Breeding of Maintainer Line and Sterile Line Brown Planthopper Resistant Rice Using Marker-assisted Selection

Hui GUO, Rui FENG, Can CHEN, Xiaoli ZHANG, Bailong LIU, Xueyi QIN*

Rice Research Institute/Guangxi Key Laboratory of Rice Genetics and Breeding, Guangxi Academy of Agricultural Sciences, Nanning 530007, China

Abstract Guangxi common wild rice variety brown planthopper highly resistant introgression line HS204 was taken as antigen donor material, hybridization, backcrossing, and molecular marker-assisted selection methods were adopted, to select maintainer line and sterile line materials, so as to provide excellent material foundation for resistance breeding of hybrid rice. Through the marker-assisted selection, it obtained 4 pieces of homozygous resistance gene maintainer line intermediate materials (100B, 101B, 102B and 103B), and 2 pieces of resistant sterile line materials (100A and 103A). All 10 combinations that have testcross with highly resistant sterile 100A showed higher level of brown planthopper resistance: 100A/R2586, 100A/ KR838, and 100A/KR527 had high resistance level, the others had low to intermediate resistance; 100A/KR527, 100A/R2586, 100A/Minghui 63,100A/Fuhui 838 and 100A/Gui 99 combinations had yield per plant significantly higher than the control group (Teyou 7118), increasing by 14.45% -49.26%. The obtained resistant lines are expected to provide a better gene source for the breeding of resistant sterile lines of hybrid rice and the obtained resistant sterile lines can be directly used in the selection of three-line hybrid rice.

Key words Rice, Brown planthopper resistant variety, Molecular marker-assisted selection, Maintainer line, Sterile line

1 Introduction

Brown planthopper [*Nemaparvatalus* (Stål), Homoptera: Delphacidae] is a major insect pests of rice in southern rice production area of China and rice production areas in Southeast Asia[1]. Brown planthopper has strong reproduction ability, short growth cycle, strong adaptability, and long distance of migration; it is the media for rice virus like grassy stunt virus, so it exerts great harm to normal growth of rice[2]. In 2001 -2010, due to the damage of brown planthopper, about 2467 $\times 10^5$ ha paddy field was affected annually, leading to annual rice loss of 1.12 $\times 10^9$ kg, increasing by 47.2% and 46.0% compared with the average value in 1990 -2000[4]. At present, brown planthopper is mainly prevented and controlled by chemical method[5], which not only increases the cost of rice production, deteriorates environmental pollution, and some pesticides can also stimulate the brown planthopper spawning and induce the drug resistance[6]. Related research indicate that exploring and using brown planthopper resistance gene and selecting and extending brown planthopper resistant rice variety is a cost-saving, safe, and effective approach[7]. The identification and localization of resistance genes are the basis of resistance breeding. By now, it has reported at least 34 brown planthopper resistance sites, in which 28 major resistance genes have been localized, dominant genes *Bph3*, *Bph14*, *Bph26* and recessive gene *bph29* have been successfully cloned[8-9], some of these genes have been applied in improvement of brown planthopper resistance genes. The identification of insect resistance is greatly influenced by environment and it consumes time and efforts. The molecular marker-assisted selection is not affected by insect pests. Combined with conventional breeding methods, the marker-assisted selection (MAS) has become an important way to breeding the brown planthopper resistant varieties[10-11]. In this study, we took Guangxi common wild rice variety brown planthopper highly resistant introgression line HS204 as antigen donor material. Through hybridization and backcrossing, combined with molecular marker-assisted selection method, we put the resistance gene into the maintainer line Longtefu B, then through testcross and backcrossing with Longtefu A, we selected resistant sterile line materials, so as to provide excellent material foundation for resistance breeding of hybrid rice.

