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ORIGINAL PAPER

Interaction of two recessive genes, *hbd2* and *hbd3*, induces hybrid breakdown in rice

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Abstract Reproductive barriers are important for the maintenance of species identity. We discovered a reproductive barrier via hybrid breakdown among the progeny of a cross between the *japonica* rice cultivar Koshihikari and the *indica* rice cultivar Habataki. Genetic analysis indicated that the hybrid breakdown is regulated by the interaction of two recessive genes: *hbd2* in Habataki and *hbd3* in Koshihikari. Linkage mapping showed that *hbd2* is located near the 100 cM region of chromosome 2 in Habataki, whereas *hbd3* is located near the 60 cM region of chromosome 11 in Koshihikari. Construction of nearly isogenic lines for *hbd2* and *Hbd3* (NIL-*hbd2* and NIL-*Hbd3*), as well as a pyramiding line (NIL-*hbd2* + *Hbd3*), confirmed that the hybrid breakdown is induced by the interaction of these two recessive genes. Our results indicate that these genes are novel for the induction of hybrid breakdown in rice.

Introduction

A species can be defined as a group of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1942). Reproductive barriers are important to distinguish species and to maintain the identity of established species. Reproductive barriers are classified into three general categories (Stebbins 1950; Smith 1989). The first is geographical isolation resulting in unique genotypes that are found in different environments. The second category includes prezygotic reproductive barriers, which prevent the formation of hybrids. Such barriers involve the development of different flowering times or incompatible pollen-tube growth between different species. The last category is postzygotic reproductive barriers, which act after hybrid formation and are classified into several subtypes. Hybrid weakness (inviability) and hybrid sterility are observed in F_1 hybrids. When these traits are observed at the F_2 generation or later, it is termed hybrid breakdown.

To date, several postzygotic reproductive barriers have been reported in rice, including hybrid weakness (Oka 1957; Amemiya and Akamine 1963; Sato and Morishima 1987), hybrid sterility (Oka 1958, 1974; Jennings 1966), and hybrid breakdown (Oka 1957, 1978; Oka and Doida 1962; Yokoo 1984; Okuno 1986; Sato and Morishima 1988; Wu et al. 1995; Li et al. 1997; Fukuoka et al. 2005). For crop plants such as rice, reproductive barriers can complicate breeding. Breeders wishing to introduce agriculturally important or desirable genes from one species into another to produce new varieties often encounter reproductive barriers that inhibit the process.

Clarifying the mechanisms behind reproductive barriers such as hybrid breakdown is important not only to understand biological speciation but also to remove the barriers as necessary for crop breeding. To elucidate the general mechanisms underlying reproductive barriers, it is necessary to identify examples of these phenomena and clone the genes involved. We discovered an example of hybrid breakdown among the progeny of a cross between Koshihikari (*Oryza sativa* ssp. *japonica*) and Habataki (*O. sativa* ssp. *indica*). Here, we show that the interaction of two

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genes, *hybrid breakdown 2* (*hbd2*) and *3* (*hbd3*), regulates hybrid breakdown in rice.

Materials and methods

Plant materials

We used the rice varieties Koshihikari (*Oryza sativa* ssp. *japonica*) and Habataki (*O. sativa* ssp. *indica*) and the progeny from a cross between the two cultivars. To create nearly isogenic lines (NILs), F_1 plants (Koshihikari/Habataki) were backcrossed with Koshihikari four times. NILs were selected from the BC_4F_2 generation (i.e., Koshihikari/Habataki/Koshihikari/Koshihikari/Koshihikari/Koshihikari) by marker-assisted selection (MAS; Yano and Sasaki 1997; Yano 2001).

