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Stability of Four New Sources of Bacterial Leaf Blight Resistance in Thailand Obtained from Indigenous Rice Varieties

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ABSTRACT

Bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases in rice production. Breeding varieties specifically for their resistance to BLB disease is therefore an efficient and cost-effective strategy. However, the resistance gene for BLB can be race and non-race specific, meaning it is often overcome by the pathogen. The identification of new sources of resistance genes for *Xoo* is crucial in rice breeding programmes. In this study, six rice varieties were assessed using six *Xoo* isolates in multiple screening conditions. The GGE biplot analysis considers both genotype (G) and genotype environment (GE) interaction effects and demonstrates GE interaction. The first two principal components (PCs) accounted for 95.46% of the total GE variation in the data. Based on lesion length and stability performance, Phaladum was the most ideal genotype against all *Xoo* isolates in the four screening conditions. The results relayed that Phaladum indigenous rice varieties could be considered as new sources of bacterial leaf blight resistance in Thailand. In the future, the BLB resistance gene in this variety will be identified in regard to mode of inheritance and used as parental line in rice breeding programmes for resistance to BLB.

Keywords: Bacterial leaf blight; GGE biplot; rice; stability; *Xanthomonas oryzae*

INTRODUCTION

Rice (*Oryza sativa* L.) is an important source of calories in South and Southeast Asia where annually 90 % of the world's rice is produced. More than 50 % of the world's population consumed rice as a staple food (Khush, 2005; Latif et al., 2011). The major diseases affecting rice yields is bacterial leaf blight (BLB) disease caused by the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). While this bacterial pathogen is infectious all year round, it is particularly so during wet season. BLB is a vascular disease that results in greyish tan to white lesions along the veins of the plants in field conditions. The wounded leaves or root during transplantation usually infected by the pathogen. Therefore, young plants at seedling stage are the most susceptible especially plants that are younger than 21 days old. (Ou, 1985; Reddy & Mohanty, 1981). However, disease incidence increases with stages of plant growth and peaking at the flowering stage. An infection could reduce rice yields by more than 50 % and even lead to a complete crop damaged when it occurs during the tillering stage (Ou, 1985). In several rice production areas in Thailand, BLB has been reported as one of the most destructive diseases in rice (Korinsak et al., 2009). Rice varieties RD6 and KDML105, aromatic rice cultivars with high cooking qualities, are widely grown in the North and Northeast of Thailand. The large-scale and long-term cultivation of these two varieties makes them easy targets for being damaged by the pathogen.

Out of all the available strategies to control BLB disease, breeding for host-plant resistance is effective way. However, BLB disease mainly infects plants in wet conditions, a factor that severely limits the breeding process and selection of plants due to seasonal confounding. However, the limited of seasonal confounding could overcome by marker-assisted selection (MAS) (Collard & Mackill, 2008). The modes of inheritance of resistance to BLB in rice were differences when evaluated in different sources of resistance (Khan, Naeem, & Iqbal, 2014; Das, Sengupta, Prasad, & Ghose, 2014). This suggests a number of different mechanisms or genes may confer resistance to BLB. So far, 40 BLB resistance genes have been reported (Khan,

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Naeem, & Iqbal, 2014). However, resistance was very often overcome by the pathogen, which in turn led to high levels of variability in infections in cultivation areas (Sreewongchai et al., 2009). In addition, more virulent populations of the pathogen emerge and resistant cultivars lose their effective resistance within a short period of time (Thakur, Shetty, & King, 1992). The incorporation of resistant genes was a continuous process to counter evolving pathogens according to the gene-for-gene hypothesis (Flor, 1971).

Mapping several resistance genes or quantitative trait loci (QTLs) shows the same location in a genome region, indicating that some of the resistance genes or QTLs are the same (Das, Sengupta, Prasad, & Ghose, 2014). The use of same resistance gene in large production areas is very often cause resistance breaking by the pathogen. Therefore, rice breeders need to search other natural sources of resistance and subsequently determine additional resistance genes in order to pyramid several genes to achieve durable resistance. New rice resistant sources against *Xoo* were identified in wild related species, namely *O. nivara, O. longistaminata* and *O. punctate*. The use of these species in rice breeding programmes has been recommended (Akhtar et al., 2011). It is important to note, however, that desirable agronomic attributes, such as photoperiod response, plant types and cooking quality, can be found lacking with genetically diverse resistance parents. The increasing use of locally available genetic resistance materials as parents can minimise this problem.

