Improvement of bacterial blight and brown planthopper resistance in an elite restorer line Huazhan of *Oryza*

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Abstract: Development of resistant varieties is one of the most economical and effective strategies to prevent rice from bacterial blight disease (BB) and brown planthopper (BPH), the two main pests jeopardizing rice production. The hybrid rice Tianyouhuazhan (TianfengA/Huazhan) has been widely used in rice production in China, but this hybrid is susceptible to BB and BPH. In this study, one BB resistance gene (*Xa23*) and two BPH resistance genes (*Bph14* and *Bph15*) were simultaneously introgressed into the restorer line Huazhan to improve the BB and BPH resistance of Tianyouhuazhan using marker-assisted backcrossing (MABC) strategy coupled with phenotypic selection. The results of identification of BB and BPH resistance revealed that almost all of the improved restorer lines and their derived hybrids showed high resistance (HR) or resistance (R) to BB and BPH. Almost all of the agronomic traits of improved restorer lines and their hybrids were similar to those of their respective original versions in field trial, except the 1000-grain weight of improved hybrids showed an extremely significant increase than that of Tianyouhuazhan. Plot yields test in 2013 and 2014 suggested that two improved hybrid combinations named as TianfengA/R43-06 and TianfengA/R43-07 had higher grain

yields per plot than Tianyouhuazhan and other improved hybrids. These results clearly indicate that pyramiding of *Xa23*, *Bph14* and *Bph15* genes is a useful approach for improving BB and BPH resistance, two hybrid combinations TianfengA/R43-06 and TianfengA/R43-07 could replace Tianyouhuazhan for extension in BB and/or BPH epidemic area in China.

Key words: Hybrid Rice; Restorer Line; Bacterial Blight Resistance; Brown Planthopper Resistance; Gene Pyramiding

1. Introduction

Rice is one of the most important staple food crops for more than half of the world's population. In China, hybrid rice has accounted for 57% of the total rice planting area and contributes 65% of the total rice yield (Yuan 2014). Bacterial blight disease (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious rice diseases for both inbred and hybrid rice in Asia and can cause a reduction of rice yield by 20% to 30% and in some severe cases by up to 80% (Mew et al. 1993; Khan et al. 2014). Meanwhile, the brown planthopper (BPH) (*Nilaparvata lugens* Stål) is one of the most destructive insect pest of rice in Asia, which not only causes direct damage to rice plants by sucking the sap from the rice phloem, but also causes indirect damage by transmitting viral diseases such as grassy stunt virus and ragged stunt virus (Jena and Kim 2010; Cheng et al. 2013). The statistic data show that the annual direct losses of rice production in China caused by the BPH can be 1 to 1.5 billion kg (Cheng et al. 2013). Not surprisingly, development of resistant variety is the most economical, environmentally safe and effective strategy to control BB and BPH. However, the dominant rice varieties planted in China are less in resistance to both BB and BPH, especially hybrid rice cultivars;

more seriously, the rate of resistant variety is gradually declining year by year because breeders pay more attention to yield and quality traits (Zhang 2009; Deng et al. 2011). Thus, there is an urgent need to improve the resistance of hybrid rice to BB and BPH.

Introgression of resistance genes into parents using marker-assisted backcrossing (MABC) strategy is a fast and highly efficient breeding method to improve diseases and insects resistance of hybrid rice. Until now, at least 39 genes related to BB resistance have been identified from cultivated and wild species of *Oryza* (Khan et al. 2014; Zhang et al. 2015). Nine of the 39 genes have been successfully cloned, i.e. *Xa1*, *xa5*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, *Xa26* and *Xa27* (Khan et al. 2014; Tian et al. 2014; Liu et al. 2015; Wang et al. 2015), but only a few genes have been widely used in the BB resistance breeding programs (Ding 2005; Zhang 2009; Rao et al. 2014). *Xa4* gene was utilized in almost all of the commercial inbred and hybrid cultivars of *indica* rice in main rice-growing regions of China; now, the resistance to BB conferred by this gene has completely been lost in most rice planting area due to monoculture of *Xa4* varieties (Zhang 2009). *Xa21* gene has been widely used also in commercial rice cultivars in China (Cao et al. 2003; Khan et al. 2014), but this gene has no application values in Guangdong and Guangxi province of China where the dominant race IV and race V can infect *Xa21* varieties (Zeng et al. 2002; Zhang 2009; He et al. 2011). *Xa7* gene, which carries high resistance to most strains of BB, was also utilized in a few BB-resistant cultivars from China and Phillipines (Vera Cruz et al. 2000; Ding 2005; Webb et al. 2010). Unfortunately, the strains virulent to *Xa7* varieties had been isolated from the diseased leaves collected from paddy fields in China and Phillipines (Liu et al. 2004, 2007). Amongst all the known BB resistance genes, *Xa23* gene identified from *Oryza rufipogon* is the most promising one for breeding BB-resistant cultivars because of its broad resistance spectrum and highly resistant level during all the growth stages (Zhang et al. 1998,

2001). In recent years, *Xa23* gene has been introduced into several restorer lines, and their hybrid combinations have been produced (Li et al. 2006a; Zhou et al. 2011). But there is no report till now that the hybrid rice cultivars with *Xa23* gene have been utilized in large scale rice production.

Up to now, 28 genes for BPH resistance have been identified (Jena and Kim 2010; Fujita et al. 2013; Huang et al. 2013; Wu et al. 2014), in which 20 genes have been mapped onto different chromosomal locations and three genes including *Bph2* (*Bph26*), *Bph3* and *Bph14*, have been cloned (Du et al. 2009; Tamura et al. 2014; Liu et al. 2015). However, only a few genes have been widely used in breeding BPH resistance varieties (Jena and Kim 2010; Fujita et al. 2013). Since 1973, four BPH-resistant varieties IR26, IR36, IR56 and IR66, which harbor *Bph1*, *bph2*, *Bph3* and *bph4*, respectively, were released in Asia and effectively alleviated the BPH damages (Cruz et al. 2011; Deng et al. 2011). Now, these varieties carrying *Bph1* or *bph2* lose resistance to BPH in China (Huang et al. 2014). Recently, new BPH-resistant lines harboring one to three genes, including *Bph6*, *Bph12*, *Bph14*, *Bph15* or *Bph18*, have been developed by marker-assisted selection (MAS), and the lines bearing two or three genes had stronger resistance to BPH than the lines bearing only one gene (Hu et al. 2012; Qiu et al. 2012; Hu et al. 2013). *Bph14* and *Bph15* identified from the wild rice *Oryza officinalis* conferred resistance to BPH biotype I, II and III (Huang et al. 2001). The *Bph14* and *Bph15* genes have been widely utilized in breeding BPH-resistant varieties in China now, and some varieties have been released in rice production, such as the male sterile lines Luohong4A and Bph68S carrying *Bph14* and *Bph15*, the restorer line R476 and its hybrid rice Guangliangyou476 carrying *Bph14* only (Xia et al. 2010; Li et al. 2011; Zhu et al. 2013a, b). These reports suggest a good future that *Bph14* and *Bph15* genes are very useful in developing BPH-resistant cultivars in China.

