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Correlation Analysis of Rice Resistance Against Black-streaked Dwarf Virus Disease (RBSDV) and Small Brown Planthopper (*Laodelphax striatellus* Fallen)

Wang Baoxiang, Wen Yibin, Liu Yan, Wang Kangjun, Dai Huimin, Liu Jinbo, Pu Hanchun, Xu Dayong *

Lianyungang Academy of Agricultural Sciences/Lianyungang Agricultural Research Institute in Jiangsu Xuhuai Region/Collaborative Innovation Center of Modern Crop Production in Jiangsu Province, Liangyungang 222006, China

Abstract To analyze the correlation of rice resistance against black-streaked dwarf virus disease (RBSDV) and small brown planthopper (SBPH; *Laodelphax striatellus* Fallen), 49 rice varieties were conduced field inoculation identification, artificial inoculation identification, antibiosis test and antixenosis test, respectively. There were more varieties with the incidence rate less than 20% in field inoculation. Field inoculation identification was greatly affected by external environment factors, and the incidence rates were significantly lower than that in artificial inoculation identification. In field inoculation and artificial inoculation identification, the incidence rates of indica rice varieties were lower than that of japonica rice varieties. Antibiosis test showed that all japonica rice varieties were susceptible to insect pests, and there were only five indica rice varieties with antibiosis value less than 81%. The antixenosis value of indica rice varieties was significantly lower than that of japonica rice varieties, and the resistance of indica rice varieties against SBPH was higher than that of japonica rice varieties. Correlation analysis of rice resistance against RBSDV and SBPH showed that the incidence rates of varieties in field identification had extremely positive correlation with its antixenosis to SBPH, but was irrelevant to antibiosis of varieties to SBPH. The incidence rates of varieties in artificial inoculation identification had significantly positive correlation with antixenosis and antibiosis of varieties to SBPH. Moreover, antixenosis of varieties had extremely positive correlation with incidence rates in both artificial inoculation identification and field inoculation identification. Therefore, insect-resistant varieties influenced virus transmitting effect of SBPH to a certain extent, and it could reduce the damage of RBSDV by cultivating resistant varieties against SBPH.

Key words Rice; RBSDV; SBPH; Correlation

Small brown planthopper (SBPH), *Laodelphax striatellus* Fallen (Homoptera: Delphacidae), is one of the insect pests causing serious damage on rice, which is widely distributed in rice planting regions in Asian countries. In rice production, in addition to feeding damage caused by SBPH, black-streaked dwarf virus disease (RBSDVD) spread by SBPH has caused more severe yield losses[1-2]. In recent years, RBSDV spread by SBPH is pandemic in Jiangsu and Zhejiang Provinces because of large scale planting of susceptible varieties, large population quantity of SBPH carrying RBSDV, as well as rice and wheat rotation cultivation measure conducive to population reproduction of SBPH[3].

Currently, some domestic scholars have carried out genetic basis research on RBSDVD and SBPH, and a batch of resistant varieties against SBPH have been found, such as RH, Mudgo, ASD7, IR36, IR64, *etc.*; nearly 20 anti-SBPH QTLs have been mapped[4-9]. Wang *et al.*[10] carried out anti-RBSDVD germplasm screening and related gene location, and screened a variety of resistant varieties, such as Kanyakumari 29, Madurai 25 and Vietnam 160, the indica rice varieties from southeast Asia. Pan *et al.*[11-12] analyzed resistance QTL of RBSDVD using recombinant inbred lines population of Zhenshan 97B/Minghui 63, and detected six QTLs in total; fine mapping of a QTL was realized, and the resistance alleles of these loci were from resistance parent Minghui 63. Wang *et al.*[13] constructed molecular linkage map using Tetep/Huaidao 6, and totally four resistance QTLs were detected. However, repeated identification shows that the incidence rates of Minghui 63 and Tetep are above 20%, so it is urgent to excavate highly resistant germplasm resources and locate related resistance genes.