DNA extraction and linkage analysis

Genomic DNA was extracted from selected individuals in each BC_1F_2 population or from the NILs using the TPS method for linkage analysis and NIL construction. For the TPS method, approximately 2 cm lengths were harvested from the rice leaf tips and ground using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) in TPS buffer [100 mM Tris–HCl (pH 8.0), 1 M KCl, and 10 mM EDTA]. After centrifuging, the supernatant was recovered and an equal volume of isopropyl alcohol was added. Isopropyl alcohol-insoluble material was recovered by centrifugation, and the pellet was washed with 75% ethanol. Thereafter, the pellet was dried and dissolved in TE [10 mM Tris–HCl (pH 8.0) and 1 mM EDTA]. The purified DNA samples were then genotyped using molecular markers. PCR-based markers, including simple-sequence repeat (SSR) markers (McCouch et al. 2002; Ware et al. 2002; http://www.gramene.org/), cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel 1993), and single nucleotide polymorphisms (SNP), which we identified by comparing the genomic DNA sequences of each parent, were used for linkage analysis and NIL production. Genotyping was carried out using PCR based on a description by Chen et al. (1997) or with the AcycloPrime-FP SNP Detection System (PerkinElmer, Wellesley, MA, USA) according to the manufacturer's protocol. Markers used for molecular mapping of *hbd2* and *hbd3* are illustrated in Fig. 3, and those used to determine the genetic background of NILs are presented in Fig. 4a.

Phenotypic evaluation

To evaluate the occurrence of hybrid breakdown, we counted the number of tillers. Weak plants had strikingly fewer tillers than normal plants. To investigate these traits, rice seeds were germinated in Petri dishes in water at 30°C for 72 h and then grown in a nursery for 1 month before transplantation into a research field. Each trait was investigated after heading.

Results

Hybrid breakdown regulated by two recessive genes, *hbd2* and *hbd3*

Introgression lines (ILs) are plant strains that possess relatively large segments of the donor parent's chromosomes with overlapping in the recurrent parental background. ILs are used to identify agriculturally important genes for breeding (Ashikari and Matsuoka 2006), whereas recombinant inbred lines (RILs), which are produced by inbreeding the progeny of a parental cross, are used for quantitative trait loci (QTL) analysis (Yano and Sasaki 1997; Yano 2001). To construct ILs and RILs, Koshihikari (*Oryza sativa* ssp. *japonica*) and Habataki (*O. sativa* ssp. *indica*) plants were crossed. In the F_2 generation, we observed the segregation of weak plants with few tillers (Fig. 1). Because both parental plants and F_1 plants were normal, we concluded that the weak plants were products of hybrid breakdown.

Because weak plants showed few tillers phenotype, we counted the number of tillers on 192 F_2 individuals (Fig. 2). Distribution data of tiller numbers in the F_2 generation showed two peaks. The small peak represented the plants with few tillers (2–6), and the large peak included plants that possessed more tillers (6–20). To determine whether the weak plants were genetically regulated, we calculated the segregation ratio of the normal versus weak plants in the F_2 population. We investigated three cases. In case 1, we divided the two peaks at the point of 4 tillers. In this case, only plants with 1–4 tillers (i.e., those obviously included in small peak) were defined as weak plants and plants that showed 5 or 6 tillers (i.e., those for which it was difficult to determine which peak they belonged to) were included in the normal plant peak. When divided at the 4-tillers point, the segregation of normal to weak plants was 178 to 14 (Table 1). This corresponds to a ratio of 15:1 ($x^2 = 0.20$) for two recessive genes, rather than 3:1 ($x^2 = 99.75$) for a single recessive gene. In case 2, we divided the two peaks at the point of 6 tillers (i.e., 1–6 tillers as weak and 7–20 tillers as normal). In this case, plants that showed 5 or 6 tillers were regarded as weak. When divided at the 6-tillers point, the segregation of normal to weak plants was 177 to 15 (Table 1). This corresponds to a ratio of 15:1 ($x^2 = 0.56$), rather than 3:1 ($x^2 = 93.89$). Finally, in case 3, plants having 7 or 8 tillers (i.e., those obviously included in

Fig. 1 Morphology of parents and weak plants. Weak plants were observed in the F_2 population of a cross between Koshihikari and Habataki. The weak plants were relatively small, with few tillers. *Bar* = 50 cm

