanya and Gnanamanickam, 2000).

The opportunities of uncovering latest alleles of currently available genes with even wide resistance spectrum can be achieved by evaluating wild species and germplasm accessions (Das et al., 2012). Although about 100 blast resistant genes besides 347 QTLs, have been identified and 20 of the identified genes have since been cloned and characterized at orderly stage (Koide et al., 2010; Sharma et al., 2012), the adaptation to some particular area is the limitation of exotic varieties usage. Therefore, indigenous rice, varieties which thrive in wide range of agro-ecological conditions with disease - insect resistance and adaptation to environment stress in particular regions should be utilized (Chang, 1976). The continues breaking down of deployed resistance varieties after few years of cultivation prompted the need for continuous evaluation. Screening for varietal resistance using artificial inoculation method may always be precise as a result of the presence of enough inoculation usage of the disease (Mew, 1984). There are four methods of Inoculation for varietal evaluation popularly used. Even though evaluation for rice resistance can be in field conditions under natural infection, the outcome may vary due to seasonal differences (Vasudevan et al., 2014). Artificial inoculation reduces such conditions. The four methods generally used to screen for resistance to this disease; needle pricking has been generally practiced in some of Asian countries up to the introduction of the clipping method (Ou, 1985). The clipping method is advantageous because after cutting the tip of the plant leaf the pathogens is straightly induced into the plant. Whereas, the dipping method, the young plant's roots are directly submerged into the inoculum before taking to the field (Mew et al., 1979).

Spraying method, the inoculum is sprayed straightly at the seedling (Mew et al., 1982). However, blast disease can be screened using any of the above inoculation methods, depending on the person (s) conducting the experiment. Thus, the clipping method has been widely use today based on its effectiveness and accuracy.

The objective of this study was aimed at screening local Thai indigenous lowland rice germplasm for genetic resistance to blast disease, if accomplished, to be used by breeders in a breeding program to produce more durable cultivars.

Materials and Methods

Plant material

A total of 347 germplasm of Thai indigenous lowland cultivated varieties of rice including 333-lowland rice varieties obtained from 21 provinces across Thailand were used in this study (Figure 1). And the eleven resistant check varieties are Azucena, IR62266, IR64, IRBB21, IRBB5, P0489, JHN, Thanya Sirin, and RD23, HY1 and RD6 NIL. While the three susceptible check varieties are KDML105, RD6, and SKN. The sources of the check varieties are Azucena, IR62266, IR64, IRBB21, IRBB5, and P0489 source from International Rice Research Institute (IRRI), whereas, JHN, and Thanya Sirin, source from National Science Technology Development Agency (NSTDA), Thailand. While KDML, RD6, SKN, RD23, and HY1 source from Rice Department of Thailand, and RD6 NIL source from the Department of Rice Project, Khon Kaen University.

Planting nursery and disease evaluation

The germplasm was screened by natural infection in rice growing season (wet season) under field condition in two years. The first year 2017, and second-year 2018 were done at the Agronomy Research Station, Khon Kaen Province, Thailand (KKU). Further experiment to validate the selected genotypes was conducted in 2018, at Nong Khai Rice Research Center, Thailand.

At KKU Research Center, the seeds were soaked for three days before sowing on September 4, 2017 and August 20, 2018. One seed per planting hill, in a plastic tray of 30-hills and 5-borders hills. All the tested varieties were trap with susceptible check varieties, RD6 and KDML105 within and around the trays. There were three replications, each replication had 35-plastic trays at planting distance of 5 cm by 5 cm between plants and 30 cm by 30 cm between rows of trays. After every 2 trial genotypes, check varieties were inserted in all 3 replications. The surrounding of the experimental area was completely trapped with spreader varieties, (susceptible check KDML105, SKN, and RD6). Completely randomized designed (CRD) was used in the design of the experiment. The first application of fertilizer at the rate 23.5 kg/ha of N, P_2O_5, K_2O was applied at 15 days after sowing (DAS). Nitrogen fertilizer was applied at 20 DAS at the rate 28.75 kg/ha of N. The disease scoring was done when KDML105 shown susceptible to disease at 39 and 31 DAS in 2017 and 2018, respectively. The different in the age in the plant of infestations, may be due to the difference in the weather condition between the two years. The plant started showing small roundish, elongated necrotic gray spots with a distinct brown margin, mostly found on the lower leaves about 1 - 2 mm length. Later moved to upper leaves covering significant portion of the leaves area up to 2 - 3 mm length. The standard evaluation method of scoring leaf blast 0 - 9 of scoring (IRRI, 2013) was employed during the exercise; 0=highly resistant, 1 - 2 = resistant. 3 - 4 = moderately resistant, 5 - 6 = moderately susceptible, 7 - 8 = susceptible, 9 = highly susceptible. The water level was maintained regularly at 8 cm throughout the period of the experiments. Digital weather recorder, Thermo-Hygrometer was used to record the average temperature and relative humidity during the course of the experiments.

