Genetic Dissection of the Breakdown of Durable Resistance in Indica Rice Variety PTB33 to Brown Planthoppers *Nilaparvata Lugens* (Stål)

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Abstract

Background

To avoid and delay the resistance breakdown of varieties against pathogens and insect pests, broad-spectrum and durable resistance by multiple genes pyramiding are expected to be one of the practical approaches. The indica rice variety PTB33 (*Oryza sativa* L.) shows high and durable resistance to the brown planthopper (BPH, *Nilaparvata lugens* Stål). However, this variety gradually lost its resistance against the recent virulence development of BPH. However, breakdown processes are not fully elucidated by individual genetic loci.

Results

Effective QTLs were explored across the whole genomic region against four BPH populations collected in Japan in 1988, 1989, 1999, and 2013 using high-density single-nucleotide polymorphism (SNP) markers obtained by genotyping-by-sequencing. Among seven genomic regions of PTB33 likely conferring BPH resistance, four QTLs, *qFSA4a*, *qFSA6*, *qFSA11*, and *qFSA12* on chromosomes 4, 6, 11, and 12, respectively, were validated as BPH resistance QTLs. The PTB33 alleles at the four QTLs positively contributed to BPH resistance. Infestation of monogenic segregating lines showed that the PTB33 alleles at *qFSA11*, *qFSA12,* and *qFSA4a* lost resistance effects at least in 1989, 1999, and 2013, respectively.

Conclusion

This study showed breakdown of durable resistance in PTB33 was explained by step-by-step losses of genetic effects at each resistance locus and probably multiple acquisitions of virulence genes in BPH in a gene-by-gene specific manner.

Background

Rice (*Oryza sativa* L.) is a major cereal crop in the Asia-Pacific region. Its stable production has recently been threatened by the brown planthopper (BPH, *Nilaparvata lugens* Stål) (Bottrell and Schoenly 2012). BPH is a monophagous insect that causes not only direct damage to plants by sucking the phloem sap with its proboscis but also causes indirect damage by transmitting viral diseases such as rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) (Cheng et al. 2013). Highly dense BPH populations in the late stage of rice cultivation result in a significant decrease in yield. Insecticides have been widely used to control BPH, but repeated exposure to insecticides has affected human health and the environment and led to the development of insecticide resistance in BPH (Thuy et al. 2012; Matsumura et al. 2014). Therefore, the development and utilization of BPH-resistant varieties are essential for reducing the damage caused by BPH from the perspective of both environmental and economic costs.

The BPH populations in Asia are proposed to be classified into the East, Southeast, and South Asia (Sogawa 1992) and supported by the recent population genomics approaches (Hu et al. 2019). These

species are unable to overwinter in Japan and migrate to Japan each year from the Chinese mainland during the early part of the rice-growing season (Kisimoto 1976). The biotypes and virulence changes of the BPH population in Japan are likely to be affected by continental East Asian BPH populations (Reviewed by Matsumura 2001).

To date, more than 40 BPH resistance genes have been reported in rice varieties and wild species (Fujita et al. 2013; Hu et al. 2016; Balachiranjeevi et al. 2019). Among the identified genes and QTLs conferring BPH resistance, eight (*BPH6*, *BPH9*, *BPH14*, *BPH17*, *BPH18*, *BPH26*, *BPH29*, and *BPH32*) have been isolated, and the molecular basis of the resistance they confer has been elucidated (Du et al. 2009; Tamura et al. 2014; Liu et al. 2015; Wang et al. 2015; Ji et al. 2016; Ren et al. 2016; Zhao et al. 2016; Guo et al. 2018) *BPH6, BPH17, BPH29, BPH32,* and *BPH26* encode the exocyst localization protein, lectin receptor-like kinase, B3 DNA-binding domain-containing protein, SCR domain protein, and coiled-coil nucleotide-binding-site leucine-rich-repeat protein (CC-NBS-LRR), respectively. These BPH resistance genes are valuable genetic resources for the development of resistant varieties.

However, varieties carrying a single resistance gene exhibit rapid decay of resistance within a few years after release owing to spatial and temporal divergence in the virulence of BPH populations. IR26, the first variety with the resistance gene *BPH1*, was developed in 1973, and its cultivation has been widely promoted in Southeast Asian countries. However, in 1975, a loss of resistance was reported (Cohen et al. 1997). Although the variety IR36 harboring the *BPH2* resistance gene was released in 1976 following the release of IR26, its resistance was not effective in the early 1980s, probably due to the changing virulence of BPH populations (Khush and Virk 2005). These results indicate that introducing only a single resistance gene permits rapid adaptation of BPH to the resistance gene, making it difficult to achieve a sustainable effect. However, it has also been reported that varieties integrating multiple resistance genes show a more enhanced and durable resistance (Horgan et al. 2019). Therefore, it is necessary to evaluate how long individual genes in durable resistance variety are effective to BPH.

The indica rice variety PTB33 (*O. sativa* L. ssp. *indica*) shows wide-spectrum resistance to BPH populations with different virulence patterns in South and Southeast Asia, including India, Taiwan, Vietnam, Indonesia, and the Philippines (Horgan et al. 2019). The BPH resistance of PTB33 can be explained by the presence of at least three loci, *BPH32, BPH2,* and *BPH17. BPH32* on chromosome 6 has been isolated using a map-based cloning approach. *BPH2* has been identified using linkage mapping (Angeles et al. 1985; Ren et al. 2016). In addition, PTB33 possesses an identical amino acid sequence to the BPH resistance gene *BPH17* on the distal end of the short arm on chromosome 4, which was isolated from the *indica* rice variety Rathu Heenati (Liu et al. 2015). Three near-isogenic lines (NILs) carrying chromosome segments derived from PTB33 at *BPH32, BPH2,* and *BPH17* exhibited higher resistance effects in the genetic background of the *japonica* rice variety Taichung 65 (T65) (Nguyen et al. 2019). However, these three genes could only partially explain the high-level resistance of PTB33. Therefore, genome-wide explorations of genes or quantitative trait locus (QTL) with other additive and epistatic genetic effects would be necessary better to understand the durable resistance and its breakdown of PTB33.

