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Evaluation of Rice Genotypes for Resistance to Brown Planthopper (*Nilaparvata lugens* Stål) Populations from the Central Region of Thailand

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ABSTRACT

Intensive cultivation of rice is believed to be one of the factors responsible for the outbreak of brown planthopper (BPH). An experiment was conducted to study the reaction of 22 rice genotypes to six populations of BPH collected from rice research centers and from farmers' fields of central Thailand. The results showed that populations of BPH from rice research centers were relatively more virulent than those from farmers' fields. Cluster analysis using the mean BPH damage score divided the genotypes into three groups. Group I contained 18 susceptible genotypes including the susceptible check TN1. Group II contained two moderately resistant genotypes, IR64 and RD31, while the third group was made up of the resistant checks, Rathu Heenati and Ptb33. Rathu Heenati and Ptb33 could be used as sources of resistance for BPH in central Thailand. However, it is important to study the reaction of more genotypes by using additional BPH populations. The differential response of genotypes to populations of rice research centers and farmers' fields of the same province also needs further investigation.

Keywords: Brown planthopper, *Nilaparvata lugens* Stål, resistance, rice

INTRODUCTION

Rice, the primary source of food for more than half of the world's population, is planted on about 11% of the world's cultivated land (Khush, 2005). About 163.46 million ha of land was used for rice production in 2012 worldwide (Food and Agriculture Organization, 2013). Most of the world's rice production is from irrigated and rain-fed lowland fields where insect pests are severe constraints. In these areas the major insect pests, which contribute to yield losses, are leafhoppers and planthoppers (Heinrichs, 1986).

Among the six kinds of planthoppers, brown planthopper (BPH; *Nilaparvata lugens* Stål) is the most important insect pest in Asia (Jena and Kim, 2010). Different reports are available about the economic importance of BPH in Asia. In China, Indonesia and Vietnam, BPH damage of rice on 9.4 million, 28,421 and 348,927 ha of land was observed in 2006, respectively (Catindig *et al*., 2009). The incidence of BPH in Thailand has been reported since 1973—outbreaks have been increasing with the expansion of irrigated rice production and the continuous cultivation of high-yielding varieties (Pongprasert and Weerapat, 1979).

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From a management viewpoint, host-plant resistance is a better way to control rice pests as it is free to the farmer, environmentally friendly and compatible with other control measures (Heinrichs, 1986). The search for resistant varieties of rice and the incorporation of resistance factors into modern varieties has been the major strategy to control BPH (Claridge and den Hollander, 1983). Numerous sources of genetic resistance to BPH are available in the rice germplasm and that resistance has been found to be compatible with other important traits; however, the development of biotypes capable of surviving on resistant plants is a major threat to the stability of varietal resistance and complicates breeding for resistance to this pest (Pathak and Khush, 1979; Heinrichs, 1986). Different factors were reported to be responsible for the breakdown of resistance and the consequent outbreak of the insect. Besides killing natural enemies of BPH, sub-lethal doses of insecticides increase the fecundity and migratory capacity of BPH (Wu *et al*., 2001; Yin *et al*., 2008). Increased plant nitrogen, as a result of higher doses of nitrogen fertilizer, increases the survival of nymphs, fecundity, population growth and the tendency for BPH outbreaks (Visarto *et al*., 2001; Lu and Heong, 2009). The fact that the fitness and population dynamics of BPH are influenced by intensive cultivation (continuous monoculture, higher doses of N fertilizer and insecticides) shows the significance of continuous monitoring of the ability of resistant varieties and the virulence of different populations of BPH. The objectives of the experiment were to study the resistance of some rice genotypes to BPH and to investigate the virulence of BPH collected from rice research centers and farmers' fields. The reaction of 22 rice genotypes to six populations of BPH from central Thailand was investigated.

MATERIALS AND METHODS

Experimental location

The experiment was conducted at the Kasetsart University, Bangkhen campus, Thailand from November 2012 to February 2013 under natural photoperiod conditions.

