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

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**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 resistance of the rice germplasm against rice blast disease under natural infection in field condition, to give faster screening for rice blast resistance as a forerunner in a breeding system. A total of 347 Thai indigenous lowland rice germplasm with 14 check varieties, were screened under a natural infection in blast hot spots field. The screening was conducted at two locations that have a history of high incidence of blast disease occurrence. A completely randomized design with 3 replications was laid out, blast disease scoring of standard evaluation system (SES) 0-9 method of IRRI were recorded. The finding revealed that two third of indigenous lowland rice accessions were classified as blast resistant in both experimental years with score lower than 2.00. Additionally, the top ten selected indigenous lowland rice accessions including LLR276, LLR397, LLR054, LLR425, LLR055, LLR057, LLR253, LLR334, LLR363, and LLR258 also showed 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*, KDML105, Neck blast, Upland short row, Natural infection

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## 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. The nine resistant check varieties are Azucena, IR62266, IR64, P0489, JHN, Thanya Sirin, RD23, HY1 and RD6 NIL. While the three susceptible check varieties are KDML105, RD6, and SKN. The sources of the check varieties Azucena, IR62266, IR64, and P0489 were from International Rice Research Institute (IRRI), whereas JHN, and Thanya Sirin, were from National Science Technology Development Agency (NSTDA), Thailand. While KDML, RD6, SKN, RD23, and HY1 are were from Rice Department of Thailand, whereas RD6 NIL was supplied by the 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 2017 and 2018 at Agronomy Research Station, Khon Kaen Province, Thailand (KKU). The validation of disease resistance, the selected genotypes, was conducted in 2018, at Nong Khai Rice Research Center, Nong Khai Province, Thailand (**Figure 1**).

At KKU Research Center, the seeds were soaked for three days before sowing on September 4, 2017 and August 20, 2018. The seedling were plant one seed per planting hill, in a plastic tray of 30 hills and 5 borders hills. All the tested varieties were trapped by inserted row of susceptible check varieties, RD6 and KDML105, 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. Check varieties was inserted every trays. The surrounding of the experimental area was completely trapped with spreader varieties (susceptible check KDML105, SKN, and RD6). Completely randomized designed (CRD) was laid out for the experiments. The first application of fertilizer at the rate 23.5 kg/ha of N, P2O5, K2O was applied at 15 days after sowing (DAS). Nitrogen fertilizer was applied at 20 DAS at the rate 28.75 kg/ha N for disease inducing. The disease scoring was done when KDML105 showed susceptiblility to disease at 39 and 31 DAS in 2017 and 2018, respectively. The different of plant infestations was due to the difference in the weather condition between 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. The standard evaluation method of scoring leaf blast 0 - 9 of scoring (IRRI, 2013) was described as 0=highly resistant, 1 - 2 = resistant, 3 - 4 = moderately resistant, 5 - 6 = moderately susceptible, 7 - 8 = susceptible, and 9 = highly susceptible. The water level in field condition 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.

At Nong Khai Rice Research Center, the screening was conducted to validate the performances of the ten selected genotypes that showed resistance to blast disease in year, 2017 at KKU. The direct seeding was performed on August 24, 2018 followed the upland short row method. The experimental was laid out in CRD with three replications in a plot size of 50 cm long and 10 cm apart. The surrounding row was planted with trap plants (KDML105). At 10 DAP, When the trap plant started to showed blast symptom, Seeds of tested genotypes were sown. Trap plants was also inserted every two rows genotypes. Disease scoring was carried out using standard evaluation system (IRRI, 2013) when the susceptible check variety started to show blast symptom.

**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. The statistix-10 software was used to analysis of variance (ANOVA), Least significant difference (LSD) was used to determine the difference of treatment means.

