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# Evaluation of Rice Germplasm for Resistance to the Small Brown Planthopper (*Laodelphax striatellus*) and Analysis of Resistance Mechanism

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**Abstract:** One hundred and thirty-eight rice accessions were screened for resistance to the small brown planthopper (SBPH) (*Laodelphax striatellus* Fallén) by the modified seedbox screening test. Twenty-five rice accessions with different levels of resistance to SBPH were detected, accounting for 18.1% of the total accessions, which included 2 highly resistant, 9 resistant and 14 moderately resistant varieties. Compared with indica rice, japonica rice was more susceptible to SBPH. Antixenosis test, antibiosis test and correlation analysis were performed to elucidate the resistance mechanism. The resistant check Rathu Heenati (RHT), highly resistant varieties Mudgo and Kasalath, and resistant variety IR36 expressed strong antixenosis and antibiosis against SBPH, indicating the close relationship between resistance level and these two resistance mechanisms in the four rice varieties. Antibiosis was the dominant resistance pattern in the resistant varieties Daorenqiao and Yangmaogu due to their high antibiosis but low antixenosis. Dular, ASD7 and Milyang 23 had relatively strong antixenosis and antibiosis, indicating the two resistance mechanisms were significant in these three varieties. The resistant DV85 expressed relatively high level of antixenosis but low antibiosis, whereas Zhaiyeqing 8 and Guiyigu conferred only moderate antibiosis and antixenosis to SBPH, suggesting tolerance in these three varieties. Antibiosis and antixenosis governed the resistance to SBPH in the moderately resistant accession 9311. Antixenosis was the main resistance type in V20A. Tolerance was considered to be an important resistance mechanism in Minghui 63 and Yangjing 9538 due to their poor antibiosis and antixenosis resistance. The above accessions with strong antibiosis or antixenosis were the ideal materials for the resistance breeding.

**Key words:** rice germplasm; the small brown planthopper; evaluation for resistance; resistance mechanism; antixenosis; antibiosis

The small brown planthopper (SBPH), *Laodelphax striatellus* Fallén (Homoptera: Delphacide), is an economically important and wide-spread insect pest of rice (*Oryza sativa* L.) in China, in which heavy infestation occurs in the middle and lower reaches of the Yangtze River and North China. In recent years, the SBPH population has been drastically rising, which leads to more and more serious damage to rice. When the outbreak occurred in Jiangsu and Anhui Provinces, China in 2004 and 2005, the insect density of SBPH reached 74.1 million per hectare and 123.6 million per hectare of rice respectively, causing 30% of yield reduction in the areas without pesticide treatment [1-3].

The adults and nymphs of SBPH in large number suck rice sap. The leaves infested by SBPH turn yellow, become wilting, and even die, resulting in yield loss and grain quality decline. In addition, this planthopper also transmits rice viral diseases such as rice black-streaked dwarf virus (RBSDV) and rice stripe virus (RSV). RSV is one of the most serious diseases and often causes more severe yield losses than the feeding damage [4-6].

Nowadays, pesticides are widely used to control the SBPH, which leads to natural enemy death, environment pollution and chemical resistance. Furthermore, the controlling effect is not satisfying due to the migration and resistance of SBPH [7-9].

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Host-plant resistance has been recognized as one of the most economic and effective measures in controlling the planthopper and rice stripe disease. Therefore, breeding rice varieties resistant to SBPH is a viable alternative to chemical methods [10-11]. The resource of resistance is the basis of breeding, however, screening for germplasm resistance to SBPH has not been reported to date.

In this experiment, we modified the standard seedbox screening test (SSST) [6] based on the SBPH trait and established a screening program for the resistance to SBPH. And 138 rice accessions were used to screen the resistance to the SBPH by the modified seedbox screening test (modified SSST) and their resistance mechanisms were preliminarily analyzed by antixenosis and antibiosis tests.

## MATERIALS AND METHODS

### Plant materials

One hundred and thirty-eight rice accessions were provided by the National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University; the China National Rice Research Institute; and Institute of Crop Sciences and Institute of Plant Protection, the Chinese Academy of Agricultural Sciences. 'Rathu Heenati (RHT)' and 'Wuyujing 3' were used as resistant and susceptible controls, respectively.

### Insect population

The SBPH population used for infestation was first provided by Institute of Plant Protection, the Chinese Academy of Agricultural Sciences, and maintained on barley for four generations before transferred to rice Wuyujing 3 in the greenhouse of Institute of Crop Sciences, the Chinese Academy of Agricultural Sciences. The population was confirmed to be nonviruliferous by dot-immunobinding assay and PCR detection [12].