Here we conducted genetic dissection of breakdown processes of BPH resistance in PTB33 by individual genetic loci by genome-wide QTL analysis using high-density SNP markers obtained by genotyping-by-sequencing (GBS) with the infestation of the four BPH populations collected in Japan in 1988, 1989, 1999, and 2013.

Results

Resistance breakdown of PTB33

Four BPH populations collected at Hadano City, Kanagawa Prefecture, Japan, in 1966 (1966-Hadano population), Chikugo City, Fukuoka Prefecture, Japan in 1989 (1989-Chikugo population), Koshi City, Kumamoto Prefecture, Japan in 1999 (1999-Koshi population), and Koshi in 2013 (2013-Koshi) were used in infestation (Table S1). The BPH resistance of PTB33 was evaluated by the rate of females with a swollen abdomen (FSA) at five days after infestation (DAI) by the 1966-Hadano, 1989-Chikugo, 1999-Koshi, and 2013-Koshi BPH populations were 0.0%, 4.0%, 16.0%, and 55.0%, respectively, suggesting that the virulence of BPH populations increases with the collection year of BPH (Fig. 1).

QTL estimation for BPH resistance

The effective resistance QTLs to the four BPH populations were investigated in hybrid progenies derived from a cross between the susceptible varieties T65 and PTB33 (Fig. S1). The B_1F_2 populations were infested by the 1966-Hadano BPH population, and the B_1F_3 populations were infested by 1989-Chikugo, 1999-Koshi, and 2013-Koshi BPH populations (Fig. 1). The B_1F_2 and B_1F_3 populations backcrossed with T65 showed continuous distributions of FSA. The population means of FSA gradually increased with the collection year of BPH, with that of 1966-Hadano, 1989-Chikugo, 1999-Koshi, and 2013-Koshi being 33.4%, 47.0%, 68.0%, and 89.1% at 3 DAI (Fig. 1a-d) and 43.4%, 55.2%, 75.2%, and 91.7%, at 5 DAI (Fig. 1e-h), respectively. When the resistance was evaluated in female adult mortality (FAM), the BPH resistance of the population similarly decreased with the collection year of BPH (Fig. S2). FAM means in the T65 backcrossed population were found to decrease with the collection years: 35.1%, 35.2%, 19.8%, and 14.0% at 3 DAI and 52.6%, 54.4%, 32.4%, and 39.6% at 5 DAI, respectively (Fig. S2).

In the PTB33 backcrossed population, the FSA of the 1966-Hadano, 1989-Chikugo, 1999-Koshi, and 2013-Koshi BPH populations were 12.1%, 11.3%, 14.6%, and 34.5% at 3 DAI (Fig. 2a-d) and 19.8%, 20.0%, 19.8%, and 54.5%, respectively, at 5 DAI (Fig. 2e-h), respectively. FAM was 45.6%, 84.8%. 89.3%, and 35.5% at 3 DAI and 84.0%, 93.9%, 93.8%, and 55.9% at 5 DAI (Fig. S3).

Next, QTLs conferring BPH resistance were elucidated by multiple QTL mapping (MQM) for FSA at 5 DAI in four BPH populations since FSA at 5 DAI showed a larger phenotypic variance could be a suitable measure for QTL analysis. The numbers of SNP markers detected by genotyping-by-sequencing were shown in Table S1. In the T65-backcrossed populations infested by the 1966-Hadano BPH population, simple marker regression detected only one significant QTL, *qFSA4a,* at 6.4 Mbp on chromosome 4. Multiple QTL mapping, including *qFSA4a* as a covariate, detected the 2nd QTL, *qFSA6,* at 2.2 Mbp on

chromosome 6. Several cycles of multiple QTL mapping, including all detected QTLs as covariates, were repeated until the updated genetic model did not improve the LOD value by more than 3.0 in the likelihood ratio test (see Methods). Collectively, multiple QTL mapping for FSA detected five QTLs (Table 1): *qFSA4a, qFSA6*, *qFSA11* at 24.3 Mbp on chromosome 11, *qFSA7a* at 1.6 Mbp on chromosome 7, and *qFSA3* at 10.3 Mbp on chromosome 3. The genetic effects of the five QTLs were estimated simultaneously: q*FSA4a* had the largest genetic effects, with 43.0% PVE and -28.8% additive effects; *qFSA6* explained 6.5% of the PVE and an additive effect of -13.9%; *qFSA11* had a PVE of 5.0% and an additive effect of 16.9%; *qFSA7a* had a PVE of 4.3% and an additive effect of -15.8%; and *qFSA3* had the lowest PVE of 4.0% and an additive effect of -9.6%.

Next, effective QTL to the 1989-Chikugo BPH at 5 DAI was explored. All of the QTLs detected by the 1966-Hadano were not detected. Instead, *qFSA4b* at 13.0 Mbp on the short arm of chromosome 4 and *qFSA12* at 23.5 Mbp on the long arm of chromosome 12 were involved in BPH resistance (Table 1). *qFSA4b* explained a PVE of 20.6% and an additive effect of -20.8%. *qFSA12* explained a PVE of 11.9% and an additive effect of -15.8%. In the BC_1F_3 population infested by the 1999-Koshi BPH population, one QTL with a PVE of 22.6% and an additive effect of -15.3% was detected at 7.4 Mbp on chromosome 4, which seemed equivalent to *qFSA4a* (Table 1). No QTLs were detected in the BC_1F_3 population infested by the 2013-Koshi BPH population.

In the PTB33 backcrossed population, no QTLs were detected for FSA at 5 DAI in any BPH population, probably due to small phenotypic variance because almost segregating plants showed strong resistance. However, at 3 DAI, several QTLs were successfully detected (Table 2). In the BC_1F_3 population infested by the 2013-Koshi BPH population, *qFSA6* at 2.4 Mbp on chromosome 6 and *qFSA8* at 18.0 Mbp on chromosome 8 were involved in BPH resistance with additive effects of -25.7%. and -14.6%, respectively).