Plant materials

In total, 22 rice genotypes, including the checks were used for the study. The genotypes Rathu Heenati and Ptb33 were included as resistant checks, while Taichung native1 (TN1) was used as a susceptible check. The detailed description of the genotypes is presented in Table 1.

BPH populations

Six populations of brown planthopper were used in the study. The populations were collected from three provinces—Bangkok, Chachoengsao and Pathum Thani—in central Thailand. Each province was represented by a BPH population both from a rice research center and a farmer's field. Bangkok was represented by the Kasetsart University Bangkhen campus as a station, and by a farmer's field. The six populations were kept in nylon screening net separately to maintain the desired numbers of each insect population.

BPH bioassay

A standard seed-box screening test was used to determine the reaction of the rice genotypes to the BPH populations. To ensure that all the seedlings were at the same growth stage for BPH infestation, each genotype was germinated on moist tissue paper in a Petri dish and seedlings of each genotype were then transplanted. After inspecting all plants for predators and other insects, approximately 10 nymphs (second to third instar) per seedling were used to infest the plants. Scoring was started when the susceptible check was killed by the nymphs. The reaction of each genotype to BPH was scored using the standard evaluation system for rice (International Rice Research Institute, 2002).

Table 1 Description of rice genotypes used in brown planthopper resistance screening

Genotype	Sub-species/type	Origin
NERICA 3	Interspecific cross	Côte d'Ivoire
NERICA 4	Interspecific cross	Côte d'Ivoire
NPT4	New plant type	the Philippines
NPT8	New plant type	the Philippines
NPT13	New plant type	the Philippines
NPT18	New plant type	the Philippines
CH1	Indica	China
CH2	Indica	China
PTT1	Indica	Thailand
CNT1	Indica	Thailand
SPR1	Indica	Thailand
KDML105	Indica	Thailand
IR64	Indica	the Philippines
AZUCENA	Tropical japonica	the Philippines
RD31	Indica	Thailand
TDK1	Indica	Laos
TDK5	Indica	Laos
CO39	Indica	India
Rathu Heenati	Indica	Sri Lanka
Mudgo	Indica	India
TN1	Indica	Taiwan
Ptb33	Indica	India

Data analysis

A randomized complete block design was used and each test cultivar was replicated three times. The mean damage-score of each cultivar was tested for cluster analysis using the NTSYSpc software (version 2.01; Exeter Software; London, UK). The SIMINT module of NTSYSpc 2.01 was selected to compute Canberra distance coefficients by using the unweighted paired group method with arithmetic average, and the SAHN module was used to execute hierarchical clustering. Principal component analysis was done using the PAST version 1.93 software (Hammer *et al*., 2001). The variance-covariance matrix was used during the principal component analysis.

RESULTS

Relative virulence of populations

The mean BPH damage-score and the relative virulence of the six BPH populations are shown in Tables 2 and 3. The damage-score ranged from 0 to 9 (Table 2). The virulence of BPH ranged from 45.45% for populations from Klong Sam Wa and Chachoengsao farms to 81.82% for the Chachoengsao Rice Research Center (Table 3).

Susceptibility of genotypes

The susceptibility also ranged from 0 to 100% (Table 3). Ptb33 and Rathu Heenati were resistant to all the BPH populations. IR64 was resistant to three and moderately resistant to the other three populations. RD31 was resistant to four and moderately resistant to two populations while

CNT1, NPT13 and NPT8 were not consistent in their reactions to the BPH populations studied. Both CNT1 and NPT13 were susceptible to the BPH population from the Chachoengsao Rice Research Center. On the other hand, CNT1 was resistant to two and moderately resistant to three BPH populations; while NPT13 was resistant to one and moderately resistant to four BPH populations. NPT8 was resistant, moderately resistant and susceptible to one, three and two populations, respectively. Mudgo was moderately resistant only to the population from the Pathum Thani farmer's field (Thanyaburi district), but susceptible to the rest of the BPH populations. Azucena, CH1, NERICA3, NERICA4 and TN1 were susceptible to all the six populations.