### Modified SSST

Considering relatively less feeding capacity of SBPH than that of brown planthopper (*Nilaparvata lugens*) or the whitebacked planthopper (*Sogatella furcifera*), we modified the SSST to suit the SBPH resistance screening. The procedure was as follows: i) at the 1.5-leaf stage, to infest rice seedlings with second- to third-instar SBPH nymphs by 15 insects per seedling; ii) to use 'Rathu Heenati' and 'Wuyujing 3' as resistant and susceptible controls, respectively; and iii) to perform scoring when about 90% seedlings of the susceptible variety Wuyujing 3 become dead after (15±1)-day infestation according to the standard evaluation systems described by IRRI [13]. The resistance scale of each accession was then calculated based on the weighted average of the seedlings tested (Table 1).

Resistances of the 138 rice accessions were evaluated by the modified SSST. Twenty-five germinated seeds were sown in a plastic pot (8 cm in diameter and 9 cm in height) with a hole in the base and two pots per accession. Generally, 28 pots, together with resistant and susceptible control varieties, were randomly placed in a 65-cm × 44-cm × 14-cm plastic seedbox with about 2-cm depth of water on the soil surface. All the plants for evaluation were grown at 26±1°C with sunlight and natural ventilation.

### Antixenosis test

#### *Antixenosis to nymphs of SBPH in the rice seedling*

According to the method described by Hiroshi et al [14], germinated seeds were sown as above with 15 seeds for each accession in a row and two rows of each accession. At the 1.5- to 2.0-leaf stage, the seedlings were transferred into the cages covered with nylon net and infested with second- to third-instar SBPH nymphs by 5 insects per seedling. The insects were counted after 24-hour infestation, and then

**Table 1. Evaluation criteria for resistance to SBPH at the rice seedling stage.**

| Damage symptom | Resistance scale | Resistance level [a] |
|---|---|---|
| No visible damage | 0 | I |
| Very slightly damage | 1 | HR |
| Partial yellowing of the first and second leaves | 3 | R |
| Pronounced yellowing, and some seedlings slight stunning | 5 | MR |
| Seedlings showing signs of severe stunning or wilting | 7 | S |
| 90% seedlings dead | 9 | HS |

[a] Resistance level: I, Immune; HR, Highly resistant; R, Resistant; MR, Moderately resistant; S, Susceptible; HS, Highly susceptible.

dispersed evenly among the seedlings after counting every day. The average number of insects on each entry was calculated and treated as the value of antixenosis test after 5-day investigation.

### *Antixenosis to SBPH adults and oviposition for rice plants at the vegetative stage*

Germinated seeds were treated as above and two rows for each accession with 10 seedlings in a row were planted. On the 30th day after sowing (DAS), the plants were transferred into the cages covered with nylon net and then their tillers were pruned and infested with gravid females at a density of 2 insects per stem. The numbers of SBPH were counted after 24-, 48-, and 72-hour infestation, respectively. The numbers of SBPH eggs on rice stems were also counted after 72-hour infestation.

### Antibiosis test

The antibiosis test was followed by the method described by Sebastian et al [15]. On the 8th day after sowing germinated seeds, seedlings were transferred to test tubes (one seedling per test tube) containing 1 mL of water at 26±1°C, and then 5 first- to second-instar nymphs were added to each tube. Ten seedlings per accession were tested and repeated for three times. The survival rate of nymphs was calculated as a measure of antibiosis. The scoring was done each day for 5 days with the initial count done at 6 hour after infestation. The antibiosis score (AS) was computed as follows:

$$AS = [(A_1\times1)+(A_2\times2)+\cdots+(A_n\times n)]\times100/(1+2+\cdots+n);$$

Where, $n$ is the days after adding SBPH nymphs and $A_n$ is the percentage of nymph survival on the $n^{th}$ day. The plants with AS values of 0 to 81% are classified as resistant and the others with AS value greater than 81% as susceptible. The less AS values show the stronger antibiosis resistance.

## RESULTS

### Identification of resistance to SBPH by modified SSST

One hundred and thirty-eight rice accessions were screened for resistance to SBPH in the seedlings by modified SSST. Two highly resistant (Mudgo and Kasalath), nine resistant (Dular, ASD7, DV85, Milyang 23, Guiyigu, IR36, Zhaiyeqing 8, Daorenqiao and Yangmaogu) and 14 moderately resistant varieties were detected, accounting for 1.4%, 6.5% and 10.1% of the total accessions, respectively. Susceptible and highly susceptible entries made up 82.0% of the total accessions (Fig. 1). The resistant varieties belonged to indica rice and the majority of japonica rice entries were susceptible, suggesting that japonica rice might be more susceptible to SBPH.