At Nong Khai Rice Research Center, the screening was conducted to validate the performances of the ten selected genotypes which showed resistance to blast disease in both experiments in two years, 2017 and 2018 at KKU and had enough seed. These same germplasms have been used by other investigators in previous study, but with different objectives. Based on this, the seeds of some of the varieties significantly reduced. After using the same germplasms for our first two experiments, fortunately, it was realized that, the ten varieties that put out good performances still have enough seeds for further experiment. The direct seeding was planted on August 24, 2018 follow the upland short row method, in a plot size of 1m-width by 5m-length and the designed used was completely randomized designed, with three replications. The surrounding was planted with trap plants (KDML105) ten days before sowing the trial genotypes at the time the trap variety started showing symptom of blast disease. Seeds were drilled per variety by rows, 2 cm. by 2 cm. between plants in row, spreaders/susceptible and resistance checks varieties were inserted between every 2 trail genotypes row as trap plants. The disease severity was seen all over the experiment after 21 days of sowing. Disease observation and scoring were carried out using standard evaluation system of IRRI (2013).

Data analysis

Varieties of rice were arranged into a category according to their responses to the disease during the observations/scoring of the experiments 2017/2018. A scattered plot analysis program was used in the data analysis. Excel 2010 software program was used to calculate the averages based on the groups, and subsequently, input averages into statistix-10 for analysis of variance (ANOVA), least significant difference (LSD) was used to determine the difference of treatment means.

Result

The screening of lowland indigenous germplasm for blast resistance through natural infection, data frequency revealed differences in responses in both test years, 2017 and 2018 (Figure 1a&b). In 2017, the total of 279 genotypes were classified as resistant with score less than 5, 66 genotypes were susceptible with score more than 5 (**Figure 1a**). In 2018, less resistant varieties were recorded than 2017, the total of 255 genotypes were categorized as resistant varieties and 90 genotypes were susceptible (**Figure 1b**). The disease reaction in the two years varied, based on the change in weather conditions during the period of the experiments (**Figure 2**).

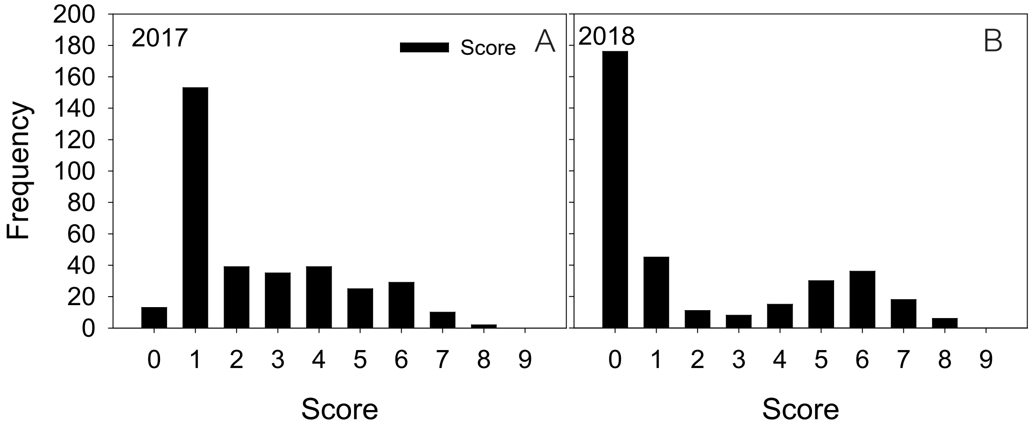

Figure 1 The Frequency of blast disease reaction in both years (2017 and 2018); highly resistant = 0, resistant = 1-2, moderately resistant = 3-4, moderately susceptible = 5-6, susceptible = 7-8, highly susceptible = 9.

