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Evaluation of Backcrossed Pyramiding Lines of the Yield-related Gene and the Bacterial Leaf Blight Resistant Genes

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Abstract. The yield-related gene, *WFP* increases grain number by increasing the primary branch number per panicle in rice. In the present study, *WFP* was introgressed from ST-12 to IRBB60, a pyramiding line having the genes *Xa4*, *xa5*, *xa13* and *Xa21* for BLB resistance in the IR24 genetic background. The pyramided lines PL-1, PL-4 and PL-5 that were included in the initial BC_2F_2 selections based on their improved PBN were selected for further agronomic evaluation and generation advance up to BC_2F_3. All three lines recorded significantly higher PBN and GN due *WFP* introgression, although the loss of at least two BLB genes during the breeding process resulted in the variable response of the lines to the different BLB races. Among the lines, only PL-5 showed a significantly higher estimate of actual yield measured in terms of panicle weight per square meter compared to IRBB60. PL-5 also exhibited resistance to five out of the six BLB races used for resistance screening. Despite the negative effects in grain size, the improved yield estimates, as well as the validated BLB resistance of PL-5 makes it a suitable candidate for cultivar adoption under the tropical rice ecosystem of Southeast Asia.

Key words: Rice, *Wealthy Farmer's Panicle* (*WFP*), bacterial leaf blight (BLB), pyramiding, high-yielding

Introduction

By 2050, the current world population is expected to reach 9.7 billion (https://www.un.org/development/desa/en/). An increase of this magnitude poses a very serious threat to the current state of global food security (http://www.fao.org/home/en/). To feed 9 billion people, global cereal production alone needs to increase by 38% in the next 34 years[1)].

Rice, wheat and maize constitute approximately 50% of the calorie source of the human diet[2)]. Among these three, rice serves as a staple for more than half of the world's population. Although rice is cultivated in more than 100 countries across the globe, more than 90% of rice is still produced and consumed in Asia (GRiSP, http://www.grisp.net/main/summary). In recent years, a drastic increase in demands for rice has also been reported in Africa[3)] (GRiSP, http://www.grisp.net/main/summary). Given the importance of rice as a staple food, increasing

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rice production would play a critical role in securing world food supply.

Marker-assisted selection (MAS) is powerful tool for an efficient breeding. Since MAS makes it possible to distinguish plants into which target genes/QTLs have been introgressed during nursery, field area and efforts for individual selection of breeding reduce. Also, target genes/QTLs can be introgressed more reliably than visually selection. MAS has been successfully used in breeding programs to improve agronomic traits, as well as biotic and abiotic stress resistance in rice. In the past decades, MAS have significantly shortened the time requirement while increasing the precision and efficacy by which target genes/QTLs are transferred across different genetic backgrounds[4, 5]. To date, several rice cultivars have been improved through MAS and these include the improved varieties developed under the Wonder Rice Initiative for Food Security and Health (WISH) project (http://motoashikari-lab.com/wp/wp-content/uploads/2018/11/WISH_catalogue_lite4.pdf).

To effectively utilize MAS in breeding programs, genes/QTLs underlying important traits need to be mapped and identified. In rice, many genes/QTLs that are related to the sink ability have been identified including *GN1a*/*OsCKX2*[6], *WFP/IPA1*[7, 8], *NAL1*/*SPIKE*/*GPS*[9, 10, 11], *APO1*/*SCM2*[12, 13, 14], *qSW2*[15], *GW2*[16], *GW6a*[17] and *GS3*[18, 19]. Conversely, several genes/QTLs that are related to the source or translocation ability of rice has also been reported[20, 21, 22] that includes *AMY2A*[23], *BAM2*[23] and *GWD1*[24].

Wealthy Farmer's Panicle (*WFP*)/*Ideal Plant Architecture 1* (*IPA1*) is a yield-related gene that encodes the plant-specific transcription factor OsSPL14[7, 8]. *OsSPL14* contains a microRNA-targeted sequence in an exon that is targeted and cleaved by OsmiR156. *OsSPL14* functions in the regulation of primary branch number and is therefore associated with grain number. The *WFP* allele from ST-12 has been reported to increase grain number per panicle in rice[7]. Advanced breeding lines of the *japonica* rice cultivar Nipponbare and several other *indica* cultivars having the *WFP* allele from ST-12 showed at least 28% increase in grain yield compared to the original varieties[7, 25].

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases of rice throughout the world. In some regions in Asia, BLB can cause yield losses of up to 50%[26]. The most effective approach to combat BLB is the use of resistant cultivars[27]. To date, many resistant genes against BLB have been identified. Huang et al. focused on four BLB resistance genes/QTLs namely *Xa4*, *xa5*, *xa13* and *Xa21*, and pyramided all four resistance genes in the IR24 genetic background to generate IRBB60[28]. IRBB60 has resistance to six races of *Xoo* from the Philippines[28]. Currently, the BLB genes *xa5*, *xa13* and *Xa21* have been identified and characterized[29, 30, 31].