Gene pyramiding is one of the most effective solutions to diseases and insects resistance

breakdown. The pyramided lines stacking multiple BPH or BB resistance genes showed broader spectrum, higher level and more durable resistance than the lines with only one gene (Singh et al. 2001; Sharma et al. 2004; Perez et al. 2008; Hu et al. 2012; Huang et al. 2012; Hu et al. 2013; Suh et al. 2013; Win et al. 2013). Moreover, new conventional varieties and hybrid combinations harboring several resistant traits could be developed by pyramiding multiple resistance genes for different traits. To date, BB and blast resistance genes have been pyramided into commercial varieties and hybrid rice parental lines through MABC (Zhan et al. 2012; Hari et al. 2013; Pan et al. 2013). But to our knowledge, there are few reports on simultaneous introgression of BB and BPH resistance in hybrid rice parental lines. Li et al (2006a, 2006b) obtained a pyramided restorer line named as R106 stacking *Xa23*, *Bph14* and *Bph15* in the genetic background of 9311 using multiple crosses and backcross, but from which there was no hybrid combination registered in China. Chen et al. (2013) pyramided *Xa23* and *Bph18* into Minghui86, Shuhui527 and Zhehui7954 by MABC, respectively, but there was no report about BB and BPH resistance of hybrid combinations from improved restorer lines till now.

Huazhan is an elite male parent for two-line hybrids (Lin et al. 2014b) and a restorer line for wild abortive cytoplasmic male sterile (WA-CMS) three-line hybrids (Yu et al. 2009; Xu 2013; Xu and Lin 2013; Lin et al. 2014a). Hybrid combinations derived from Huazhan, such as Tianyouhuazhan, Yueyouhuazhan, Rongyouhuazhan, Xinrongyouhuazhan, Wuyouhuazhan, Ciliangyouhuazhan, Longliangyouhuazhan and Jingliangyouhuazhan, have been registered by National and/or Provincial Rice Variety Authorization Committee and widely planted in China due to their high yield potential, wide adaptability and good grain quality. In particular, Tianyouhuazhan (a hybrid of TianfengA and Huazhan) has been widely grown in the south and the middle-lower Yangtze River regions of China, and has been designated as check variety in national and some provincial regional trials, because of its

wide adaptability and steady yields. However, Huazhan and Tianyouhuazhan are susceptible to BB and BPH, which lead to limited application area in rice production (Yu et al. 2009; Li et al. 2012). In this study, we successfully pyramided *Xa23*, *Bph14* and *Bph15* genes into Huazhan using MABC strategy coupled with phenotypic selection, and obtained new restorer lines and their hybrids with improvement in both BB and BPH resistance.

2. Materials and Methods

2.1. Rice materials

R106 (Li et al. 2006a, 2006b), derived from 9311///9311/CBB23//1826/B5, was used as resistant control for BB inoculation and the donor of BB resistance gene (*Xa23*) and BPH resistance genes (*Bph14* and *Bph15*). Huazhan, which is widely utilized as a male parent in hybrid rice breeding, but susceptible to BB and BPH (Li et al. 2012), was used as the recipient of three resistance genes and the recurrent parent. TianfengA (Chen et al. 2006), a WA-CMS line, was used as the female parent to produce hybrid combinations. Tianyouhuazhan (Yu et al. 2009) was used as a control hybrid rice variety. B5 (Huang et al. 2001) and TN1 was used as a highly resistant and susceptible control for BPH infestation, respectively.

2.2. Molecular markers and breeding scheme

M-*Xa23*, located on chromosome 11, was a codominant sequence tagged site (STS) marker of 0.4 cM apart from the *Xa23* locus (Gao et al. 2010). Bph14-2dom, a dominant and functional molecular marker used to detect *Bph14* gene before 2014, was designed in this study by comparing the sequence of *Bph14* with the sequence of Nipponbare in NCBI. The DNA oligo primers for amplifying

Bph14-2dom were Bph14-2domF (5'-AGACCAGAAGCTATTCGGACTCC-3') and Bph14-2domR (5'-TGTGAAGGGTGTGTGTGTGTCAC-3'). Bph14P/Bph14N, the codominant and functional molecular markers used to detect *Bph14* gene in 2014, were designed according to the promoter and LRR regions of *Bph14* gene (Zhou et al. 2013). MS5, located on chromosome 4, is a codominant SSR marker with the genetic distance of 0.3 cM from *Bph15* gene (Yang et al. 2004). The *Xa23*, *Bph14* and *Bph15* genes were simultaneously introgressed into an elite restorer line Huazhan following the MABC procedure (Fig.1) combined with phenotypic selection.

Fig. 1 MABC procedure for developing new restorer lines improved in BB and BPH resistance.

2.3. *Xoo* strain and evaluation of BB resistance at booting stage

2.3.1. *Xoo* strain: Zhe173 collected from China belonged to IV pathotype, provided by Hunan Rice Institute. PXO86 and PXO99 are representative pathotypes in Philippines, provided by Shanghai Jiao Tong University. The other nine strains collected from China, named as YN18, YN1, GD414, HeN11, ScYc-6, YN7, YN11, FuJ and YN24, are representatives of nine virulent pathotypes belonging

to R1 to R9 (Liu et al. 2007), provided by Nanjing Agricultural University.

2.3.2. Incubation and inoculation of *Xoo* strain: twelve representative strains belonging to different pathotypes were incubated in NA liquid medium with shaking at 160 r/min and at 28 °C for 24 to 36 hours (Wu et al. 2007). The bacterial cell suspensions with concentrations of 3×10^8 cfu/mL were used to inoculate the rice plants by leaf-clipping method (Wu et al. 2007). The rice materials were sowed at Langli Experimental Station of our institute (Changsha, Hunan province, China) on June 15, 2014, and then 30 plants of each line were transplanted in field in space of 16.7cm $\times$ 20cm on July 12, 2014. Each material was inoculated for ten flag leaves at booting stage, and the LL (lesion lengths) was measured 21 days post-inoculation, then reaction of rice plant to BB was determined by the mean LL as described by Huang et al. (2012).

2.4. Propagation of BPH nymphs and evaluation of BPH resistance at seedling stage

2.4.1. Propagation of BPH nymphs: The BPH insects used for infestation were collected from early season rice fields at Changsha, Hunan province, China in June, 2014, and maintained on plants of rice variety TN1 at vigorous tillering stage.