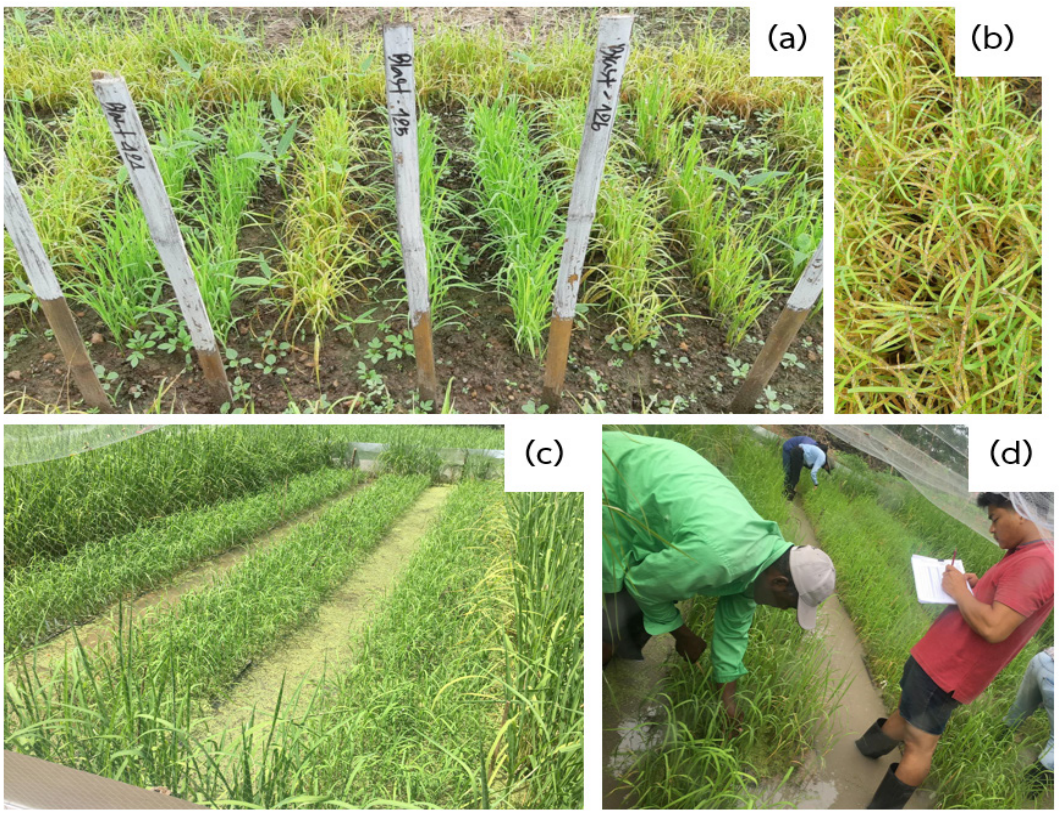



**Figure 1** Evaluation of blast disease, (a) upland short row experiment at Nong Khai, (b) blast disease symptoms of KDML105 of upland short row at Nong Khai, (c) field experiment at KKU and (d) blast disease scoring at KKU

## Result

The screening of lowland indigenous germplasm for blast resistance through natural infection, histogram revealed differences responses between the two year (**Figure 2**). In 2017, the total of 279 genotypes were classified as resistant with score less than 5, while 66 genotypes were susceptible with higher score than 5 (**Figure 2a**). In 2018, less resistant varieties were recorded than in 2017, the total of 255 genotypes was categorized as resistant varieties and 90 genotypes were susceptible (**Figure 2b**). The disease reaction in the two years varied, based on the change in weather conditions during the period of the experiments (**Figure 3**).

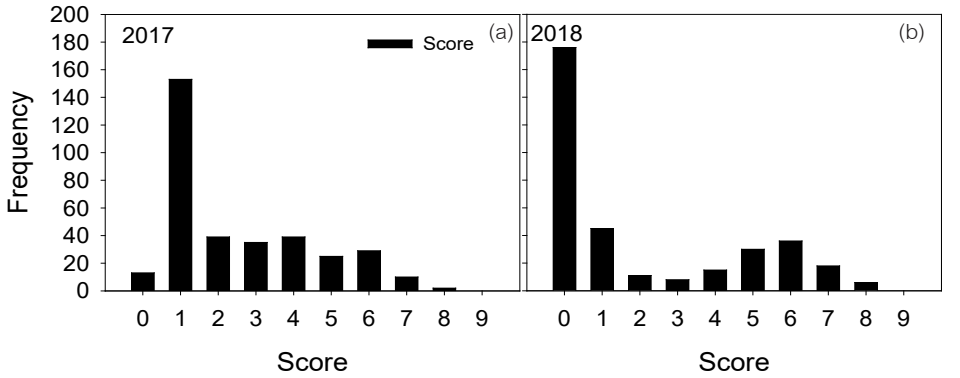



**Figure 2** Histogram of blast disease score of the years 2017(a) and 2018 (b); highly resistant = 0, resistant = 1-2, moderately resistant = 3-4, moderately susceptible = 5-6, susceptible = 7-8, highly susceptible = 9.