### Antixenosis against SBPH nymphs in the rice seedlings

Eighteen rice accessions, including 3 highly resistant (RHT, Mudgo and Kasalath), 9 resistant (Dular, ASD7, DV85, Milyang 23, Guiyigu, IR36, Zhaiyeqing 8, Daorenqiao and Yangmaogu), 4 moderately resistant (9311, V20A, Yangjing 9538 and Minghui 63) and 2 highly susceptible (Wuyujing 3 and 06381) entries were tested for antixenosis to SBPH nymphs. The number of nymphs on the seedlings with different scales of resistance ranged from 1.8 to 8.3. Furthermore, the antixenosis values of rice entries with the identical resistance scale, such as Daorenqiao, IR36 and Yangmaogu also varied at a certain extent (Table 2).

The susceptible varieties Wuyujing 3 and 06381 were the least antixenosis-resistant varieties, and moderately resistant variety Yangjing 9538, and resistant varieties Guiyigu, Zhaiyeqing 8, Daorenqiao and Yangmaogu showed the low level of antixenosis

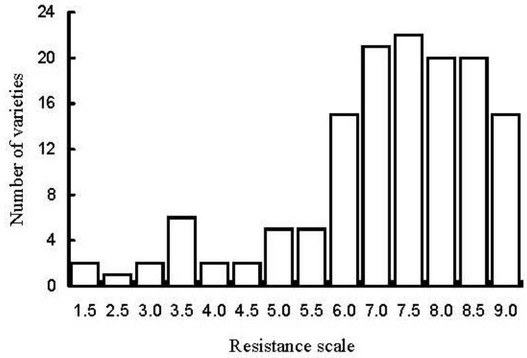


**Fig. 1. Distribution of SBPH resistance scales in the 138 rice accessions.**

**Table 2. Reaction of antixenosis to nymphs of SBPH in the seedlings of 18 rice varieties.**

| Variety | Value of antixenosis | Variety | Value of antixenosis |
|---|---|---|---|
| Wuyujing 3 | 8.3±0.28 a | 9311 | 4.3±0.44 def |
| 06381 | 7.7±0.19 a | Dular | 3.9±0.38 efg |
| Yangjing 9538 | 6.4±0.32 b | Milyang 23 | 3.5±0.51 fg |
| Yangmaogu | 6.1±0.28 b | DV85 | 3.4±0.39 g |
| Daorenqiao | 5.5±0.35 bc | IR36 | 3.2±0.35 gh |
| Minghui 63 | 5.1±0.42 cd | ASD7 | 3.2±0.24 gh |
| Guiyigu | 4.5±0.42 cde | Mudgo | 2.4±0.18 hi |
| Zhaiyeqing 8 | 4.5±0.25 cde | Kasalath | 2.2±0.18 i |
| V20A | 4.3±0.49 def | Rathu Heenati (RHT) | 1.8±0.22 i |

Means followed by the common letters indicate no significant difference among the varieties at the 5% level by the Duncan's test.

resistance to SBPH by statistical analysis. The relationship between resistance scale and antixenosis value was not significant in the above four resistant varieties by correlation analysis ($r$=0.5052, $P > 0.05$), indicating that the antixenosis was not the dominant resistance type in these four varieties. RHT, Kasalath and Mudgo were the strongest antixenosis resistant varieties, followed by ASD7, IR36, DV85, Milyang 23 and Dular. The significant relationship between the resistance scale and the antixenosis value ($r$=0.8351, $P < 0.05$) confirmed that the antixenosis was a significant resistance mechanism in these eight entries.

## Antixenosis of rice plants at the vegetative stage to SBPH adults and oviposition

Ten rice varieties, including RHT, Mudgo, Kasalath, Dular, Milyang 23, Yangmaogu, Yangjing 9538, Nipponbare, Wuyujing 3 and 06381, were used for detecting the antixenosis to adults and oviposition. The antixenosis resistance in the varieties with different resistance levels varied at a considerable extent. There existed significant relationship between the resistance level of host and the number of SBPH adults with a correlation coefficient ($r$) of 0.9424 ($P <$ 0.05) and also between the number of insects and the number of eggs laid by SBPH adults ($r$=0.8351, $P <$ 0.05). SBPH adults markedly preferred to settlement and oviposition on the susceptible plants, with 3.3 insects and 88.9 eggs per stem in susceptible variety Wuyujing 3 but 0.5 insects and 10.6 eggs per stem in resistant variety RHT (Fig. 2 and Fig. 3).