2.4.2. Evaluation of BPH resistance: Modified bulk seedling test was used to evaluate BPH resistance of improved restorer lines and their hybrids (Li et al. 2006b). All rice materials were germinated in an incubator at 25°C on August 19, 2014 and the seedlings with similar and healthy growth of each material were transplanted after 7 days in 60 cm (length) $\times$ 40 cm (width) $\times$ 10 cm (height) plastic trays in greenhouse. Each material included three replications, and each replication contained 24 plants transplanted in two rows; the space of every replication was 8 cm, the row space within a replication was 4 cm. At three-leaf stage, the seedlings were infested with the 2nd ~ 3rd-instar

nymphs on density of eight to ten insects per seedling. When 95% of seedlings of TN1 were withered, the BPH resistance was evaluated according to Liu et al. (2002): resistance score = 0 and resistance level = immune (IM) if dead seedling rate $\leq 1.0\%$; resistance score = 1 and resistance level = high resistance (HR) if dead seedling rate between $1.1\% \sim 10.0\%$; resistance score = 3 and resistance level = resistance (R) if dead seedling rate between $10.1\% \sim 30.0\%$; resistance score = 5 and resistance level = medium resistance (MR) if dead seedling rate between $30.1\% \sim 50.0\%$; resistance score = 7 and resistance level = medium susceptible (MS) if dead seedling rate between $50.1\% \sim 70.0\%$; resistance score = 9 and resistance level = susceptible (S) if dead seedling rate $\geq 70.1\%$.

2.5. Evaluation of agronomic trait and plot yield

To evaluate the agronomic traits under natural field condition, variety comparative test was continuously conducted in 2013 and 2014 at Langli Experimental Station of our institute (Changsha, Hunan province, China). The improved hybrids and Tianyouhuazhan (check hybrid) were sowed on June 15, and then transplanted in fields on July 12 of every year. Each hybrid contained three plots, with each plot layout in randomized complete block design that contained 50 plants in five rows (17 cm within-row space and 20 cm between-row space) in 2013; in addition, each plot consisted of 100 plants in ten rows in 2014. The improved restorer lines and Huazhan were sowed on May 29 and then transplanted on June 25, 2014, whereas the other field designs were the same as those in 2013. Normal water and fertilizer management, disease and pest control were conducted. The heading dates of the tested lines were recorded in fields, and the days from seeding to heading (DSH) was calculated based on the sowing and heading date. The plant height (PH) of three normal plants in middle of the central row in each plot were measured at fully mature stage, and then these three plants were harvested to

evaluate other agronomic traits in laboratory, including panicle length (PL), panicles per plant (PP), spikelet per panicle (SP), seed set (SS), 1000-grain weight (KW) and yield per plant (YP). The left plants of each plot were harvested and mixed with the grains of the three plants to assess grain yield per plot (GYP). The software DPS (V14.10) was used to determine significance of differences in agronomic traits and plot yields by least significant difference (LSD) method.

3. Results

3.1. Introgression of BB and BPH resistance genes

The entire breeding flowchart is shown in Fig.1. The BB- and BPH-resistant improvement of Huazhan was initiated in the spring of 2011 in Hainan, China, using Huazhan as a recurrent parent and R106 as the donor of *Xa23*, *Bph14* and *Bph15* genes. By use of MAS cooperated with phenotypic selection, three plants carrying three resistance genes were selected from 51 plants on the BC₁F₁ generation, two individuals with three introgressive genes were picked from 35 individuals on the BC₂F₁ generation, and three plants possessing the target genes were screened from 42 plants on the BC₃F₁ generation, successively and respectively. In order to obtain lines homozygous in these three genes, 300 plants on the BC₂F₂ generation were planted in Hainan, China in the spring of 2013, and 12 individuals harboring *Xa23*, *Bph14* and *Bph15* genes were selected out by M-*Xa23*, Bph14-2dom and MS5 markers respectively, among which *Xa23* and *Bph15* genes were homozygous (Fig.2a-c); 300 BC₃F₂ plants from three BC₃F₁ plants were grown in Changsha in the summer of 2013, and three individuals with *Bph14* gene, homozygous *Bph15* gene and homozygous *Xa23* gene were picked up using the same DNA markers coupled with phenotypic selection. In the spring of 2014, twelve improved lines including R43-01 ~ R43-12 were identified to be homozygous in BB and BPH

resistance genes on the BC₂F₄ generation (Fig.2e), and three improved lines (R43-13 ~ R43-15) homozygous in three resistance genes were selected out on the BC₃F₃ generation by use of M-Xa23, Bph14P/ Bph14N and MS5 marker respectively (Fig.2d).

Fig. 2 Detection of *Xa23*, *Bph14* and *Bph15* genes on the generation of BC₂F₂ (a, b, c), BC₃F₃ (d) and BC₂F₄ (e). (a): a STS marker M-Xa23 was used for detecting *Xa23*; (b): a dominant and functional marker Bph14-2dom was used for detecting *Bph14*; (c): a SSR marker MS5 was used for detecting *Bph15*. Lane R and H are R106 and Huazhan, respectively. Lanes 1 to 22 represent 22 individual plants of improved restorer lines. For *Xa23* gene, lanes 1, 4, 7, 9, 17, 18 and 19 are homozygous, and lane 2, 3, 5, 10, 12, 14, 15, 16, 21 and 22 are heterozygous; for *Bph14* gene, all the lanes have except for lane 1, 4, 7 and 20; for *Bph15* gene, lane 3, 5, 10, 11, 14 and 18 are homozygous, and lane 4, 7, 8, 9, 12, 13, 15, 16 and 22 are heterozygous. (d) and (e): a codominant and functional marker Bph14N/ Bph14P were used for detecting *Bph14*. Lane R and H are R106 and Huazhan, respectively. Lane 1-1 to 3-2 and Lane 5-1 to 7-2 represent 44 individuals from 6 improved restorer lines; lane 1-2, 1-6, 1-7, 1-9, 2-2, 2-5, 2-9, 2-10 and 5-1 ~ 7-2 are homozygous, lane 1-1, 1-5, 1-8, 2-1, 2-6, 2-7, 2-8 and 3-2 are heterozygous.

3.2. Evaluation of bacterial blight resistance

The results of BB resistance on basis of the mean LL are shown in Table 1 and Table 2. TianfengA

was found to be resistant to only three *Xoo* strains including YN7, YN11 and YN18, and be moderately or highly susceptible to the other nine *Xoo* strains with average LL ranging from 10.92 to 17.80 cm; both Huazhan and Tianyouhuazhan showed medium resistant (MR), resistant (R) or highly resistant (HR) level of resistance to only four *Xoo* strains such as ScYc6, YN7, YN11 and YN18, and showed medium susceptible (MS), susceptible (S) or highly susceptible (HS) to the other eight *Xoo* strains, with mean LL ranging from 6.88 to 21.18 cm and 8.64 to 17.58 cm, respectively. However, R106 and fifteen improved restorer lines with *Xa23* gene showed very high level of resistance to all twelve *Xoo* strains with LL below 1.0 cm, and the fifteen hybrids from improved restorer lines exhibited R or HR level of resistance to twelve *Xoo* strains with LL under 3.0 cm. Amongst fifteen improved hybrid combinations, TianfengA/R43-04, TianfengA/R43-03 and TianfengA/R43-07 were with higher level of resistance to BB than other hybrids (Table 2; Fig.3). Overall, the improved restorer lines showed slightly higher level of resistance to BB than their corresponding hybrids. With the introgression of *Xa23* gene, the resistance levels of improved restorer lines and their hybrids were promoted from at least MS to R or HR for the eight *Xoo* strains, and the resistance levels for the other four *Xoo* strains were sustained HR or promoted to HR. It indicates that the introgression of *Xa23* gene to restorer line is an effective method to promote BB resistance of hybrids.