In this study, we aimed to pyramid *WFP* from ST-12 to IRBB60, a rice cultivar having four genes for BLB resistance. The resulting pyramided lines were evaluated for yield and yield components, as well as for their resistance to BLB races from Vietnam, Myanmar and India. Results of the preliminary assessment of the agronomic performance of advanced backcrosses lines identified PL-5, a pyramiding line with higher yield compared and comparative BLB resistance relative to the recipient parent IRBB60.

Materials and Methods

Marker-assisted introgression of WFP in the pyramiding line IRBB60

IRBB60, IR24, ST-12 and three pyramiding lines (PL-1, PL-4 and PL-5) at the BC_2F_4 generation were used in the study. IRBB60 is a pyramiding line having the four bacterial leaf blight (BLB) resistant genes, *Xa4*, *xa5*, *xa13* and *Xa21* in the genetic background of IR24[28], whereas ST-12 is the donor of the yield-related gene, *WFP*[7]. Phenotype and marker-assisted backcrossing of the initial hybrids generated from crosses between IRBB60 and ST-12 was carried out up to the BC_2F_4 generation (Fig. 1). An SSR marker (forward primer: GCGGTAACAAACCAACCAACC, reverse primer: AAAGCAGGACACAGTCACACAGG) was used to monitor the introgression of *WFP* in the IRBB60 background. In 2016, six BC_2F_3 lines (PL-1, PL-2, PL-4, PL-5, PL-6 and PL-7) were selected for preliminary evaluation of yield and yield components in the field. Based on this preliminary investigation, three pyramiding lines namely PL-1, PL-4 and PL-5 were selected and advanced for further performance evaluation in the field.

All test materials were raised in the greenhouse for four weeks before transplanting them in a rice paddy at the Togo Field Center for Research and Education of Nagoya University in Aichi, Japan (35º06' north, 137º04' east) in 2017. The seedlings were transplanted separately in 3 rows (1.2 m) at a density of 22.2 plants/m^2 and at a plant spacing of 30cm x 15cm. All materials were grown in three replications in randomized blocks and under low fertilizer conditions i.e. 300 kg K-P-N (16-12-14)/ha only before transplanting.

Evaluation of the pyramiding lines for agronomic traits

Before sampling panicles from all materials, data on the days to heading (DH), plant height (PH) and panicle length (PL) of five plants per block were recorded from

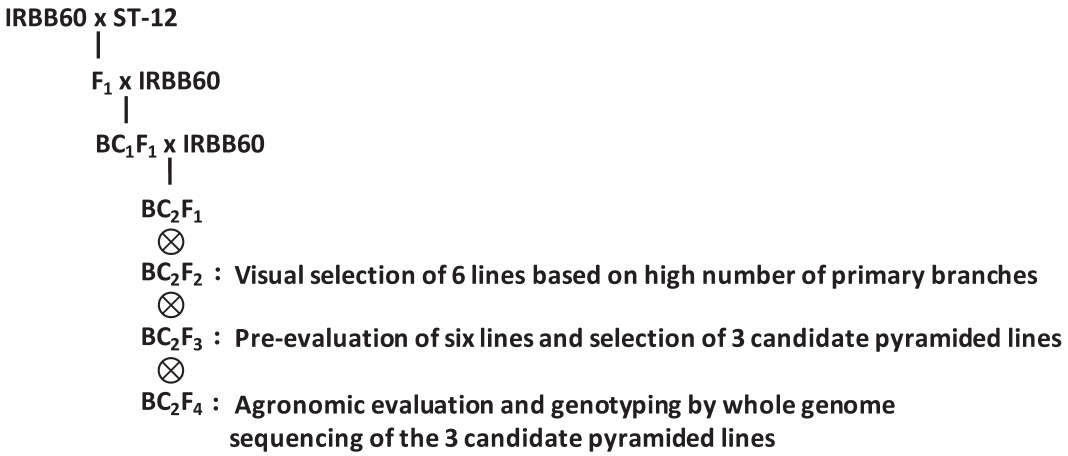

Fig. 1. Process of developing the pyramiding lines. X mark in a circle means selfing. The introgression of *WFP* from ST-12 was confirmed by MAS in BC_1F_1, BC_2F_1 and BC_2F_3.

plant stands in the paddy. At 40-45 days after heading, all panicles from five plants from each block were sampled. After sampling, panicle weight per plant (PW), panicle number per plant (PN), primary branch number per main panicle (PBN) and grain number per main panicle (GN) were measured. To estimate the actual yield of each pyramiding line in the field, panicle weight per plant was measured and converted to panicle weight per square meter (PWm^2). Only plants within rows were sampled to avoid border effects. All panicles were dried for a week in an incubator set at 42°C. To determine fertility ratio (FR), the three largest panicles including the main panicle were chosen from each plant. FR was calculated by dividing the number of filled spikelets by the total number of spikelets, multiplied by 100. The grain weight and number of filled spikelets of the three largest panicles were measured and converted to 1000-grains weight per main three panicles (TGW). Grain shape based on grain length (GL), width (GW) and thickness (GT) was determined from ten randomly selected spikelets in each plant. For the statistical analyses, plants showing maximum and minimum PW in each plot were excluded.