In Thailand, Chanlakhon, Thammabenjapon, & Sanitchon (2012) identified 14 indigenous lowland rice varieties as resistant to three BLB isolates by completing the same resistance checks (IRBB5, IR64 and IR62266). Out of those, Hom Mali Nin, Pet-Retree, LG6822 and Phaladum were considered to have high potentials for the resistance breeding programme. However, an effective breeding for BLB resistance relies on a good understanding of the relationship between host, pathogen and environment.

The resistances testing in difference pathogen isolates and over conditions have significant fluctuations in disease reaction due to GE interaction. The durable resistance varieties could be identified by stability analysis strategies (Jenns, Leonard, & Moll, 1982). In the presence of GE interaction, superior genotypes could be identified using genotypic means across conditions (Kang, 1992). Moreover, the most resistant variety, the most virulent pathogen and the particular environment for differentiating resistance levels among varieties could also be employed by genotype and genotype by environment (GGE) biplot analysis (Yan & Falk, 2002).

The GGE biplot analysis of plant traits focused on identifying a stable genotype for deployment as well as suitable areas for subsequent commercial grain production (Lakew, Tariku, Alem, & Bitew, 2014), stability of disease resistance (Idowu, Salami, Ajayi, Akinwale, & Sere, 2013; Tabien, Samonte, Abalos, & San Gabriel, 2008; Yan & Falk, 2002), stability of F1 hybrid (Akter et al., 2015), cooking quality (Fotokian & Agahi, 2014), and anthocyanin content (Somsana, Wattana, Suriharn, & Sanitchon, 2013). Therefore, the analysis of disease reaction also aims to determine suitable donor parents for the trait and to identify BLB isolates that will be useful in further screening for resistance breeding program. The objectives of this study were (a) to confirm the response of Thai indigenous rice varieties to BLB disease and (b) to identify genotypes with high stability and broad resistance to BLB disease through the use of the GGE biplot method.

MATERIALS AND METHODS

Rice Varieties and BLB Isolates

Four indigenous low land rice varieties, Hom Mali Nin (HMLN), Pet-Retree (PRT), LG6822 and Phaladum (PLD) were identified as BLB resistant (Chanlakhon, Thammabenjapon, & Sanitchon, 2012). These four varieties, together with two susceptible varieties, RD6 and Kao Dok Mali 105 (KDML105), were used as materials to screen against six *Xoo* isolates: two isolates from Ubon Ratchatani (BB2009-758 and BB2009-786), two isolates from Udon Thani (BB2009-928 and BB2009-1066), and two isolates from Khon Kaen (BB2009-1099 and BB2009-1111) (Fig. 1).

Rice Growing Conditions and Inoculum Preparation

Growing conditions were tested using two different approaches: solution and soil cultures. Solution cultures required the seeds to be sown individually in small holes in styrofoam plates with a nylon net providing support underneath. The styrofoam plates floated on water without nutrient solution in rectangular plastic for three days.

Afterwards the plastic trays were filled with nutrient solution following the protocol described by Yoshida, Forno, Cock, & Gomez (1976). The nutrient solution was renewed every four days until 21 days after sowing (DAS) for disease evaluation.

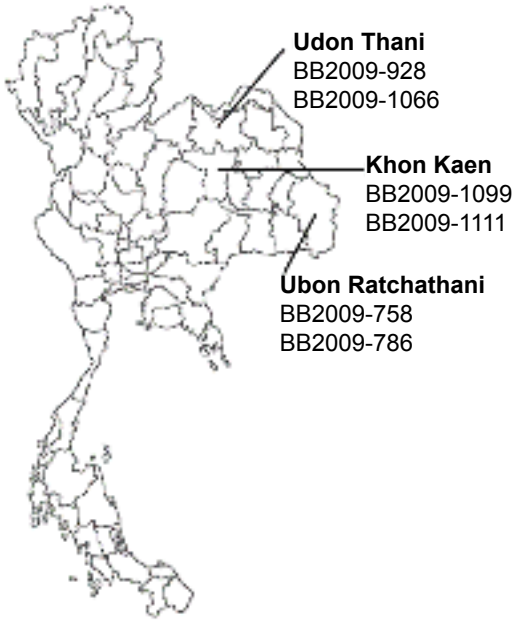

Fig 1. Distribution of sources of six *Xanthomonas oryzae pv. oryzae* isolates used for artificial inoculation in greenhouse conditions

To produce the soil cultures, the procedure described by Gregorio, Dharmawansa, & Mendoza (1997) was applied, with slight modifications. Two-day old seedlings were transferred into 4.7 x 4.7 x 5.0 cm^3 plastic pots filled with soil and soaked in a water tank, with one seedling per pot (four pots/variety). The pots were fertilised with 156 kg ha^{-1} of nitrogen at 15 DAS until inoculation.