When the reactions of the genotypes to BPH collected from the rice research centers and farmers' fields were compared, IR64 was moderately resistant to the Chachoengsao Rice Research Center population but resistant to the Chachoengsao farmer's field population (Bang Nam Priao district). The Thai rice varieties CNT1, PTT1 and KDML105 were susceptible to the Chachoengsao Rice Research Center population but moderately resistant to the Chachoengsao farmer's field population. However, PTT1 and SPR1 which were susceptible to the Khlong Sam

Table 2 Mean damage-scores of brown planthopper (BPH) in the six populations of the 22 tested genotypes of rice.

Genotype	BPH population					
	PTT_R	CCS_R	BKK_R	PTT_F	CCS_F	BKK_F
Azucena	9	9	9	9	9	7
CH1	7	9	9	9	9	9
CH2	7	9	9	7	3	7
CNT1	3	7	5	3	5	5
CO39	7	7	7	9	5	3
IR64	5	5	3	5	3	1
KDML105	7	9	7	9	5	9
Mudgo	9	9	9	5	9	7
NERICA3	7	9	9	9	7	9
NERICA4	9	9	7	7	7	7
NPT13	5	7	5	5	3	5
NPT18	7	9	9	5	7	5
NPT4	7	7	9	7	9	5
NPT8	7	7	5	5	3	5
PtB33	0	1	3	3	1	0
PTT1	7	7	5	9	5	7
Rathu Heenati	1	1	3	3	1	3
RD31	5	3	3	5	1	3
SPR1	7	9	5	9	7	7
TDK1	7	9	5	7	5	5
TDK5	7	9	9	9	9	5
TN1	9	9	9	9	9	9

PTT_R = Pathum Thani Rice Research Center, CCS_R = Chachoengsao Rice Research Center, BKK_F = Khlong SamWa farmer's field, CCS_F = Chachoengsao farmer's field, BKK_R = Kasetsart University-Bangkhen, PTT_F = Pathum Thani farmer's field.

Wa farmer's field population were moderately resistant to the Kasetsart University Bangkhen population.

Cluster and principal component analyses

Cluster analysis of the genotypes using the mean BPH resistance (damage) score showed appreciable diversity among the genotypes used. It divided the test genotypes into three groups at about 30% similarity level. Group I contained 18 genotypes including the susceptible check TN1. Group II contained two genotypes, IR64 and RD31, while the third group was made up of the two resistant checks Rathu Heenati and Ptb33 (Figure 1).

The principal component (PC) analysis showed that the first and the second axes explained 76.88 and 8.18% of the variation, respectively. Together, the first two axes explained 85.1% of the total variation. The first PC was very important in separating the genotypes studied (Figure 2).

DISCUSSION

Varietal resistance to insects is the most practical and economical control measure (Brar

Table 3 Relative virulence of the six brown planthopper (BPH) populations and susceptibility of the 22 tested rice genotypes.

Genotype	BPH population						
	PTT_R	CCS_R	BKK_R	PTT_F	CCS_F	BKK_F	Susceptibility (%)
PtB33	R	R	R	R	R	R	0
Rathu Heenati	R	R	R	R	R	R	0
RD31	MR	R	R	MR	R	R	0
IR64	MR	MR	R	MR	R	R	0
CNT1	R	S	MR	R	MR	MR	16.7
NPT13	MR	S	MR	MR	R	MR	16.7
NPT8	S	S	MR	MR	R	MR	33.3
TDK1	S	S	MR	S	MR	MR	50
CO39	S	S	S	S	MR	R	66.7
NPT18	S	S	S	MR	S	MR	66.7
PTT1	S	S	MR	S	MR	S	66.7
CH2	S	S	S	S	R	S	83.3
KDML105	S	S	S	S	MR	S	83.3
Mudgo	S	S	S	MR	S	S	83.3
NPT4	S	S	S	S	S	MR	83.3
SPR1	S	S	MR	S	S	S	83.3
TDK5	S	S	S	S	S	MR	83.3
Azucena	S	S	S	S	S	S	100
CH1	S	S	S	S	S	S	100
NERICA 3	S	S	S	S	S	S	100
NERICA 4	S	S	S	S	S	S	100
TN1	S	S	S	S	S	S	100
Virulence (%)	72.7	81.8	54.5	59.1	45.5	45.5	