Table 1 BB resistance levels of the fifteen improved restorer lines, gene donor and recurrent parent^a

Line ^a	<i>Xoo</i> strain											
	Zhe173	PXO86	PXO99	FuJ	GD414	HeN11	ScYc6	YN1	YN7	YN11	YN18	YN24
R43-01	0.20 ^b (HR) ^c	0.20 (HR)	0.13 (HR)	0.12 (HR)	0.15 (HR)	0.25 (HR)	0.12 (HR)	0.13 (HR)	0.10 (HR)	0.13 (HR)	0.10 (HR)	0.33 (HR)
R43-02	0.12 (HR)	0.25 (HR)	0.12 (HR)	0.13 (HR)	0.22 (HR)	0.18 (HR)	0.12 (HR)	0.12 (HR)	0.15 (HR)	0.13 (HR)	0.17 (HR)	0.17 (HR)
R43-03	0.13 (HR)	0.18 (HR)	0.15 (HR)	0.13 (HR)	0.18 (HR)	0.13 (HR)	0.10 (HR)	0.10 (HR)	0.13 (HR)	0.15 (HR)	0.12 (HR)	0.27 (HR)
R43-04	0.27 (HR)	0.17 (HR)	0.12 (HR)	0.12 (HR)	0.17 (HR)	0.20 (HR)	0.18 (HR)	0.17 (HR)	0.10 (HR)	0.12 (HR)	0.12 (HR)	0.27 (HR)
R43-05	0.12 (HR)	0.25 (HR)	0.10 (HR)	0.10 (HR)	0.10 (HR)	0.17 (HR)	0.10 (HR)	0.13 (HR)	0.12 (HR)	0.15 (HR)	0.13 (HR)	0.27 (HR)
R43-06	0.18 (HR)	0.15 (HR)	0.15 (HR)	0.20 (HR)	0.18 (HR)	0.22 (HR)	0.12 (HR)	0.13 (HR)	0.10 (HR)	0.10 (HR)	0.10 (HR)	0.28 (HR)
R43-07	0.13 (HR)	0.18 (HR)	0.15 (HR)	0.20 (HR)	0.12 (HR)	0.18 (HR)	0.13 (HR)	0.12 (HR)	0.13 (HR)	0.12 (HR)	0.13 (HR)	0.35 (HR)
R43-08	0.22 (HR)	0.40 (HR)	0.13 (HR)	0.18 (HR)	0.10 (HR)	0.63 (HR)	0.10 (HR)	0.15 (HR)	0.10 (HR)	0.13 (HR)	0.10 (HR)	0.33 (HR)
R43-09	0.12 (HR)	0.15 (HR)	0.10 (HR)	0.13 (HR)	0.12 (HR)	0.17 (HR)	0.15 (HR)	0.12 (HR)	0.17 (HR)	0.13 (HR)	0.12 (HR)	0.32 (HR)
R43-10	0.12 (HR)	0.23 (HR)	0.12 (HR)	0.10 (HR)	0.10 (HR)	0.15 (HR)	0.10 (HR)	0.10 (HR)	0.12 (HR)	0.13 (HR)	0.12 (HR)	0.33 (HR)
R43-11	0.12 (HR)	0.13 (HR)	0.12 (HR)	0.15 (HR)	0.12 (HR)	0.13 (HR)	0.18 (HR)	0.17 (HR)	0.13 (HR)	0.13 (HR)	0.12 (HR)	0.25 (HR)
R43-12	0.35 (HR)	0.28 (HR)	0.15 (HR)	0.12 (HR)	0.12 (HR)	0.18 (HR)	0.15 (HR)	0.13 (HR)	0.18 (HR)	0.13 (HR)	0.12 (HR)	0.30 (HR)
R43-13	0.15 (HR)	0.22 (HR)	0.15 (HR)	0.20 (HR)	0.13 (HR)	0.23 (HR)	0.10 (HR)	0.13 (HR)	0.12 (HR)	0.12 (HR)	0.12 (HR)	0.27 (HR)
R43-14	0.17 (HR)	0.18 (HR)	0.12 (HR)	0.15 (HR)	0.10 (HR)	0.15 (HR)	0.15 (HR)	0.13 (HR)	0.10 (HR)	0.15 (HR)	0.15 (HR)	0.33 (HR)
R43-15	0.13 (HR)	0.18 (HR)	0.10 (HR)	0.13 (HR)	0.13 (HR)	0.12 (HR)	0.17 (HR)	0.15 (HR)	0.13 (HR)	0.15 (HR)	0.12 (HR)	0.15 (HR)
R106	0.68 (HR)	0.50 (HR)	0.36 (HR)	0.38 (HR)	0.32 (HR)	0.30 (HR)	0.26 (HR)	0.34 (HR)	0.40 (HR)	0.26 (HR)	0.34 (HR)	0.32 (HR)
Huazhan	6.88 (MS)	11.60 (MS)	9.68 (MS)	21.18 (HS)	7.22 (MS)	10.82 (MS)	4.17 (MR)	7.02 (MS)	4.20 (MR)	1.55 (R)	1.45 (R)	12.92 (S)

^aFor the improved lines, R43-01 ~ R43-12 were on generation of BC₂F₅, R43-13 ~ R43-15 were on generation of BC₃F₄. ^b The lesion length (cm) was averaged over five replications. ^c The resistance levels to BB were scaled according to Huang et al (2012).