The severity of RBSDVD outbreak is closely associated with the population density of SBPH carrying RBSDV[14-15], and effective control of SBPH population can indirectly control the virus disease spread by SBPH. Therefore, it is particularly important to study the correlation of rice resistance against both RBSDVD and SBPH. We conducted resistance tests of rice varieties against RBSDVD and SBPH, respectively, and analyzed the correlation between these two kinds of resistance, in order to provide important reference for clarifying resistance mechanism of rice against RBSDVD and seeking a new resistance pathway to solve actual production problems.

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* Corresponding author. E-mail: xudayong3030@sina.com

1 Materials and Methods

1.1 Materials Totally 49 rice varieties were used in the test, including 19 indica rice varieties and 30 japonica rice varieties (Tab. 1).

1.2 Methods

1.2.1 Natural inoculation identification of RBSDVD in fields. Materials were planted in Huangchuan experimental field in Donghai County, and the plots surrounded by wheat fields were selected, to ensure adequate insect sources. On May 15, 2013 (3 weeks before wheat harvest), 80 seeds of each variety were sown in a row, with the row length of 50 cm and row spacing of 10 cm. Seedlings were transplanted individually from June 20 to June 25, with the row and plant spacing of 15 cm × 20 cm, 40 holes each variety (line). The plots were under conventional water and fertilizer management, without application of any pesticides. The population density of SBPH was investigated through plant-flapping method[10]. The results showed that the density of SBPH at seedling stage was 2.032 million specimen/667 m^2 in experimental plots, and the initial population quantity was huge.

The incidence rate was statistically calculated at peak tillering stage of rice. Diseased plants at this stage were featured by very obvious dwarf, breakthrough of heart leaf out of leaf sheath; in some plants heart leaf stretched out of lower leaf pillow in helical form, and partial leaves appeared curling at tip. Healthy rice plants had vigorous tiller and strong vitality, but did not elongate yet. There were very obvious contrast between diseased plants and healthy plants. The mean of incidence rates of two repeats was adopted as the anti-RBSDVD phenotypic value of varieties for statistics.

1.2.2 Artificial inoculation identification of RBSDVD. Diseased rice plants at peak tillering stage in field or laboratory were transplanted to plastic drums with the specification of 20.5 cm (length) × 13.5 cm (width) × 13.5 cm (height), and a hole was reserved on the bottom of each plastic drum. Plastic drums were then transferred into plastic boxes, and 1 – 2 instar nymphs of SBHP were accessed into the box for 72 h. The insect quantity was three times of the identified seedlings.

After presoaking and germination-accelerating, the seeds of parent, resistant varieties and susceptible varieties were sown in a straight line in plastic boxes with the specification of 70.5 cm × 50.5 cm × 41.5 cm, 50 seeds each variety, and soil layer was maintained about 3 cm. Seedlings were thinned when grown to 2.5 – 3.0 leaves, weak or sick seedlings were eliminated, and 40 healthy seedlings with consistent growth were retained. Each plastic box was sealed with gauze at the top. The mean of incidence rates of two repeats was adopted as the anti-RBSDVD phenotypic value of varieties for statistics.

1.2.3 Antixenosis test. Antixenosis test was performed according to the method by Nemoto *et al.*[16] with slight improvement. The germination-accelerating rice seeds were sown in plastic boxes with the specification of 65 cm × 44 cm × 14 cm, a row each variety and 25 plants each row. All varieties were arranged randomly, with two repeats. When seedlings were grown to 1.5-2.0 leaves, weak seedlings were eliminated, and 15 seedlings were retained in each row; 2 – 3 instars nymphs of SBPH were inoculated onto seedlings, five nymphs each seedling, and incubated at (26 ± 1) ℃ under natural light. The insect number of individual plants was investigated once every day after 24 h, and insects were repelled after investigation, to ensure uniform distribution as much as possible. The average insect number of individual plant of each variety was calculated after 5 d, which was used as the antixenosis test value.