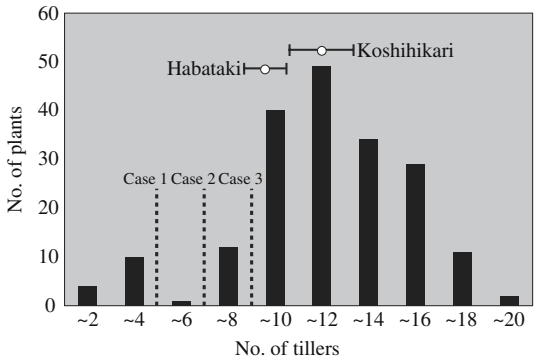

Fig. 2 Distribution of number of tillers in the F_2 population of a cross between Koshihikari and Habataki

Table 1 Segregation for hybrid breakdown in F_2 progeny

Tiller number	No. of plants			χ^2 (3:1)	χ^2 (15:1)
	Normal	Weak	Total		
Case 1	178	14	192	99.75***	0.20^{NS}
Case 2	177	15	192	93.89***	0.56^{NS}
Case 3	165	27	192	37.36***	18.69***

Case 1: normal is determined 5–20 tillers and weak is 1–4 tillers

Case 2: normal is determined 7–20 tillers and weak is 1–6 tillers

Case 3: normal is determined 9–20 tillers and weak is 1–8 tillers

NS not significant; *** $P < 0.01$

the normal peak) were regarded as weak plants. When divided at the 8-tillers point, the segregation of normal to weak plants was 165 to 27, which did not correspond to a ratio of 15:1 ($x^2 = 18.69$) and 3:1 ($x^2 = 37.36$). These results suggest the possibility that the weak phenotype was regulated by the interaction of two recessive genes when we divided the phenotypes at 4 or 6 tillers. Because the parental lines and their F_1 progeny were normal, we hypothesized that Koshihikari and Habataki each possess one recessive gene associated with hybrid breakdown, and that F_2 plants with both recessive genes would exhibit the weak phenotype.

To confirm the hypothesis that the inheritance of two recessive genes leads to hybrid breakdown, further genetic analyses were performed. First, three weak plants from the F_2 generation were crossed with each parental variety. Given that we hypothesized that weak plants possess two recessive genes and that one is from Koshihikari and the other from Habataki, crossing with each parent causes the segregation of only one recessive gene. Therefore, the segregation ratio of normal and weak plants among the BC_1F_2 progenies were expected to fit 3:1 ratio. First, weak plants were crossed with Koshihikari to obtain BC_1F_2 progenies. The segregation of normal and weak plants among the BC_1F_2 progenies were 74:22, 75:21, and 67:29, all corresponding to a 3:1 ratio (Table 2). Each phenotype of BC_1F_2 was confirmed by inspecting the BC_1F_3 progenies. This result indicated that one recessive gene regulating hybrid breakdown occurred in the Habataki genome. This recessive gene from Habataki was named *hybrid breakdown 2* (*hbd2*). The name *hbd1* (*hybrid breakdown 1*) was used for a previously detected gene involved in hybrid breakdown between Koshihikari and Nonabokura (Yano, personal communication). Similarly, three weak F_2 plants were crossed with Habataki, and segregating progenies (i.e., normal and weak BC_1F_2 plants) were obtained at a ratio of 70:26, 73:23, and 71:25, all corresponding to a 3:1 ratio (Table 2). Each phenotype was confirmed by observing the

Table 2 Segregation for hybrid breakdown in BC_1F_2 progenies

	No. of plants			χ^2 (3:1)
	Normal	Weak	Total	
Weak plant 1 × Koshihikari	74	22	96	0.13^{NS}
Weak plant 2 × Koshihikari	75	21	96	0.46^{NS}
Weak plant 3 × Koshihikari	67	29	96	1.13^{NS}
Weak plant 1 × Habtaki	70	26	96	0.13^{NS}
Weak plant 2 × Habtaki	73	23	96	0.01^{NS}
Weak plant 3 × Habtaki	71	25	96	0.01^{NS}

NS not significant

BC_1F_3 progenies. This indicated that the Koshihikari genome also possessed one recessive gene involved in hybrid breakdown. This recessive gene from Koshihikari was named *hybrid breakdown 3* (*hbd3*). In this way, we confirmed that the weak plants resulted from an interaction between two recessive genes: *hbd2* from the Habataki parental plant and *hbd3* from the Koshihikari parental plant.