Evaluation of the pyramided lines for bacterial leaf blight resistance

Six races of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causing bacterial leaf blight (BLB) from Vietnam (HAU01043, HAU0209, HAU02021-2 and HAU02024-6)[32], Myanmar (HKM13)[33] and India (IND2-3) were used to evaluate the resistance of the experimental materials to the pathogen. At booting stage, the plants were inoculated with *Xoo* using the leaf clipping method[34]. A preliminary test was conducted using IR24 to confirm the pathogenicity and virulence of the six BLB races. Immediately after that, all the experimental materials including IR24 as the susceptible control and IRBB60 as the resistant control, were screened against the pathogen. After two weeks from inoculation, lesion lengths were measured, and the resistance/susceptibility of each material was evaluated. Classification of the response of materials as resistant, moderate, susceptible or partially resistant was carried out based on a comparison to the response of the susceptible reference IR24 and the resistant reference IRBB60 using Tukey's HSD (Table 1). Screening for BLB resistance including maintenance and culture of the pathogen was

Table 1. Classification of the response of materials to the BLB races based on a comparison to the response of the susceptible reference IR24 (S) and the resistant reference IRBB60 (R).

Statistical difference to IR24 (S)	Statistical difference to IRBB60 (R)	Response
Significant*	Not significant	Resistant
Significant	Significant	Moderate
Not significant	Significant	Susceptible
Not significant	Not significant	Partially resistant**

*; significant according to Tukey's HSD ($p<0.05$).
**; only in the case that evaluation value is between the values of the references.

carried out in Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University.

Genotyping-by-sequencing analysis of the pyramiding lines

Genotyping-by-sequencing (GBS) was conducted for the three pyramiding lines at the BC_2F_4 generation using the Illumina Miseq sequencing system and Illumina Miseq Reagent Kit v3 (150 cycles)[35, 36]. Three plants from each of the pyramiding line, as well as IRBB60 and ST-12 were genotyped by GBS for whole genome. After sequencing, the reads were trimmed, and genotypes were called and filtered using TASSEL-GBS[37]. SNPs with minor allele frequency (MAF) of >0.02, as well as lines with >0.2 lost genotype data and with >0.125 degree of heterozygous sites were removed from genotyping. Finally, imputation and error correction were carried out with FSFHap in TASSEL 4.0[38].

Statistical analysis

T-test and Tukey's HSD were performed using the free statistical software R (https://www.r-project.org/). A significant difference in Tukey's HSD was set at $p<0.05$. R was also used for the randomization of blocks in the field design.

Result

Phenotypic evaluation of the three pyramiding lines for yield components

The pyramiding lines PL-1, PL-2, PL-4, PL-5, PL-6 and PL-7 in the genetic background of IRBB60 were selected visually from a BC_2F_2 population for their high primary branching number. The introgression of *WFP* from ST-12 in these six lines at the BC_2F_3 generation was confirmed by MAS. Evaluation for agronomic and yield-related traits showed that except for PL-6, all the pyramiding lines had significantly more PBN than IRBB60 (Table 2). PL-6 and PL-7 recorded significant lower PN (panicle number per plant) than IRBB60. The reduction of PN is the negative effect for actual yield, hence both PL-6 and PL-7 were removed from the candidate lines. Similarly, PL-2 was removed from the candidate lines because of comprehensive gross morphology. Based on the overall agronomic performance and gross morphology of the pyramiding lines, we selected PL-1, PL-4 and PL-5 for further generation advance and agronomic evaluation (Fig. 2).

At the BC_2F_4 generation, PL-1, PL-4 and PL-5 were evaluated for yield components including DH, PH, PL, PN, GN, PBN, FR, TGW, GL, GW and GT (Table 3). Compared to IRBB60, PL-1 recorded a significant 4-day delay in a heading, whereas PL-5 was significantly early by 4 days. Heading date in PL-4 was equivalent to that of IRBB60. PN decreased by 0.33 to 1.33 in all pyramiding lines compared to IRBB60. GN (grain number per main panicle) and PBN (primary branch number per main panicle) of the three pyramiding lines were significantly higher than those of IRBB60, suggesting the positive effects of *WFP* introgression from ST-12. The PBN of ST-12 was twice higher than that of IRBB60 and IR24. FRs (fertility ratio per three main panicles) of IRBB60, IR24 and PL-5 showed high values, while those of ST-12, PL-1 and PL-4 were low, below 77%. Among the three pyramiding lines, PL-5 showed the highest FR (92.56 ± 0.40%). The FRs of PL-1 and PL-4 were 76.84 ± 2.37% and 72.64 ± 0.61% respectively, which were equivalent to that of ST-12.