1.2.4 Antibiosis test. Antibiosis test was conducted following the method by Duan *et al.*[6]. An individual seedling (the sixth day seedling after seed germination) was placed in a tube, and inoculated with five individuals of 1 – 2 instars nymphs. The number of dead insects was statistically calculated at 6 d post inoculation once every day for consecutive 5 d, and the survival rate of nymphs was calculated.

Antibiosis score (AS; %): $AS = [(A_1 \times 1) + (A_2 \times 2) + \cdots + (A_n \times n)]/(1 + 2 + \cdots + n)$.

Here, n stands for the days after insect inoculation; An stands for the survival rate of SBPH nymphs at that day. Ten seedlings of each variety were tested. AS < 81% represented resistance and AS > 81% represented susceptibility. The lower AS value represented stronger antibiosis resistance, and vice versa. The test was performed at (25 ± 1) ℃.

1.3 Detection of virus carried rate of SBPH Referred to the method by Zhou *et al.*[17], RBSDV of 100 specimen of SBPH was detected by RT-PCR. The results showed that the virus carried rate of RBSDV reached 5% on average, indicating virus source was sufficient.

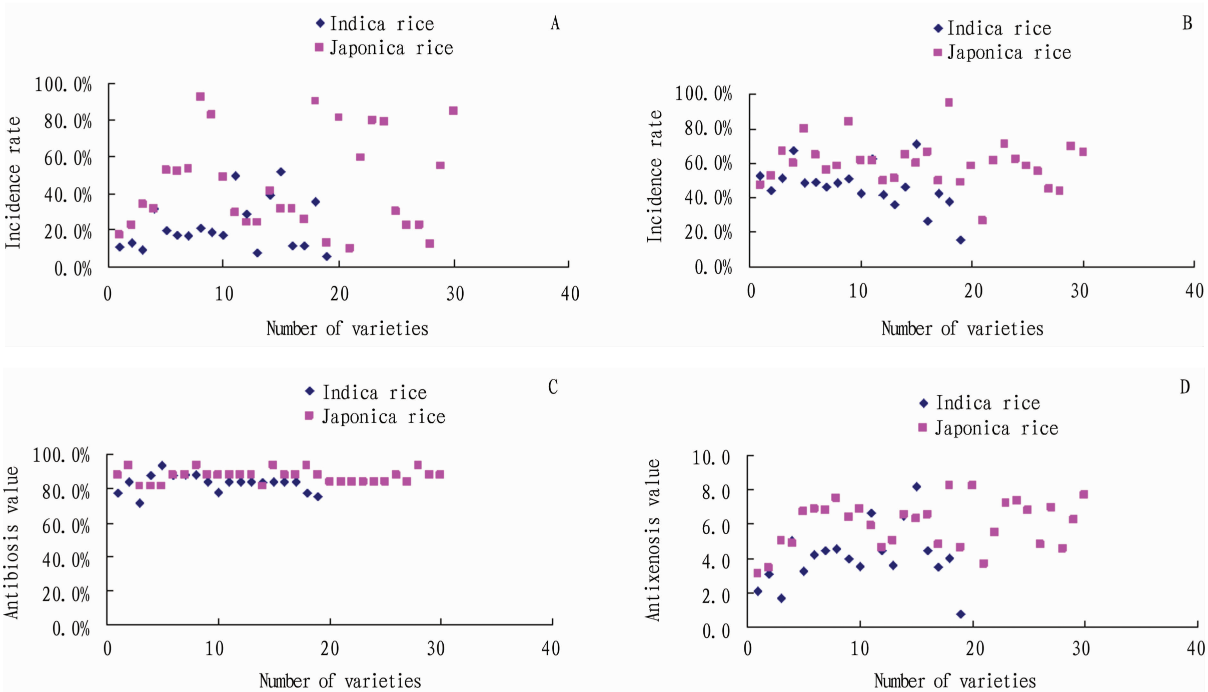
1.4 Data analysis Experimental results at different groups were analyzed using SPSS (Statistical Product and Service Solutions) software.

