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Development of near-isogenic lines for *BPH25*(t) and *BPH26*(t), which confer resistance to the brown planthopper, *Nilaparvata lugens* (Stål.) in *indica* rice 'ADR52'

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The brown planthopper [BPH; *Nilaparvata lugens* (Stål.)] is one of the most destructive insect pests in Asian rice-growing areas. Two genes conferring resistance to BPH, *BPH25*(t) and *BPH26*(t) derived from a BPH-resistant *indica* rice cultivar, *Oryza sativa* ADR52, have been identified. However, they are linked to genes conferring late heading and hybrid spikelet sterility. To eliminate these unfavorable traits (linkage drag), we generated BC_6F_1 populations carrying *BPH25*(t) or *BPH26*(t) in a BPH-susceptible *japonica* cultivar, Taichung 65, through marker-assisted selection. We selected three near-isogenic lines (NILs) carrying *BPH25*(t) without late heading date and one NIL carrying *BPH26*(t) without spikelet sterility from BC_6F_2 progeny that showed between 96.3 and 99.8% of the Taichung 65 genetic background through whole-genome survey. In antibiosis testing, the rates of surviving insects and of females with swollen abdomens were lower on the NILs than on Taichung 65, indicating that *bph25*(t) and *Bph26*(t) alleles from ADR52 controlled the resistance to BPH. The NILs will serve as useful resources for (1) monitoring BPH virulence and for (2) increasing resistance to BPH.

Key Words: rice, brown planthopper resistance, *BPH25*(t), *BPH26*(t), near-isogenic line, linkage drag, marker-assisted selection.

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is one of the most serious and destructive pests of rice throughout Asian rice-growing areas. BPH causes significant yield losses in susceptible cultivars every year (Khush 1979). Heavy infestations cause complete drying and death of plants, a condition known as "hopperburn". The development of a novel BPH resistant cultivar is thought to be the most effective manner to combat this pest. A number of major BPH-resistance genes have been identified from *indica* rice cultivars (Chen *et al.* 2006, Kabir and Khush 1988, Khush 1979, Khush *et al*. 1985, Nemoto *et al.* 1989, Sun *et al.* 2005). The development of near-isogenic lines (NILs) and pyramided lines (PYLs) carrying one or several resistance genes has been performed (Jairin *et al*. 2009, Sharma *et al*. 2004) and is a most effective manner to facilitate the use of the BPH-resistance genes or of quantitative trait loci (QTLs) for future rice improvement.

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Hybridization of rice cultivars frequently results in various reproductive barriers, such as hybrid sterility, hybrid breakdown, inviability, certation, and chlorosis. Hybrid breakdown is also observed in intraspecific crosses between *japonica* and *indica* cultivars (Kubo and Yoshimura 2005). The *indica* parent IR24 has a sterility allele, named *hsa1*–IR, located on the long arm of chromosome 12. Hybrids carrying *hsa1*–IR in the *japonica* cultivar Asominori genetic background exhibit spikelet sterility due to the abortion of female gametes. Heading date is also an important factor for the adaptation of novel rice cultivars to local environments. However, some rice accessions from tropical and subtropical regions carry recessive alleles for photosensitivity on the short arm of chromosome 6 (Dung *et al.* 1998, Hagiwara *et al.* 2009, Monna *et al.* 2000). Plants carrying the homozygous recessive alleles or QTLs experience delayed heading under natural field conditions in Japan. When these unfavorable loci are linked to a desired locus, both traits are inherited. Thus, preventing this "linkage drag", which can lead to massive reductions in crop yield, is critical.

In our previous study, which involved linkage analysis using a BC_3F_2 population carrying BPH resistance genes from the BPH-resistant *indica* cultivar *Oryza sativa* ADR52,

we identified two BPH resistance loci: *BPH20*(t), on the short arm of chromosome 6, and *BPH21*(t), on the long arm of chromosome 12 (Myint *et al.* 2005). Because these names conflicted with previous nomenclature (see the next paragraph for details), we renamed the genes *BPH25*(t) and *BPH26*(t), respectively. We developed pre-NILs carrying *BPH25*(t), *BPH26*(t), or both loci; however, they show delayed heading and hybrid sterility, suggesting that they are linked to a heading date–related gene and a hybrid sterility–related gene, respectively. Therefore, the *BPH25*(t) pre-NIL and the *BPH26*(t) pre-NIL were unsuitable as donors of BPH-resistance genes. The objectives of the present study were to develop more advanced NILs by the removal of the unfavorable loci from the pre-NILs.

Here, we follow the new gene nomenclature system for rice that has been proposed by McCouch and CGSNL (2008), and renamed the BPH resistance loci to avoid confusion with other recently reported loci. We had previously reported two BPH resistance alleles derived from *indica* cultivar ADR52 and tentatively named them *bph20*(t) and *Bph21*(t) (Myint *et al*. 2005). However, Rahman *et al*. (2009) had used the same gene names, *Bph20*(t) and *Bph21*(t), for a different donor species, (*O*. *minuta*, Acc. No. 101141), and these were definitely different loci. In addition, *Bph22*(t) and *Bph23*(t) (Ram *et al*. 2010) and *Bph24*(t) (Deen *et al*. 2010) have recently been reported. To avoid confusion with previous gene names among the different BPH resistance loci, we have proposed the following new gene names: *BPH25*(t) instead of *bph20*(t), and *BPH26*(t) instead of *Bph21*(t). These names follow the new gene nomenclature system for rice.

