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Screening of Rice Genotypes for Resistance to the Brown Planthopper, *Nilaparvata lugens* Stål

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In order to breed rice cultivars for resistance to the brown planthopper, *Nilaparvata lugens* Stål (BPH) in Bangladesh, were evaluated for resistance in greenhouse screening tests. Over a period of six years (2005–2010), 1,767 entries/cultivars were screened using the plant hopper screening (PHS) system. The results showed 87 donors possessing different levels of resistance to the BPH. One exotic cultivar was highly resistant to the BPH and 86 materials showed medium resistance (tolerance) to the BPH. The rest of the materials including germplasm, F_2, exotic, IRBPHN (International Rice Brown Planthopper Nursery) and advanced lines were susceptible. Most of entries coming from the International Rice Research Institute (IRRI) via the IRBPHN were moderately resistant.

Keywords: rice, germplasm, screening, *Nilaparvata lugens,* resistance

Introduction

The human population is rapidly approaching seven billion and more than one half depend on rice as their food staple (IRRI 2010). The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is a serious insect pest of rice, especially in tropical Asia, where rice crops are continuously cultivated (Gurr et al. 2011). A major tool for managing the BPH is the integration of host plant resistance with natural enemy conservation and cultural practices. However, field resistance levels are limited by the rapidity with which the delphacids, especially *N. lugens*, overcomes resistance genes through the development of biotypes (Horgan 2009). In recent years, BPH infestations have intensified across Asia in response to resurgence inducing insecticides resulting, in heavy rice yield losses (Normile 2008; Matsumura and Sanada-Morimura 2010). Both BPH nymphs and adults directly damage rice plants through sucking the cell sap from base (stem) of the plants. The BPH also transmits viruses such as rice ragged stunt (RRSV) and rice grassy stunt (RGSV) (Hibino 1989, 1996) which cause severe losses. From 2005 to 2006, more than 485,000 ha of rice in southern Vietnam were severely affected by viral diseases seem-

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ingly spread by BPH, resulting in the loss of 828,000 tons of rice valued at US$120 million (Du et al. 2007).

The BPH has been able to rapidly adapt to resistant rice varieties and an array of pesticide chemistries (Liu et al. 2005; Yang et al. 2005; Yin et al. 2008). Rice entomologists and breeders have frequently observed that rice varieties may be resistant to *N. lugens* in one geographic region but susceptible in another and rice varieties that were previously resistant may become susceptible over time. Thus there is a need for new genes to replace the genes which the BPH has adapted to through biotype development. The use of resistant rice varieties is the most economical and efficient method for controlling the BPH (Alam and Cohen 1998; Renganayaki et al. 2002), therefore it is imperative to identify BPH-resistance genes from diverse sources and incorporate them into rice cultivars by the use of modern molecular tools. With a view to widening the genetic base so as to enable the reliable use of BPH resistance, the identification of a larger number of cultivars with BPH resistance to the known biotypes in Bangladesh is necessary. Thus we undertook a screening evaluation to determine the reaction of different cultivars against Bangladeshi BPH biotype to identify cultivars that can be used as donors in the rice breeding program.

Materials and Methods

Insect rearing

We used the method described by IRRI (Pathak and Khush 1977) to rear the BPH. The source insects were collected from the field and continuously rearing in greenhouse for screening purpose that infested cultivation variety of rice in the field in Bangladesh. The insects were reared on 40- to 50-day-old rice plants (susceptible variety BR3) inside a 0.5 × 0.5 × 1.0 m cage. This cage consisted of a steel frame covered with a fine mesh wire screen. The cage bottom was open and setting in water. Potted plants were changed as needed. Each cage could accommodate several potted plants that could support 2,000 to 3,000 late-instar BPH nymphs. The original colony per cage was started by 30–40 gravid adults. Eggs of about the same day age were obtained by placing the plants in a cage with gravid adults for two days.