Molecular mapping of *hbd2* and *hbd3*

To elucidate the molecular mechanism underlying hybrid breakdown, one must clone the genes involved and conduct a functional analysis. As an initial step toward this goal, we sought to pinpoint the chromosomal positions of the genes involved using molecular mapping. The 288 BC_1F_2 plants generated from the cross between weak plants and Koshihikari (Table 2) were used to map *hbd2*. Using molecular markers to test the entire rice genome, *hbd2* was mapped between S21164 and RM526 around the 100 cM region of the long arm of chromosome 2 in Habataki (Fig. 3a). The molecular marker RM6617 co-segregated with *hbd2*. Similarly, 288 BC_1F_2 plants from the cross between weak plants and Habataki (Table 2) were used to construct a linkage map of *hbd3*. The gene *hbd3* was mapped between S790A and RM1355 around the 60 cM region of the long arm of chromosome 11 in Koshihiakri (Fig. 3b). The molecular marker RM6272 co-segregated with *hbd3*.

Construction of NILs confirmed the genetic interaction between *hbd2* and *hbd3*

Our segregation and linkage mapping data showed that Habataki possesses the recessive version of *hbd2* on chromosome 2, whereas Koshihikari contains the dominant version, *Hbd2*. Likewise, Habataki contains the dominant version of *Hbd3* on chromosome 11, whereas Koshihikari possesses the recessive version (*hbd3*; Fig. 4b-1, b-2, see the graphical genotypes for Koshihikari and Habataki). According to our present data, hybrid breakdown results from the combined inheritance of *hbd2* on the long arm of chromosome 2 from Habataki and *hbd3* on the long arm of chromosome 11 from Koshihikari. To confirm whether this combination of recessive genes is enough to induce hybrid breakdown, we constructed nearly isogenic lines (NILs). The use of NILs, which carry small chromosomal segments that contain a target gene from the donor in a unique genomic background, and pyramiding lines facilitates the analysis of interactions between different genes, as well as their effects. Our NILs were produced by repeatedly crossing a donor parent (Habataki) with a recurrent parent (Koshihikari) in combination with marker assisted selection (MAS). Markers used to construction of NILs are indicated in Fig. 4a. By comparing the traits between an NIL carrying a target gene and its recurrent parent, one can evaluate the gene and its interactions.

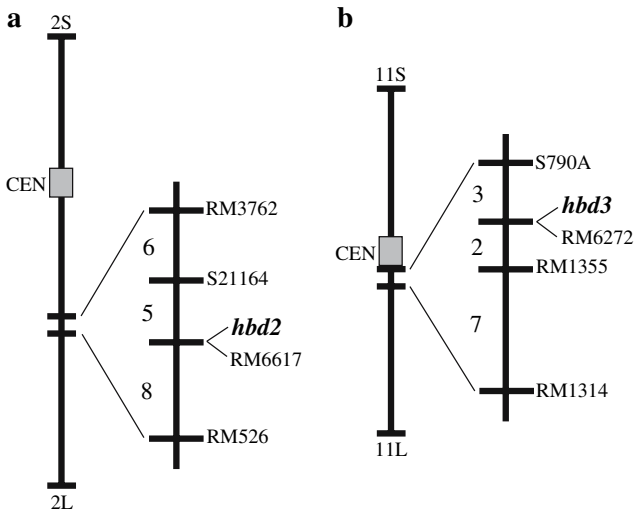

Fig. 3 Linkage maps of *hbd2* and *hbd3*. **a** The *hbd2* is located on the long arm of chromosome 2. **b** The *hbd3* is located on the long arm of chromosome 11. *CEN* indicates a centromere

An NIL carrying a chromosomal segment from the *hbd2* region of Habataki chromosome 2 in the Koshihikari background was selected for analysis (NIL-*hbd2*; Fig. 4b-3). NIL-*hbd2*, which possesses the two recessive genes (i.e., the Habataki *hbd2* allele on chromosome 2 and the Koshihikari *hbd3* allele on chromosome 11), exhibited a weak phenotype. This result confirms that the recessive genes *hbd2* from Habataki is sufficient to trigger hybrid breakdown in the Koshihikari genomic background.