Materials and Methods

Development of the NILs

Fig. 1 shows the breeding scheme used for the development of NILs carrying the BPH resistance genes. An F_2 population derived from a cross between the susceptible *japonica* cultivar Taichung 65 (T65) and the resistant *indica* cultivar ADR52 was previously used for QTL analysis (Sonoda *et al.* 2003). The F_1 plants from this cross were backcrossed with T65 to generate a BC_1F_1 population. Ten BC_1F_1 plants were then backcrossed with T65 as the recurrent parent to develop 10 BC_2F_1 populations. A total of 93 BC_2F_1 plants were generated and selected through marker-assisted selection (MAS) using the simple sequence repeat (SSR) markers in Supplemental Table 1. The 27 selected BC_2F_1 plants were backcrossed with T65 to develop BC_3F_1 populations that were heterozygous for the targeted QTL regions on chromosomes 6 and 12. A total of 321 BC_3F_1 individuals were generated and 52 of these that retained the chromosomal segments on chromosomes 6 and 12 were selected by MAS. The selected BC_3F_1 individuals were backcrossed with T65 to develop BC_4F_1 plants carrying the BPH-

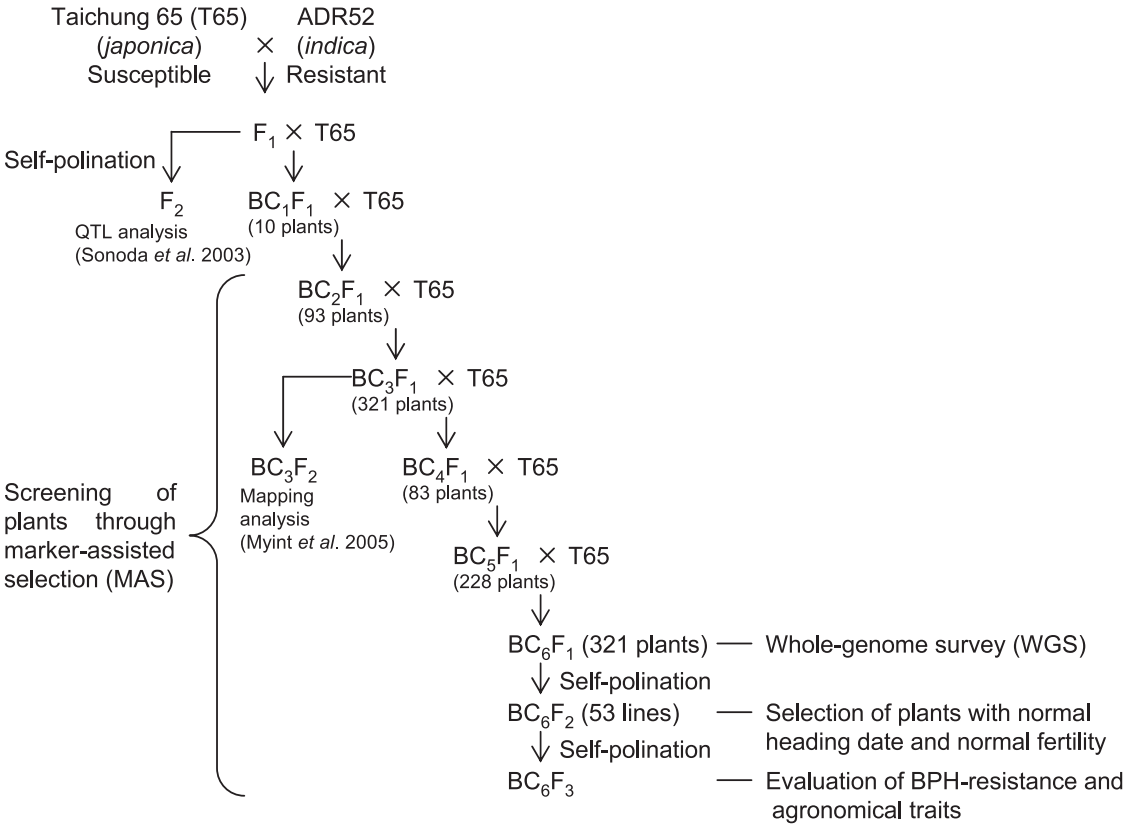

Fig. 1. Breeding scheme for development of NILs carrying *BPH25*(t) or *BPH26*(t).

resistance genes, *BPH25*(t) and *BPH26*(t). A total of 83 BC_4F_1 individuals were generated, and 19 carrying the ADR52 alleles at *BPH25*(t), *BPH26*(t), or both loci were selected from the BC_4F_1 population by MAS. The selected BC_4F_1 individuals were backcrossed with T65 to develop BC_5F_1 plants carrying *BPH25*(t), *BPH26*(t), or both loci. A total of 228 BC_5F_1 individuals were generated, and 41 carrying ADR52 alleles at *BPH25*(t), *BPH26*(t), or both loci were selected from the BC_5F_1 population by MAS. Finally, 321 BC_6F_1 individuals were generated, and 176 carrying ADR52 alleles at *BPH25*(t), *BPH26*(t), or both loci were selected by MAS. We then self-pollinated 53 of those BC_6F_1 plants. From the resulting BC_6F_2 progeny, we selected three lines carrying *BPH25*(t) without late heading date and a line carrying *BPH26*(t) with normal spikelet fertility as candidate NILs. The BC_6F_3 individuals homozygous for ADR52 or T65 alleles and heterozygous individuals were evaluated for their resistance to BPH.

SSR markers and sequence-tagged site markers

To select individuals carrying the BPH resistance genes, we used 9 SSR markers near the *BPH25*(t) or *BPH26*(t) loci (Supplemental Table 1). Seven of these markers (*RM3353*, *RM588*, *RM8101*, *RM309*, *RM28449*, *RM3813*, and *RM5479*) have previously been reported (International Rice Genome Sequencing Project 2005, McCouch *et al*. 2002, Temnykh *et al*. 2001). In addition, we developed two new SSR markers (*S00310* and *S20103*) near the *BPH25*(t) or *BPH26*(t) loci based on the Nipponbare genome sequence from the International Rice Genome Sequencing Project. For MAS of the population carrying *BPH25*(t) from ADR52, we used the markers flanking *BPH25*(t) on chromosome 6, namely *S00310*, *RM3353*, *RM588* and *RM8101* (Myint *et al*. 2005). For MAS of the population carrying *BPH26*(t) from ADR52, we used the markers flanking *BPH26*(t) on chromosome 12, namely *RM309*, *RM28449*, *RM3813*, *S20103* and *RM5479*. For linkage analysis, we used the SSR markers *RM225* and *RM6306* (IRGSP 2005, McCouch *et al*. 2002, Temnykh *et al*. 2001) and three sequence-tagged site (STS) markers (*sHd1*, *sRFT1* and *sHd3a*) on the short arm of chromosome 6. We designed these additional STS markers using sequence information for the heading date–related genes *Hd1*, *RFT1* and *Hd3a* that were identified by Yano *et al.* (2000) and Kojima *et al.* (2002).