QTL identification in T65 genetic background

The suggested QTLs for FSA were identified using flanking SSR markers by the infestation of the 1966-Hadano BPH population at 5 DAI. In the B_1F_3 population derived from backcrossing with T65, plants heterozygous at the QTL and homozygous for T65 or PTB33 at the background QTL were self-pollinated to obtain B_1F_4 populations as the monogenic segregating populations (Fig. 3, Table S3). LOD peaks at *qFSA4a*, *qFSA6*, *qFSA11*, and *qFSA12* exceeded over more than a 5% empirical threshold in the corresponding MSP: *qFSA4a* was repeatedly detected in B_1F_4 population #102, and the PTB33 allele was estimated to reduce 33.2% of FSA at 5 DAI; *qFSA6* was identified in population B_1F_4 #114, with an additive effect of -26.5% for the PTB33 allele. Similarly, *qFSA11* and *qFSA12* were identified with additive effects of -13.2% and -11.5% for the PTB33 allele in B_1F_4 populations #109 and #110, respectively. The remaining three QTLs (*qFSA3*, *qFSA4b,* and *qFSA7a*) were not identified in these populations.

Assessment of genetic effects at QTLs responding to insect population

The reduction of genetic effects at the *qFSA4a*, *qFSA6*, *qFSA11,* and *qFSA12* to developing virulence of the four BPH populations was statistically measured by LOD_i, LOD scores attributed from interaction component between QTLs and BPH populations (Table 3). The phenotypic values and genotypes of the B_1F_2 populations infested by 1966-Hadano BPH and the B_1F_3 populations infested by 1999-Koshi BPH were mixed and simultaneously solved to estimate LOD_i. The LOD_i score at *qFSA4a* was higher than the 5% significant threshold, implying that genetic effects at *qFSA4a* were different by 1966-Hadano and 1999-Koshi BPH populations. This difference is likely due to the shrinkage of the additive effect of FSA from -28.8% in the 1966-Hadano BPH to -15.3% in the 1999-Koshi BPH (Tables 1 and 3).

The LOD_i scores at *qFSA11* did not show apparent at 5% significance level but showed significance at 10% level in population size in this study. The additive effects of the PTB33 allele at *qFSA11* were -16.9% or zero (null hypothesis) by the 1966-Hadano or 1999-Koshi BPH, respectively. The LOD_i scores at *qFSA12* implied that genetic effects were different by the BPH populations at a 10% significance level. The additive effect at the *qFSA12* was significantly estimated with -11.5% for the PTB33 allele in the B_1F_4 monogenic segregating population infested by the 1966-Hadano BPH infestation (Table S3). However, *qFSA12* was not detected in the B_1F_2 generation, probably because residuals were not sufficient enough to detect *qFSA12* under simultaneous estimation with *qFSA4a*, *qFSA6*, *qFSA11*, *qFSA7a*, and *qFSA3* in this population size. On the other hand, the interaction of *qFSA6* by the BPH populations was not apparent, while *qFSA6* conferred resistance by the infestation of the 1966-Hadano BPH but not to the 1999-Koshi BPH population (Table 3).

The genetic effects of the PTB33 alleles at the *qFSA4a*, *qFSA6*, *qFSA11,* and *qFSA12* to the four BPH populations were clarified the B_1F_5 plants homozygous for T65 (T) and PTB33 (P) were obtained from a single heterozygous MSP of each QTL (Fig. 4) and infested by the four BPH populations. Female insects with medium swollen abdomens (MS) and non-swollen abdomens (NS) were evaluated as those with reduced growth and insects with swollen abdomen as those with normal (N) growth. Infestation with 1966-Hadano BPH revealed an apparent resistance effect of *qFSA4a*, *qFSA11*, and *qFSA12* on homozygous plants for PTB33, as compared with homozygous plants for T65 in Fisher's exact test. However, the resistance effect of the *PTB33* allele at *qFSA11* was lost in the 1989-Chikugo insect population, while the other QTLs *qFSA4a* and *qFSA12* were still effective. The PTB33 allele at *qFSA12* lost resistance effect, whereasone at *qFSA4a* remained to the 1999-Koshi BPH population. However, all four QTLs lost resistance to the 2013-Koshi insect population. Therefore it was concluded that the PTB33 alleles at *qFSA11*, *qFSA12,* and *qFSA4a* lost resistance effects in 1989, 1999, and 2013, respectively.

Discussion

This study detected four QTLs conferring BPH resistance in backcrossed progenies between a resistant variety PTB33 and a susceptible variety T65 infested by four BPH populations with developing virulence by year in Japan. It has been reported that varieties harboring a single resistance QTL/gene with major

genetic effects rapidly promote genetic changes or adaptations of BPH populations and result in resistance breakdown of resistant variety. In contrast, a pyramiding of multiple resistance genes enables resistance to BPH to persist for a longer duration (Horgan et al. 2019). Multiple BPH resistance genes might cause resistance of PTB33 to BPH populations from most South and Southeast Asian countries (Horgan et al. 2015). However, the BPH resistance of PTB33 gradually reduced across years (Fig. 1). Nevertheless, PTB33 was still resistant to the recently collected BPH populations (2013-Koshi BPH population). These results suggest that the persistent resistance of PTB33 to BPH is mediated by the presence of multiple BPH resistance genes.