Screening procedures

Screening for resistance to the BPH was conducted at the seedling stage in the greenhouse. The screening procedures standardized at IRRI and described by Heinrichs et al. (1985) were adopted in this study. Each test variety or line was seeded in 20-cm-long rows in a seed box (60 × 45 × 10 cm). Rows were 5 cm apart. A row of the susceptible check variety (BR3) and a resistant check variety (T27A) was planted randomly in the seed boxes. At the sixth day after seeding, plants were thinned to 20 to 30 seedlings per row. The seed boxes were placed on a galvanized iron tray on a table inside a screened room in the greenhouse. To provide suitable humidity for insect survival and avoid the disturbance of watering on the tested insects, we maintained a depth of about 5 cm standing water in the tray.

The seedlings were infested at the one- to two-leaf stage (about 7 days after seeding) by uniformly scattering a large number of 2nd to 3rd instar BPH nymphs on them. The seed boxes were covered with nylon nets after infestation. An average of 5–7 insects per seedling constituted an optimum population to differentiate the resistant level of tested lines. The damage rating was taken when about 90% of the plants of the susceptible check variety were killed, usually about 5 to 7 days after infestation. The varieties were rated using the standard evaluation system for rice (Table S1*) (IRRI 1988).

We first conducted an initial evaluation of 1,767 varieties consisting of 1,210 domestic (mostly old indigenous cultivars) varieties, 155 overseas introduced varieties/lines as exotic, and 402 as IRBPH coming from IRRI), using one replicate per variety. The varieties whose resistance fell into grade 0 to 5 were selected for further evaluation, using the same technique but with 3 to 4 replicates per variety/entry. All of the screening was conducted in Bangladesh during the period 2005 to 2010.

Statistical analysis

The experimental design used was completely randomized design (CRD) with four replications. The scores obtained in all varieties in the damaged rating analysis were then pooled for constructing a single data matrix. The data were subjected to statistical analysis to obtain information on the mean performance and variability existing among the genotypes. The raw values of 1,767 genotypes for a single trait were subjected to cluster analysis and principal component analysis (PCA) using the computer software NTSYS pcv2.02i (Rholf 1992).

Results

A total of 1,767 rice cultivars were initially screened during the six year-period, 2005–2010 (Table 1). Of these 770 sources of germplasm were collected from the Bangladesh Rice Research Institute (BRRI) Genetic Resources and Seed Division and 390 advanced lines from the BRRI Breeding Division. The rest of the entries were supplied by IRRI, either through the Tropical Agriculture Research Center or directly as entries of the IRBPHN (International Rice Brown Planthopper Nursery). The reaction of the rice varieties from various sources to the Bangladesh BPH population infestation is presented in Table 1. One highly resistant, 86 moderately resistant (Table 2) and 1,680 susceptible and highly susceptible (cultivars were identified accounting for 0.13%, 4.8% and 95.07% of the total cultivars, respectively).

Of the 1,212 genotypes of BRRI germplasm, most (99.1%) were susceptible to the BPH and only 11 (0.9%) entries were moderately resistant to the BPH (Table 1). One hundred and fifty-five exotic materials were tested (collected from different research institutes and countries). One variety (0.7%) was resistant, seven (4.6%) were moderately resistant and 147 (94.7%) were susceptible to the BPH. Of the 402 IRBPHN cultivars 70

* Further details about the supplementary data can be found at the end of the article.

Table 1. Number of rice cultivars rated for brown planthopper (*Nilaparvata lugens* Stål) resistance, 2005–2010

Country or group of cultivars	Reaction to BPH			Total
	R	M	S	
Bangladesh, Genetic Resources and Seed Division, BRRI, Germplasm	0	9	1,201	1,212
Exotic materials	1	7	147	155
IRBPHN, IRRI, Phillipines	0	70	332	402
Total	1	86	1,680	1,767

R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible

(17%) were moderately resistant and 332 (83%) susceptible. In contrast to the BRRI germplasm entries the IRBPHN group possessed a high frequency of moderately resistant accessions.