Whole-genome survey of the NILs using SSR and STS markers

We analyzed the NILs using SSR and STS markers scattered throughout the rice chromosomes (McCouch *et al*. 2002) to characterize their genetic background and to identify the positions of the targeted genes. We used 172 of the 240 tested SSR markers that showed polymorphism between T65 and ADR52 at the marker loci in different regions of the 12 chromosomes for a whole-genome survey (WGS) of the selected NILs that contained *BPH25*(t), *BPH26*(t), or both. Using the genotype data for these NILs, we graphically displayed the genotypes of each line according to the concept of graphical genotypes proposed by Young and Tanksley (1989). We calculated the physical distances between each pair of markers on the basis of the Nipponbare genome sequence in the Rice Annotation Project database.

Evaluation of heading date and spikelet fertility

We evaluated the heading date and spikelet fertility in the BC_6F_2 populations and T65. The heading dates were distinctly segregated in the specific populations, with only two kinds of phenotypes (normal heading plants that headed 100 to 107 days after sowing and were similar to T65 in term of heading date and delayed-heading plants that headed more than 135 days after sowing). We defined spikelet fertility as the number of filled grains divided by the total number of filled and unfilled spikelets. We classified plants with less than approximately 40% filled spikelets as highly sterile, and classified plants with more than approximately 80% filled spikelets as fertile.

Evaluation of BPH resistance

We evaluated the BPH resistance of the *BPH25*(t) and *BPH26*(t) NILs by means of infestation with BPH, as previously described (Myint *et al.* 2009a). The virulence of laboratory strains of BPH, collected in Japan between 1966 and 2005, was evaluated using rice differential varieties (Myint *et al.* 2009b). The BPH strain collected in 1966 (Hatano-66) is avirulent on all the tested rice cultivars including ADR52, but is virulent on T65. The insect population has been maintained by means of continuous rearing at 25 ± 1°C under a 16-h light, 8-h dark photoperiod. Five brachypterous females were released within 24 h after their emergence onto a single rice plant that had been sown 1 month earlier. The antibiosis scores were recorded 5 days after infestation (DAI). The resistance level of the NILs was described using the rate of surviving insects (RSI) and the rate of females with swollen abdomens (RFWSA). Plants with RSI and RFWSA <30% were categorized as resistant to BPH, and plants with RSI and RFWSA >70% were categorized as susceptible.

Evaluation of agronomic traits

For evaluation of culm length (CL), panicle length (PL), seed number per panicle (SNPP), percentage of ripened grains (PRG), and 1000-grain weight (TGW), we prepared *BPH25*(t) NIL (BC_6F_2) plants that would be heterozygous at *BPH25*(t), and *BPH26*(t) NIL (BC_6F_2) plants that would be heterozygous at *BPH26*(t). We then genotyped *BPH25*(t) and *BPH26*(t) in BC_6F_3 individuals. We compared the agronomic traits of the NILs homozygous for the ADR52 allele with those of NILs homozygous for the T65 allele and those of heterozygous plants. We defined CL as the average length from the soil surface to the spike neck of the main tiller. PL was the average length from the spike neck to the panicle tip of the main tiller. SNPP was measured as the average number of seeds per mature panicle. After husking, we screened the ripened grains with a sieve (1.8-mm mesh size, Kyushukett Co., Ltd.). PRG was calculated as the number of

ripened grains divided by the total number of grains. TGW was calculated as the average weight of 1000 ripened grains.

Results

Development of a BPH25(t) NIL that lacks the late-heading phenotype

We chose 25 BC_6F_1 plants that would be heterozygous at the *BPH25*(t) locus as candidate NILs. Among the 25 BC_6F_2 populations, 21 showed segregation for heading date, while the remaining 4 populations were fixed for normal heading date. Among the 21 segregating populations, 16 showed normal-heading and late-heading plants segregated in an approximately 3:1 ratio (214:78; $\chi^2 = 0.46$; $P = 0.4976$; Supplemental Table 2), indicating that heading date was controlled by a single Mendelian factor. We performed linkage analysis using five SSR markers (*S00310*, *RM3353*, *RM588*, *RM8101* and *RM225*) and three STS markers (*sRFT1*, *sHd3a* and *sHd1*) located on the short arm of chromosome 6 (Supplemental Table 1). All tested plants were homozygous for the T65 allele at *sHd1*, which links to selection SSR markers and is outside the region between *S00310* and *sHd3a*. This suggests that a heading date–related gene, tentatively named *hdX*(t), was located between *sHd3a* and *RM225*, separated by 0.34 cM from both loci (Fig. 2A). In the three BC_6F_2 populations with normal heading date, the allele for normal heading date was fixed (Supplemental Table 2). Three of the BC_6F_1 parental plants (BC_6F_1 4-4, BC_6F_1 31-4 and BC_6F_1 34-2) were heterozygous at *S00310* and *RM588*, but homozygous for the T65 allele at *sHd3a*, *hdX*(t) and *RM225* (Fig. 3). Table 1 shows that these lines covered at least 96.3% of the T65 genome. We therefore selected these three lines as candidate *BPH25*(t) NILs.