PTT_R = Pathum Thani Rice Research Center, CCS_R = Chachoengsao Rice Research Center, BKK_F = Khlong SamWa farmer's field, CCS_F = Chachoengsao farmer's field, BKK_R = Kasetsart University-Bangkhen, PTT_F = Pathum Thani farmer's field, S= susceptible, MR= moderately resistant, R= resistant.

et al., 2009). The development of new biotypes after the release of improved genotypes of rice with a single major insect resistance gene poses a great problem as the new biotypes have acquired virulence to the specific resistance gene (Fujita *et al*., 2009). This calls for the identification of several resistance genes (Brar *et al.*, 2009) and the sequential release of varieties with major genes

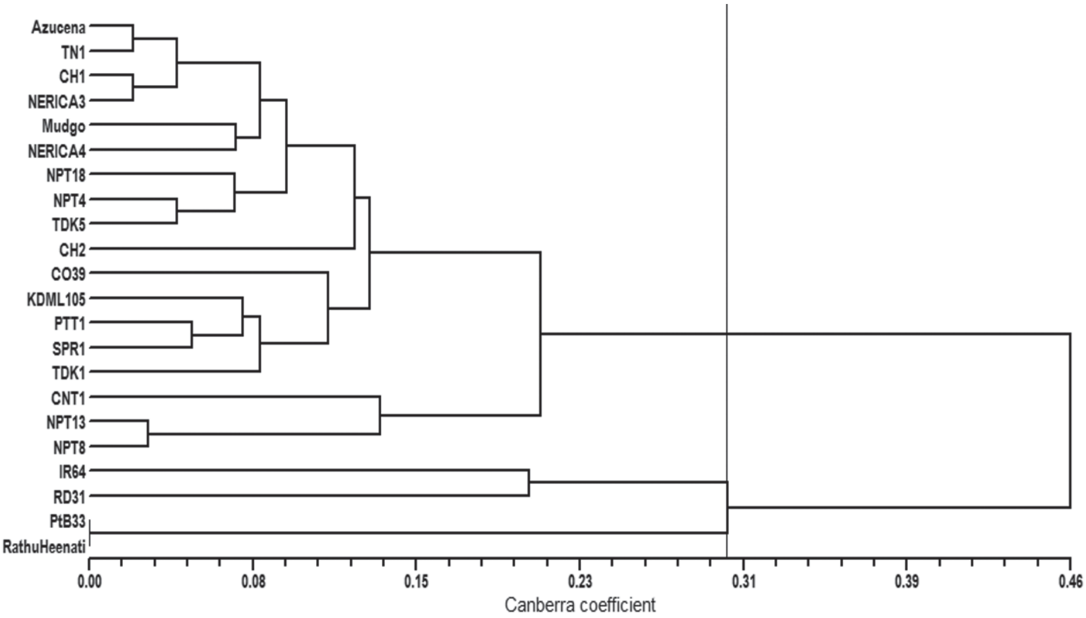

Figure 1 Dendrogram of 22 rice genotypes based on the reaction to six BPH populations and constructed by means of paired group algorithm.