2 Materials and methods

2.1 Experimental materials The donor parent was brown planthopper highly resistant HS204, and the brown planthopper resistance gene came from common wild rice in Guangxi and was near-isogenic line selected through many years of hybridization. The receptor parent was the three-line maintainer line Longtefu B. Ptb33 was the pest-resistance control (CK-R), and TN1 was the pest-sensitive control (CK-S). The restorer lines for testcross included Gui 99, Minghui 63, Zhonghui 6, Yanhui 559, R7954, and Fuhui 838; self-fertile restorer line: R2586; self-fertile brown planthopper resistant restorer lines included KR838, KR527, and KR30. The test insect pests came from mixed biotype collected in field of Nanning in Guangxi. They were stored in net room and bred with TN1 for reproduction. They were mainly brown plan-

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* Corresponding author. E-mail: qxueyi@gxaas.net

thopper biotype II, and formed with Vietnam Jiulongjiang type, Bengal type, and biotype I to the mixed biotype.

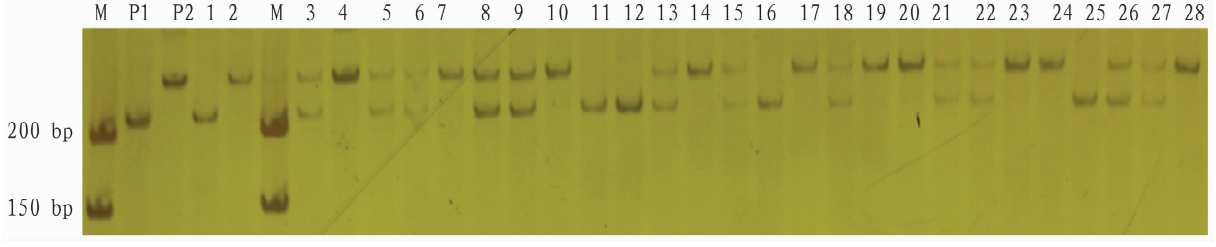
2.2 Detection molecular marker Antigen HS204 brown planthopper resistant genes were located between chromosome molecular marker M4-10 and M4-68. In two parents, SSR marker and M4-10 were polymorphic. With reference to CTAB extraction method, DNA samples were extracted from rice leaves. Using M4-10 (F: 5'-GTGTAGCTGCTAGGCCGAAC-3'; R: 5'-TTCCTTTCGCTACGTTGGAC-3') closely linked with brown planthopper resistant genes, trace and detection were carried out for resistance gene *Bph*(*t*) in segregated population, and strains carrying target genes were selected. The molecular marker primer was synthesized by Beijing Sunbiotech Co., Ltd., and DNA polymerase and dNTPs were bought from Sangon Bitech (Shanghai) Co., Ltd. PCR amplification, electrophoresis and silver staining were carried out with reference to the method of Li Jinbo *et al.* [10], the total volume of PCR amplification was 10 μL, the reaction system contained 10 × PCR Buffer (1 μL), 10 μl dNTP 0.2 μL, 5 U/μLTaq DNA polymerase 0.1 μL, 10 μM upstream and downstream primers (0.2 μL separately), DNA template 1 μL, ddH_2O, adjusted to total volume 10 μL.

2.3 Identification of brown planthopper resistance With the reference to the seedling group screening method[12], proper amount of each strain to be identified was selected, vernalized and drilled in plate, 20 – 25 seeds were sown in each row, when the seedlings grew to 2 – 3 leaves, removed the small and weak seedlings, and kept the healthy and strong seedlings, put 5 – 8 pieces of 2 – 3 instar nymphs for each plant, and made investigation in 7 days after the death of the pest-sensitive control TN1. The pest resistance level was rated according to standard of International Rice Research Institute (IRRI): level 1: no harm or the first leaf slightly turns yellow; level 3: the first and second leaves turn yellow; level 5: the first to the third leaves turn yellow or the plant is dwarfed; level 7: the plant starts to wither; level 9: the plant die. Finally, the average resistance level of each strain was calculated using the following formula: average resistance = Σ (number of plants of each resistance level × corresponding resistance level) / total number of plants. The average resistance level: high resistance (HR): 1.0 – 1.9; resistance (R): 2.0 – 3.9; moderate resistance (MR): 4.0 – 5.9; moderately sensitive (MR): 6.0 – 7.9; highly sensitive (HS): 8.0 – 9.0.