2 Results and Analysis

2.1 Resistance analysis of varieties against RBSDVD and SBPH Field inoculation and artificial inoculation tests of 49 varieties showed that the incidence rates in artificial inoculation were higher than that in field inoculation, and there was only a variety with the incidence rate lower than 20% (RH). There were more varieties with incidence rates lower than 20% in field inoculation, which had great difference with phenotypic value of artificial inoculation, indicating field inoculation was greatly affected by external environmental factors (Tab. 1, Fig. 1A and Fig. 1B) and the incidence rates were significantly lower than artificial inoculation. Moreover, the incidence rates of indica rice varieties were significantly lower than that of japonica rice varieties in both field inoculation and artificial inoculation (Tab. 1, Fig. 1A and Fig. 1B).

Tab. 1 Resistance responses of different rice varieties against RBSDVD and SBPH

Variety	Type	Source	Incidence rate in field inoculation	Incidence rate in artificial inoculation	Antibiosis value	Antixenosis value
IR58	Indica rice	IRRI	10.6%	52.5%	77.3%	2.1
IR29	Indica rice	IRRI	12.5%	43.8%	84.0%	3.1
Kasalath	Indica rice	India	8.8%	51.3%	72.0%	1.7
Mancang 515	Indica rice	Fujian	31.3%	67.5%	88.0%	5.0
Longtepu B	Indica rice	Fujian	19.4%	48.8%	93.3%	3.3
Shijihuangzhan	Indica rice	Fujian	17.1%	48.8%	88.0%	4.2
Sankecun	Indica rice	Sichuan	16.9%	46.3%	88.0%	4.5
Chengdu'ai 3	Indica rice	Sichuan	21.3%	48.8%	88.0%	4.6
Aituogu 151	Indica rice	Sichuan	18.8%	51.3%	84.0%	4.0
Chengnongshuijing	Indica rice	Sichuan	17.5%	42.5%	77.3%	3.5
Maweinian	Indica rice	Guizhou	50.0%	62.5%	84.0%	6.6
Xiaobaimi	Indica rice	Guizhou	28.8%	41.3%	84.0%	4.5
Xiangwanxian1	Indica rice	Hunan	7.5%	36.3%	84.0%	3.6
Nantehao	Indica rice	Jiangxi	39.4%	46.3%	84.0%	6.5
Xiangzaoxian 7	Indica rice	Hunan	51.9%	71.3%	84.0%	8.1
Muda	Indica rice	Malaysia	10.9%	26.3%	84.0%	4.5
Gropak U1	Indica rice	Indonesia	11.6%	42.5%	84.0%	3.6
Major	Indica rice	Indonesia	35.4%	37.5%	77.3%	4.0
RH	Indica rice	Vietnam	5.0%	15.0%	74.7%	0.8
Shoufenguang	Japonica rice	Japan	17.5%	47.5%	88.0%	3.1
Yangnuo 2	Japonica rice	Taihu lake area	22.5%	52.5%	93.3%	3.5
Suzhouxuan	Japonica rice	Taihu lake area	33.8%	67.5%	81.3%	5.1
Huangdao	Japonica rice	Taihu lake area	31.9%	60.0%	81.3%	4.9
Shuangfeng 2	Japonica rice	Taihu lake area	52.5%	80.0%	81.3%	6.7
Huhui 628	Japonica rice	Zhejiang	52.1%	65.0%	88.0%	6.9
Wuyoudao 1	Japonica rice	Heilongjiang	53.8%	56.3%	88.0%	6.8
Hejiang 19	Japonica rice	Heilongjiang	92.9%	58.8%	93.3%	7.5
Xingguo	Japonica rice	Jilin	82.9%	83.8%	88.0%	6.4
Chimao	Japonica rice	Jilin	48.8%	61.3%	88.0%	6.9
Qitoubaigu	Japonica rice	Yunnan	29.4%	61.3%	88.0%	5.9
Gongju 73	Japonica rice	Yunnan	23.8%	50.0%	88.0%	4.7
Yanjing 5	Japonica rice	Jiangsu	23.8%	51.3%	88.0%	5.0
Gaoyangdiandao Dahongmang	Japonica rice	Hebei	41.0%	65.0%	81.3%	6.5
Shuiyuan 300 li	Japonica rice	Hebei	31.9%	60.0%	93.3%	6.3
Putaohuang	Japonica rice	Tianjin	31.9%	66.3%	88.0%	6.6
Zhonghua 8	Japonica rice	Beijing	25.5%	50.0%	88.0%	4.8
Beijing Jiangmidao	Japonica rice	Beijing	90.0%	95.0%	93.3%	8.2
Jijing 14	Japonica rice	Hebei	12.5%	48.8%	88.0%	4.7
Si Lau(A)	Japonica rice	Malaysia	81.3%	58.8%	84.0%	8.2
Radi Pagalou	Japonica rice	Malaysia	9.8%	26.3%	84.0%	3.7
Jingnong 1	Japonica rice	Beijing	59.4%	61.3%	84.0%	5.5
Aen Petu	Japonica rice	Indonesia	79.5%	71.3%	84.0%	7.2
Djerambangan Bulu	Japonica rice	Indonesia	78.8%	62.5%	84.0%	7.4
Qundut	Japonica rice	Philippines	30.4%	58.8%	84.0%	6.8
xx-4	Japonica rice	Thailand	22.5%	55.0%	88.0%	4.8
Ku101	Japonica rice	Thailand	22.9%	45.0%	84.0%	7.0
GZ 2175-5-6	Japonica rice	Egypt	12.2%	43.8%	93.3%	4.6
E 425	Japonica rice	Senegal	55.0%	70.0%	88.0%	6.2
Wuyujing 3	Japonica rice	Jiangsu	85.0%	66.3%	88.0%	7.7