Development of a BPH26(t) NIL that lacks the hybrid-sterility phenotype

We established 9 BC_6F_1 plants that would be heterozygous at *BPH26*(t) as candidate NILs. Among the 9 BC_6F_2 populations, 8 showed segregation for spikelet fertility, and only the remaining 1 population was fixed for normal spikelet fertility (Supplemental Table 3). In 4 of the 8 segregating populations, the normally fertile plants and the highly sterile plants were segregated in an approximately 3:1 ratio. We performed linkage analysis of the segregating populations using six SSR markers (*RM309*, *RM28449*, *RM3813*, *S20103*, *RM5479* and *RM6306*) located on the long arm of chromosome 12 (Supplemental Table 1). We observed segregation distortion at all the SSR marker loci except *RM6306*. At the marker (*RM5479*) closest to the spikelet sterility locus, hybrid sterility was co-segregated with the homozygote for the ADR52 allele, whereas normal spikelet fertility was co-segregated with the homozygote for the T65 allele and with the heterozygous plants. Linkage analysis showed that the hybrid sterility-related gene, tentatively named *hsY*(t), was located between *RM5479* and *RM6306*, separated from these loci by 1.44 and 10.18 cM, respectively

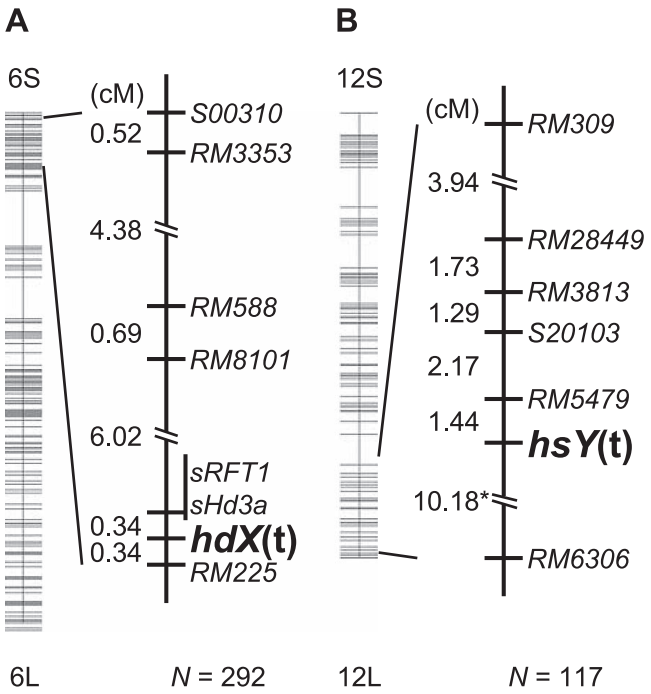

Fig. 2. Linkage maps showing the location of (A) the heading date–related gene *hdX*(t) and (B) the hybrid sterility-related gene *hsY*(t). Horizontal bars represent the positions of the SSR and STS markers used for the linkage analysis. *This value was calculated using 47 individuals. Framework maps are quoted from Harushima *et al*. (1998). Numbers between horizontal bars indicate map distances (cM).

(Fig. 2B). In one line of the BC_6F_2 populations carrying *BPH26*(t), the progeny showed normal spikelet fertility (Supplemental Table 3). The parental plant (BC_6F_1 27-4) of this population was heterozygous at *RM309* and *S20103*, and was homozygous for the T65 allele at *RM5479*, *hsY*(t), and *RM6306* (Fig. 3D). Table 1 shows that this line covered at least 98.0% of the T65 genome. This line was selected as the candidate *BPH26*(t) NIL.

Surveying the genetic backgrounds of the BPH25(t) and BPH26(t) NILs

We analyzed the selected NILs in detail to characterize their genetic backgrounds through WGS with respect to the BC_6F_1 plants (Fig. 3). In the BC_6F_1 4-4 plant, the size of the substituted segment from ADR52 on chromosome 6 was estimated as 1.4 to 1.7 Mbp based on the loci of two flanking SSR markers (*S00310* and *RM588*; Fig. 3A). Another substituted segment was detected between *RM6051* and *RM205* on chromosome 9, with a size of 10.1 to 12.5 Mbp. In the BC_6F_1 31-4 plant, the size of the substituted segment was estimated as 1.5 to 2.9 Mbp based on the loci of *S00310* and *RM8101* on chromosome 6 (Fig. 3B). Another substituted segment was detected at *RM4997* on chromosome 8, with a size of less than 0.5 Mbp. The BC_6F_1 34-2 plant was heterozygous at *S00310* and *RM588* on chromosome 6, but was homozygous for the T65 allele at *RM19267* (Fig. 3C). Further analysis showed that the BC_6F_1 34-2 plant was heterozygous at

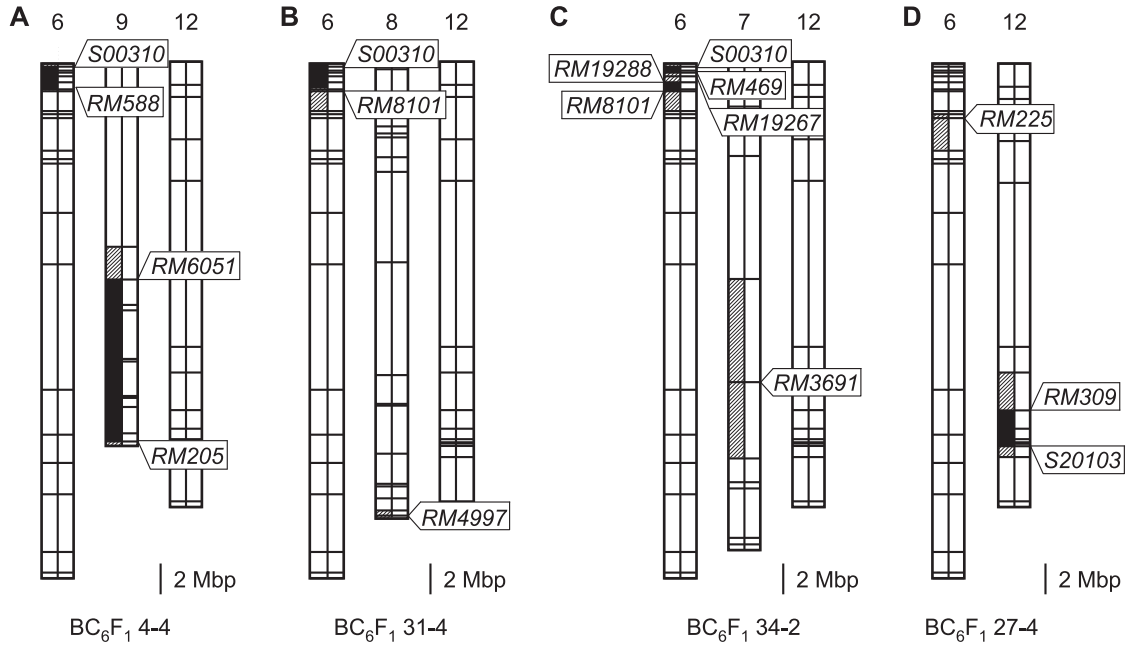

Fig. 3. Graphical genotypes of the *BPH25*(t) and the *BPH26*(t) NILs selected using MAS. The bars indicate the retained chromosome segments in the BC_6F_1 lines (A) 4-4, (B) 31-4, (C) 34-2, and (D) 27-4. The horizontal lines in the bars show the locations of the polymorphic SSR markers used in the MAS and WGS; numbers above the bars represent the chromosome number. White and black bars indicate chromosomal regions from the T65 and ADR52 alleles, respectively. Cross-hatched bars indicate intervals in which a recombination has occurred.