Additionally, an NIL carrying a chromosomal segment from the *Hbd3* region of Habataki chromosome 11 alone in the Koshihikari background was also constructed (NIL-*Hbd3*; Fig. 4b-4). NIL-*Hbd3*, which possesses the Koshihikari *Hbd2* allele and the Habataki *Hbd3* allele, did not exhibit a weak phenotype. Moreover, pyramiding NILs from a cross between NIL-*hbd2* and NIL-*Hbd3* (NIL-*hbd2* + *Hbd3*) (Fig. 4b-5), which possesses the Habataki *hbd2* and *Hbd3* alleles, grew normally. This indicated that *Hbd3* from Habataki is enough to mask the deleterious effect of *hbd2* in Koshihikari background. These data support the idea that the inheritance of both *hbd2* from Habataki and *hbd3* from Koshihikari is necessary and sufficient for the induction of hybrid breakdown.

Phenotypic evaluation of hybrid breakdown

Because NILs have unique genomic backgrounds except for their target regions, comparisons between NILs and their recurrent parents indicate the effects of a gene of interest. A small reduction in plant height was observed for NIL-*hbd2* (106.3 cm) compared to Koshihikari (117.5 cm; Fig. 5a),

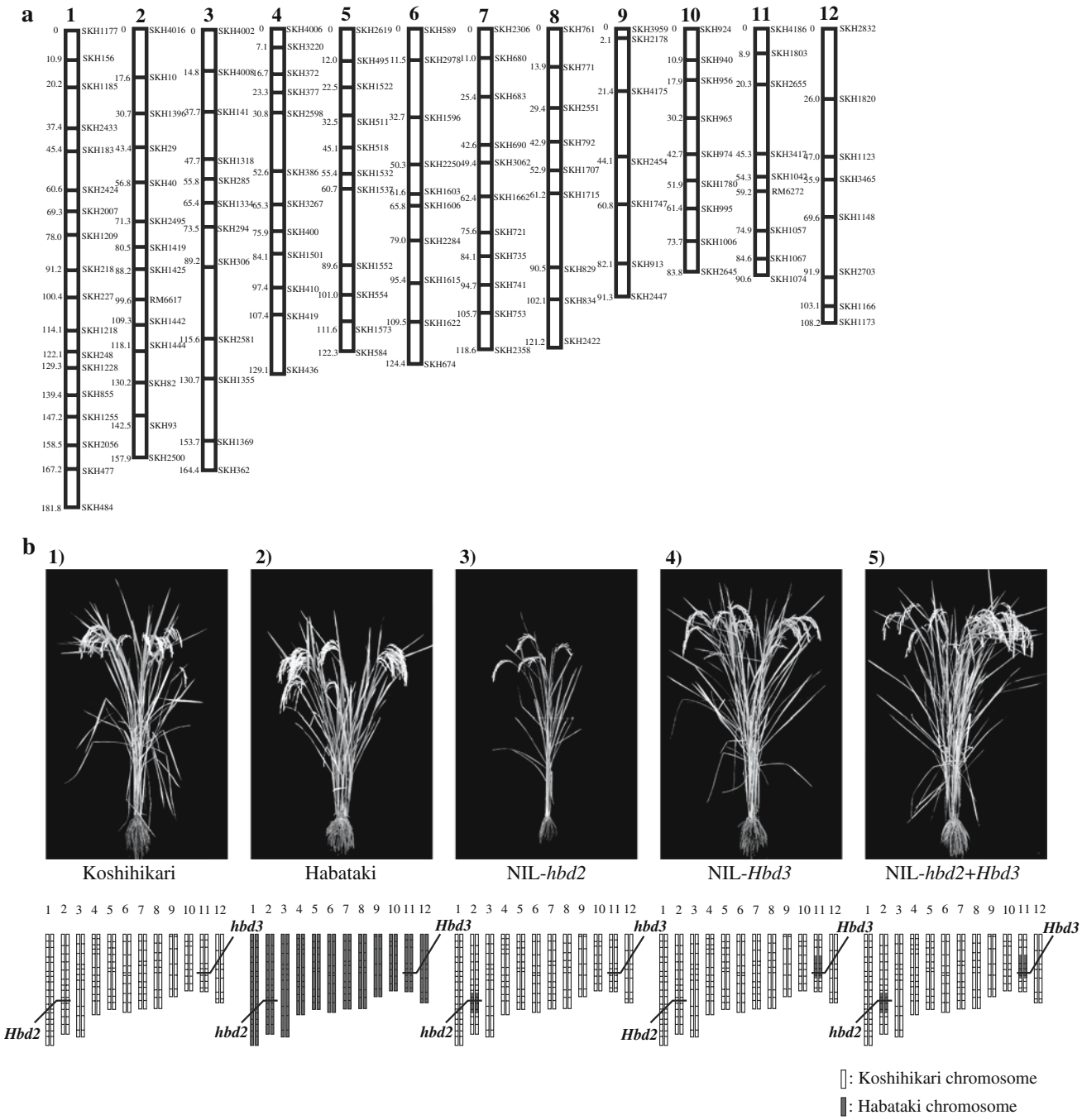