To prepare the inoculum, six isolates of *Xoo* were stored with glycerol at a constant of -20°C. Next, *Xoo* isolates were regrown on nutrient agar at 27-30°C for 48 hours. Each bacterial colony was suspended in sterilised, distilled water and adjusted to concentrations of 10^9 cfu ml^{-1} (OD = 0.3 at 600 nm) by spectrophotometer.

Assessments of BLB Resistance

Assessments of BLB resistance were performed from 2013 to 2015 under greenhouse conditions in different seasons and with different screening methods at Khon Kean University in Thailand. The rice varieties HMLN, PRT, PLD, LG6822, RD6 and KDML105 were tested against six *Xoo* isolates under soil culture conditions in the 2013 rainy season, under solution culture conditions in the 2014 winter season, and under soil and solution culture conditions in the 2015 summer season by factorial analysis in a completely randomised design (CRD) with three replications. In all experiments, seeds were sown and remained untouched for 21 days. Afterwards, artificial inoculation was completed using the leaf-clipping method (Sun et al., 2004). Fourteen days after inoculation (DAI), the plant reaction to BLB was examined based on the lesion length in individual plants.

Data Analysis

The lesion length data for six varieties of six isolates in four conditions was used to analyse the variance (ANOVA) to determine the effects of both genotype (G) and environment (E), and their interactions. The mean lesion length data was graphically analysed to interpret GE interaction using the GGE biplot software (Yan, 2001), and then PC1 and PC2 were described to account for the main and minor affects respectively using R v 2.10 (R Development Core Team, 2010).

RESULTS AND DISCUSSION

The analysis of the variance in lesion length (in cm) at 14 DAI in six varieties using six *Xoo* isolates under greenhouse conditions in 2013, 2014 and 2015 is shown in Table 1. Significant ($P<0.01$) differences were found among varieties, isolates and their interaction for BLB disease lesions (Table 1). The mean lesion lengths for all rice varieties, isolates and conditions are shown in Table 2. Out of the six rice varieties, PLD showed the shortest lesion length (1.2 cm), followed by LG6822, PRT and HMLN (2.7, 2.8 and 3.3 cm respectively). In contrast, the longest lesion length was observed in RD6 and KDML105 (8.4 and 7.1 cm). PLD's short lesion length suggests that this variety has a broad resistance to several *Xoo* isolates. It is therefore suitable as a resistance donor parent in the breeding programme. Host-plant resistance is the most economic and sustainable strategy for controlling BLB disease. According to BLB's genetic control, resistance is governed by several genes or QTLs, with major resistance genes consist of *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*. The latter, *Xa21*, is the most broad-spectrum resistance to *Xoo* strains, inherited from the wild rice species *O. longestaminata*. It was widely used in rice breeding programmes (Khush, Mackill, & Sidhu, 1989).

Table 1. Summary of ANOVA tests for bacterial leaf blight disease response as measured by lesions length (cm) at 14 days after inoculation in six varieties used six Xanthomonas oryzae pv. oryzae isolates in a greenhouse environment in 2013, 2014 and 2015

SOVa	Soil culture 2013		Solution culture 2014		Soil culture 2015		Solution culture 2015	
	df[b]	MS[c]	df	MS	df	MS	df	MS
Isolate(I)	5	52.5**	5	8.49**	5	37.6*	5	53.8**
Varieties(V)	5	868.8**	4	1.91*	5	210.5**	5	26.9**
I x V	25	17.1**	20	0.82ns	25	25.2**	25	22.9**
Error	58	7.4	48	0.59	56	8.04	57	4.40

Remarks: a) SOV= source of variation, b) df= degree of freedom, c) MS= mean squares, ns, * and ** = non-significant, significant at p<0.05 and p<0.01, respectively

Table 2. Mean lesion lengths in rice varieties and Xanthomonas oryzae pv. oryzae isolates under greenhouse conditions from 2013 to 2015