The selected cultivars resistant to BPH biotypes I, II, III, and IV and the tested Bangladesh population are presented in Table S2. All cultivars with resistance genes to biotypes in other countries showed a susceptible reaction to the Bangladesh population.

Discussion

Out of 1,767 tested genotypes collected from different sources only 86 were found to be moderately resistant to the BPH (Table 2). Almost all the traditional varieties in Bangladesh were susceptible to the BPH. In contrast to domestic and advanced lines, the IRBPHN possessed high frequency of moderately resistant/moderately susceptible genotypes (81.4% at scale 3–5). IRRI has a wide range of resistance sources for BPH. The incorporation of resistance to the improved Bangladesh rice varieties is essential because our data showed that the frequency of the domestic rice varieties resistant to BPH was quite low in Bangladesh.

Jena and Kim (2010) reported that Mudgo, ASD7, Raghu Hematic, Babawee, ARC10550, Swarnalata, T12, Chin Saba, Balamawee are resistant donors. But the present study showed that those varieties had no resistance to the BPH at the seedling stage in green house screening. Thus this study suggested that the Bangladesh BPH population may be a new biotype with high virulence.

Our observation suggested that rapid change of BPH biotype in Bangladesh may be due to the increased amount of insecticides applied by farmers. Outbreaks of BPH are common phenomena in Bangladesh nowadays. We believe that the out breaks of BPH in Bangladesh are due to the overuse of resurgence – inducing insecticides as was described by Chelliah (1987) in India that are very much true for Bangladesh, too.

This study indicated that a major portion of the tested rice genotypes are susceptible to the Bangladesh BPH population. It is predicted that this may have occurred due to the resistance breaking ability of the Bangladesh BPH population. The resistance-breaking ability of recently collected BPH in Korea was similar to that of the South Asian BPH populations, which occurred in Bangladesh, Sri Lanka, and southern India, based on virulence to

Table 2. Classification of selected rice cultivars according to their reaction to the Bangladesh brown planthopper (*Nilaparvata lugens* Stål) population in evaluations conducted from 2005 to 2010