Fig. 4 Construction of NILs and confirmation of genetic interaction between *hbd2* and *hbd3*. **a** The chromosome map of markers used to determine genetic background of each NIL. Markers with SKH—at the top of the name are newly constructed SNP markers for this study. **b** Plant morphologies and graphical genotypes of (*1*) Koshihikari, (*2*) Habataki, (*3*) NIL-*hbd2*, (*4*) NIL-*Hbd3*, and (*5*) NIL-*hbd2* + *Hbd3*

whereas a large reduction in the number of tillers was also observed (4.3 tillers for NIL-*hbd2* versus 11.6 tillers for Koshihikari; Fig. 5b). In contrast, no significant differences in plant height or tiller number were observed among NIL-*Hbd3*, NIL-*hbd2* + *Hbd3*, and Koshihikari. These results confirmed that the plants carrying a single recessive gene (either *hbd2* from Habataki or *hbd3* from Koshihikari) did not exhibit a weak phenotype, whereas the plants carrying both recessive genes (i.e., *hbd2* and *hbd3*) had lower numbers of tillers and exhibited weak phenotypes.

Discussion

In the process of constructing ILs and RILs from a cross between Koshihikari (*Oryza sativa* ssp. *japonica*) and

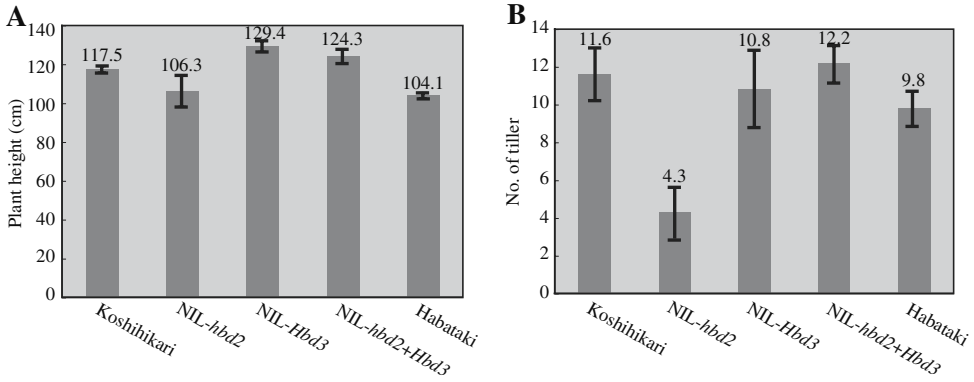

Fig. 5 Comparison of (**a**) plant height and (**b**) number of tillers among Koshihikari, Habataki, and the nearly isogenic lines (NILs)