The temperature and humidity and amount of rainfall throughout the experiments were showed in **figure 2**. The average temperature and relative humidity were recorded at 28°C and 85% in both years. The distribution of rainfall during growing period was slightly better in 2017 than 2018 which affected constant relative humidity and temperature in 2017 than 2018 (**Figure 2**). The high relative humidity was effective for disease incident and the well distribution of rainfall was suitable condition for blast disease.

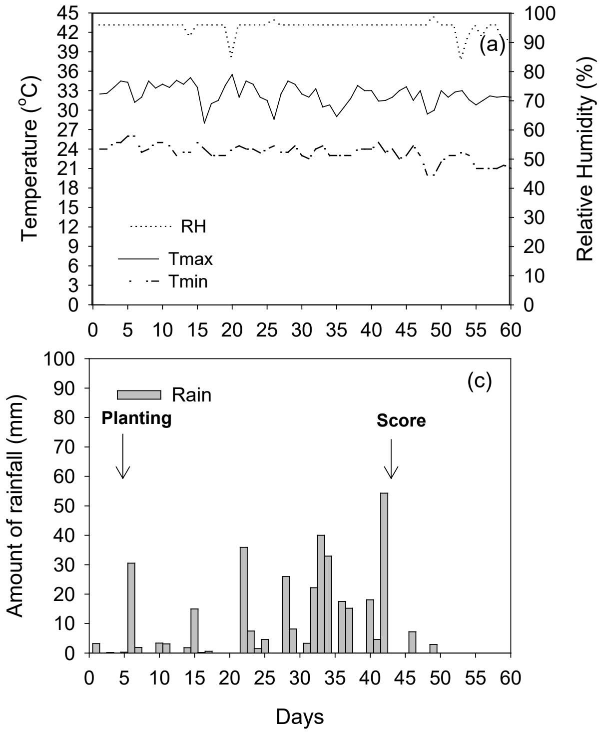

Figure 2 Rainfall, RH%, minimum, maximum temperature during field evaluation at KKU in 2017 and 2018; RH%, minimum, maximum in 2017 (a), RH%, minimum, maximum in 2018 (b), amount of rainfall 2017 (c), amount of rainfall 2018 (d).

Due to the variations of disease reactions in the two years, selection of resistant genotype based on one year may not be accurate enough, therefore, the combined data from both experiments were required to determine the resistance genotype. The relationship of blast resistant score was plot (**Figure 3**). Resistance check varieties, as well as susceptible check varieties, maintained their status respectively (Figure 3). Rice varieties, LLR054, LLR055, LLR150, LLR252, LLR278, LLR300, LLR363, LLR364, LLR397, LLR422 and LLR425 were highly resistant with score of 0.00 in both experimental years. While disease score of susceptible genotypes ranged from 5.00 to 9.00, LLR019, LLR042, LLR043, LLR080, LLR084, LLR085 and LLR367 varieties were highly susceptible with score of 6.00 to 8.00. Moreover, 101 genotypes were classified as resistance in both 2017 and 2018 (score < 2) (Figure 3). Moreover, the high correlation (r = 0.763**) of two years' data indicated that resistant genotypes were invariability (**Figure 3**).