Table 1. Rates of surviving insects and females with a swollen abdomen and coverage (%) of T65 genome on the *BPH25*(t) and *BPH26*(t) NILs

Population	Origin	Resistance gene[a]		RSI[b]	RFWSA[b]	Coverage (%) of T65 genome[c]
		BPH25(t)	*BPH26*(t)			
BPH25(t)						
BC_6F_2 3-11	BC_6F_1 4-4	A	T	53.3 ± 17.6[abc]	13.3 ± 6.7[a]	96.3–97.0
BC_6F_2 20-3	BC_6F_1 31-4	A	T	72.0 ± 10.2[bc]	20.0 ± 8.9[a]	99.1–99.6
BC_6F_2 23-5	BC_6F_1 34-2	A	T	65.0 ± 17.1[abc]	25.0 ± 12.6[a]	96.3–99.8
BPH26(t)						
BC_6F_2 63-8	BC_6F_1 27-4	T	A	12.0 ± 12.0[a]	4.0 ± 4.0[a]	98.0–99.3
ADR52		A	A	26.7 ± 17.6[ab]	6.7 ± 6.7[a]	
T65		T	T	100.0 ± 0.0[c]	86.7 ± 6.7[b]	

[a] T and A indicate loci homozygous for the T65 and ADR52 alleles, respectively. The genotypes of *BPH25*(t) and *BPH26*(t) in the BC_6F_2 individuals were confirmed using two sets of flanking markers: *S00310–RM588* and *RM28449–RM3813*, respectively.

[b] Values in a column (mean ± S.E.) followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer multiple comparison test).

[c] The coverage was calculated as the percentage of the total physical length of the segments from T65 over the total length of the rice nuclear genome. These physical lengths were estimated on the basis of the Nipponbare genome sequence.

RM469 and *RM19288* on chromosome 6. These results suggest that two substituted segments from ADR52 were retained as a result of recombination events between *RM469* and *RM19267* and between *RM19267* and *RM19288*. The size of the shorter segment from ADR52 was estimated as 0.4 to 0.8 Mbp based on the loci of *S00310* and *RM469*. The size of the longer segment from ADR52 was estimated as 0.5 to 2.1 Mbp based on the loci of *RM19288* and *RM8101*. Another substituted segment was detected at *RM3691* on chromosome 7, with a size of less than 11.2 Mbp. In the BC_6F_1 27-4 plant, the size of the substituted segment on chromosome 12 was estimated as 2.3 to 5.3 Mbp based on the loci of *RM309* and *S20103* (Fig. 3D). Another substituted segment was detected at *RM225* on chromosome 6, with a size of less than 2.5 Mbp, but the location was separated from the predicted region of *BPH25*(t). Although *RM225* is linked the *hdX*(t), all progenies of the BC_6F_1 27-4 showed the normal heading date (data not shown), indicating that *hdX*(t) from ADR52 is removed in the BC_6F_1 27-4.

Evaluation of the RSI and RFWSA of the BPH25(t) and BPH26(t)NILs

To confirm the BPH resistance of the newly-developed *BPH25*(t) and *BPH26*(t) NILs, we conducted an antibiosis test using the Hatano-66 strain of BPH. We compared the levels of resistance to BPH of the progeny of the *BPH25*(t)

and *BPH26*(t) NILs that were homozygous for the ADR52 allele or the T65 allele, or that were heterozygous, with the resistance of ADR52 and T65 (Fig. 4). The RSI values of the BC_6F_2 progeny of *BPH25*(t) NILs homozygous for the ADR52 allele (BC_6F_2 3-11, 20-3 and 23-5) were lower than those of the progeny of NILs homozygous for the T65 allele (BC_6F_2 3-1, 20-2 and 23-3) or of heterozygous NILs (BC_6F_2 3-6, 20-1 and 23-1), but the differences were not significant. The RFWSA values of BC_6F_2 3-11, 20-3 and 23-5 were all significantly lower than those of the other BC_6F_2 lines. Although the RSI values of BC_6F_2 3-11, 20-3 and 23-5 were lower than the RSI value of T65, the difference was not significant; in contrast the RFWSA values of these NILs were significantly lower than the value of T65 (Table 1). These results demonstrate that the *BPH25*(t) allele inherited from ADR52, *bph25*(t), controlled the BPH resistance in a recessive-like manner.

The RSI and RFWSA values of the BC_6F_2 progeny of the *BPH26*(t) NIL homozygous for the ADR52 allele (BC_6F_2 63-8) and in the heterozygote (BC_6F_2 63-1) were significantly lower than those of the NIL homozygous for T65 (BC_6F_2 63-6) (Fig. 4). The RSI and RFWSA values of BC_6F_2 63-8 were both significantly lower than those of T65, and were not significantly different from those of ADR52 (Table 1). These results demonstrate that the *BPH26*(t) allele inherited from ADR52 controlled the BPH resistance in a dominant manner. The level of BPH resistance of the *BPH26*(t) NIL was higher than that of the *BPH25*(t) NILs, but the differences were generally not significant (Table 1).