Habataki (*O. sativa* ssp. *indica*) plants, we discovered an example of hybrid breakdown that prevented the plants from growing normally (i.e., not like the parental varieties; Fig. 1). Using genetic analysis, we identified two recessive genes, *hbd2* in Habataki and *hbd3* in Koshihikari, which control hybrid breakdown. Linkage analysis indicated that *hbd2* is located around the 100 cM region of the long arm of chromosome 2 in Habataki (Fig. 3a), whereas *hbd3* is located around the 60 cM region of the long arm of chromosome 11 in Koshihikari (Fig. 3b). Plants possessing a combination of these two recessive genes (*hbd2/hbd2* and *hbd3/hbd3*) exhibit abnormal tiller growth (Figs. 4, 5); however, plants with other genetic combinations (*Hbd2/Hbd2* and *hbd3/hbd3*, *hbd2/hbd2* and *Hbd3/Hbd3*, *Hbd2/Hbd2* and *Hbd3Hbd3*) exhibit normal tiller growth. Genetic analyses and NIL comparisons indicated that co-inheritance of these two recessive alleles is sufficient to cause hybrid breakdown.

Among the F_2 progeny from the cross between Koshihikari and Habataki, two types of weak plants were observed: severe and mild (Fig. 1). The phenotype of NIL-*hbd2* plants (Fig. 4b-3) is relatively mild when compared to the weak plants observed in the F_2 population (Fig. 1). The hybrid breakdown can be explained by the interaction of two genes; however, two recessive genes are likely not sufficient to determine the degree of weakness. Therefore, other factors that affect the severity of hybrid breakdown may be involved.

Among the reports of hybrid breakdown in rice involving a weak phenotype, three identified the chromosomal locations of the genes that caused the hybrid breakdown. Fukuoka et al. (1998) reported that two genes, *hwd1* located around the 3 cM region of chromosome 10, and *hwd2* located around the 50 cM region of chromosome 7, were involved in hybrid breakdown at the F_2 and subsequent generations from a cross between the Sasanishiki cultivar (*O. sativa* ssp. *japonica*) and a Thai upland rice cultivar, Col. No. 15. In addition, Kubo and Yoshimura (2002) reported that two genes, *hwe1* located around the 50 cM region of chromosome 12, and *hwe2* located around the 40 cM region of chromosome 1, controlled hybrid breakdown at the F_2 generation or later following a cross between the cultivars Asominori (*O. sativa* ssp. *japonica*) and IR24 (*O. sativa* ssp. *indica*). Finally, Fukuoka et al. (2005) reported that two genes, *hwg1* located around the 80 cM region of chromosome 6, and *hwg2* located around the 90 cM region of chromosome 11, were involved in hybrid breakdown at the F_2 generation or later of progeny resulting from a cross between the Sasanishiki cultivar (*O. sativa* ssp. *japonica*) and an accession from Assam, India, ARC10303. Because the chromosomal positions that we found do not match these previously reported genes, *hbd2* and *hbd3* are likely novel genes involved in hybrid breakdown in rice.

The establishment of ILs and NILs enables an evaluation of the effect of QTLs, as well as fine mapping and cloning of QTL genes (Yano 2001; Ashikari et al. 2005; Ashikari and Matsuoka 2006). Hybrid breakdown prevents these types of analysis because plant weakness can mask other phenotypes. The genetic analysis indicated that the *hbd2* allele from the Habataki chromosome induces hybrid breakdown in a Koshihikari background. Indeed, an NIL with a Habataki chromosomal segment containing the *hbd2* gene (NIL-*hbd2*) in Koshihikari exhibited a weak phenotype (Fig. 4b-3). This phenotype inhibits a precise evaluation of other QTLs linked to *hbd2* due to the effect of hybrid breakdown. To overcome this problem, we also constructed an NIL containing a Habataki chromosomal segment from the *Hbd3* region (NIL-*Hbd3*; Fig. 4b-4). We then crossed NIL-*Hbd3* with NIL-*hbd2* to construct NIL-*hbd2* + *Hbd3*, and this line did not exhibit a weak phenotype despite the fact that it contains the *hbd2* gene (Fig. 4b-5). Since NIL-*hbd2* + *Hbd3* can mask the deleterious effects of *hbd2*, NIL-*hbd2* + *Hbd3* can be used for the evaluation of other QTLs linked to *hbd2*. Moreover, crossing NIL-*hbd2* + *Hbd3* and NIL-*Hbd3* also permits fine mapping of the QTLs linked with *hbd2*. This demonstrates that the deleterious effects of hybrid breakdown in the NILs can be overcome by a simple pyramiding strategy.