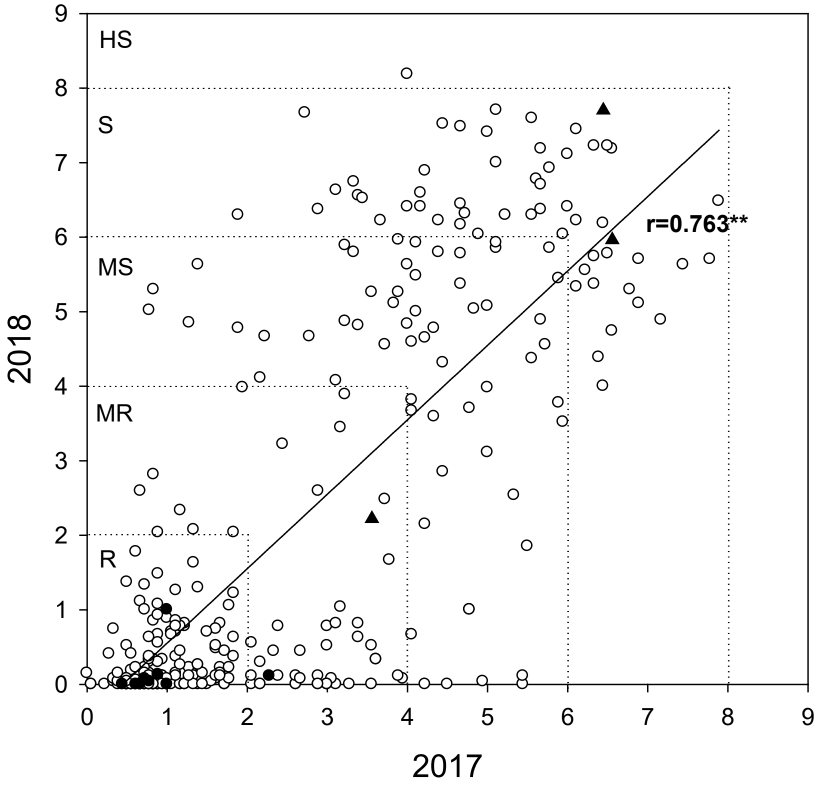

Figure 3 Relationship of blast score of 2017and 2018 at KKU; R=resistant, MS=moderately resistant, MS=moderately susceptible, S=susceptible, HS=highly susceptible. Black circle = resistance check varieties, black triangle= susceptible check varieties.

The indigenous lowland rice varieties, which demonstrated consistent resistant in both experiments of natural infection and have enough seeds, were selected to confirm the resistance in blast incidents hot spots field conditions. The ten selected genotypes (LLR276, LLR397, LLR054, LLR425, LLR055, LLR057, LLR253, LLR334, LLR363 and LLR258) were evaluated under field condition using upland short row method at Nong Khai. The results demonstrated that the blast disease at field was severe due to KDML105 variety had disease score of 9 which resulted in to complete of the seedling after 17 days of sowing. The ten selected indigenous lowland rice varieties; LLR276, LLR397, LLR054, LLR425, LLR055, LLR057, LLR253, LLR334, LLR363 and LLR258 were resistant to leaf blast with disease score ranged from 1.33 to 4.00 (**Table 1**). The resistance check variety, HY71 shown resistance with disease score of 3.33 (**Table 1**). The results indicate that the ten selected indigenous lowland rice genotypes were stable in their resistance to leaf blast disease. Moreover, in this study, we used RD6 introgression line "RD6 NIL" for blast evaluation together. The results found that the RD6 NIL was highness resistance than the others one (**Table 1**).

Table 1. Responses of 10 selected rice varieties in field evaluations of leaf blast at Nong Khai province in 2018

Entry	Variety	Score	
1	LLR276	3.33	bc
2	LLR397	4.00	b
3	LLR054	2.33	bcde
4	LLR425	2.67	bcd
5	LLR055	2.00	cde
6	LLR057	1.67	cde
7	LLR253	1.33	de
8	LLR334	3.00	bcd
9	LLR363	1.67	cde
10	LLR258	3.33	bc
11	KDML105	9.00	a
12	RD6 NIL	0.67	e
13	HY1	3.33	bc
	F-test	**	
	CV(%)	38.78	

** = significant different at P<0.01. The different letter within column showed significant different.

Discussion

The North-eastern Thailand contributes major area of rice production land in the country. Thai aromatic rice varieties, KDML105 and RD6 covered more than 87% of the rice cultivated area. However, both varieties are susceptible to blast pathogen (Aoki and Miyagawa, 2000). The disease occurrence can be on two major times (leaf and neck blast) corresponding to the bimodal rain pattern in this particular area (Jongdee et al., 2004). Continuous usage of susceptible varieties has led to the sources of inoculum in this particular area. The incorporation of resistant varieties is way to mitigate the problem. However, the usage of exotic germplasm is limited by their adaptability and genetics drag of non-agronomic attribute. Indigenous rice is widely distributed throughout the country by ethnic groups and field crop growers, making them further adaptable to biotic threats in these areas.

In this investigation, 347 lowland rice varieties were screened for resistance to blast disease, under field condition in disease target areas. The results reveal that the blast disease reactions of genotypes were slight difference between the two test years. There was a change in the weather condition, with the raised temperature and declined relative humidity in 2018 as compared to 2017 (Figure 2), a condition that is not favourable for the blast disease development. This may be attributed to less rainfall, excessive sunshine which could cause increasing temperature and decreasing humidity. The good distribution of rainfall after planting affected to immovable humidity and temperature in 2017 than 2018 which led to good disease scoring (**Figure 2**).