Table 2 BB resistance levels of the fifteen improved hybrid combinations, hybrid check Tianyouhuazhan and its parents^a

Line or hybrid ^a	<i>Xoo</i> strain											
	Zhe173	PXO86	PXO99	FuJ	GD414	HeN11	ScYc6	YN1	YN7	YN11	YN18	YN24
TianfengA/R43-01	0.88 ^b (HR) ^c	1.62 (R)	0.60 (HR)	0.40 (HR)	0.60 (HR)	0.66 (HR)	0.46 (HR)	0.56 (HR)	0.26 (HR)	0.30 (HR)	0.30 (HR)	0.66 (HR)
TianfengA/R43-02	0.68 (HR)	1.02 (R)	0.48 (HR)	0.36 (HR)	0.32 (HR)	0.44 (HR)	0.74 (HR)	0.36 (HR)	0.20 (HR)	0.26 (HR)	0.30 (HR)	0.42 (HR)
TianfengA/R43-03	0.84 (HR)	1.34 (R)	0.52 (HR)	0.30 (HR)	0.42 (HR)	0.46 (HR)	0.32 (HR)	0.46 (HR)	0.28 (HR)	0.28 (HR)	0.22 (HR)	0.54 (HR)
TianfengA/R43-04	0.96 (HR)	0.78 (HR)	0.66 (HR)	0.32 (HR)	0.48 (HR)	0.64 (HR)	0.34 (HR)	0.74 (HR)	0.20 (HR)	0.18 (HR)	0.14 (HR)	0.52 (HR)
TianfengA/R43-05	0.76 (HR)	1.60 (R)	0.40 (HR)	0.32 (HR)	0.34 (HR)	0.60 (HR)	0.50 (HR)	0.42 (HR)	0.24 (HR)	0.24 (HR)	0.36 (HR)	0.40 (HR)
TianfengA/R43-06	0.72 (HR)	1.64 (R)	0.64 (HR)	0.38 (HR)	0.48 (HR)	0.56 (HR)	0.68 (HR)	0.44 (HR)	0.40 (HR)	0.34 (HR)	0.24 (HR)	0.50 (HR)
TianfengA/R43-07	0.74 (HR)	1.36 (R)	0.30 (HR)	0.24 (HR)	0.26 (HR)	0.66 (HR)	0.38 (HR)	0.62 (HR)	0.16 (HR)	0.38 (HR)	0.20 (HR)	0.40 (HR)
TianfengA/R43-08	1.10 (R)	2.35 (R)	0.55 (HR)	0.43 (HR)	0.45 (HR)	0.38 (HR)	0.45 (HR)	0.63 (HR)	0.48 (HR)	0.38 (HR)	0.30 (HR)	0.75 (HR)
TianfengA/R43-09	0.86 (HR)	2.26 (R)	0.48 (HR)	0.64 (HR)	0.50 (HR)	0.56 (HR)	0.46 (HR)	0.82 (HR)	0.24 (HR)	0.40 (HR)	0.50 (HR)	0.46 (HR)
TianfengA/R43-10	0.64 (HR)	2.44 (R)	0.42 (HR)	0.44 (HR)	0.38 (HR)	0.44 (HR)	0.56 (HR)	0.48 (HR)	0.24 (HR)	0.26 (HR)	0.16 (HR)	0.44 (HR)
TianfengA/R43-11	0.44 (HR)	2.52 (R)	0.52 (HR)	0.92 (HR)	0.34 (HR)	0.66 (HR)	0.60 (HR)	0.66 (HR)	0.38 (HR)	0.40 (HR)	0.22 (HR)	0.58 (HR)
TianfengA/R43-12	1.54 (R)	2.16 (R)	1.34 (R)	0.46 (HR)	0.30 (HR)	0.86 (HR)	0.54 (HR)	0.58 (HR)	0.24 (HR)	0.36 (HR)	0.36 (HR)	0.86 (HR)
TianfengA/R43-13	1.28 (R)	2.24 (R)	1.32 (R)	0.50 (HR)	0.60 (HR)	1.22 (R)	0.68 (HR)	1.84 (R)	0.34 (HR)	0.46 (HR)	0.28 (HR)	0.64 (HR)
TianfengA/R43-14	1.20 (R)	1.52 (R)	0.44 (HR)	0.42 (HR)	0.54 (HR)	0.70 (HR)	0.38 (HR)	0.78 (HR)	0.26 (HR)	0.32 (HR)	0.32 (HR)	0.64 (HR)
TianfengA/R43-15	1.42 (R)	2.72 (R)	0.50 (HR)	0.28 (HR)	0.36 (HR)	0.54 (HR)	0.36 (HR)	1.62 (R)	0.42 (HR)	0.14 (HR)	0.14 (HR)	0.64 (HR)
Tianyouhuazhan	11.06 (MS)	14.34 (S)	9.52 (MS)	17.58 (S)	11.26 (S)	8.80 (MS)	3.50 (MR)	8.64 (MS)	0.42 (HR)	0.56 (HR)	0.66 (HR)	17.20 (S)
TianfengA	15.00 (S)	16.02 (S)	11.02 (MS)	16.82 (S)	10.92 (MS)	17.12 (S)	17.80 (S)	16.84 (S)	0.48 (HR)	0.52 (HR)	0.54 (HR)	15.60 (S)
Huazhan	6.88 (MS)	11.60 (MS)	9.68 (MS)	21.18 (HS)	7.22 (MS)	10.82 (MS)	4.17 (MR)	7.02 (MS)	4.20 (MR)	1.55 (R)	1.45 (R)	12.92 (S)

^a The hybrids TianfengA/R43-01 ~ TianfengA/R43-15 were generated using R43-01 to R43-15 as male parent crossed with TianfengA, respectively. ^b The lesion length (cm) was averaged over five replications. ^c The resistance levels to BB were scaled according to Huang et al (2012)

Fig.3 Comparison of BB resistance to 12 Xoo strain between the improved hybrid rice TianfengA/R43-7 and the control Tianyouhuazhan. CK represents control hybrid Tianyouhuazhan, H43-07 represents the improved hybrid combination TianfengA/R43-07.

3.3. Evaluation of brown planthopper resistance

The results of BPH resistance based on average resistance scores are displayed in Fig.4. The average BPH resistance scores of fifteen improved restorer lines homozygous in *Bph14* and *Bph15* loci were less than 3.0, whereas the average resistance score of Huazhan was 6.3 (Fig.4a). It suggested that the BPH resistance of improved restorer lines was significantly stronger than that of Huazhan. Amongst fifteen improved restorer lines, five lines including R43-01, R43-02, R43-05, R43-11 and R43-12 were highly resistant to BPH with the average scores less than 1.0 (Fig.4a), which were similar to the highly resistant control variety B5. All the improved hybrids except TianfengA/R43-09 (resistance score = 3.7) were also less than 3.0, while the average resistance scores of TianfengA and Tianyouhuazhan were 5.7 and 5.0, respectively (Fig.4b). This indicated that the fifteen improved hybrids had much higher resistance level than Tianyouhuazhan. In the fifteen improved hybrid combinations, four hybrids including TianfengA/R43-01, TianfengA/R43-02, TianfengA/R43-04 and TianfengA/R43-12 were highly resistant to BPH with the average scores less than 1.0 (Fig.4b; Fig.5). Furthermore, BPH bioassay also showed that the improved restorer lines had slightly higher or similar resistance to BPH than their corresponding hybrids (Fig.4b). The higher the resistance level of a restorer line was, the

higher the resistance level of its corresponding hybrid was. For example, the mean resistance scores of restorer lines R43-03 and R43-08 were 1.33 and 1.67 respectively, and the resistance scores of the corresponding hybrids TianfengA/R43-03 and TianfengA/R43-08 were 1.67 and 2.33 respectively. These findings indicated that pyramiding of *Bph14* and *Bph15* genes in genetic background of Huazhan could significantly improve BPH resistance of this restorer line and its hybrid, and the resistance of restorer line was positively correlative to that of its hybrid.