In PWm^2, PL-5 recorded a significantly higher yield (845.50 ± 57.20 g/m^2) than IRBB60 (782.42 ± 47.90 g/m^2), with the rate of increase in PL-5 reaching up to 8.1% of IRBB60. ST-12, the donor of *WFP*, had significantly lower PWm^2 (712.4 ± 43.5 g/m^2) than IRBB60 (Fig. 3). The panicle weight of PL-1 and PL-4 were statistically equivalent to that of IRBB60.

Table 2. Yield-components of the pyramiding lines at BC_2F_3 in the IRBB60 background.

	PH (cm)	PL (cm)	PN	GN	PBN
IRBB60	97.00 ± 4.36 ac*	26.40 ± 0.51 ab	11.33 ± 0.58 a	203.33 ± 17.79 a	12.33 ± 0.58 a
ST-12	115.00 ± 7.55 bd	25.43 ± 1.33 b	7.00 ± 1.00 bc	280.67 ± 43.35 ab	21.00 ± 4.00 bc
PL-1	119.33 ± 3.21 b	29.26 ± 0.61 a	8.67 ± 0.85 ac	314.67 ± 66.83 ab	23.33 ± 1.53 b
PL-2	98.50 ± 6.36 ac	25.48 ± 1.52 ab	11.00 ± 1.41 ad	281.00 ± 31.11 ab	23.00 ± 1.41 b
PL-4	114.33 ± 2.52 bd	26.40 ± 1.03 ab	11.33 ± 1.53 a	312.67 ± 33.56 ab	24.67 ± 0.58 b
PL-5	102.33 ± 5.13 acd	23.43 ± 0.99 ab	8.33 ± 1.53 ab	274.33 ± 15.53 ab	20.33 ± 1.53 bc
PL-6	89.33 ± 6.43 a	24.90 ± 1.73 ab	5.33 ± 0.58 b	204.00 ± 33.05 a	17.00 ± 1.00 ac
PL-7	103.67 ± 1.15 cd	25.30 ± 1.63 b	7.67 ± 1.53 bcd	354.33 ± 62.01 b	21.00 ± 1.00 bc

Numbers represent average ± standard deviation.

PH=plant height; PL=panicle length; PN=panicle number per plant; GN=grain number per main panicle; PBN=primary branch number per main panicle.

*; different letters indicate significant differences at $p<0.05$ by Tukey's HSD.

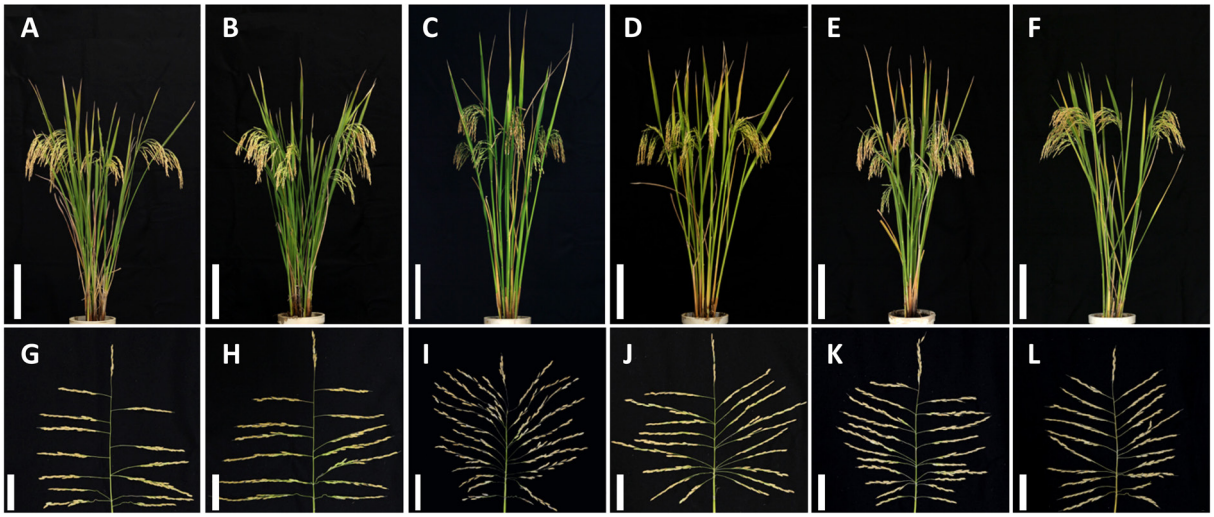

Fig. 2. Gross morphology of the plants and panicles of the experimental materials. A-F: Gross plant morphology at the ripening stage, G-L: Gross panicle morphology at the ripening stage. A and G: IRBB60, B and H: IR24, C and I: ST-12, D and J: PL-1, E and K: PL-4 and F and L: PL-5. Bar = 20cm (A–F), 5cm (G–L).