Note: A. Incidence rates of varieties in field inoculation; B. Incidence rates of varieties in artificial inoculation; C. Antibiosis value; D. Antixenosis value

Fig. 1 Resistance values of different varieties against RBSDVD and SBPH

Tab. 2 Correlation analysis of incidence rate in field identification, incidence rate in artificial identification, antibiosis value and antixenosis value

Items	Incidence rate in field identification	Incidence rate in artificial identification	Antibiosis value	Antixenosis value
Incidence rate in field identification	1	0.728 **	0.249	0.813 **
Incidence rate in artificial identification		1	0.302 *	0.700 **
Antibiosis value			1	0.382 **
Antixenosis value				1

Note: * and ** represent significant and extremely significant differences at 0.05 and 0.01 levels, respectively.

Antibiosis test results of rice varieties to SBPH showed that there were only five indica rice varieties with the antibiosis value less than 81%, and the antibiosis value of japonica rice varieties was higher than 81%, indicating japonica rice varieties were all susceptible to SBPH. The antixenosis values of indica rice varieties were significantly lower than that of japonica rice varieties (Tab. 1, Fig. 1C and Fig. 1D), indicating the resistance of indica rice varieties to SBPH was higher than that of japonica rice varieties.

2.2 Correlation analysis of rice resistance against RBSDVD and SBPH Correlation analysis of rice resistance against RBSDVD and SBPH was conducted using SPSS software, and the results showed that the incidence rates of varieties in field identification had extremely positive correlation with antixenosis to SBPH but were irrelevant to antibiosis of varieties to SBPH, indicating field inoculation was easily affected by antixenosis of varieties to SBPH. In addition, the incidence rate in field inoculation identification had significantly positive correlation with that in artificial inoculation identification, indicating the results of these two identification methods were consistent.

The incidence rate of varieties in artificial inoculation identification had significantly positive correlation with antixenosis and antibiosis to SBPH, so the antixenosis and antibiosis of varieties to SBPH affected artificial identification results. Besides, antixenosis had extremely positive correlation with incidence rate in both field inoculation and artificial inoculation, indicating antixenosis of varieties to SBPH was an important factor affecting identification results.