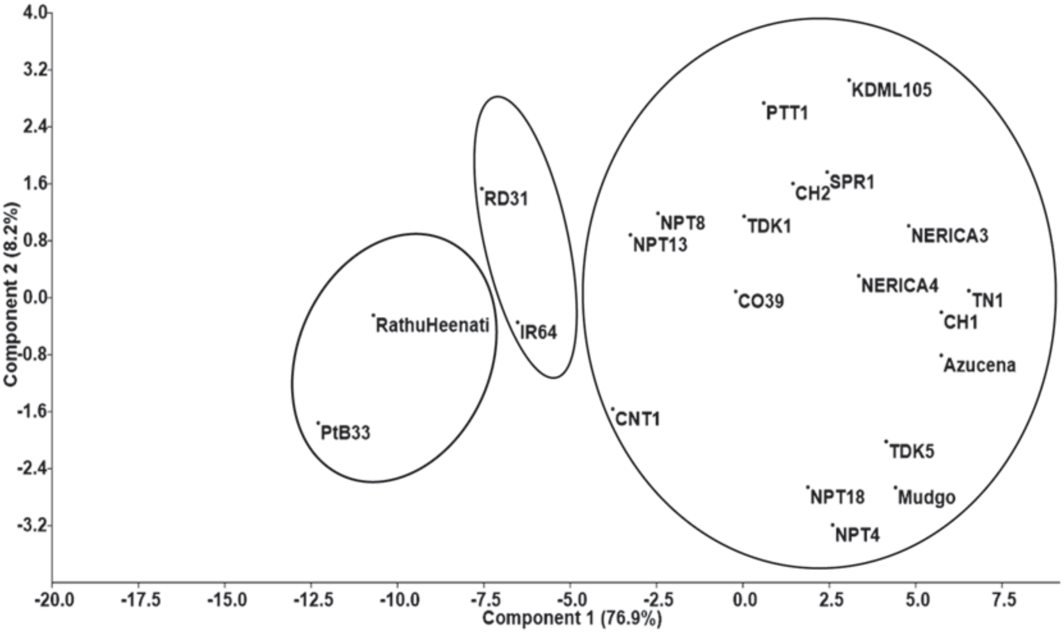

Figure 2 Scattered distribution of the 22 genotypes on the first two principal component axes.

(Khush, 1979) to cope with the development of new biotype populations. Great emphasis has been given to organic farming as chemicals have adverse effects on the environment and nontarget insects, and even on people. Therefore, using BPH-resistant varieties is consistent with this system since no chemicals are involved in the control of the insect. Furthermore, it is an economical practice for farmers in developing countries because they are not expected to incur extra costs once they get the resistant varieties.

An attempt was made to determine the reaction of 22 rice genotypes to six BPH populations collected from three provinces on the central plain of Thailand. The results showed appreciable differences among the populations used for the study. The population from the Chachoengsao Rice Research Center was found to be the most virulent compared to the others, as 18 (81.8%) of the rice genotypes were susceptible to this population. The next virulent population was that of the Pathum Thani Rice Research Center which was virulent with 16 (72.7%) of the rice genotypes. The results also showed differences in virulence of BPH populations collected from the rice research centers and farmers' fields of the same province. In this regard, the virulence of BPH from the Chachoengsao Rice Research Center and farm was 81.8 and 45.5%, respectively; while that of the Pathum Thani Rice Research Center and farm site were 72.7 and 59.1%, respectively. Similarly, the virulence of the BPH population from the Kasetsart University Bangkhen campus (representing a Bangkok research station) was 54.5%, while that of the farmer's field (Khlong Sam Wa district) was 45.5%. In all cases, the populations collected from the rice research centers were relatively more virulent than the associated field sample. Since the rice research centers are dealing with a number of rice genotypes compared to farmers, the BPH populations of the rice research centers may have had the chance to adapt to more genotypes (hence having greater virulence to more genotypes) than populations from the farmers' fields. Claridge *et al*. (1982) reported the occurrence of closely adapted BPH populations to different locally grown varieties within a small geographical area (about 100 km apart) in Sri Lanka.