Table 3. Yield-components of the pyramiding lines at BC_2F_4 in the IRBB60 background.

Line Name	DH (days)	PH (cm)	PL (cm)	PN	GN	PBN
IRBB60	105.00 ± 0.50	94.67 ± 1.55	23.89 ± 0.44	10.44 ± 0.65	160.22 ± 9.47	12.00 ± 0.37
IR24	109.44 ± 0.33**	83.89 ± 1.38**	22.22 ± 0.58**	10.33 ± 0.80	161.33 ± 10.07	12.78 ± 0.37*
ST-12	95.78 ± 0.33**	101.89 ± 1.14**	23.78 ± 0.53	8.89 ± 0.50**	283.11 ± 15.61**	22.33 ± 0.96**
PL-1	109.11 ± 0.44**	103.33 ± 2.39**	25.78 ± 1.14*	9.11 ± 0.93	219.89 ± 16.68**	20.11 ± 0.65**
PL-4	105.22 ± 0.53	96.67 ± 1.98	23.22 ± 0.50	9.44 ± 0.41*	231.78 ± 10.14**	21.44 ± 1.40**
PL-5	101.11 ± 0.67**	95.89 ± 1.71	22.00 ± 0.24**	10.11 ± 0.80	239.89 ± 9.29**	20.56 ± 0.75**

Line Name	FR (%)	TGW (g)	GL (mm)	GW (mm)	GT (mm)
IRBB60	94.90 ± 0.26	26.45 ± 0.08	9.46 ± 0.02	2.41 ± 0.01	1.96 ± 0.01
IR24	88.14 ± 0.79**	25.65 ± 0.11**	9.11 ± 0.03**	2.52 ± 0.01**	1.97 ± 0.01*
ST-12	72.75 ± 1.14**	23.73 ± 0.11**	9.87 ± 0.02**	2.33 ± 0.01**	1.94 ± 0.01**
PL-1	76.84 ± 2.37**	27.55 ± 0.40**	9.56 ± 0.06**	2.45 ± 0.02**	1.91 ± 0.02**
PL-4	72.64 ± 0.61**	24.40 ± 0.09**	9.47 ± 0.02	2.41 ± 0.01	1.86 ± 0.01**
PL-5	92.56 ± 0.40**	20.83 ± 0.11**	8.60 ± 0.01**	2.22 ± 0.01**	1.79 ± 0.01**

Numbers represent average ± standard error.
DH=days to heading; PH=plant height; PL=panicle length; PN=panicle number per plant; GN=grain number per main panicle; PBN=primary branch number per main panicle; FR=fertility ratio per three largest panicles in a plant; TGW=1000-grain weight per three largest panicles in a plant; GL=grain length; GW=grain width; GT=grain thickness.
*, **; indicate significant difference from IRBB60 at $p<0.05$ and $p<0.01$ by t-test, respectively.

Based on these results, the high yield of PL-5 can be attributed to the increase in GN and PBN. In spite of PL-5 having 49.7% higher GN than IRBB60, PWm^2 of PL-5 was only 8.1% higher. As a major cause of this phenomenon, we focused on grain size. The grain size is expected to affect TGW (1000-grains weight per main three panicles). Compared to the TGW of IRBB60 (26.45 ± 0.08 g), the TGW of PL-5 (20.83 ± 0.11 g) was significantly lower (Table 3). Similarly, PL-5 recorded smaller grains in terms of GL (8.60 ± 0.01 mm), GW (2.22 ± 0.01 mm) and GT (1.79 ± 0.01 mm) compared to those of IRBB60 (9.46 ± 0.02 mm, 2.41 ± 0.01 mm, 1.96 ± 0.01 mm) (Table 3). The smaller grains of PL-5 negatively affected its yield.