3 Discussion

Interaction among virus transmitting media, virus and plant leads to occurrence of viral diseases. Much less is known about resistance mechanism against RBSDVD, but related researches have been developed in the study of other viral diseases. Nemoto H., a Japanese scholar, studied rice stripe virus disease (RSVD) spread by SBPH, and found that moderate resistant varieties IR50 and Tadukan had resistance against virus and media insects; he put forward that rice resistance against insects could reduce the spread of RSVD; the resistance of rice against RSVD included the resistance against rice stripe virus (RSV) and the resistance against media SBPH, while the resistance against virus included resistance against virus infection and tolerance to virus[16]. In addition, Tobayashi[18] and Takita *et al.*[19] suggested that the resistance of rice variety IR42 against Tungro virus mainly caused by the resistance to media insects.

In this study, 49 rice varieties from different regions were conducted field inoculation identification, artificial inoculation identification, antixenosis and antibiosis test, respectively. The results showed that the incidence rates of indica rice varieties were significantly lower than that of japonica rice varieties in both field inoculation and artificial inoculation; in antixenosis and antibiosis tests, the resistance of indica rice varieties to SBPH was stronger than that of japonica rice varieties. Therefore, resistance of indica and japonica rice varieties against RBSDVD and virus transmitting media SBPH had certain correlation. Correlation analysis of incidence rate in field inoculation, incidence rate in artificial inoculation, antixenosis value and antibiosis value showed that the incidence rates of varieties had extremely positive correlation with their antixenosis to SBPH; the incidence rates of varieties in field identification had no association with antibiosis of varieties to SBPH, indicating field inoculation was easily affected by antixenosis of varieties to SBPH. Thus, antixenosis of varieties to SBPH is an important factor affecting field identification results. In artificial inoculation identification, the incidence rates of varieties had significantly positive correlation with their antixenosis and antibiosis to SBPH, and the antixenosis and antibiosis of varieties to SBPH affected artificial identification results. Therefore, insect-resistant varieties influence virus transmitting effect of SBPH to a certain extent, and it could reduce the occurrence of RBSDVD by cultivating SBPH-resistant varieties.

Indica rice and japonica rice generally perform different resistance responses to the same viral disease. Yamaguchi *et al.*[20] and Washio *et al.*[21-23] put forward that the resistance source of RSV widely existed in indica rice, javanica and upland rice in Japan, while no resistance source existed in other japonica rice varieties. The incidence rates of indica rice varieties were lower than that of japonica rice varieties in field inoculation and artificial inoculation. Besides, the resistance of indica rice varieties against SBPH was higher than that of japonica rice varieties, indicating stronger resistance of indica rice varieties against RBSDVD than japonica rice varieties possibly attributed to stronger resistance of indica rice varieties to virus transmitting media SBPH. The paper provided an important research clue and basis for correlation of rice resistance against RBSDVD and SBPH.

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prevention and control according to a unified deployment, so that orchards free of prevention measures will not be the infection target of fruit fly. In orchards with comprehensive measures, repel plants of fruit fly can be planted in orchard, or repellent and attractants can be suspended, or luring plants can be planted near orchard.

4 Conclusions

As a top fruit with obvious health care function, blueberry price is firm and market demand is continuously raised. However, the researches on occurrence, development regularity and control of pests and diseases lag behind. The study lasted more than three years, and conducted comprehensive and systematic investigation in major blueberry export base in Guizhou. A total of 21 kinds of pests and diseases on blueberry were found. Although current occurrence of pests and diseases is light, without large scale damage, we need to prepare in advance. In-depth and systematic survey should be conducted in typical orchards in different blueberry planting regions, to understand species of common pests and diseases and their occurrence scope and regularity. Targeted prevention and control should be implemented timely when monitoring and forecasting is well done. Meanwhile, standardized and reasonable cultivation techniques and agronomic measures should be combined together. Overall, correct measures and solid prevention and control work against diseases and pests can effectively improve the yield and quality of blueberry in China, providing a broad development prospect in high-end market and export.

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