The spreading of disease score was easy way to primary selection for extremely resistance or susceptible in breeding program. In this study, two third of indigenous lowland rice germplasm shown resistant ability to blast disease in both experimental years (Figure 1A&B), indicated that those have been selected beyond disease stress in this particular area. The varying reactions of varieties were as a result of the diversity of present resistant genes (Vasudevan et al., 2014). Moreover, the disease prevalence resulting from natural infection might demonstrate to be inadequate, due to the variation in the pathogen population, perhaps, an insufficient inoculum of pathogens (Hoque and Mansfield, 2005). Additionally, variability and insufficient inoculum or isolates in particular area of evaluation has serious effect on the outcome of the overall performances of the tested varieties, also infection can get serious base on so many factors such as the presence of enough inoculum, low-temperature at 26-27 ^{0} C, relative humidity at 85-90% (Sere, 2011).

The rice accessions gathered from 21 potential rice growing provinces of Thailand show a broad geographical diversity. Almost, 74% of the accessions screened demonstrated resistance to the natural infection field evaluation. Different resistance patterns were seen among these accessions when infected with natural infection in field condition. However, the susceptible check variety RD6 showed resistance at KKU in the two years of experiments (**Figure 3**). It may be caused by RD6 was not exposed to an adequate pathogen inoculum. In fact, breakdown of the resistance is attributed to poor pre-release challenge by an adequate pathogen population (Zeigler et al., 1994). For instance, Serea et al (2002), conducted experiments, in 2000 trial indicated no leaf blast, and neck blast appeared only on one variety. Whereas, in 2001, only two entries were scored at level 5 and no neck blast was observed. While, in 2002, all 15 lines were ‘resistant’ to leaf blast and only one line presented 38% of neck blast. Additionally, a lack of disease (or a small amount of disease) in experimental plots may also be due to a lack or small quantity of virulent races at the site (Chen et al., 1995)

The future study was carried out to confirm the resistant ability of those resistant genotypes. Due to the large number of resistant genotypes from field experiment at KKU in both years, we identified the genotypes which showed consistency of resistant in both years using scuttle plot (**Figure 3**). The ten identified genotypes with enough seeds and disease score of 0.0 to 0.83 were selected and evaluated at blast incidents hot spots in field conditions at Nong Khai province. The selected top ten resistance varieties, dropped in resistance ability to pathogen strain at Nong Khai’s experimental sites with 1.33 to 4.00 blast disease score (**Table 1**). Thus, they maintained their resistant status in the two different location. This validated the existing of increasing variations and different types of blast isolates in the pathogen population (Vasudevan et al., 2014; Chen et al., 1995).

The severity of host plant disease phenotypes depends on interactions between various plant resistance genes (De-Young and Innes, 2006). The trends of disease responses against the strain of rice blast ranged from highly resistant to highly susceptible which was realized in these germplasms, indicating the availability of race specific genes/alleles as well as their combinations. Taking in to consideration the apparent lack of durable major gene to confer disease resistance, varieties demonstrating such partial resistance could be used as major donors for identification and characterization of weaker, better still, and more durable sources of resistance (DeYoung and Innes, 2006). Moreover, RD6 NIL, the RD6 introgression with 4 blast resistant QTL on Chromosome 1, 2, 11 and 12 shown high resistant ability than all test varieties to blast disease in Nong Khai. This result indicated that multiple resistant gene can be cope of disease problem in this particular area (Koide et al., 2010).

Additional follow up screening are needed to validate the greenhouse resistance of these accessions, as their responses might vary from those of the field situations (Aram et al., 2013; Arshad et al., 2008; Shafaullah et al., 2011). However, we have realized that the number of genotypes for subsequent evaluation, in order to give additional reliable data, might fall short to the stability of resistance in the identical germplasm by natural infection in similar time period. We, however, suggesting for more field trial or screening, that will involve different isolates for inoculation, shall be administered.

Conclusion

Thai indigenous lowland rice genotypes displayed a variation in their responses to blast disease under natural infection conditions. Two third of genotypes were classified as blast disease resistant. Ten out of those including, LLR276, LLR397, LLR054, LLR425, LLR055, LLR057, LLR253, LLR334, LLR363 and LLR258 were classified as durable blast resistant, and are possible emerging sources of blast resistance varieties in Thailand, which will provide a major source of blast resistant genes for upcoming rice breeding programs.