Evaluation of agronomic traits of the BPH25(t) and BPH26(t) NILs

We selected each NIL (BC_6F_2) that has no retaining segment from ADR52 except for targeted resistance gene. We then measured five agronomic traits (CL, PL, SNPP, PRG and TGW) in order to evaluate the morphological differences between T65 and the *BPH25*(t) and the *BPH26*(t) NILs that were homozygous for the ADR52 or T65 alleles or that were heterozygous (Table 2). No traits in the *BPH25*(t) NIL homozygotes and heterozygotes were significantly different from their values in T65. On the other hand, CL was significantly higher in the ADR52 *BPH26*(t) homozygotes and in the heterozygotes than in the T65 homozygotes. CL in the ADR52 *BPH26*(t) homozygotes and in the heterozygotes was slightly higher than that in T65, and CL in the T65 *BPH26*(t) homozygotes were slightly lower than that in T65, but the differences were not significant. TGW in the *BPH26*(t) NIL was generally significantly higher than those in the *BPH25*(t) NIL and in T65. The values of the other traits in the *BPH26*(t) NIL were not significantly different from those in the *BPH25*(t) NIL and T65. These results indicate that the genes concerned with agronomically important traits were not inherited from ADR52 in the *BPH25*(t) NIL, but that genes concerned with increased CL and TGW were inherited from ADR52 in the *BPH26*(t) NIL.

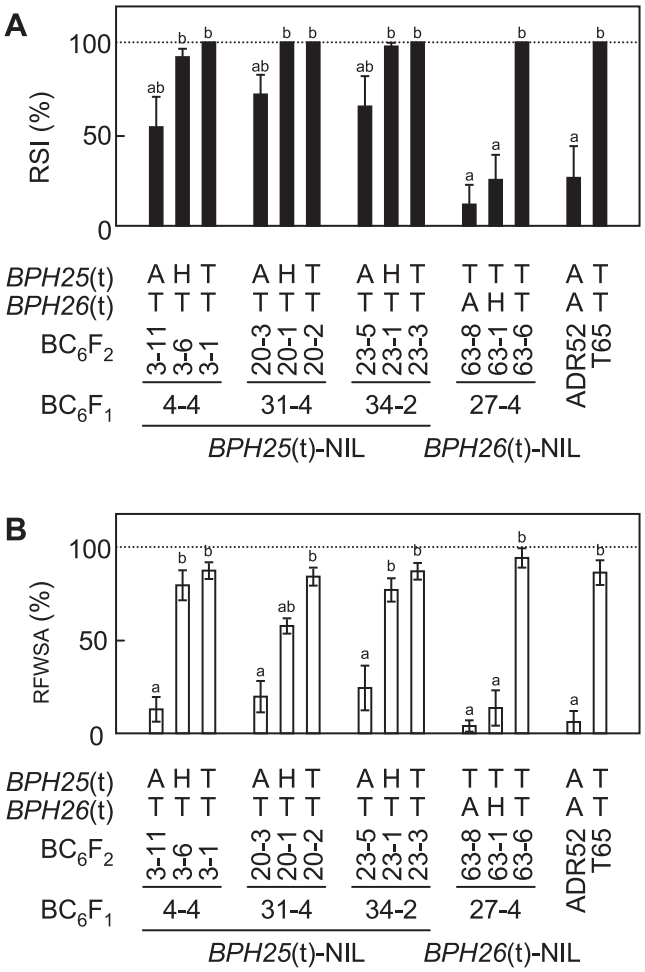

Fig. 4. The survival of BPH on newly developed NILs carrying *BPH25*(t) and *BPH26*(t) in a T65 genetic background: (A) rate of surviving insects (RSI) and (B) rate of females with swollen abdomens (RFWSA). Data represent the means and standard errors of 3 to 10 samples. Bars labeled with the same letters are not significantly different ($P < 0.05$, Tukey–Kramer multiple-comparison test). T, A, and H indicate loci homozygous for the T65 allele, homozygous for the ADR52 allele, and heterozygous, respectively.

Discussion

In this study, we found that ADR52 has both the late-heading allele *hdX*(t) near *BPH25*(t) and the hybrid spikelet-sterility allele *hsY*(t) near *BPH26*(t). We successfully developed three *BPH25*(t) and one *BPH26*(t) NILs with a T65 genetic background by the removal of these unfavorable loci by means of MAS. These NILs showed BPH resistance combined with normal heading date and normal spikelet fertility (Fig. 4, Supplemental Tables 2, 3).

Our linkage analysis indicated that *hdX*(t) is linked to *BPH25*(t) on the short arm of chromosome 6, and is located between *sHd3a* and *RM225* (Fig. 2A). Several studies have reported the existence of various heading date–related genes (*Hd3a*, *Hd3b*, and *RFT1*) and QTLs (*qDF6.1*, *qDF6.2*, *qDF6.3*, *qDF6.4* and *qDF6.5*) on the short arm of

Table 2. Evaluation of agronomic traits in the *BPH25*(t) and *BPH26*(t) NILs, and comparison with the values for T65

Population	Origin	Resistance gene[a]		$N = 15$		$N = 5$		
		BPH25(t)	*BPH26*(t)	CL (cm)[b]	PL (cm)[b]	SNPP[b]	PRG (%)[b]	TGW (g)[b]
BPH25(t)–NIL								
BC_6F_2 23-10	BC_6F_1 34-2	A	T	88.1 ± 0.9[b]	22.7 ± 0.3[a]	121.4 ± 5.9[a]	89.0 ± 1.2[a]	23.8 ± 0.1[a]
		H	T	91.8 ± 0.9[ab]	23.3 ± 0.4[a]	120.2 ± 5.3[a]	90.4 ± 1.7[a]	24.2 ± 0.1[a]
		T	T	88.3 ± 0.8[b]	23.7 ± 0.3[a]	126.8 ± 3.5[a]	88.6 ± 1.6[a]	24.2 ± 0.1[ab]
BPH26(t)–NIL								
BC_6F_2 63-4	BC_6F_1 27-4	T	A	93.9 ± 1.0[a]	24.1 ± 0.4[a]	126.6 ± 5.0[a]	88.2 ± 1.6[a]	25.0 ± 0.2[bc]
		T	H	94.1 ± 1.0[a]	23.8 ± 0.3[a]	124.4 ± 5.9[a]	90.9 ± 1.2[a]	25.2 ± 0.2[c]
		T	T	87.9 ± 1.1[b]	23.6 ± 0.4[a]	119.7 ± 4.1[a]	90.5 ± 0.4[a]	25.3 ± 0.1[c]
T65		T	T	89.1 ± 1.7[ab]	23.7 ± 0.6[a]	124.2 ± 7.5[a]	86.2 ± 1.1[a]	23.5 ± 0.1[a]

[a] T, A, and H indicate loci homozygous for the T65 allele, homozygous for the ADR52 allele, and heterozygous respectively. The genotypes of *BPH25*(t) and *BPH26*(t) in the BC_6F_2 individuals were confirmed using two sets of flanking markers: *S00310–RM588* and *RM28449–RM3813*, respectively.