Conditions	Isolates	Varieties						
		RD6	KDML105	LG6822	PRT	HMLN	PLD	Mean
Soil culture 2013	B786	10.7	12.6	0.6	0.9	1.3	1.1	**4.5**
	B928	11.4	5.2	1.0	0.4	0.7	0.8	**3.2**
	B1111	18.2	18.5	3.5	3.4	2.0	1.2	**7.8**
	B1066	14.8	16.8	0.1	2.2	1.7	0.5	**6.0**
	B1099	19.1	14.5	0.6	4.3	0.3	2.0	**6.8**
	B758	21.7	15.8	1.0	1.7	1.8	0.4	**7.1**
	Mean	**16.0**	**13.9**	**1.1**	**2.1**	**1.3**	**1.0**	**5.9**
Solution culture 2014	B786	0.9	1.1	ND*	0.7	1.1	1	**1.0**
	B928	0.5	0.4	ND	0.2	0.2	0.3	**0.3**
	B1111	0.9	1.6	ND	1	1.2	0.6	**1.1**
	B1066	4	3.2	ND	1.7	1.5	0.3	**2.1**
	B1099	0.2	0.2	ND	0.1	0.2	0.3	**0.2**
	B758	1.6	0.7	ND	0.4	1.1	0.3	**0.8**
	Mean	**1.4**	**1.2**	**ND**	**0.7**	**0.9**	**0.5**	**0.9**
Soil culture 2015	B786	8	3.2	0.5	7	8	6.2	**5.5**
	B928	10.8	12.7	11.5	4	7.8	4.4	**8.5**
	B1111	9.8	9.4	2.8	3.5	8	1.9	**5.9**
	B1066	13.1	14.3	4.3	8.7	11.6	0.2	**8.7**
	B1099	16.6	8.5	5.7	7.4	8.9	0.2	**7.9**
	B758	10.8	8.5	7.8	9.1	11.7	0.2	**8.0**
	Mean	**11.5**	**9.4**	**5.4**	**6.6**	**9.3**	**2.2**	**7.4**
Solution culture 2015	B786	12.7	1.4	1	0.5	1.1	0.3	**2.8**
	B928	1.7	1.2	1.2	0.6	0.7	0.4	**1.0**
	B1111	2.2	0.7	2.1	0.7	2.8	1.8	**1.7**
	B1066	1.8	2.4	1.1	0.5	2.4	0.5	**1.5**
	B1099	1.3	0.6	0.7	1	0.6	0.5	**0.8**
	B758	4.9	15	2.5	5.4	2	4	**5.6**
	Mean	**4.1**	**3.6**	**1.4**	**1.5**	**1.6**	**1.3**	**2.2**
Overall mean		**8.4**	**7.1**	**2.7**	**2.8**	**3.3**	**1.2**	**4.2**

Remarks: ND= non determined

To date, several breeding programmes to improve BLB resistance in Thai rice varieties have been developed (Korinsak et al., 2009; Korinsak, Sirithanya, & Toojinda, 2009; Win et al., 2012; Pinta, Toojinda, Thummabenjapone, & Sanitchon, 2013). In most cases, researchers utilised the resistance lines from introduced varieties, such as IRBB21, IR24, IRBB5 and IR62266. Limitations of this approach included undesirable agronomic attributes and instability of resistance. In contrast, screening indigenous rice varieties are adaptive to local areas and possess desirable agronomic attributes.

Different virulent reactions were observed when examining *Xoo* isolates from different rice varieties. The BB2009-758 isolate from Ubon Ratchatani, BB2009-1066 isolate from Udon Thani and BB2009-1111 isolate from Khon Kaen were more virulent, indicating that these varieties transmit infections to other varieties and in different cultivation conditions. However, some isolates are more virulent in specific conditions, such as BB2009-928 isolate from Udon Thani. This isolate was more virulent in soil cultures in the summer of 2015 than others, suggesting that this isolate was a specific pathogen. However, PLD reported the shortest lesion length in all *Xoo* isolates (Table 2), indicating that PLD has a different BLB resistance gene.

Das, Sengupta, Prasad, & Ghose (2014) have previously reported on divergent genes for BLB resistance. In this study, the reaction to *Xoo* isolates from HMLN, PRT, LG6822 and PLD are different (see Table 1 and 2), indicating different resistance genes. PLD, the indigenous lowland rice variety was collected from Yasothorn province in Northeast Thailand, possessed the broadest resistance, followed by PRT, LG6822 and HMLN, respectively. However, BLB infections were vary from varieties to varieties based on seasonal and screening conditions. The pathogen favours wet conditions (Table 2) and large-scale field tests, which limits breeding programmes' success due to environmental factors. Ultimately, soil cultures in rainy seasons are most suited to BLB pathogenic testing to distinguish susceptible and resistant varieties than other cultures and seasons (Table 2). Artificial inoculation under controlled conditions is neither labour- nor space-intensive and therefore is ideal for large-scale year round screening.