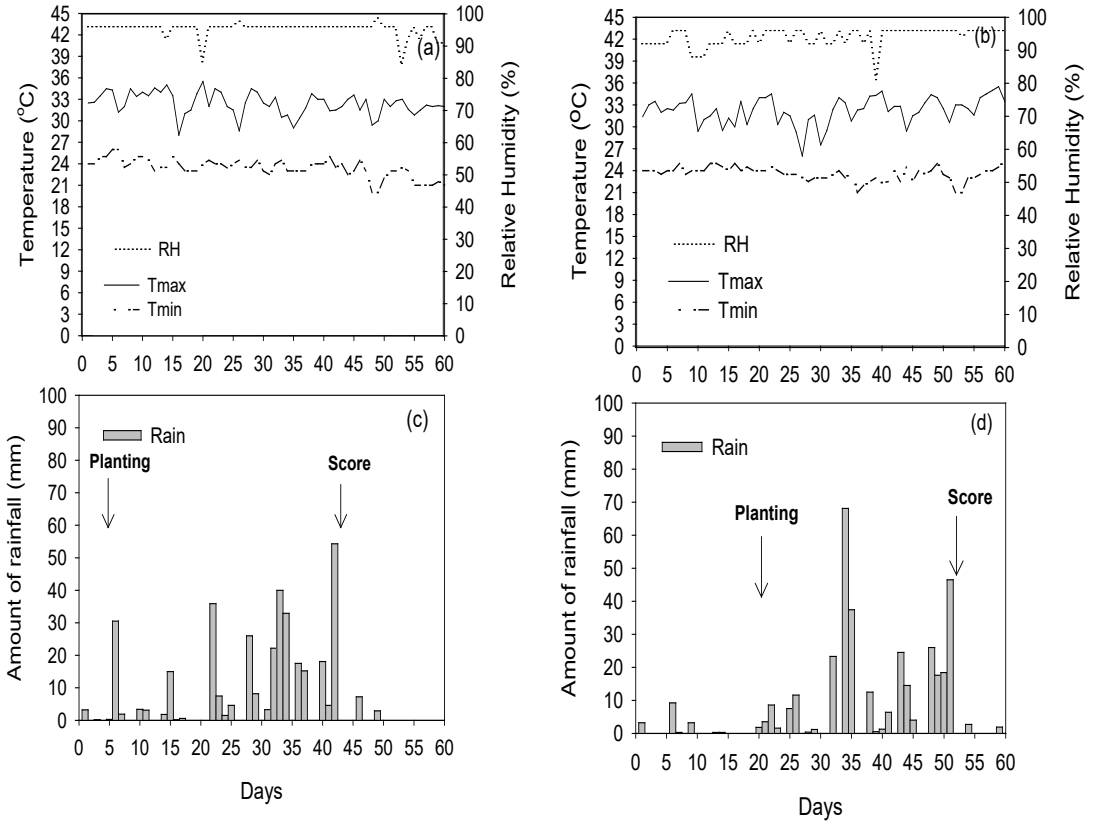



**Figure 3** Whether data at KKU in 2017 and 2018: minimum and maximum temperature and relative humidity in 2017(a) and 2018(b)

The correlation of blast resistant score from the two years was plot (**Figure 4**). Resistance check varieties, as well as susceptible check varieties, maintained their status respectively (**Figure 4**). 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. In addition, 101 genotypes were classified as resistance in both 2017 and 2018 (score < 2) (**Figure 4**). Moreover, the high correlation (r = 0.763**) of two years data indicated that resistant genotypes were invariability (**Figure 4**).

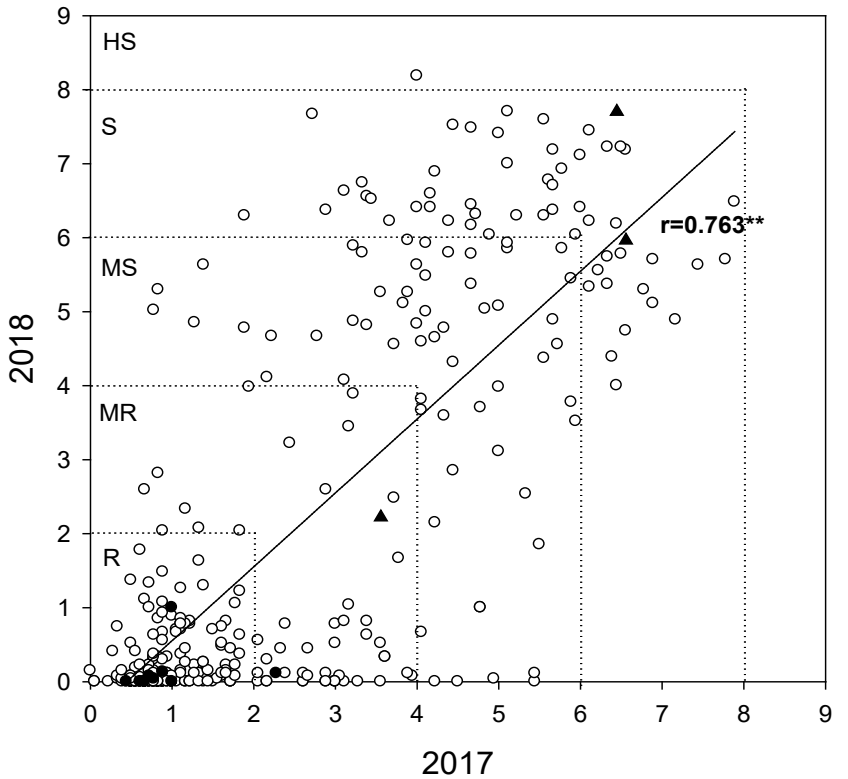



**Figure 4** Relationship of blast score of 2017and 2018 at KKU; R=resistant, MS=moderately resistant, MS=moderately susceptible, S=susceptible, HS=highly susceptible. Black circle = resistance check varieties, black triangle= susceptible check varieties.