Fig.4 The BPH resistance scores of the improved restorer lines, improved hybrids, control hybrid and their parents. (a) The resistance scores of fifteen improved restorer lines and recurrent parent Huazhan in the modified bulk seedling test in greenhouse. (b) The resistance scores of the improved hybrids from improved restorer lines, control hybrid and their parents in the modified bulk seedling test. HZ represents the recurrent parent Huazhan, TYHZ represents the hybrid Tianyouhuazhan. R43-01 ~ R43-15 represent the fifteen improved restorer lines, and H43-01 ~ H43-15 represent the fifteen improved hybrid combinations derived from TianfengA crossing with R43-01 ~ R43-15, respectively.

Fig.5 Performance of BPH resistance level of 15 improved hybrids, control hybrid Tianyouhuazhan and their parents at seedling stage. TN1 was used as BPH susceptible control, B5 as BPH resistant control, TYHZ (Tianyouhuazhan) as check hybrid. H43-01 ~ H43-15 represent 15 improved hybrids derived from 15 improved restorer lines, respectively.

3.4. Evaluation of agronomic traits

Eight agronomic traits of Huazhan, Tianyouhuazhan, fifteen improved restorer lines and their hybrids were evaluated. The results showed that most of the improved restorer lines were superior to the recurrent parent Huazhan in some agronomic traits like PL, SP, KW, etc. (Table 3). For example, almost all of the improved restorer lines displayed significant increase ($P<0.01$) in KW compared with the recurrent parent Huazhan (Table 3). Accordingly, the KW of all improved hybrid combinations were significantly heavier ($P<0.01$) than that of Tianyouhuazhan (Table 4), which displayed higher yield potential. However, most of the other agronomic traits of improved hybrid combinations were quite similar to those of the control Tianyouhuazhan. There was no significant difference ($P>0.05$) in PP, PL, SS and YP between all improved hybrid combinations and Tianyouhuazhan (Table 4). Compared with the control hybrid Tianyouhuazhan, one hybrid TianfengA/R43-11 displayed longer DSH ($P<0.01$), two hybrids TianfengA/R43-11 ($P<0.01$) and TianfengA/R43-12 ($P<0.05$) showed higher PH, one hybrid TianfengA/R43-04 was of less SP ($P<0.05$) (Table 4). Overall, there was no obviously negative effect on agronomic traits while simultaneously pyramiding BB and BPH resistance genes into Huazhan.

Table 3 Comparison of agronomic traits between fifteen improved restorer lines and recurrent parent Huazhan

Line	Days from seeding to heading	Plant height (cm)	Panicles per plant	Panicle length (cm)	Spikelet per panicle	Seed set (%)	1000-grain weight (g)	yield per plant (g)
R43-01	88.7±1.2	100.1±3.4**	11.8 ±2.1	22.6 ±0.8**	160.4 ±12.5**	90.5 ±1.8	23.2 ±0.5**	34.2 ±6.3
R43-02	88.7±0.6	99.9±2.9**	11.0 ±3.5	22.2 ±0.7**	152.3 ±13.6*	90.8 ±2.8	25.2 ±0.3**	34.1 ±12.4
R43-03	89.0±1.0	98.5±2.9**	10.3 ±2.5	22.5 ±1.1**	154.3 ±15.4*	92.1 ±2.8	23.2 ±0.2**	33.8 ±7.6
R43-04	88.3±0.6	104.7±1.6**	10.6 ±2.8	22.1 ±1.2**	171.0 ±25.5**	87.7 ±5.0*	24.0 ±0.4**	32.3 ±9.4
R43-05	89.3±0.6	100.1±2.5**	13.2 ±3.4	21.4 ±0.7**	142.9 ±14.3	90.2 ±4.2	23.5 ±0.4**	30.7 ±9.4
R43-06	90.0±1.0	102.3±2.6**	11.2 ±4.3	22.4 ±1.4**	157.5 ±8.7**	88.3 ±5.6	24.0 ±0.5**	30.4 ±10.6
R43-07	89.7±1.2	100.3±4.0**	10.3 ±2.2	22.1 ±1.1**	142.0 ±23.7	88.6 ±2.0	24.6 ±0.6**	28.5 ±6.4
R43-08	89.3±0.6	96.7±1.5*	11.0 ±2.6	22.5 ±0.9**	166.2 ±11.3**	85.9 ±4.3**	23.6 ±0.4**	32.8 ±6.7
R43-09	88.7±0.6	92.3±2.3	13.6 ±2.2	22.4 ±1.3**	164.3 ±13.9**	86.4 ±3.6**	23.5 ±0.4**	38.0 ±7.8*
R43-10	90.3±0.6	96.9±3.7*	11.6 ±2.3	22.3 ±1.1**	153.1 ±14.7*	90.8 ±2.8	23.7 ±0.2**	33.6 ±4.8
R43-11	91.3±1.2*	100.9±3.6**	9.9 ±2.5	20.2 ±0.6	143.1 ±20.5	89.9 ±2.8	25.9 ±0.7**	28.6 ±9.3
R43-12	88.0±1.0	101.9±4.2**	12.4 ±3.2	22.1 ±0.5**	147.9 ±15.6*	91.3 ±1.4	25.0 ±0.5**	34.5 ±7.0
R43-13	89.3±0.6	100.5±1.1**	11.7 ±2.3	20.7 ±0.6*	160.9 ±19.8**	89.6 ±4.0	22.2 ±0.5	27.1 ±8.5
R43-14	89.3±1.2	99.0±5.6**	11.1 ±2.8	21.6 ±1.4**	163.2 ±19.4**	86.7 ±3.9**	23.2 ±0.6**	30.0 ±5.2
R43-15	89.3±0.6	95.5±4.4	11.7 ±3.8	22.2 ±0.9**	157.1 ±23.8**	91.0 ±2.4	23.6 ±0.4**	31.0 ±8.0
Huazhan	89.3±0.6	93.2±3.3	12.0 ±2.1	19.6 ±0.5	129.4 ±21.3	91.8 ±1.9	21.7 ±0.2	27.9 ±4.1

All agronomic traits data were mean of three plants over three replicates. * and ** mean significant difference at 0.05 and 0.01 level respectively.