[b] Values of a parameter (mean ± S.E.) followed by the same letter are not significantly different ($P < 0.01$, Tukey-Kramer multiple comparison test).

chromosome 6 in *indica* cultivars and *O. rufipogon* (Hagiwara *et al.* 2009, Kojima *et al.* 2002, Monna *et al.* 2002). Among these reported genes and QTLs, *hdX*(t) is located in the same region as *qDF6.3*, which is derived from the *indica* cultivar Patpaku, and *qDF6.5*, which is derived from *O. rufipogon*, Acc. W593. *qDF6.5* repressed heading, whereas *qDF6.3* promoted heading (Hagiwara *et al*. 2009). The delay of heading date in the *hdX*(t) NIL may therefore be associated with the repressed heading caused by *qDF6.5*.

Our linkage analysis also showed that *hsY*(t) was located between *RM5479* and *RM6306* (Fig. 2B). Previous research showed a hybrid sterility allele, *hsa1*–IR, on the long arm of chromosome 12 in the *indica* cultivar IR24 (Kubo and Yoshimura 2005). Furthermore, two hybrid sterility alleles, *hsa2*–As and *hsa3*–As, are found on chromosomes 8 and 9, respectively, in the *japonica* cultivar Asominori. The *hsa1*–IR plants homozygous for *hsa2*–As and *hsa3*–As show female sterility. This finding was similar to the sterile phenotype observed in the *hsY*(t) NIL here. Furthermore, the pollen grains of the plants with hybrid spikelet sterility had normal form (data not shown). Our linkage analysis therefore suggests that *hsY*(t) is located in the same chromosomal region as *hsa1*.

In several *indica* cultivars, loci related to BPH resistance have been found at chromosomal positions near *BPH25*(t) and *BPH26*(t), respectively. Two BPH-resistance genes (*Bph3*, derived from 'Rathu Heenati' and 'PTB33' and *bph4*, derived from 'Babawee') were previously reported on the short arm of chromosome 6 (Jairin *et al.* 2007, 2010). On the other hand, three BPH-resistance genes (*Bph1*, derived from the *indica* cultivar Mudgo; *bph2*, derived from the *indica* cultivar ASD7; and *Bph9*, derived from the *indica* cultivar Pokkali) have been reported on the long arm of chromosome 12 (Murata *et al.* 2000, Sharma *et al.* 2004). These findings raise the possibility that using these cultivars as donors of BPH resistance genes will encounter the same problem as here with linkage drag related to late heading and hybrid spikelet sterility. Our information on chromosomal position of *hdX*(t) and *hsY*(t) may be applicable to the development of advanced NILs carrying these resistance loci by the removal of the linkage drag.

In our previous study, the virulence of 4 BPH strains collected in 1966, 1989, 1999 and 2005 have been evaluated using 7 rice differential varieties carrying different BPH-resistance genes, such as ADR52 carrying *bph25*(t) and *Bph26*(t), Mudgo carrying *Bph1*, ASD7 carrying *bph2*, Rathu Heenati carrying *Bph3* and *Bph17*, Babawee carrying *bph4*, Chin Saba carrying *bph8*, Balamawee carrying *Bph9* (Myint *et al*. 2009b). For example, although all the tested varieties are resistant to the BPH strain collected in 1966 (Hatano-66), Mudgo, ASD7, Babawee, and Chin Saba are susceptible to a BPH strain collected in 2005 (2005BPH). Thus, the effects of these major resistance genes in the rice differential varieties to the BPH strains are becoming clearer. However, the influence of the different genetic background in each variety remains unclear. We therefore propose that these newly developed NILs with a T65 genetic background will be useful for monitoring the virulence of Asian BPH populations as new differential lines for the specific BPH-resistance genes, *BPH25*(t) and *BPH26*(t).

These NILs are also important as donors for the development of PYLs carrying both *BPH25*(t) and *BPH26*(t). Using the Hatano-66 were collected in 1966, pre-NILs carrying either *BPH25*(t) or *BPH26*(t) and pre-PYLs carrying both *BPH25*(t) and *BPH26*(t) were resistant to the BPH strain (Myint *et al*. 2009a). The pre-NILs are susceptible to the 2005BPH, but the pre-PYLs are resistant to the 2005BPH. This finding shows that PYLs with both *BPH25*(t) and *BPH26*(t) had an epistatic effect on BPH resistance, indicating that *BPH25*(t)/*BPH26*(t) PYLs will be essential materials for the development of more durable resistance to BPH. Here, we obtained both *BPH25*(t) and *BPH26*(t) NILs that show BPH resistance combined with normal heading date and normal spikelet fertility. It should therefore be easy and

reliable to develop *BPH25*(t)/*BPH26*(t) PYLs by means of hybridization between these NILs.

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Literature Cited

Chen, J.W., L. Wang, X.F. Pang and Q.H. Pan (2006) Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph19*(t). Mol. Genet. Genomics 275: 321–329.

Deen, R., K. Ramesh, S.K. Gautam, Y.K. Rao, V.J. Lakshmi, B.C. Viraktamath, D.S. Brar and T. Ram (2010) Identification of new gene for BPH resistance introgressed *O. rufipogon*. Rice Genet. Newsl. 25: 70–72.

Dung, L.V., T. Inukai and Y. Sano (1998) Dissection of a major QTL for photoperiod sensitivity in rice: its association with a gene expressed in an age-dependent manner. Theor. Appl. Genet. 97: 714–720.