2.4 Detection of agronomic traits of testcross combination The testcross was made for resistant sterile line and restorer line obtained through transformed, Teyou 7118 was taken as control group. In each block, one combination was planted as per 5 rows and 10 plants, repeated three times, collected field data of 3 – 8 plants of the third row in each block. Main indicators included plant height, number of effective panicles, panicle length, number of grains per panicle, seed setting rate, and 1000-grain weight.

3 Results and analyses

3.1 Breeding and improvement of brown planthopper maintainer line In 2011, early rice adopted Longtefu B as female parent and brown planthopper resistant HS204 to hybridize. In the same year, late rice adopted Longtefu B as recurrent parent to backcross. In 2012, early rice was detected using marker M4-10 and M4-68 closely linked with the resistance gene, selected single plant containing target gene to make backcrossing again. Later, combined with molecular marker assisted selection, seedling resistance identification, and field observation of agronomic traits, selected single plant conforming to breeding target to make self-pollination. Finally, it obtained 4 pieces of homozygous resistant gene maintainer line intermediate materials (100B, 101B, 102B and 103B). Fig. 1 shows 28 single plants randomly selected from segregated population of improved Longtefu B using marker detection.



Note: M: DL500 DNA Marker; P1: donor parent HS204; P2: receptor parent Longtefu B; 1 – 28: BC_2F_2 of segregated population.

Fig. 1 Results of molecular marker M4-10 detection of BC_2F_2 generation single plant

3.2 Breeding of brown planthopper sterile line In order to shorten the breeding time, the BC_2F_3 generation improved maintainer line was tested and selected for sterility maintaining ability from the late 2013. Using testcross with Longtefu A corresponding to the maintainer line to be improved, the next rice adopted strains with infertility rate above 99.99%, made the molecular marker, and selected resistant strains with excellent agronomic traits to conduct continuous backcrossing. By 2016, the early rice obtained BC_5F_1 generation resistant sterile materials 100A and 103A, and obtained BC_2F_8 generation resistant maintainer line 100B and 103B.

3.3 Identification of improved maintainer line and testcross combination brown planthopper resistance After several years of continuous breeding, by 2015, the improved resistance maintainer line had reached BC_2F_7 generation, and the agronomic traits were basically stable. In the seedling resistance identification of 2014 and 2015 consecutive years (Table 1 and Fig. 2), the resistance level of improved maintainer lines 100B and 103B was lower than level 2, reached the high resistance level. This indicated that

the improved maintainer lines 100B and 103B had stable resistance to brown planthopper, and the agronomic traits were basically stable after many generations of self-pollination.