We focused on the QTL conferring FSA at 5 DAI in the T65 backcrossed population (Table 1). *qFSA4a*, *qFSA6*, *qFSA11*, *qFSA7a*, and *qFSA3* were detected in the infestation of the 1966-Hadano BPH population. *qFSA4b* and *qFSA12*, which were not detected in the 1966-Hadano BPH population, were detected in the infestation of the 1989-Chikugo BPH population. In addition, *qFSA4a* was detected in the infestation of the 1999-Koshi BPH population. No QTLs were detected in the infestation of the 2013-Koshi BPH population. These suggest that PTB33 may have changed the QTL conferring BPH resistance in the four BPH populations. In the PTB33 backcrossed population, *qFSA6* and *qFSA8* were detected in the infestation of the 2013-Koshi BPH population at 3DAI (Table 2). *qFSA8* was only detected in the PTB33 backcrossed population, likely due to interaction with other QTLs in the genetic background of PTB33. Identification of *qFSA7b* and *qFSA8* is necessary for further analysis in the appropriate genetic backgrounds. To date, most of BPH resistance loci were contained in a cluster of four chromosomal regions designated clusters A, B, C, and D on the long arm of chromosome 12, the short arm of chromosome 4, the short arm of chromosome 6, and the long arm of chromosome 4, respectively (Fujita et al. 2013). *qFSA4a*, *qFSA6*, and *qFSA12* were detected in clusters B, C, and A, respectively. *qFSA4a*, *qFSA6*, and *qFSA12* were inferred to be identical to *BPH17*, *BPH32*, and *BPH2*, respectively, on previous genetic studies using PTB33. *qFSA11* is a novel locus of BPH resistance.

Individual genetic loci in this study dissected the resistance breakdown of PTB33. Since BPH are unable to overwinter, the biotypes and virulence changes of BPH are likely to be affected by continental East Asian BPH populations in Japan (Matsumura 2001). The PTB33 allele at *qFSA12* lost resistance between 1989 and 1999 in Japan (Fig. 4). *qFSA12* was located on Fujita's cluster A containing *BPH1* and *BPH2*. The BPH immigrating into Japan became virulent to a resistance gene *BPH1* around 1988-1990 (Sogawa, 1992), and the BPH population rapidly became virulent to *BPH2* beginning in 1997 (Tanaka and Matsumura 2000). Therefore, *qFSA12* is likely to be identical to *BPH1* or *BPH2* corresponding to the previous reports. A recessive virulence gene to *BPH1*, *vBPH1*, was identified on the linkage map of BPH, indicating BPH resistance resulted from *BPH1* occurred in a gene-for-gene manner with the resistance gene in a host rice plant and the avirulence gene in a pest insect BPH (Kobayashi et al. 2014). The PTB33 allele at *qFSA4a* was found to lost resistance between 1999 and 2013 (Fig. 4). Horgan et al. (2015) reported that a single BPH resistance gene was insufficient to resist the current BPH populations increasing their virulence. The evolution of the virulence gene would explain the recently increasing virulence to at least *qFSA4a* or *BPH17*. The PTB33 allele at *qFSA11* would lose resistance as early as 1989 in Japan, although a BPH resistance gene has not been detected around this region. In the initial

breakdown process to the BPH resistance gene between 1966 and 1989, not only *BPH1* and *BPH2* but also other unknown genetic loci, including *qFSA11,* might have already lost their resistance effect.

Conclusion

This study suggested that reduced genetic effects at each resistance locus explain the breakdown of durable resistance in PTB33 due to acquiring multiple virulence genes in BPH. The virulence mechanisms of BPH need to be clarified in a gene-by-gene manner using BPH resistance gene NILs in further studies.

Materials and Methods

Plant materials

A susceptible *japonica* variety, T65, and a resistant *indica* variety, PTB33, were used as female and male parents, respectively, to produce F_1 hybrids. The F_1 plants were backcrossed with T65 or PTB33 to produce the B_1F_1 population (Fig. S1). The B_1F_2 and B_1F_3 populations were grown in 2017 and 2018 for QTL detection. For QTL identification, the B_1F_3 plants heterozygous at a single QTL and homozygous for T65 or PTB33 at the other QTLs were selected and self-pollinated to obtain The B_1F_4 populations. B_1F_5 segregants homozygous for T65 and PTB33 were used to assess the genetic effects of QTLs by BPH populations.

Insect populations

Four BPH populations were collected at Hadano City, Kanagawa Prefecture, Japan, in 1966 (1966-Hadano population), Chikugo City, Fukuoka Prefecture, Japan in 1989 (1989-Chikugo population), Koshi City, Kumamoto Prefecture, Japan in 1999 (1999-Koshi population), and Koshi in 2013 (2013-Koshi, Table S1). The collected populations were continuously reared using the *japonica* variety Reiho at the Kyushu-Okinawa Agricultural Research Center (KARC), Koshi City, Kumamoto Prefecture, Japan, at 25°C for 16 h under light and 8 hours dark cycle conditions. BPH resistance was evaluated using the 1966-Hadano BPH population in the B_1F_2, B_1F_4, and B_1F_5 populations and the remaining four BPH populations in the B_1F_3 and B_1F_5 populations (Fig. S1).

Evaluation of BPH resistance

The antibiosis effects of rice on BPH were determined by assessing female adult mortality (FAM) and the rate of females with a swollen abdomen (FSA) of BPH. First, a single seed was sown in a 215 ml plastic cup and grown for approximately 80 days. Five adult females within 48 h after emergence were used to infest a single plant in a transparent tube. FAM and FSA on three and five days after infestation (3 DAI and 5 DAI) were examined as the percentage of dead insects and the percentage of insects with swollen abdomens, respectively, of the total insects on a single plant. The degree of FSA was evaluated at three levels: massively swollen abdomen (S) if extremely large, non-swollen abdomen (NS) if a swollen

abdomen was not observed, and a medium swollen abdomen (MS) if a moderately swollen abdomen was noted between S and NS.

Genotyping-by-sequencing

For extraction of total genomic DNA, 20–40 mg of young leaf samples frozen in liquid nitrogen were ground in a 2.0 ml plastic tube using a Multibeads shocker (Yasui Kikai, Osaka, Japan). Total genomic DNA was extracted using the protocol described by Mayjonade et al. (2016). 10 mg of genomic DNA digested with the restriction enzymes *Kpn*I and *Msp*I was used for library preparation of GBS (Poland et al. 2012). Library sequencing was performed using the MiSeq platform (Illumina, San Diego, CA, USA).

Short read sequences (in .fastq format) of the GBS library were processed using the Tassel5 software (Bradbury et al. 2007) to conduct genotyping calling. Imputation of missing genotypes or incorrect genotype correction was conducted using a hidden Markov model with the low-coverage biallelic impute (LB-Impute) software (Fragoso et al. 2016).