Evaluation of the pyramiding line for resistance to bacterial leaf blight

The response of the experimental materials when challenged with BLB races from Vietnam (HAU01043, HAU0209, HAU02021-2 and HAU02024-6), Myanmar (HKM13) and India (IND2-3) are shown in Fig. 4. IRBB60 showed strong resistance to the six BLB races, with lesions lengths ranging from 0 to 1.2 cm. In contrast,

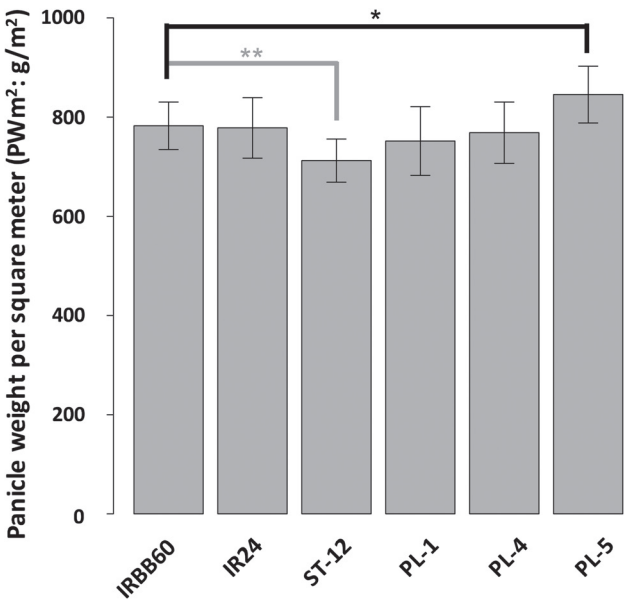

Fig. 3. Panicle weight per square meter (PWm^2) of the experimental materials. * and ** indicate significant difference from IRBB60 at $p<0.05$ and $p<0.01$, respectively, by t-test. Error bars show standard error.

IR24 and ST-12 were susceptible to the six BLB races, with lesions lengths ranging from 13.3 to 25.2 cm in IR24 and from 10.5 to 26.8 cm in ST-12. The pyramiding lines PL-1, PL-4 and PL-5 showed differential responses to the six BLB races. PL-1 exhibited resistance to the five BLB races from Vietnam (HAU0209, HAU02021-2 and HAU02024-6), Myanmar (HKM13) and India (IND2-3), and moderate to HAU01043. PL-4 exhibited resistance to HAU0209, moderate to IND2-3, partially resistance to HKM13 and susceptibility to HAU01043, HAU02021-2 and HAU02024-6. PL-5 exhibited resistance to the five BLB races from Vietnam (HAU01043, HAU0209, HAU02021-2 and HAU02024-6) and India (IND2-3), and partially resistance to HKM13 from Myanmar.

Confirmation of introgression of targeted genes with genotyping-by-sequencing

GBS analysis validated the introduction of the *WFP* allele from ST-12 and the segregation of the four BLB resistant genes of IRBB60 in each of the pyramiding lines at BC_2F_3 (Table 4). All pyramiding lines carry the *Xa21* allele from IRBB60 and the *xa13* allele from ST-12. PL-1 and PL-4 have the *Xa4* allele from IRBB60 but not PL-5 which has the *Xa4* from ST-12. The *xa5* allele from IRBB60 was introgressed in the homozygous form in PL-5 but occurs in a heterozygous form in PL-4. PL-1 has the ST-12 allele for *xa5*.

Discussion

The pyramiding lines (BC_2F_4) reported in the present study were developed from the cross between ST-12 (used as the donor of *WFP*) and IRBB60 (used as the recipient parent with BLB resistance). Introgression of *WFP* from ST-12 in the PLs was confirmed by MAS and GBS (Fig. 1, Table 4). As a result, we developed PL-5, a pyramiding line with higher yield compared to the recipient parent, IRBB60 (Fig. 3). The high yield of PL-5 can be attributed to the increase in both GN and PBN (Table 2, Table 3) due to the introgression of *WFP* from ST-12. Miura et al. reported that the introgression of *WFP* from ST-12 can significantly increase both GN and PBN in lines having the genetic background of the *japonica* rice cultivar Nipponbare[7]. Similarly, introgression of *WFP* has been reported to increase GN and PBN of several rice cultivars with *indica* genetic backgrounds[25]. In the present study, the positive effects of *WFP* from ST-12 was also observed in the genetic background of IRBB60, suggesting that *WFP* expression can increase GN and PBN in both *japonica* and *indica* rice backgrounds, and that *WFP* from ST-12 is a useful genetic resource that can be used to significantly improve the yield of rice.

PL-5 recorded 49.73% higher GN and 71.33% higher PBN compared to IRBB60. Despite the significant increase in GN and PBN, PL-5 recorded a remarkably low increase

Table 4. Genotypes in targeted genes/QTL in the three pyramiding lines in the previous generation (BC_2F_3).

	Chromosome	RAP-ID	QTL location*	IRBB60	ST-12	PL-1	PL-4	PL-5	Exp**
Xa4	11	-	Around 28.5Mb	A	B	A	A	B	A
xa5	5	Os05g0107700	-	A	B	B	H	A	A
xa13	8	Os08g0535200	-	A	B	B	B	B	A
Xa21	11	Os11g0559200	-	A	B	A	A	A	A
WFP	8	Os08g0509600	-	A	B	B	B	B	B

*; physical distance showed in RAP-db site (https://rapdb.dna.affrc.go.jp/).
**; expected genotypes.