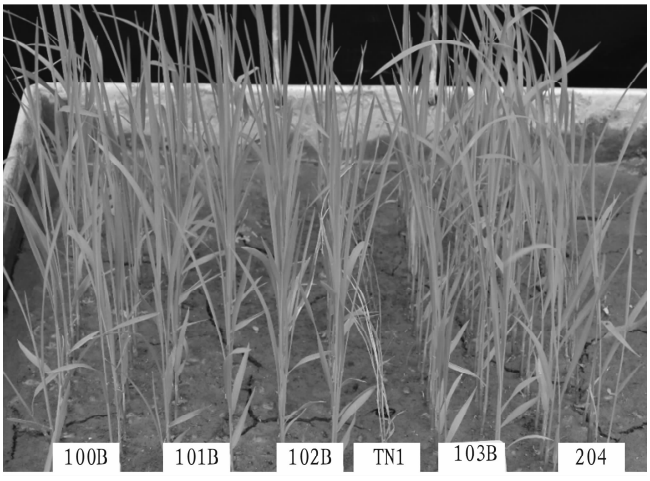


Fig. 2 Identification of improved maintainer line brown planthopper resistance

From Table 2, it can be known that the resistance of sterile line 100A to brown planthopper was close to high resistance level, and its testcross combinations showed different resistance level. The testcross hybrid with the restorer lines Gui 99, Minghui 63, Zhonghui 6, Yanhui 559, and R7954 (No. 1 – 5) reached the resistance level, the testcross hybrid with the restorer line R2586 (self-pollination) was close to high resistance level, the testcross hybrid with the restorer line Fuhui 838 reached moderate resistance level. The resistance levels of the testcross hybrid with self-pollination resistant restorer lines KR838, KR527 and KR30 (No. 8 – 10) was lower than 3, 2 of which reached the high resistance level. The testcross hybrid with self-pollination sterile line Chuang IA (highly sensitive to brown planthopper), Minghui 63 and Zhonghui 6 (No. 11 – 12) showed high sensitiveness to brown planthopper. The different resistance level of these combinations indicated that the resistance gene may have effective expression in the different context of hybrid rice, while the difference in resistance level indicated that there is difference in resistance expression, and both parents have resistance and can effectively improve the resistance level of the hybrid combinations.

Table 1 Identification of brown planthopper resistance of 2014 – 2015 seedlings

Year	Line	Number of seedlings	Resistance level					Average resistance level
			1	3	5	7	9	
2014	100B	38	29	8	0	0	1	1.63
	103B	40	23	17	0	0	0	1.85
	CK-R	20	14	2	1	0	3	2.60
	CK-S	18	0	0	0	0	18	9.00
2015	100B	37	28	9	0	0	0	1.49
	103B	43	28	15	0	0	0	1.70
	CK-R	21	14	2	1	1	3	2.81
	CK-S	20	0	0	0	0	20	9.00

Table 2 Identification results of brown planthopper resistance of combinations in seedling stage

No.	Combination	Average resistance level	Resistance level
1	100A/Gui 99	3.32 ± 0.27	Resistant
2	100A/Minghui 63	3.56 ± 0.50	Resistant
3	100A/Zhonghui 6	2.81 ± 0.25	Resistant
4	100A/Yanhui 559	3.75 ± 0.46	Resistant
5	100A/R7954	2.52 ± 0.28	Resistant
6	100A/R2586	1.79 ± 0.14	Highly resistant
7	100A/Fuhui 838	4.19 ± 0.30	Moderately resistant
8	100A/KR838	1.88 ± 0.14	Highly resistant
9	100A/KR527	1.95 ± 0.25	Highly resistant
10	100A/KR30	2.27 ± 0.33	Resistant
11	Chuang IA/Minghui 63	9.00 ± 0.00	Highly sensitive
12	Chuang I A/Zhonghui 6	8.9 ± 0.17	Highly sensitive
13	100A	1.49 ± 0.02	Highly resistant
14	TN1	9.00 ± 0.00	Highly sensitive

3.4 Comparison of agronomic traits of combinations From Table 3, it can be seen that the number of effective panicles of single plant of only combination 100A/Gui 99 in 10 combinations was significantly higher than the control group; except 100A/Yanhui 559 had no significant difference with the control group, the actual grains per panicle of rest combinations were significantly or extremely significantly lower than the control group; the single plant yield of combinations 100A/KR527, 100A/R2586, 100A/Minghui 63, 100A/Fuhui 838 and 100A/Gui 99 was significantly higher than the control group, with the growth rate of 14.45% –

49.26%, indicating that most combinations have excellent yield level in the same planting conditions.