QTL analysis

Genome-wide LOD values were scanned using marker regression with R/qtl (Broman et al. 2003). The LOD threshold at the 5% significance level was determined based on 1000 permutation tests. The DNA marker with the highest significant LOD score was included in the genetic model, and another QTL was explored under the assumption that multiple additive QTLs simultaneously control genetic values without interactions in multiple QTL mapping (MQM). When a new marker increased the LOD value to 3.0, the added genetic locus was accepted as a QTL.

Genotyping by SSR markers

Total genomic DNA was extracted according to the method described by Dellaporta et al. (1983), with minor modifications. Primer sequences of the polymerase chain reaction (PCR)-based markers used in this study are listed in Table S2. Each 15-mL reaction mixture consisted of 50 mM KCl, 10 mM Tris (pH 9.0), 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.2 mM primers, 0.75 units of *Taq* polymerase (Takara, Otsu, Japan), and 10 ng genomic DNA template. PCR was performed using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The cycling profile consisted of an initial denaturation step at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s; and a final elongation step at 72 °C for 7 min. Amplified products were electrophoresed on a 4% agarose gel in 0.5× TBE buffer.

Interaction between QTL and BPH population

Interactions between QTL and BPH populations were calculated as described by Broman et al. (2009). Briefly, the 1966-Hadano and 1999-Koshi BPH populations were coded as 0 and 1 as covariates for input into the linear models. The effect of BPH populations was partitioned by the additive effect and interaction between the QTL and BPH populations. We assumed hypotheses of the null model (H_0), the additive model (H_a) assuming a single QTL and BPH populations, and the full model (H_f) including the

additive effect of a single QTL and BPH, and interaction between the QTL and BPH. The $\log_{10}$ likelihood ratio (LOD) comparing H_f and H_0, LOD_f, represents evidence for the QTL, BPH, and interaction. The other LOD score, LOD_a, comparing H_a and H_0 provides evidence for the additive effects of QTL and BPH. LOD_i is defined as the difference between LOD_f and LOD_a, $LOD_i = LOD_f - LOD_a$, implying evidence of interaction between QTL and the BPH population. The LOD threshold at the 5% significance level was determined based on 1000 times permutation test.

Abbreviations

BPH: brown planthoppers; DAI: days after infestation; FAM: female adult mortality; FSA: females with a swollen abdomen; GBS: Genotyping-by-sequencing; LOD: logarithm of the odds; MQM: multiple QTL mapping; MS: medium swollen abdomen; MSP: monogenic segregating population; NIL: Nearly isogenic line; NS: non-swollen abdomen; QTL: Quantitative trait locus; S: massively swollen abdomen.

Declarations

Acknowledgments

Library of next-generation sequencing was conducted with Illumina Miseq at the Center for Advanced Instrumental and Educational Supports, Faculty of Agriculture, Kyushu University. Insect rearing and antibiosis tests were conducted at the Biotron Application Center, Kyushu University.

Authors' Contributions

DF, YY, and HY designed the experiments. DF and ST developed plant materials. SS-M and MM collected and reared the BPH populations. ST performed the experiments. ST and YY analyzed the data. ST, YY, DF, and HY wrote the manuscript. All authors reviewed the manuscript.

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Availability of Data and Materials

The data sets supporting this article are included in the article and in the additional files. Short-read sequences of genotyping-by-sequencing are deposited to the DNA databank of Japan (DDBJ) Read Archive submission number DRA012495.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Competing interests

The authors declare no competing interests.

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Tables

Table 1. QTLs for females with a swollen abdomen at five days after infestation detected in the population derived from backcrossing with T65.

BPH populations	QTL	Chr.	Position (Mbp) [a]	LOD	Additive effect (%) [b]	Dominance effect (%)	PVE (%) [c]
1966-Hadano	*qFSA4a*	4	6.4	22.4	-28.8	-23.9	43.0
	qFSA6	6	2.2	5.1	-13.9	7.5	6.5
	qFSA11	11	24.3	4.1	-16.9	9.0	5.0
	qFSA7a	7	1.6	3.5	-15.8	11.9	4.3
	qFSA3	3	10.3	3.3	-9.6	-4.2	4.0
1989-Chikugo	*qFSA4b*	4	13.0	6.2	-20.8	-5.6	20.6
	qFSA12	12	23.5	3.8	-15.8	-13.7	11.9
1999-Koshi	*qFSA4a*	4	7.4	5.6	-15.3	-11.9	22.6

[a] The physical positions in Os-Nipponbare-Reference-IRGSP-1.0 pseudomolecules.

[b] Positive value represents PTB33 allele increases genetic values of a trait.

[c] Percentage of phenotypic variance explained by the QTL.

Table 2. QTLs for females with a swollen abdomen at three days after infestation detected in the population derived from backcrossing with PTB33.

BPH populations	QTL	Chr.	Position (Mbp)[a]	LOD	Additive effect (%)[b]	Dominance effect (%)[b]	PEV (%)[c]
2013-Koshi	*qFSA6*	6	2.4	6.2	-25.7	1.1	22.9
	qFSA8	8	18.0	3.3	-14.6	-15.1	11.3

[a] The physical positions in Os-Nipponbare-Reference-IRGSP-1.0 pseudomolecules.

[b] Positive value represents PTB33 allele increases genetic values of a trait.

[c] Percentage of phenotypic variance explained by the QTL.

Table 3. Interaction between the major QTLs and the BPH populations collected in 1966 and 1999.

Insect population	Analysis type	Additive effect at QTLs [a]							Citation
		qFSA4a (6.04)		*qFSA6* (19.70)		*qFSA11* (23.20)		*qFSA12* (23.40)	
1966-Hadano	MQM	-28.8	*	-13.9	*	-16.9	*	*ns*	Table 1
1999-Koshi	MQM	-15.3	*	*ns*		*ns*		*ns*	Table 1
1966-Hadano / 1999-Koshi	Interaction [b]	*		*ns*		+		+	

[a] Positive value represents the PTB33 allele increases genetic values estimated in multiple QTL mapping (MQM) for QTL detection. The physical positions (Mbp) in the Os-Nipponbare-Reference-IRGSP-1.0 pseudomolecules at the SNP markers at the QTL peaks are shown in parentheses. The empirical LOD threshold at the 5% significance level was determined based on 1,000 permutation tests.