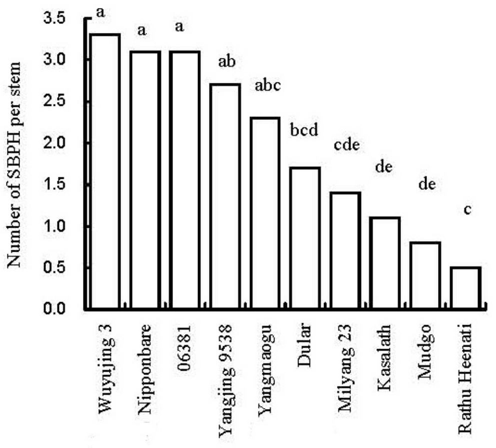


**Fig. 2. Antixenosis of adults of SBPH for plants at the vegetative stage.**

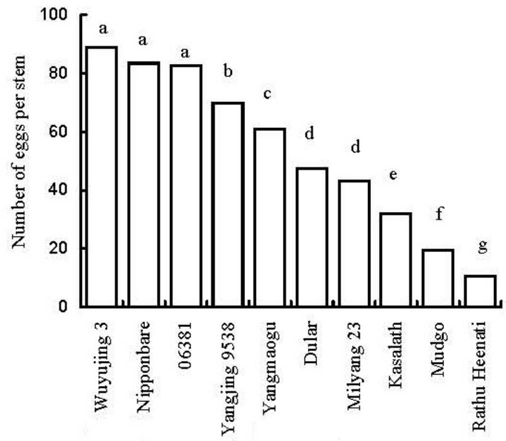


**Fig. 3. Number of eggs laid by SBPH on rice varieties.**

## Antibiosis against SBPH nymphs

Eighteen rice varieties were used for the antibiosis test. The results showed that the antibiosis scores (ASs) of the accessions with different resistance levels varied considerably, ranging from 64.3% to 91.4%. The ASs of 3 highly resistant and 9 resistant varieties were less than 81.0%, and those of moderately resistant V20A, Minghui 63 and Yangjing 9538 were more than 81.0%. RHT ranked as the first in the antibiosis resistance to SBPH, with an AS of 64.3%, followed by Mudgo, IR36, Daorenqiao, Yangmaogu and Kasalath, with the ASs of 67.1%, 67.7%, 68.1%, 68.7% and 69.1%, respectively. Rice variety 06381 was the least antibiosis-resistant variety with an AS of 91.4% (Table 3). There existed a close relationship between resistance scale and AS in varieties RHT, Mudgo and Kasalath, with a correlation coefficient of 0.8985 ($P < 0.05$), also in

**Table 3. Reaction of antibiosis to nymphs of SBPH in the seedlings of 18 rice varieties.**

| Variety | AS (%) | Variety | AS (%) |
|---|---|---|---|
| 06381 | 91.4±3.12 a | Milyang 23 | 71.5±4.92 efg |
| Wuyujing 3 | 89.9±2.85 a | ASD7 | 70.5±5.53 efg |
| Yangjing 9538 | 82.5±3.67 b | Dular | 70.1±4.37 efg |
| Minghui 63 | 81.7±4.52 b | Kasalath | 69.1±2.42 fg |
| V20A | 81.2±4.86 bc | Yangmaogu | 68.7±3.65 fgh |
| DV85 | 79.5±5.13 bc | Daorenqiao | 68.1±3.53 fgh |
| 9311 | 77.1±4.26 cd | IR36 | 67.7±2.42 fgh |
| Guiyigu | 74.6±3.08 de | Mudgo | 67.1±2.28 gh |
| Zhaiyeqing 8 | 72.3±3.54 ef | RHT | 64.3±2.39 h |

Means followed by the common letters indicates no significant difference among the varieties at the 5% level by the Duncan's test.

Daorenqiao, Yangmaogu, ASD7 and Zhaiyeqing 8, with a correlation coefficient of 0.8891 ($P < 0.05$), indicating that the antibiosis was a significant resistance pattern in the above seven entries.