Table 3 Performance of agronomic traits of combinations

Combination	Plant height//cm	Number of effective panicles	Actual grains per panicle	1000-grain weight//g	Seed setting rate//%	Single plant yield//g
100A/Gui 99	112.1 ± 2.2	10.1 ± 1.9*	192.1 ± 21.3**	24.3 ± 0.6**	83.3 ± 3.7	50.6 ± 5.6**
100A/Minghui 63	127.4 ± 1.6	6.9 ± 1.2	226.9 ± 19.8**	27.1 ± 0.8**	88.3 ± 2.0	39.1 ± 3.8*
100A/Zhonghui 6	117.7 ± 1.5	7.1 ± 1.1	187.8 ± 12.4**	28.0 ± 0.4	79.5 ± 4.2**	34.5 ± 4.2
100A/Yanhui 559	97.1 ± 1.6	8.6 ± 0.9	154.3 ± 10.1	27.6 ± 0.3	81.4 ± 2.9**	36.3 ± 6.4
100A/R7954	124.2 ± 2.0	7.1 ± 1.1	207.9 ± 8.3**	25.5 ± 0.6**	80.5 ± 3.3**	37.2 ± 3.9
100A/R2586	114.2 ± 2.0	7.4 ± 1.1	224.6 ± 13.1**	25.3 ± 0.9**	79.4 ± 2.6**	39.9 ± 4.3*
100A/Fuhui 838	120.1 ± 1.9	8.2 ± 1.1	212.1 ± 17.5**	27.9 ± 0.6	86.2 ± 3.6	47.0 ± 5.3**
100A/KR30	122.3 ± 1.2	6.6 ± 0.7	194.4 ± 12.5**	23.2 ± 0.5**	79.8 ± 3.1**	28.0 ± 4.4
100A/KR838	95.6 ± 1.6	7.3 ± 1.0	179.1 ± 18.0*	26.3 ± 0.4**	83.1 ± 1.8	33.8 ± 3.4
100A/KR527	125.1 ± 1.7	7.5 ± 1.3	231.1 ± 22.3**	24.2 ± 0.7**	82.3 ± 3.1*	38.8 ± 5.0*
Teyou 7118 (CK)	115.5 ± 1.3	8.6 ± 1.2	143.1 ± 9.3	27.7 ± 0.4	86.1 ± 2.5	33.9 ± 4.6

Note: * denotes significant difference at 0.05 level, and ** denotes significant difference at 0.01 level.

4 Conclusions and discussions

At present, the molecular marker-assisted selection (MAS) has been widely applied in rice resistance breeding and has successfully cultivated a good number of excellent disease and insect pest resistant lines. Liu Kaiyu *et al.*[13-14], combining methods of backcrossing, molecular marker-assisted selection and insect pest resistance identification, introduced brown planthopper resistant genes *Bph3* and *Bph24*(t) into main cultivar hybrid rice restorer lines Guanghong 998, 9311, R15, Minghui 63, and R29; besides, it obtained bacterial blight and brown planthopper resistant *Xa21Xa23Bph24*(*t*) and *Xa23Bph24*(*t*), and dual resistant lines reached the high level of resistance to rice bacterial blight and reached highly sensitive level in the resistance to the brown plant hopper. Using the molecular marker-assisted marker selection method, Hu Jie *et al.*[15] introduced brown planthopper resistant genes *Bph14* and *Bph15* into parents of Mingyou 63 and Zhenshan 97B of Shanyou 63, and found that when both parents contain the resistant genes, the resistance level was higher than only one parent contains the resistant gene, which is similar with our research results. The wild rice is rich in excellent genes, and the genetic diversity of common wild rice in Guangxi is very rich both in population and intraspecific level[16]. In bacterial leaf-blight, rice blast, bacterial stripe, brown planthopper, and *Sogatella furcifera*, it also has rich resources[17-20]. At the same time, some important genes such as *Xa23*, *Bph14* and *Bph15* have played an essential role in production. Selection of excellent antigen is the first issue to be solved in resistance breeding. In this study, the antigen came from Guangxi common wild rice highly resistant introgression line HS204. Using this antigen, our research team has made resistance improvement in many restorer lines[21], which showed excellent performance. Through several years of continuous identification and analysis, we determined that this antigen contains dominant brown planthopper resistant genes with genetic stability. Using this antigen as the donor, through backcrossing and hybridization with the maintainer line Longtefu B, using the molecular marker-assisted selection and seedling identification, we rapidly screened homozygous resistant lines. In the low generation of improved descendant, we started to combine lines with better agronomic traits with sterile line Longtefu A, made backcrossing for lines that have maintenance ability for sterile lines, and combined with the molecular marker-assisted selection and seedling stage identification, we obtained the improved sterile line with stable resistance after several years of backcrossing. The obtained resistant lines and improved intermediate materials are expected to provide excellent gene sources for breeding of hybrid rice sterile lines, and can be directly used in the selection of three-line hybrid rice.

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application, and accurately transmit the measurement data to remote management platform. The PLR of the network is low, and it can meet the needs of actual operation.

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