[b] * and + represent significant LOD_j score at 5% and 10% significance level.

Figures

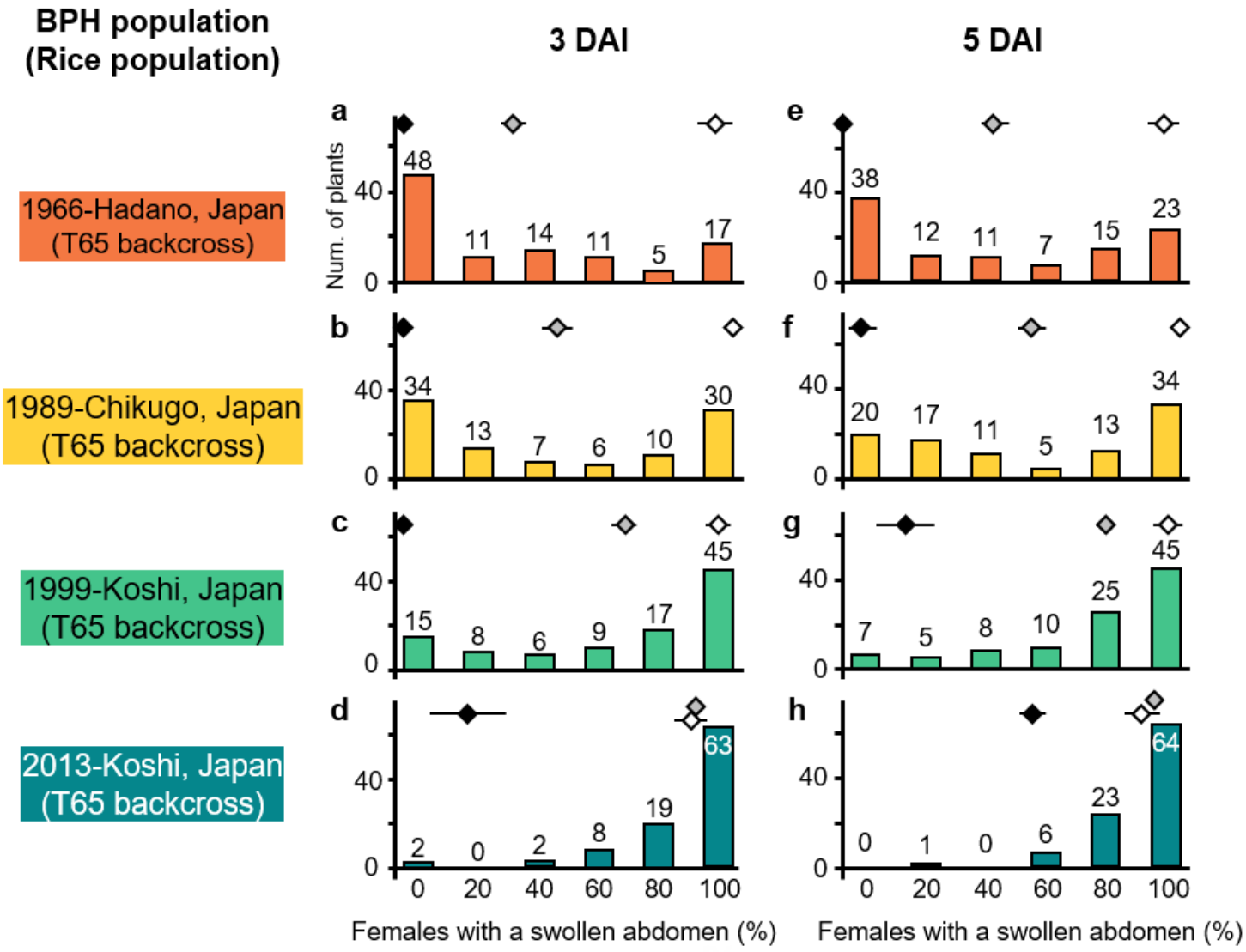

Figure 1

Frequency distribution of females with a swollen abdomen (%) in the four populations derived from backcrossing with T65 at 3 (a-d) and five days after infestation (e-h). Each of the four populations was infested by 1966-Hadano BPH (a, e), 1989-Chikugo BPH (b, f), 1999-Koshi BPH (c, g), and 2013-Koshi BPH (d, h). Black, white, and grey diamonds indicate the mean in PTB33, T65, and the population, respectively. Error bars indicate the standard error.