Cultuvar	Registance score*	Seed source	Cultivar	Resistance score*	Seed source
IR 71033-62-15-B	3	IRRI	IR 81178-29-2-3-2	5	IRRI
IR 72890-81-3-2-2	5	IRRI	IR 81935-33-1-2-1	5	IRRI
IR 75870-7-9-1-6-2-2-1-B	5	IRRI	IR 82823-B-SDO 5	5	IRRI
IR 77032-47-2-3-3	3	IRRI	IR 83142-12	5	IRRI
IR 77298-5-6	1	IRRI	IR 73006-8-2-2-1	5	IRRI
IR 77356-13-3-6-1-1-B	3	IRRI	IR 73545-3-1-1-2	5	IRRI
IR 77380-59-1-4-3-2-B	5	IRRI	IR 76926-5-1-1-4	5	IRRI
IR 77666-B-26-1-1-3-1-7-5-AJY 5	3	IRRI	IR 77186-34-2-3-3	3	IRRI
IR 77674-B-20-1-2-1-3-6-4-AJY 1	3	IRRI	IR 77674-3B-8-1-3-13-2-AJY 2	5	IRRI
IR 78091-120-3-2-2-3	3	IRRI	IR 77674-3B-8-2-2-8-2-AJY 6	5	IRRI
IR 78091-6-2-3-1-1	1	IRRI	IR 77674-3B-8-2-2-8-3-AJY 2	5	IRRI
IR 78119-24-1-2-2-2	3	IRRI	IR 79253-55-14-6	5	IRRI
IR 78126-1-2-1	1	IRRI	IR 79504-48-1-6-2	5	IRRI
IR 78126-59-1-5-1-2	1	IRRI	IR 79505-51-2-2-2	5	IRRI
IR 78545-49-2-2-2	1	IRRI	IR 79648-35-2-1-1	5	IRRI
IR 78545-57-2-1-3	3	IRRI	IR 80402-88-3-1-3	5	IRRI
IR 78555-68-3-3-3	3	IRRI	IR 80404-28-2-3-2	5	IRRI
IR 78566-1-2-1-2	3	IRRI	IR 80674-81-1-2-3	3	IRRI
IR 78581-12-3-2-2	3	IRRI	IR 80679-3-2-2-2	5	IRRI
IR 78629-57-3-3-2	1	IRRI	IR 80694-44-1-2-2	3	IRRI
IR 78806-B-B-19-3-1-AJY 1	1	IRRI	IR 80894-66-3-2-3	5	IRRI
IR 79201-49-1-1-1	3	IRRI	IR 80894-95-1-1-1	5	IRRI
IR 79203-132-1-2-2	5	IRRI	IR 81852-29-3-3-3	5	IRRI
IR 79204-84-2-2-3	3	IRRI	IR 83140-51	3	IRRI
IR 79242-5-1-1-5	5	IRRI	IR 83140-56	5	IRRI
IR 80255-82-1-3-2-1	5	IRRI	IR 83142-76	3	IRRI
IR 80285-34-3-3-2	3	IRRI	IR 84194-139	5	IRRI
IR 80694-150-3-2-2	5	IRRI	IR 84675-1-7-2	3	IRRI
IR 80860-53-1-3	5	IRRI	IR69726-29-1-2-2-2 (MATATAG 2)	5	IRRI
IR 80904-27-2-2-3	3	IRRI	IR71604-4-1-4-7-10-2-1-3	5	IRRI
IR 80919-57-2-2-1	5	IRRI	IR72890-70-2-3-3	3	IRRI
IR 81166-60-3-1-2	5	IRRI	IR73718-194-2-2-3	5	IRRI
Tangul	5	BRRI	IR74271-41-2-1	3	IRRI
Digha	5	BRRI	IR74271-68-3-2	3	IRRI
IR77958-10-1-18	5	IRRI	IR77479-8-3-2-1	1	IRRI

*1 = resistant (R); 3 = moderately resistant (MR); 5 = moderately susceptible (MS)

the resistance genes in Mudgo and ASD7 and no virulence to Rathu Heenati *(Bph3)* (IRRI 1975; Verma et al. 1979; Smith 2005). The first resistant variety with *Bph*1, IR26, was released in 1973 (Khush 1971) and it was widely accepted in the Philippines, Indonesia, and Vietnam but became susceptible in 1976–77 because of the development of BPH biotype II. By that time, varieties IR36 and IR38 with the *bph2* gene had been developed and released (Khush 1977). So from this study it is suspected that the tested BPH population may be a new biotype but more studies are needed to confirm this.

With the lack of BPH resistant donors and the threat of the development of new biotypes we suggest that the Bangladesh rice program follow the recommendations of IRRI Rice Planthopper Project (http://ricehoppers.net/about-2/). They suggest that the search for sustainable ways to manage BPH outbreaks is now along several fronts. First is to identify genes and develop rice varieties that will have sustainable resistance to planthoppers. Tolerant varieties with many minor genes provide more durable resistance than single major gene varieties. Second is to develop ecological engineering methods that will restore and enhance important ecosystem services to provide adequate crop health. This includes the conservation of biocontrol agents and prohibiting the use of natural enemy destroying resurgence-inducing insecticides which is currently the major reason for outbreaks of the BPH in Southeast Asia and likely in Bangladesh. Third is to understand farmers' decision making and to develop communication strategies to motivate policy decision makers and farmers to adopt sustainable practices.

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Supplements

Supplementary data associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Supplementary Table S1. Standard evaluation system for rating damage by the brown planthopper (*Nilaparvata lugens* Stål) to rice (IRRI 1988)

Supplementary Table S2. Classification of selected rice cultivars according to their reaction to four BPH biotypes (according to the literature) (Kaneda et al. 1981) and to the Bangladesh BPH population