The indigenous lowland rice varieties, which demonstrated consistent resistance in 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 Rice Research Center. The results demonstrated that the blast disease under field condition was severe as the KDML105 variety had a disease score of 9 resulted a complete death of seedling at 17 DAS (**Figure 1a**). The ten selected indigenous lowland rice varieties were resistant to leaf blast with disease score ranged from 1.33 to 4.00 (**Table 1**). The resistance check variety, HY71 showed resistance with a disease score of 3.33. The results indicated that the ten selected indigenous lowland rice genotypes were stable in their resistance to leaf blast disease. Moreover, in this study, the RD6 introgression line "RD6 NIL" was developed via blast resistance QTLs pyramiding showed was greater in resistance than others genotypes (**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 the major area of rice production in 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). Leaf and neck blast are normally occur in vegetative and reproductive phase respectively which correspond 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. Utilization of resistant varieties is a possible way to mitigate the blast problem. However, the usage of exotic germplasm is limited by their adaptability and genetic drag of undisirable 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 indigenous lowland rice varieties were screened for resistance to blast disease, under field condition in disease high spot areas. The results reveal that the blast disease reactions of genotypes were slight difference between the two tested 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 might contributed the less rainfall, excessive sunshine which could cause increasing in temperature and decreasing of relative humidity. The good distribution of rainfall after planting affected a favourable condition of high humidity with a low air circulation in 2017 than 2018 which led to a better disease score (**Figure 2**).

A good pattern of disease distribution could selection for extremely resistance or susceptible variety/line in breeding program. In this study, two third of indigenous lowland rice germplasm showed resistant ability to blast disease in both experimental years (**Figure 2**), indicated that those The indigenous lowland rice varieties, which demonstrated consistent resistance in 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 Rice Research Center. The results demonstrated that the blast disease under field condition was severe as the KDML105 variety had a disease score of 9 resulted a complete death of seedling at 17 DAS (**Figure 1a**). The ten selected indigenous lowland rice varieties were resistant to leaf blast with disease score ranged from 1.33 to 4.00 (**Table 1**). The resistance check variety, HY71 showed resistance with a disease score of 3.33. The results indicated that the ten selected indigenous lowland rice genotypes were stable in their resistance to leaf blast disease. Moreover, in this study, the RD6 introgression line “RD6 NIL” was developed via blast resistance QTLs pyramiding showed was greater in resistance than others genotypes (**Table 1**). accessions were selected beyond disease stress in this particular area. Variation in disease response depicted the diversity of germplasm as well as diversity of genes (Vasudevan et al., 2014). Moreover, the disease prevalence resulting from natural infection demonstrated an inadequate, due to the variation in the pathogen population, that possibly caused by an insufficient inoculum of pathogens (Hoque and Mansfield, 2005). Besides, some other factors such as low-temperature of 26-27 0 C and 85-90% of relative humidity could contribute variety to show their performance in resistance (Sere, 2011).

The rice accessions gathered from 21 potential rice growing provinces of Thailand showed 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 moderate resistance at KKU in both two years of experiments (**Figure 4**). It might cause by an inadequate of inoculum. In fact, resistance breakdown is contributed by an adequate pathogen population (Zeigler et al., 1994). A lack of disease (or a small amount of disease) in experimental plots may cause by a lack or small

quantity of virulent races at the site (Chen et al., 1995)

The evaluation found that the consistency of ten resistant genotypes with disease score of 0.0 to 0.83 in both years (**Figure 4**) were confirmed by the high severe field experiment at Nong Khai. At this site, the selected top ten resistance varieties dropped their resistance ability with the range of 1.33 to 4.00 disease score (**Table 1**). However, ten genotypes were able to maintain their resistance status in the two different locations compared to resistance check. 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. The consideration of 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 durable sources of resistance (DeYoung and Innes, 2006). Interestingly, the RD6 NIL, the RD6 introgression with 4 blast resistant QTLs on Chromosome 1, 2, 11 and 12 showed a high resistant ability to blast disease than all test varieties. This result indicated that multiple resistant gene can be coping of disease problem in this particular area (Koide et al., 2010, Pinta et al., 2013, Suwannual et al., 2017).

## 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.

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