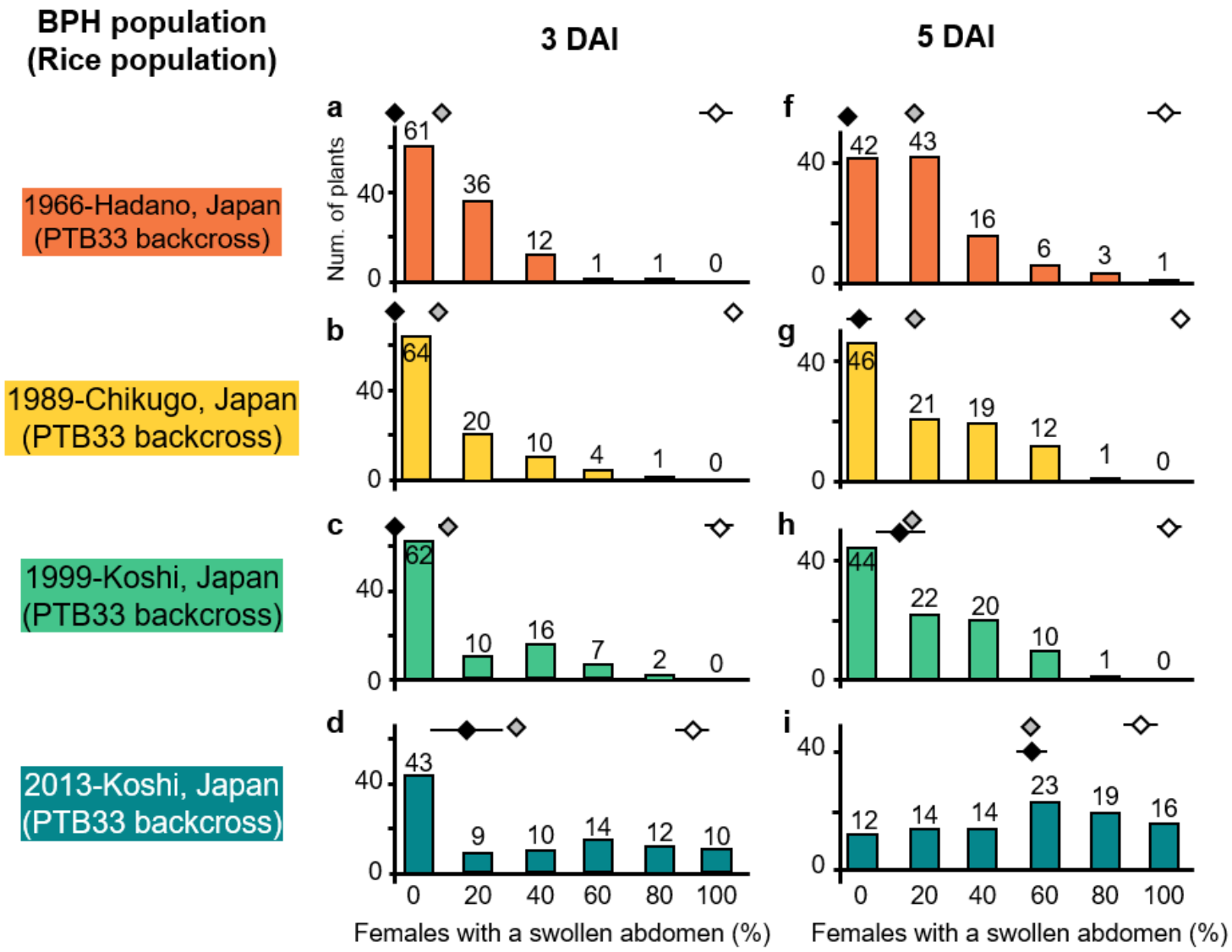

Figure 2

Frequency distribution of females with a swollen abdomen (%) in the four populations derived from backcrossing with PTB33 at 3 (a-d) and five days after infestation (e-h). Each of the four populations was infested by the BPH populations of 1966-Hadano (a, e), 1989-Chikugo (b, f), 1999-Koshi (c, g), and 2013-Koshi (d, h). Black, white, and grey diamonds indicate the mean in PTB33, T65, and the segregating population, respectively. Error bars indicate standard error.