Modified SSST, antixenosis test and antibiosis test in combination with correlation analysis were performed to elucidate the primary resistance mechanism. The highly resistant varieties such as RHT, Mudgo and Kasalath and resistant variety IR36 expressed strong antixenosis and antibiosis against SBPH, indicating that the resistances in the four rice varieties were mainly conferred by the two mechanisms. The antibiosis was the dominant resistance type in the resistant varieties Daorenqiao and Yangmaogu due to their high level of antibiosis but low antixenosis. Dular, ASD7 and Milyang 23 had relatively strong antixenosis and antibiosis, indicating that the two mechanisms contributed to the resistance in these three varieties. The resistant variety DV85 expressed relatively high level of antixenosis but low antibiosis, whereas Zhaiyeqing 8 and Guiyigu conferred only moderate antibiosis and antixenosis to SBPH, suggesting a good pest tolerance in these three varieties. Antibiosis and antixenosis governed the resistance to SBPH in the moderately resistant accession 9311. In addition, antixenosis was the main resistance type in V20A. Tolerance was considered to be an important resistance mechanism in Minghui 63 and Yangjing 9538 due to their poor antibiosis and antixenosis resistance. The above accessions with strong antibiosis or antixenosis were the ideal materials for breeding. Wuyujing 3 and 06381 were preferred host for SBPH and susceptible controls due to their least-level antixenosis and antibiosis resistance.

## DISCUSSION

SSST is a classic and rapid method for screening of resistance to brown planthopper, the whitebacked planthopper and green rice leafhopper (*Nephotettix cincticeps*) at a large scale. In this experiment, modified SSST was developed to identify accessions for resistance to SBPH by reasonable alterations of SSST due to less feeding capacity of SBPH. Based on a number of experimental data, the modified SSST was effective and suitable for SBPH resistance screening of a mass of rice germplasm. The majority of leading japonica varieties in Jiangsu, Zhejiang and Anhui Provinces and North China were susceptible to SBPH, which became a significant reason for SBPH outbreaks in these regions.

SBPH outbreak once occurred in China, Japan and Korea in 1950s and 1960s, causing considerable yield losses. When SBPH broke out in Jiangsu Province, China in 1995, the 1000-grain weight and plant height of severely infested rice reduced 8.92 g and 33.0 cm, respectively, the panicle length and the number of grains per panicle reduced 5.49 cm and 28.5, respectively, and the quantities of a moiety of blighted grains and fully blighted ones rose 36.3% and 3.20%, respectively, resulting in 41.5% of yield reduction [16]. The rice infested by SBPH accounted for 64.4% of the total rice area when its outbreak took place in Hebei Province and Tianjin Municipality, China in 1958, causing 20–30%, even 50–100% of yield losses [17]. Breeding for resistance to SBPH was carried out at one time in Korea [18-20], and subsequently related studies became rare as the infestation of SBPH waned. However, the strong stress resistance and diversity of the SBPH population increased the viability, and the infection of symbiotic bacteria resulted in the higher fecundity [21-22]. Additionally, the favorable climatic conditions, lack of resistant varieties and diverse planting patterns in the recent years increased the survival and development rate of SBPH populations. The interaction of these factors led to the population outbreak, resulting in outbreak of RSV and serious damage to rice production.

RSV could be availably controlled by extirpating SBPH or minimizing its population. Resistance to SBPH and RSV was proved to be closely related in many varieties [23], indicating that SBPH resistance genes and RSV resistance genes could be linked in these varieties. Some resistant varieties detected by the modified SSST, such as Kasalath, DV85, IR36, Zhaiyeqing 8, Daorenqiao and so on were also highly resistant to RSV [24]. It is an effective measure for governing SBPH through host resistance. The varieties with antixenosis against viruliferous SBPH may markedly decrease the chance of sucking and feeding, for instance, the accessions releasing volatile repulsive chemicals could repulse SBPH's settlement and probing and thus greatly reduced the chance of transmitting RSV. Furthermore, even if the entries with antixenosis are likely to increase the planthopper's tentative probing, the chance of transmission of RSV may be still reduced for that successful transfer of RSV needs more than 30 minutes of successive sucking [25]. The antibiosis resistant varieties can cause pests to have abnormal growth and development, thereby decreasing feeding, while tolerance seldom affects insect feeding. Therefore, an understanding of the mechanisms of resistance will be very useful to develop varieties resistant to SBPH and/or RSV.

With the increasing in direct and indirect damages, together with lack of systematic studies on rice resistance to SBPH, it will be a severe threat to rice production in case that the outbreak occurred in other regions. Therefore, it is considerably significant to conduct identification of germplasm resources for resistance to SBPH, resistance heredity and breeding for resistant varieties.

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