Acknowledgments

This research work was sponsored by the Thailand International Cooperation Agency (TICA), Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. Many thanks and appreciations to the Nong Khai Rice Research Center for upland short row experimental support.

Reference

Aoki, A. and Miyagawa, S. 2000. Changes of rice yield of rain-fed paddy field for 20 years in Don Daeng village of north-east Thailand. Japan Journal of Tropical Agriculture 44: 51-52.

Aram, P., Nadali, B.J., Nadali, B. and Gorbanali, N. 2013. Leaf blast resistance of rice different genotypes in blast nursery. Int J Agric Crop Sci 5:1307–1313

Arshad, H.M.I., Khan, J.A. and Jamil, F.F. 2008. Screening of rice germplasm against blast and brown spot disease. Pak J Phytopath 20: 52–57.

Candole, B.L., Siebenmorgen, T.J., Lee, F.N. and Cartwright, R. D. 2000. Effect of rice blast and sheath blight on physical properties of selected rice cultivars. Cereal Chemistry 77: 535-540. Plant Disease, 77: 535–540.

Chadha, S. and Gopalakrishna, T. 2005. Retro transposon-microsatellite amplified polymorphism (REMAP) markers for genetic diversity assessment of the rice blast pathogen (*Magnaporthe grisea)*. Genome 2005. 48: 943-945.

Chang, T.T. 1976. The origin, evolution, cultivation, dissemination and diversification of Asia and African rice. Euphytica 25:425-441.

Chen, D.H., Zeigler, R.S. and Leung, H. N. R. 1995. Population structure of *Pyricularia grisea* at two screening sites in the Philippines. Phytopathol. 85: 1011-1020. Plant Disease 85: 1011–1020.

Crill, P., Ham, Y.S. and Beachell, H.M. 1981. The rice blast disease in Korea and its control with rice production and gene rotation. Korean. J. Breed. 13 : 106-114. Plant Breeding, 13: 106–114.

Das, A., Soubam, D., Singh, P.K., Thakur, S., Singh, N.K. and Sharma, T.R. 2012. A novel blast resistance gene, *Pi54rh* cloned from wild species of rice, *Oryza rhizomatis* confers broad spectrum resistance to *Magnaporthe oryzae.* Functional and Integrative Genomics 12**:** 215-228.

De Young, B. J. and Innes, R.W. 2006. Plant NBS-LRR proteins in pathogen sensing and host defense. Nat. Immunol 7: 1243–1249.

Faivre-Rampant, O., Geniès, L., Piffanelli, P. and Tharreau, D. 2013. Transmission of rice blast from seeds to adult plants in a non-systemic way. Plant Pathology, 62:879–887.

FAO STAT - Agriculture 2019. Food and Agriculture data www.fenix.fao.org/faostart/internal/en/#home January 2019

Hoque, M.E. and Mansfield, J.W. 2005. A simple and reliable method for pathogenicity tests of bacterial disease of rice. Bangladesh Journal of Botany, 34:11–16.

IRRI, 2006. Breeding for disease resistance in rice: Blast.

International Rice Research Institute (IRRI), 2013. Standard Evaluation System for Rice, 5th edition, Manila, Philippines.

Jongdee, B., Pantuwan, G., Fukai, S. and Fischer, K. 2004. Improving drought tolerance in rainfed lowland rice: an example from Thailand. New directions for a diverse planet. In: Proceedings of the 4th international crop science congress, 26 Sep–1 Oct 2004, Brisbane, Australia, p 14. Accessed 21 Feb 2017

Khush, G.S. 1997. Origin, dispersal, cultivation and variation of rice, Plant Molecular Biology**,** 35: 25-34.

Koide, Y., Kawasaki, A., Telebanco-Yanorial, M.J., Hairmansis, A., Nguyet, N.T.M., Bigirimana. J., Fujita, D., Kobayashi, N. and Fukuta, Y. 2010. Development of pyramided lines with two resistance genes, *Pish* and *Pib* for blast disease (*Magnaporthe oryzae* B. Couch) in rice (*Oryza sativa* L.). Plant Breeding, 129: 670-675.