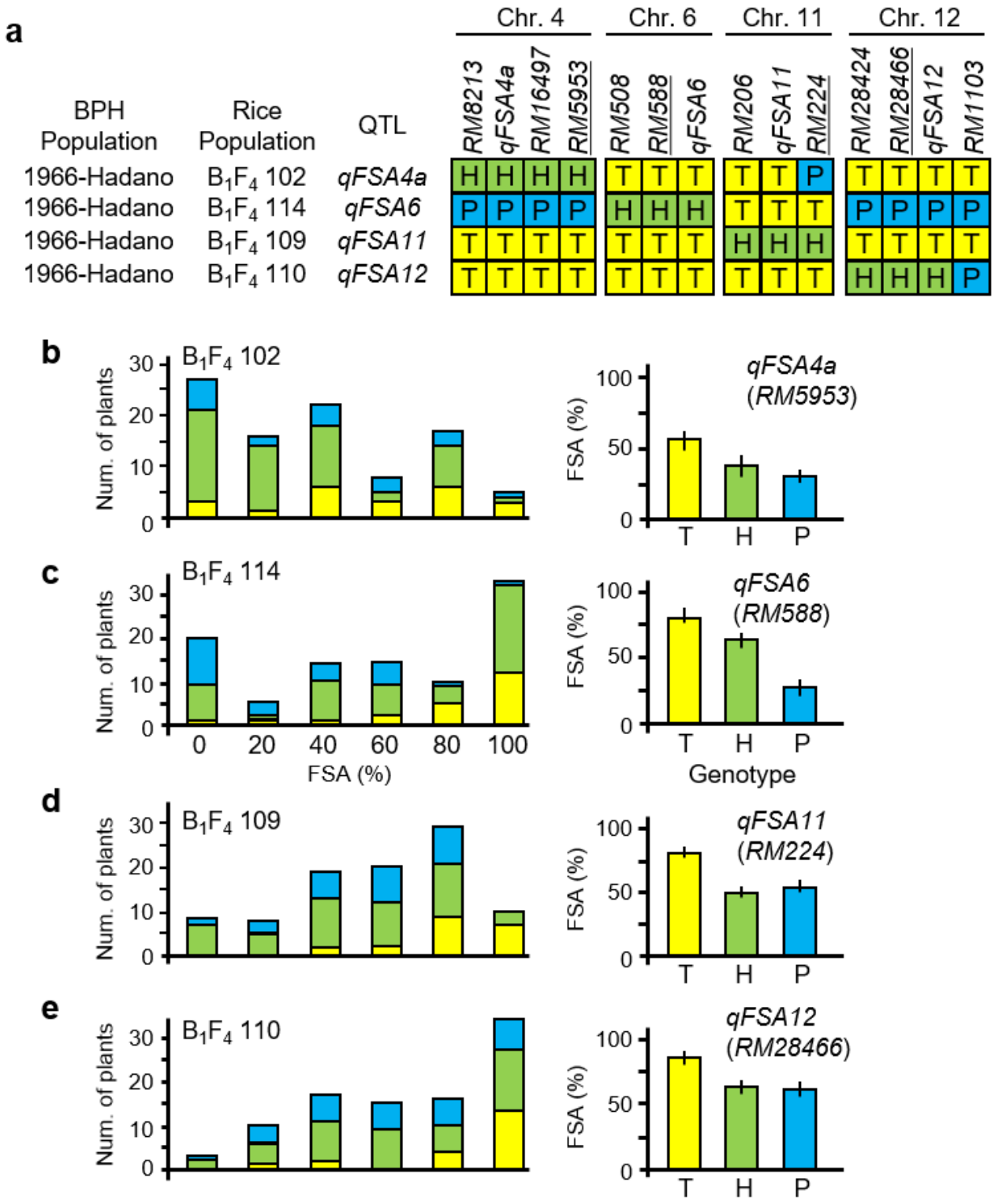

Figure 3

Validation of QTLs using monogenic segregating lines of single QTL regions. (a) Genotypes of parental plants of B1F4 populations in the genetic background of T65 in the QTL validation. T, P, and H indicate homozygous for T65, homozygous for PTB33, and heterozygous genotypes, respectively. (b-e) QTL analyses in the B1F4 populations segregating at a single QTL among qFSA4a (b), qFSA6 (c), qFSA11 (d),

and qFSA12 (e). Frequency distribution of females with a swollen abdomen (%) (FSA) in the B1F4 population (left) and average FSA in the homozygous plants for T65 (T) and PTB33 (B).

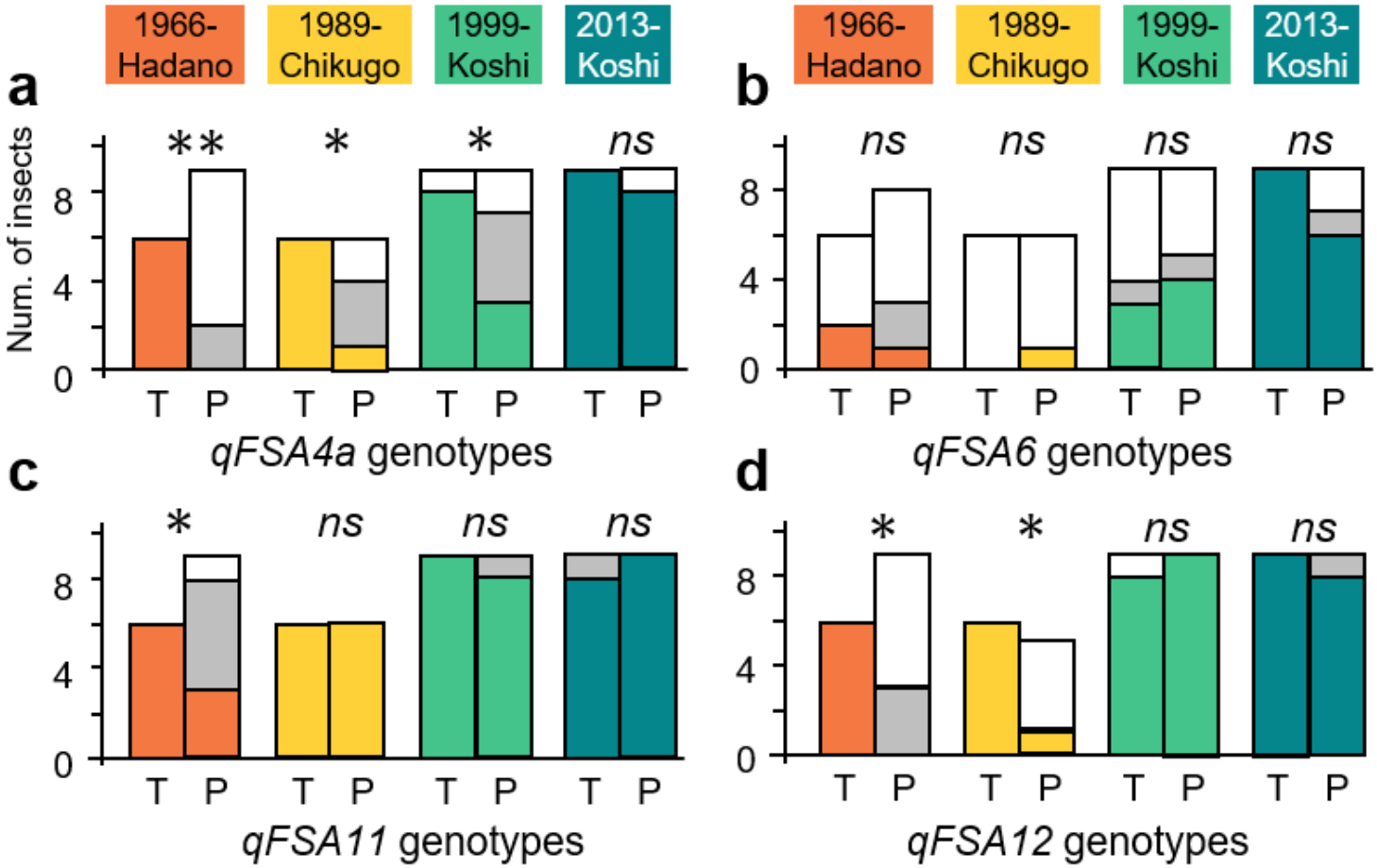

Figure 4

Assessment of a genetic effect of each QTL by brown planthopper populations. (a-d) Numbers of female insects with massive swollen (colored), medium swollen (grey), and non-swollen abdomen (white) in monogenic segregating lines homozygous for T65 (T) or PTB33 (P) at qFSA4a (a), qFSA6 (b), qFSA11 (c), and qFSA12 (d) infested by 1966-Hadano (red), 1989-Chikugo (yellow), 1999-Koshi (pale green), and 2013-Koshi (deep green). The numbers of female insects with a massively swollen abdomen as a non-resistance effect and those with medium and non-swollen abdomens as resistance effects were tested in Fisher's exact test (2 x 2 contingency table) by genotypes of the monogenic segregating lines homozygous for T65 or PTB33 at 5% significance level. * and ns represent significant and non-significant.

Supplementary Files

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