Table 4 Comparison of agronomic traits between fifteen improved hybrids and the control hybrid Tianyouhuazhan

Hybrid	Days from seeding to heading	Plant height (cm)	Panicles per plant	Panicle length (cm)	Spikelet per panicle	Seed set (%)	1000-grain weight (g)	yield per plant (g)
TianfengA/R43-01	83.3±0.6	108.9±3.6	10.2±1.6	23.9±1.2	178.9±19.8	87.1±3.6	26.9±0.3**	36.4±9.3
TianfengA/R43-02	84.0±1.0	107.4±3.0	10.6±2.2	23.8±1.6	167.6±26.7	82.5±5.1	28.3±0.2**	38.5±12.3
TianfengA/R43-03	83.3±0.6	106.4±3.5	11.0±1.0	22.9±1.1	178.0±22.5	86.2±2.8	27.7±0.5**	39.4±6.7
TianfengA/R43-04	82.7±1.2	110.1±3.1	12.0±3.0	22.5±0.6	154.8±18.5*	86.4±4.8	28.0±0.6**	35.4±7.9
TianfengA/R43-05	83.7±0.6	109.2±2.5	11.6±1.9	23.5±1.9	175.6±29.9	84.8±6.3	27.0±0.3**	40.2±7.4
TianfengA/R43-06	84.3±0.6	112.2±3.5	11.1±1.8	23.6±1.2	181.8±26.6	84.0±3.4	27.6±0.3**	38.6±8.4
TianfengA/R43-07	83.7±1.2	109.0±2.3	11.3±2.0	23.1±1.1	165.7±23.6	82.6±3.9	27.5±0.4**	36.8±10.9
TianfengA/R43-08	83.3±0.6	108.2±7.0	11.8±1.6	23.2±1.2	167.3±19.8	84.9±4.1	27.8±0.7**	39.4±8.9
TianfengA/R43-09	82.7±0.6	107.1±3.9	12.1±1.3	23.9±0.9	176.6±22.4	85.7±1.3	28.0±0.3**	43.1±7.9
TianfengA/R43-10	83.3±0.6	109.4±3.7	11.3±2.1	23.5±1.2	168.1±28.4	84.9±2.8	27.7±0.2**	40.9±11.7
TianfengA/R43-11	85.7±1.2**	118.2±4.5**	12.0±1.7	23.5±1.6	185.8±36.6	81.3±3.6	29.1±0.7**	49.5±12.1
TianfengA/R43-12	82.7±0.6	113.4±2.8*	11.6±2.2	23.9±1.2	173.1±22.9	87.7±3.5	27.8±0.2**	42.8±10.2
TianfengA/R43-13	83.7±0.6	105.9±5.1	11.8±1.1	23.9±1.1	168.0±26.0	85.0±4.5	27.0±0.4**	40.8±8.2
TianfengA/R43-14	83.0±1.0	109.7±4.1	10.6±4.0	23.8±1.3	185.0±29.7	80.5±10.5	26.7±0.8**	37.9±17.5
TianfengA/R43-15	84.0±1.0	107.8±3.2	11.7±1.2	23.4±0.6	166.3±17.4	85.1±2.6	27.6±0.3**	42.1±8.1
Tianyouhuazhan	83.7±0.6	108.7±3.1	11.4±3.4	23.2±0.9	192.1±28.5	85.0±4.0	25.9±0.4	39.8±10.9

All agronomic traits data were mean of three plants over three replicates. * and ** mean significant difference at 0.05 and 0.01 level respectively.

According to the results of plot yield test in 2013, only three hybrid combinations TianfengA/R43-06, TianfengA/R43-13 and TianfengA/R43-10 increased in GYP by 3.04%, 2.15% and 1.90% respectively (Fig.6a), but the increases over control hybrid Tianyouhuazhan were not significant ($P>0.05$). In 2014, eight combinations (TianfengA/R43-07, TianfengA/R43-05, TianfengA/R43-04, TianfengA/R43-01, TianfengA/R43-08, TianfengA/R43-09, TianfengA/R43-11 and TianfengA/R43-06) showed increases in GYP over Tianyouhuazhan, among which the TianfengA/R43-07 had the highest increase in GYP (+6.7%), but the increase was not significant when comparing with the control (Fig.6b). Based on the integrated results of GYP in two years, two improved hybrids TianfengA/R43-06 and TianfengA/R43-07 had constantly higher GYP than the control Tianyouhuazhan and other improved hybrids. In consideration of R or HR level of resistance to both BB and BPH of these two hybrid combinations, the hybrids TianfengA/R43-06 and TianfengA/R43-07 were considered as two promising hybrid combinations.

Fig.6 Comparison of GYP amongst fifteen improved hybrid combinations and Tianyouhuazhan in 2013 and 2014. (a) Comparison of GYP of 50 plants per plot in 2013. (b) Comparison of GYP of 100 plants

per plot in 2014. TYHZ stands for the control hybrid Tianyouhuazhan. H43-01 ~ H43-15 represent the fifteen improved hybrids TianfengA/R43-01~ TianfengA/R43-15 respectively. GYP: grain yield per plot.

4. Discussion

Gene pyramiding has been used not only to breed broad-spectrum and durable resistant varieties by assembling multiple resistance genes for one trait, but also to develop multiple resistant rice varieties by stacking multiple resistance genes for different traits (Zhan et al. 2012; Hari et al. 2013; Pan et al. 2013). 9311 and Minghui63 were two main restorer lines greatly used in hybrid rice production in China in the past twenty years (Xie et al. 1996; Deng 2005; Zou et al. 2008). Hybrid combinations from 9311 and Minghui63 had high yield potential and wide adaptability, but were susceptible to main diseases and insects (Xie et al. 1996; Deng 2005; Zou et al. 2008). To date, it was reported that BB and BPH resistance of these two restorer lines and their hybrids were improved by MABC coupled with phenotypic selection respectively (Zhou et al. 2003; Zhang et al. 2006; Hu et al. 2012; Hu et al. 2013), but the yield potential and grain quality of hybrids from these two restorer lines were limited due to release more than twenty years ago. Huazhan is an elite male parent in the current rice production, and its hybrid combination Tianyouhuazhan have high yield potential, wide adaptability and good grain quality, but are susceptible to BB and BPH (Yu et al. 2009; Li et al. 2012). So far there are no reports about simultaneous improvement of BB and BPH resistance of Huazhan and its hybrids. In this study, one BB resistant gene (*Xa23*) and two BPH resistant genes (*Bph14* and *Bph15*) were simultaneously pyramided into Huazhan through MABC strategy over four years (Fig.1), and a series of improved restorer lines and their hybrids with excellent agronomic traits and superior resistance to both BB and BPH were obtained (Table 1; Table 2; Fig.4; Fig.5). Furthermore, TianfengA/R43-06 and TianfengA/R43-07 with higher yield potential and stronger resistance to both

BB and BPH than the control hybrid Tianyouhuazhan and other improved hybrids were selected out as two promising hybrid combinations (Fig.6).