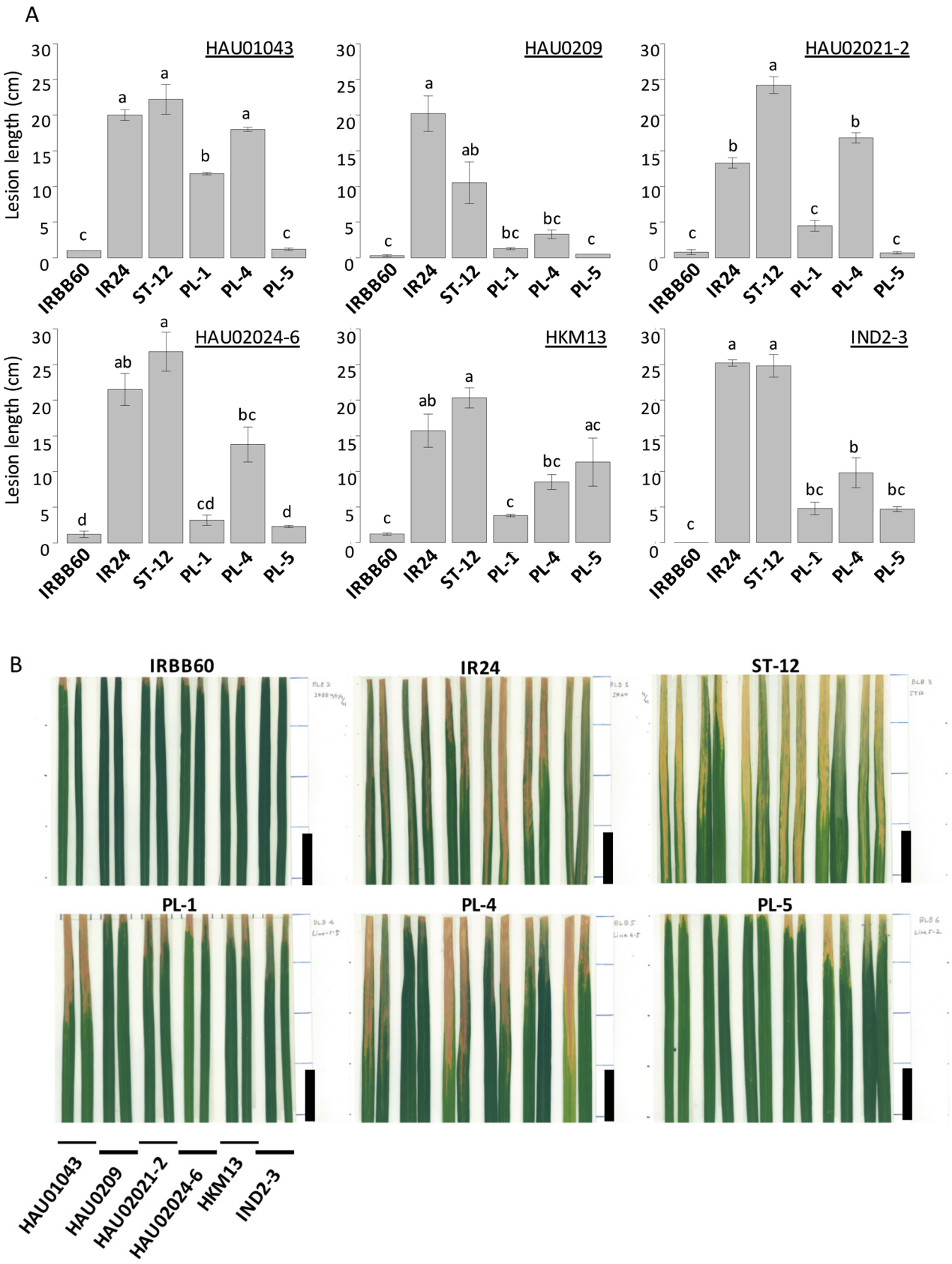

Fig. 4. The response of the experimental materials to the six races of Xoo. A: Lesion length on leaves of the experimental materials. Different letters indicate significance at $p<0.05$ by Tukey's HSD. Error bars show standard error. B: Lesions in leaves infected by the six BLB races. The reaction of the experimental materials to the BLB races is shown in two leaves each. The order of BLB races in each panel is HAU01043, HAU0209, HAU02021-2, HAU02024-6, HKM13 and IND2-3 from left to right. The inoculation site corresponds to the top of each panel. Bars = 5cm.

of 8.06% in yield (PWm^2) compared to IRBB60. A similar phenomenon was observed in PL-1 and PL-4. Rice yield has four important components namely PN, GN, FR and TGW. In PL-5, the main negative factor was TGW, which was 21.25% lower than IRBB60. Based on the results of the current study, an improvement in TGW can further increase rice yield. In PL-1, the main negative factors were PN and FR which were 12.74% and 19.03% lower than IRBB60, respectively. In PL-4, the negative factors were PN, FR and TGW which were 9.58%, 23.46% and 7.75% lower than IRBB60, respectively.