Hagiwara, W.E., N. Uwatoko, A. Sasaki, K. Matsubara, H. Nagano, K. Onishi and Y. Sano (2009) Diversification in flowering time due to tandem FT-like gene duplication, generating novel Mendelian factors in wild and cultivated rice. Mol. Ecol. 18: 1537–1549.

Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto, S.Y. Lin, B.A. Antonio, A. Parco *et al*. (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148: 479–494.

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436: 793–800.

Jairin, J., S. Teangdeerith, P. Leelagud, K. Phengrat, A. Vanavichit and T. Toojinda (2007) Physical mapping of *Bph3*, a brown planthopper resistance locus in rice. Mj. Int. J. Sci. Tech. 1: 166–177.

Jairin, J., S. Teangdeerith, P. Leelagud, J. Kothcharerk, K. Sansen and M. Yi (2009) Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection. Field Crops Res. 110: 263–271.

Jairin, J., K. Sansen, W. Wonboon and J. Kothcharerk (2010) Detection of a brown planthopper resistance gene *bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice. Breed. Sci. 60: 71–75.

Kabir, M.A. and G.S. Khush (1988) Genetic analysis of resistance to brown planthopper in rice (*Oryza sativa* L.). Plant Breed. 100: 54–58.

Khush, G.S. (1979) Genetics and breeding for resistance to brown planthopper. *In*: International Rice Research Institute (eds.) Brown Planthopper: Threat to Rice Production in Asia, International Rice Research Institute, Los Baños, Philippines, pp. 321–332.

Khush, G.S., A.N.M. Rezaul Karim and E.R. Angeles (1985) Genetics of resistance of rice cultivar ARC 10550 to Bangladesh brown planthopper biotype. J. Genet. 64: 121–125.

Kojima, S., Y. Takahashi, Y. Kobayashi, L. Monna, T. Sasaki, T. Araki and M. Yano (2002) *Hd3a*, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. Plant Cell Physiol. 43: 1096–1105.

Kubo, T. and A. Yoshimura (2005) Epistasis underlying female sterility detected in hybrid breakdown in a Japonica-Indica cross of rice (*Oryza sativa* L.). Theor. Appl. Genet. 110: 346–355.

McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing *et al*. (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9: 199–207.

McCouch, S.R. and CGSNL (2008) Gene nomenclature system for rice. Rice 1: 72–84

Monna, L., Y. Sano and M. Yano (2000) Genetic mapping of a rice photoperiod sensitivity gene Enhancer-Se1. Rice Genet. Newsl. 17: 40–42.

Monna, L., X. Lin, S. Kojima, T. Sasaki and M. Yano (2002) Genetic dissection of a genomic region for a quantitative trait locus, *Hd3*, into two loci, *Hd3a* and *Hd3b*, controlling heading date in rice. Theor. Appl. Genet. 104: 772–778.

Murata, K., M. Fujiwara, H. Murai, S. Takumi, N. Mori and C. Nakamura (2000) *Bph9*, a dominant brown planthopper resistance gene, is located on the long arm of rice chromosome 12. Rice Genet. Newsl. 17: 84–86.

Myint, K.K.M., T. Sonoda, M. Matsumura, A. Yoshimura and H. Yasui (2005) Genetic basis of antibiosis to brown planthopper, *Nilaparvata lugens* Stål., in rice. *In*: Abstracts of 5th International Rice Genetics Symposium, International Rice Research Institute, Manila, p. 103.

Myint, K.K.M., M. Matsumura, M. Takagi and H. Yasui (2009a) Demographic parameters of long-term laboratory strains of the brown planthopper, *Nilaparvata lugens* StåI, (Homoptera: Delphacidae) on resistance genes, *bph20*(t) and *Bph21*(t) in rice. J. Fac. Agric., Kyushu Univ. 54: 159–164.

Myint, K.K.M., H. Yasui, M. Takagi and M. Matsumura (2009b) Virulence of long-term laboratory populations of the brown planthopper, *Nilaparvata lugens* (StåI), and whitebacked planthopper, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae), on rice differential varieties. Appl. Entomol. Zool. 44: 149–153.

Nemoto, H., R. Ikeda and C. Kaneda (1989) New genes for resistance to brown planthopper, *Nilaparvata lugens* StåI, in rice. Jpn. J. Breed. 39: 23–28.

Pathak, P.K. and E.A. Heinrichs (1982) Selection of biotype populations 2 and 3 of *Nilaparvata lugens* by exposure to resistant rice varieties. Environ. Entomol. 11: 85–90.

Rahman, M.L., W. Jiang, S.H. Chu, Y. Qiao, T.H. Ham, M.O. Woo, J. Lee, M.S. Khanam, J.H. Chin, J.U. Jeung *et al*. (2009) High-resolution mapping of two rice brown planthopper resistance genes, *Bph20*(t) and *Bph21*(t), originating from *Oryza minuta*. Theor. Appl. Genet. 119: 1237–1246.

Ram, T., R. Deen, S.K. Gautam, K. Ramesh, Y.K. Rao and D.S. Brar (2010) Identification of new genes for brown planthopper resistance in rice introgressed from *O. glaberrima* and *O. minuta*. Rice Genet. Newsl. 25: 67–69.

Sharma, P.N., A. Torii, S. Takumi, N. Mori and C. Nakamura (2004) Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stål) resistance genes *Bph1* and *Bph2* on rice chromosome 12. Hereditas 140: 61–69.

Sonoda, T., A. Yoshimura and H. Yasui (2003) Detection of QTLs for antibiosis to brown planthopper, *Nilaparvata lugens* Stål, in rice, *Oryza sativa* L. Rice Genet. Newsl. 20: 83–85.

Sun, L., C. Su, C. Wang, H. Zhai and J. Wan (2005) Mapping of a major resistance gene to the brown planthopper in the rice Cultivar Rathu Heenati. Breed. Sci. 55: 391–396.

Temnykh, S., G. DeClerck, A. Lukashova, L. Lipovich, S. Cartinhour and S. McCouch (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res. 11: 1441–1452.

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y. Umehara, Y. Nagamura *et al*. (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12: 2473–2483.

Young, N.D. and S.D. Tanksley (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl. Genet. 77: 95–101.