Molecular improvement of BB resistance focused on conventional varieties and hybrid rice parental lines. Zhou et al. (2003) introgressed *Xa21* gene into a restorer line 9311 and obtained an improved restorer line and its hybrids which showed MR or R level of resistance to BB. Ding (2005) developed new hybrid combinations carrying *Xa7* gene with high resistance to BB. Zhang et al. (2006) used MAS for pyramiding *Xa7* and *Xa21* into Minghui63. The results of BB resistance identification showed that the improved Minghui63 and its hybrids were very highly resistant to ten *Xoo* strains, but susceptible to PXO99 (Race P6). Zeng et al. (2009) obtained a conventional variety Baixiangzhan containing *xa5* gene with high resistance to V pathotype. Sundaram et al. (2008, 2009) pyramided *xa5*, *xa13* and *Xa21* genes into Samba Mahsuri and Triguna, and developed improved varieties with significantly higher resistance to BB than the original versions. Suh et al. (2013) obtained improved Mangeumbyeo lines carrying *Xa4*, *xa5* and *Xa21* genes, which showed HR level of resistance to BB. Win et al. (2013) simultaneously incorporated *xa5*, *Xa21* and *xa33* into a high quality and aromatic variety MK-75, and obtained improved lines with high resistance to BB. However, among BB resistance genes greatly used in rice breeding, *xa5* and *xa13* are recessive genes that are difficult to apply in hybrid rice breeding. *Xa4* gene has incomplete dominance nature and its resistance level is affected by the genetic background. *Xa21* gene generally expresses resistance at late tillering stage, and its resistance level is also affected by the genetic background. *Xa7* gene cannot confer resistance to PXO99 strain. *Xa23* gene will have greater application prospect in breeding BB resistant hybrid rice because this gene has broad spectrum resistance, and shows very high level resistance over all the growth stage (Zhang et al. 1998, 2001). Li et al. (2006a) and Zhou et al. (2011) respectively introduced

Xa23 gene to two popular restorer lines 9311 and Minghui63. These improved restorer lines and their hybrids exhibited HR or R level of resistance to all *Xoo* strains from China, India, Philippines and Japan, suggesting that the expression of *Xa23* gene is not affected by genetic background of recipient. In this study, we found that fifteen improved restorer lines and their hybrids with *Xa23* gene were highly resistant to all twelve strains belonging to different pathotypes from China and Philippines (Table 1; Table 2), that further indicated the genetic background could not affect the function of *Xa23* gene, and demonstrated the great application value of *Xa23* gene. The next step towards the utilization of BB-resistant restorer lines and their hybrids carrying *Xa23* will be playing a crucial role in controlling BB in China and other Asian countries.

IRRI was the earliest research institute of carrying out molecular breeding of BPH resistance, and developed BPH-resistant varieties IR26, IR36, IR56 and IR66, which harbored *Bph1*, *bph2*, *Bph3* and *bph4* respectively (Cruz et al. 2011; Deng et al. 2011) and were used intensively in southeast Asia. Then, Sharma et al. (2004) obtained new *japonica* lines carrying *Bph1* and *bph2* with resistance to BPH. Results of BPH bioassays showed that the pyramided lines with two resistance genes were more significantly resistant to BPH than those with single resistant gene. China began to implement molecular breeding of BPH resistance at beginning of this century. Yang et al. (2010) and Liu et al. (2013) introgressed *Bph3* and *Bph24* into maintainer lines and restorer lines respectively, and the improved lines showed significantly higher resistance to BPH than their original parents. Qiu et al. (2012) developed improved Nipponbare lines carrying *Bph6* and *Bph12*, which showed R or HR level of resistance to BPH. Hu et al. (2012, 2013) obtained improved Minghui63, improved 9311 and their hybrids carrying both *Bph14* and *Bph15* gene using MABC strategy. BPH bioassay found out that the improved restorer lines with homozygous *Bph14* and *Bph15* gene showed higher level of resistance

than their hybrids. In consideration of biosafety and biodiversity, the utilization of *Bph14* and *Bph15* gene in rice breeding was limited in China (Fujita et al. 2013). Numerous studies revealed that the BPH resistance level conferred by *Bph14* and *Bph15* gene was related to genetic background of recipient. The improved 9311 carrying homozygous *Bph14* and *Bph15* had slightly higher or similar resistance to BPH than the heterozygous genotypes, suggesting that these two genes were of almost dominance or dominance on background of 9311 (Hu et al. 2013). Hu et al. (2012) also found that the *Bph14* and *Bph15* genes were of incomplete dominance on background of Minghui63. Yan et al. (2014) reported that the improved restorer line carrying homozygous *Bph14* and *Bph15* gene had high resistance to BPH, but its hybrids derived from this improved line were highly susceptible to BPH, suggesting that the *Bph14* and *Bph15* gene were not dominant on background of R1005. In this study, fifteen improved restorer lines and their hybrids displayed HR or R level to BPH except hybrid TianfengA/R43-09 displayed MR level; generally speaking, the resistance levels of hybrids heterozygous on *Bph14* and *Bph15* gene fluctuated up or down around the resistance levels of corresponding restorer lines homozygous on *Bph14* and *Bph15* gene (Fig.4; Fig.5). That fortunately indicates these two genes are of dominance on genetic background of Huazhan. However, the improved restorer lines and their hybrids were evaluated on BPH resistance only at seedlings stage in this study, and it is still unknown about adult-plant resistance to BPH of these improved lines and hybrids. So the BPH resistance of adult-plant or field resistance of these improved lines and hybrids needs to be evaluated in the future.

In order to develop new rice variety with strong resistance to diseases and insects, good grain quality and high yield potential, it is needed to evaluate agronomic traits of improved lines on the process of improving insects and diseases resistance. Sundaram et al. (2008) showed that the improved Samba Mahsuri carrying *xa5*, *xa13* and *Xa21* was superior or similar in agronomic traits to Samba

Mahsuri. Hu et al. (2012) suggested that the agronomic traits of improved Minghui63 and its hybrids were superior or similar to those of Minghui63 and its hybrids. The improved 9311 and R1005 carrying two or three BPH resistance genes were also superior or similar in agronomic traits to 9311 and R1005 respectively, but the agronomic traits of their hybrids were not evaluated (Hu et al. 2013; Yan et al. 2014). Chen et al. (2013) also obtained the improved Zhehui7954 and its hybrids pyramiding *Xa23* and *Bph18* that their agronomic traits were superior or similar to the original version. In this study, the introgression of BB and BPH resistance genes into the elite restorer line Huazhan does not bring apparent yield penalty. Conversely, the 1000-grain weight of improved restorer lines and their hybrids are significantly heavier ($P < 0.01$) than those of Huazhan and Tianyouhuazhan respectively (Table 3; Table 4), demonstrating that the yield potential was increased after improvement. We are believed that the BB and BPH resistant hybrids developed in this study will be of great advantage in BB and/or BPH endemic areas.

5. Conclusion

Fifteen improved restorer lines, designated as R43-01~R43-15, have been developed by introgressing one BB resistance gene *Xa23* and two BPH resistance genes *Bph14* and *Bph15* into an elite restorer line Huazhan through MABC and phenotypic selection approaches. Bioassays indicate that the fifteen pyramided lines and their hybrids are obviously improved in both BB and BPH resistance comparing with those of their respective original versions. Two improved hybrid combinations TianfengA/R43-06 and TianfengA/R43-07 are selected out from fifteen improved hybrids due to higher yield potential and broader-spectrum and stronger resistance to both BB and BPH than Tianyouhuazhan.

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