The low TGW of PL-5 may be unrelated to the function of *WFP*. PL-5 has smaller grains, giving it lower TGW than IRBB60. The donor parent ST-12 do not have small grains, and *WFP*/*IPA1* from ST-12 has not been reported to be involved in the regulation of grain size. We hypothesize that the genetic background of PL-5 which resulted from random recombination between the ST-12 and IRBB60 genome contributed to its smaller grains.

Miura et al. suggested that *WFP* from ST-12 is involved in the regulation of shoot branching in the vegetative stage[7]. It was also suggested that *WFP*/*IPA1* from the *japonica* cultivar Aikawa 1 and from the *japonica* cultivar Shaoniejing reduces tiller number[7, 8]. While the presence of *WFP*/*IPA1* allele may increase GN, it may have negative effects on PN. In the present study, an increase in GN due to *WFP*/*IPA1* introgression resulted in a concomitant decrease in PN. To suppress any reduction in PN due to *WFP* introgression, it is necessary to modify factor(s) related to controlling PN specifically in the vegetative stage.

It is considered that the low FR of PL-1 and PL-4 occurred as a result of the inability of the source to keep up with the improved sink ability of both lines as conferred by *WFP*. In PL-5, a remarkable reduction in grain size has been confirmed that allowed the source to cope with the increase in sink. In the context of sink-source relationship, *WFP* is a sink ability-related gene. Improvement of the sink is necessary to improve yield, but this requires a concomitant improvement in the source[39]. Efficient breeding for high yield in rice requires elucidation of the genetic factors regulating the ability of the source to generate photosynthetic assimilates that will fill the sink. Improving the photosynthetic ability of plant tissues with source functions may be the key to breaking through the yield potential of the current high-yield varieties[40].

The present study aimed to pyramid *WFP* with four BLB resistant genes already present in the IRBB60 background. The three selected pyramiding lines however, had *WFP* and at least two BLB resistant genes each (Fig. 4, Table 4). The incomplete maintenance of all four BLB resistance genes in the pyramiding line accounts for the variability in the response of each line to each of the BLB races. While the BLB resistant genes are not linked, *xa13* is linked to *WFP* at approximately 1.5 Mb distance downstream. In the process of developing these pyramiding lines, genotyping was carried out to monitor the introgression of only *WFP*. To ensure that all four BLB resistant genes are maintained in the pyramided line, these genes should also be monitored in the backcrossed and selfed generations by MAS.

Despite losing at least two BLB resistant genes, PL-5 remains resistant to four BLB races from Vietnam and a race from India, whereas PL-1 is resistant to three races from Vietnam and races from Myanmar and India. These results indicate the both PL-1 and PL-5 can be cultivated in areas with a reported incidence of the specific BLB infection. Overall, the significantly improved yield of PL-5 in combination with its resistance to five known BLB races from Vietnam and India makes this pyramiding line a viable candidate for high yield line with adoption in tropical rice ecosystems in Southeast Asia.

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収量関連遺伝子と白葉枯病抵抗性遺伝子の戻し交配によるピラミディング系統の評価

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要旨

イネ収量関連遺伝子 *WFP* は、一次枝梗数増加による着粒数増加をもたらす。IR24 に白葉枯病抵抗性遺伝子群（*Xa4*、*xa5*、*xa13*、*Xa21*）を集積した IRBB60 へ、ST-12 が持つ *WFP* の導入を行った。IRBB60 を反復親とした戻し交雑後代 BC_2F_2 と BC_2F_3 世代で、DNA マーカーの利用と一次枝梗数を含む収量形質の評価から、3 ピラミディング系統（PL-1、PL-4、PL-5）を選抜した。BC_2F_4 において、これらは *WFP* による一次枝梗数と着粒数の増加を示したが、4 白葉枯病抵抗性遺伝子の集積が不完全で、白葉枯病レース群への反応の差が見られた。しかし、PL-5 は IRBB60 よりも収量増加が見込まれ、今回供試した白葉枯病レース（ベトナム、ミャンマー、インド由来 6 レース）の内 5 レースに対して抵抗性を示したことから、PL-5 は東南アジアの熱帯地域での展開を見込んだ候補系統となり得ると考えられた。

キーワード：イネ、*WFP* 遺伝子、白葉枯病抵抗性、ピラミディング、高収量性