Out of the four conditions tested, the 2015 soil culture had the longest lesion length and the 2014 solution culture had the shortest. Interestingly, the 2013 soil culture showed a higher ability to discriminate amongst varieties than the others. Table 2 suggests that these conditions can be used to select *Xoo* isolates as well as variety in a rice breeding programme. The lesion length for each variety differed among culture conditions due to the correlation of the severity of disease and GXE interaction (Table 1). PLD shows a stable lesion length with a simple regression value of b=0.17. This value lies below the values shown by other varieties (Fig. 2) and indicates that this variety possesses a broad resistance to BLB. However, BLB disease incidence affected by the GE interaction led to confounding results. Therefore, a GGE biplot analysis can also be employed to identify durable resistance, the most virulent pathogen and the particular environment that can be used to distinguish resistance levels among varieties (Yan & Falk, 2002).

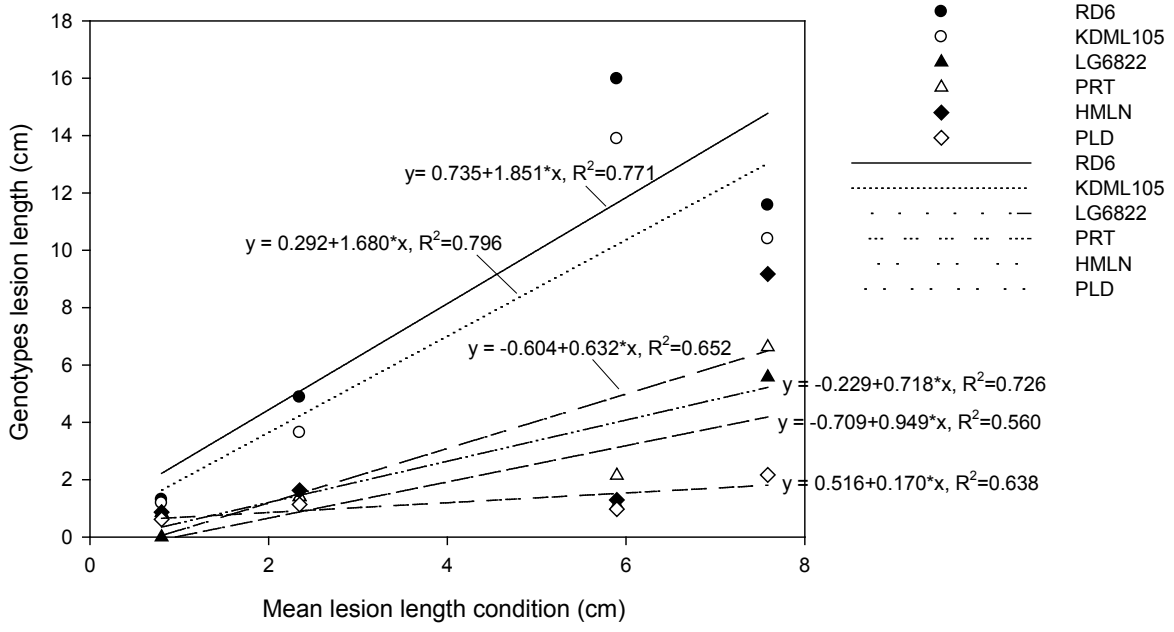

Fig 2. Simple linear regression of disease incidence for different severity of rice varieties under greenhouse conditions