Ptb33 and Rathu Heenati were resistant to all the six BPH populations. The nature of resistance in Ptb33 is reported to be digenic and conditioned by *Bph3* and *bhp2* genes (Angeles *et al*., 1986) whereas the *Bph3* gene is the source of resistance for Rathu Heenati (Lakshminarayana and Khush, 1977). Jairin *et al*. (2007a) reported that Rathu Heenati (*Bph3*) is highly resistant to a number of BPH populations in Thailand. Both IR64 and RD31 could be regarded as moderately resistant genotypes. Moderate resistance of IR64 to the BPH population of Pathum Thani was also reported previously (Jairin *et al*., 2005). A major resistance gene (*Bph1*) was reported to be incorporated from Mudgo into IR64 (Jena and Kim, 2010). However, in this study, Mudgo was found to be susceptible to all of the BPH populations except the one from the Pathum Thani farmer's field (moderately resistant). Multiple quantitative trait loci conferring BPH resistance were detected using a mapping population derived from a cross of IR64 and Azucena (Alam and Cohen, 1998; Ramalingam *et al*., 2003; Soundararajan *et al*., 2004) most of which derived from IR64 (Alam and Cohen, 1998). In addition, IR64 has been reported to have slight-to-moderate levels of antibiosis, antixenosis and tolerance (Cohen *et al*., 1997). This explains why IR64 was resistant or moderately resistant to the populations investigated while its parent Mudgo was susceptible.

The genotypes CNT1, NPT13 and NPT8 were not consistent in their reaction to the BPH populations studied. On the one hand, they had become resistant and moderately resistant to some populations; on the other hand, they were still susceptible to one or two of the populations. Both CNT1 and NPT13 were susceptible to the population from the Chachoengsao Rice Research Center which was found to be the most virulent according to this study. CNT1, once resistant to BPH, is now losing its resistance. Jairin *et al*. (2007b) also reported the breakdown of the resistance of this variety through the occurrence of virulent BPH biotypes. Therefore, the production of this variety is not dependable. The genotypes NERICA3, NERICA4, Azucena, CH1 and TN1 were susceptible to all the six populations. NERICA (New Rice for Africa) genotypes were developed by interspecific hybridization of African rice *O. glaberrima* and Asian rice *O. sativa* (Samado *et al*., 2008) under African environmental conditions. BPH is not common in Africa (Catindig *et al*., 2009) and therefore, it is unlikely to introduce resistance to BPH in African varieties without operational selective pressure of the insect. TN1 was included as a susceptible check as it does not have a resistance gene to any of the BPH biotypes identified so far (Jena and Kim, 2010).

Cluster analysis divided the test genotypes into three groups. Group I could be regarded as the susceptible group and contained breeding line varieties developed by interspecific crosses (NERICAs), genotypes once resistant but susceptible nowadays, like CNT1, SPR1 and Mudgo (Khush, 1979; Jairin *et al*., 2007b), and traditional varieties without known resistance genes like KDML105, Azucena and TN1 (Cohen *et al*., 1997; Jairin *et al*., 2005; Jena and Kim, 2010). Group II contained two genotypes, IR64 and RD31, with moderate resistance. In areas with low insecticide use and the presence of potential actions of natural enemies, high levels of *N. lugens* resistance may not be necessary (Cohen *et al*., 1997; Bottrell and Schoenly, 2012). Therefore, in such areas on the central plain of Thailand, IR64 and RD31 could be used with caution. The third group, the resistant group, was made up of Rathu Heenati and Ptb33. These two genotypes could clearly be used as parents in breeding for BPH resistance.

The principal component analysis showed that the first PC was very important in separating the genotypes studied based on their reaction to the BPH populations; clearly showing a pattern whereby the genotypes on the left side of the two dimensional plane (Figure 2) are highly resistant and the resistance decreases toward the right hand side of the plane.

CONCLUSION

Monitoring the virulence of BPH and the ability of resistance genes may be necessary as there is a possibility of development of new BPH biotypes and the breakdown of major resistance genes as well. The cluster and PC analyses were in agreement in explaining the variability contained in the rice genotypes. Rathu Heenati and Ptb33 could be used as sources of resistance for BPH in central Thailand to curb the eminent development of new BPH biotypes and outbreaks of the insect. Genotypes IR64 and RD31 could be used with caution. However, it is important to study the reactions of more genotypes by rearranging their positions in the seed box and using additional BPH populations. Identification of biotypes could also be considered. The differential responses of genotypes to populations from the rice research centers and farmers' fields of the same province also need further investigation.

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