Korinsak, S.P. 2010. Identification of blast resistance QTLs in two rice RIL populations and marker assisted selection for pyramiding of four QTLs in RD6 rice variety. Dissertation, Kasetsart University.

Lavanya, B. and Gnanamanickam. S.S. 2000. Molecular tools forcharacterization of rice blast pathogen (*Magnaporthe grisea)* population and molecular marker-assisted breeding for diseaseresistance. Current Science, 78: 248-257.

Mackhill, D.J. 1992. Inheritance of blast resistance in near isogenic lines of rice. Phytopathology, 82: 746-749.

Manandhar, H.K., Shrestha, K. and Amatya, P. 1992. Seed-borne diseases. In: Mathur, S.B., Amatya, P., Shrestha, K. and Manandhar,H.K. (eds.) Plant Diseases, Seed Production and Seed Health Testing in Nepal. Danish Government, Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark pp: 59. Plant Disease, 59–74.

Mew, T.W., Mew, I.C. and Huang, J S. 1984. Scanning electron microscopy of virulent and avirulent strains of *Xanthomonas campestris pv. oryzae* on rice leaves. Phytopathology, 74:635-641.

Mew, T.W., Vera Cruz, C. M. and Reyes, R. C. 1982. Interaction of *Xanthomonas campestris pv. oryzae* and a resistant rice cultivar. Phytopathology, 72:786-789.

Mew, T.W., Vera Cruz, C. M., Reyes, R. C., and Zaragoza, B. A. 1979. Study on kresek (wilt) of the rice bacterial blight syndrome. IRRI Research. Pap. Ser. 39: 8-11.

Oerke, E.C., Dehne, H.W., Schönbeck, F. and Weber, A. 1994. Crop Production and Crop Protection - Estimated Crop Losses in Major Food and Cash Crops. Elsevier, Netherlands.

Ou, S. H. 1985. Rice Diseases. Kew, England: Commonw. Mycol. Inst. 380 pp. 2nd ed.

Padmanabhan, S.Y. 1965. Estimating losses from rice blast in India. In The rice blast diseases, 203-221. Baltimore, Maryland; Johns Hopkins Press.

Piotti, E., Rigano, M.M., Rodino, D., Rodolfi, M., Castiglione and S., Picco, A.M. 2005. Genetic Structure of *Pyricularia grisea* (Cooke) Sacc. Isolates from Italian Paddy Fields. Phytopathology, 153:80-86.

Rao, K. M. 1994. Rice Blast Disease. Daya Publishing House, Delhi, India.

Reddy, A. P. K. and Bonman, J. M. 1987. Recent epidemics of rice blast in India and Egypt. Plant Disease, 71: 850.

Sere, Y., Sy, A.A., Sie, M., Akator, S.K., Onasanya, A., Kabore, B., Conde, C.K. and Traore, M. 2011. Importance of varietal improvement for blast disease control in Africa. JIRCAS Working Report No 70: 77-90 ISSN 1341-710X. Plant Breeding, 70: 77–90.

Shafaullah, K., Khan, N.A., Salim-il, Y. and Mahmood, Y. 2011. Response of rice germplasm to blast disease under field conditions. Pakistan Journal of Phytopathology, 23:52–55.

Sharma, T.R., Rai, A.K., Gupta, S.K., Vijayan, J., Devann, B.N. and Ray, S. 2012. Rice Blast Management Through Host-

Plant Resistance: Retrospect and Prospects. Agricultural Research, 1:37-52.

Shim, H.S., Hong, S.J., Yeh, W.H., Han, S.S. and Sung, J. M. 2005. Damage analysis of rice panicle blast on disease occurrence time and severity. Plant Pathology, 21: 87-92.

Thruston, H. D. (2nd ed.). 1998. Tropical plant diseases. APS Press. American Phytopathological Society, pp: 31-40.

Vasudevan, K., Vera Cruz, C.M., Gruissem, W., and Bhullar, N.K. 2014. Large scale germplasm screening for identification of novel rice blast resistance sources. Frontiers in Plant Science, 5:1–9.

Zeigler, R.S., Tohme, J., Nelson, R., Levy, M., and Correa-Victoria, F.J. 1994. Lineage exclusion: A proposal for linking blast population analysis to resistance breeding. p 267–292. In: Zeigler RS, Leong SA and Teng PS eds. Rice Blast Disease. CAB.