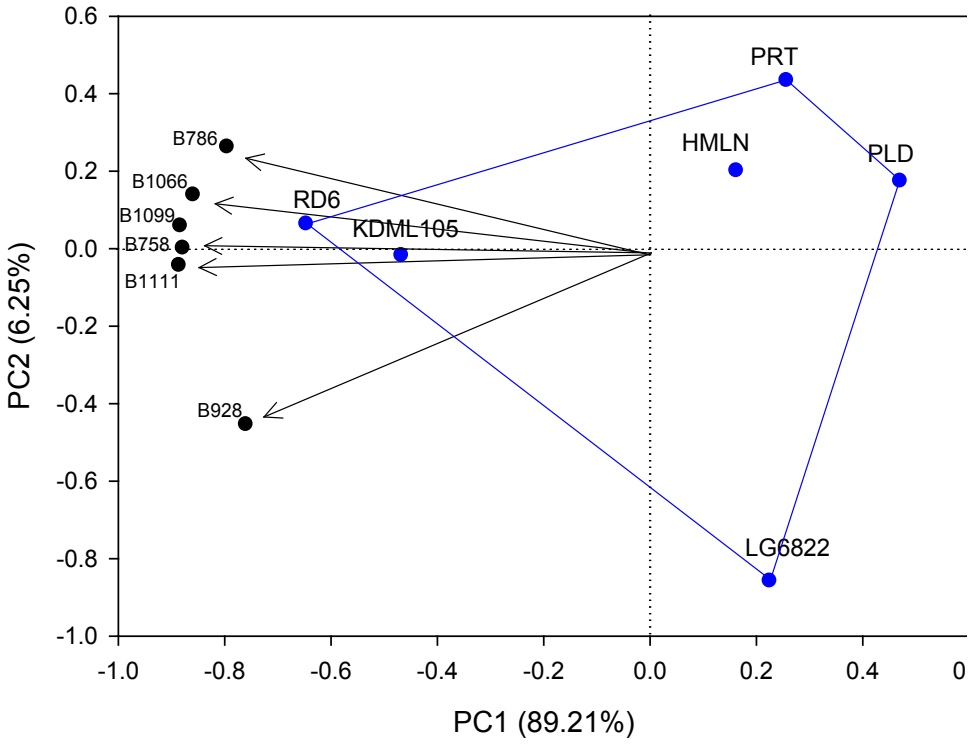

Fig 3. GGE biplot based on BLB lesions length in six rice varieties and six *Xanthomonas oryzae pv. oryzae* isolates. The average environmental axis (AEA) is the straight line that passes through the origin (0.0) and coordinate of the culture condition mean

The GGE biplot analysis shows the grouping of six varieties based on their mean lesion length and stability (Fig. 3) over three screening conditions (2014 soil culture was omitted due to missing data for variety LG6822). Following this, the varieties can be divided into two groups. The first group is represented by *Xoo* isolates and the varieties with the longest lesion length or highest susceptibility, RD6 and KDML105. The second group consists of varieties PLD, PRT, LG6822 and HMLN, which had a shorter lesion length or less resistance to BLB disease. In this graph, the first two PCs accounted for 95.46% (PC1=89.21%, PC2=6.25%) of the total GGE variation among the data (Figure 3). PC1 consistently shows higher values than PC2 with regards to lesion length for the six isolates, demonstrating that the lesion length is majorly affected by genotype rather than GE interaction effects. The varieties located closer to the X-axis show a more stable resistance. In this study, PLD was found to be the best genotype out of all the *Xoo* isolates in the three screening conditions. Based on lesion length and stability performance, PLD came first, followed by PRT, HMLN and LG6822, while RD6 and KDML105 performed the worst.

The GGE biplot was used to identify disease resistance and associated stability in several crops, such as soybean (Twizeyimana et al., 2008), chick pea (Sharma et al., 2012), wheat (Gitonga, Ojwang, Macharia, & Njau, 2016), mung bean (Alam, Somta, Jompuk, Chatwachirawong, & Srinives, 2014) and rice (Idowu, Salami, Ajayi, Akinwale, & Sere, 2013; Tabien, Samonte, Abalos, & San Gabriel, 2008). Moreover, genotype and GE interaction, graphically represented using a GGE biplot to assess the genotypes and environment, play an important role in variety selection (Yan, 2001; Yan & Hunt, 2002). In this study, the GGE biplot analysis identifies ideal conditions and isolates for BLB screening, and also highlights the PLD rice variety as the most stable new source of BLB resistance in Thailand.

CONCLUSION

The overall results of this study confirmed the preliminary results published by Chanlakhon, Thammabenjapon, & Sanitchon (2012) that some of Thai indigenous rice varieties are potential to be a source of BLB resistance. The varieties demonstrate a stable resistance to BLB for all seasonal and screening conditions. This indicates that the resistance varieties, including PLD, PRT, LG6822 and HMLN, are reliable. The four varieties provide an opportunity for breeding with resistance to BLB in Thailand. Further research is needed to identify the genes responsible for resistance in these varieties in order to integrate divergent sources of resistance into commercial cultivation.

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