Clarifying the causes of hybrid breakdown contributes to our understanding about the mechanism of speciation. The

process of speciation requires that organisms establish and maintain reproductive barriers against other species (Mayr 1942). Although postzygotic reproductive barriers restrict gene flow if organisms occur sympatrically, they play an important role in speciation and genetic differentiation. The theory of postzygotic reproductive barrier development was proposed independently by Dobzhansky (1937) and Muller (1940), and is now called the Dobzhansky–Muller model. In this model, the development of postzygotic reproductive barriers results from an interaction between two or more genes. Supporting this theory, previous studies have shown that reproductive barriers in rice, including hybrid weakness (Oka 1957; Amemiya and Akamine 1963; Sato and Morishima 1987), hybrid sterility (Oka 1958, 1974; Jennings 1966), and hybrid breakdown (Oka 1957, 1978; Oka and Doida 1962; Yokoo 1984; Okuno 1986; Sato and Morishima 1988; Wu et al. 1995; Li et al. 1997; Fukuoka et al. 2005), are controlled by two or more genes. The hybrid breakdown observed between Koshihikari and Habataki fits the Dobzhansky–Muller model because it also involves the interaction of two genes, the *hbd2* gene from Habataki and the *hbd3* gene from Koshihikari.

The next step in elucidating the molecular mechanism of speciation involves cloning the genes that contribute to the reproductive barriers. Currently, few reports describe the cloning of genes related to reproductive barriers, apart from one in *Xiphophorus* fish (Wittbrodt et al. 1989) and four in *Drosophila* (Ting et al. 1998; Barbash et al. 2003; Presgraves et al. 2003; Brideau et al. 2006). Although several genes related to reproductive barriers in plants, including rice, have been identified, none have been cloned. Once the genes causing reproductive barriers have been cloned, their interactions must be analyzed. Two theories have been proposed to explain how reproductive barriers result from genetic interactions. One is a biochemical model in which deleterious effects from interacting proteins cause reproductive barriers. The other is a genetic model in which the loss of alternative copies of duplicated genes leads to reproductive barriers. Thus far, only one set of genes causing reproductive barriers has been cloned (Brideau et al. 2006); although the precise biological function and mechanism of interaction between these genes causing the reproductive barrier are still unclear, they are not duplicated genes. In yeast, however, it has been proposed that an alternative loss of duplicated genes from a common ancestral chromosomal segment leads to reproductive barriers (Scannell et al. 2006). Because this inheritance model can accommodate recessive genes, we considered whether the hybrid breakdown presented here might be explained by this model. Guyot and Keller (2004) and Yu et al. (2005) have reported examples of rice ancestral chromosomal duplication; however, according to these reports, no chromosomal duplications occur between the regions containing *hbd2* and *hbd3*. Although a possibility yet exists that duplication of a very small chromosomal segment between the *hbd2* and *hbd3* regions may have occurred, another mechanism may be a more likely cause of hybrid breakdown. Even though numerous studies have been performed in various organisms, the mechanistic or molecular basis of the interaction involved in the creation of reproductive barriers is still unknown. Therefore, cloning of the *hbd2* and *hbd3* genes is currently under way, and an analysis of their interaction at the molecular level will be performed.

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References

Amemiya A, Akamine H (1963) Biochemical genetic studies on the root growth inhibiting complementary lethal genes on rice plant. Bull Nat Inst Agric Sci Ser D 10:139–226

Ashikari M, Matsuoka M (2006) Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends Plant Sci 11:344–350

Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. Science 309:741–745

Barbash DA, Siino DF, Tarone AM, Roote J (2003) A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. Proc Natl Acad Sci USA 100:5302–5307

Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA (2006) Two Dobzhansky–Muller genes interact to cause hybrid lethality in *Drosophila*. Science 314:1292–1295

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Dobzhansky T (1937) Genetics and the origin of species. Columbia University Press, New York

Fukuoka S, Namai H, Okuno K (1998) RFLP mapping of the genes controlling hybrid breakdown in rice. Theor Appl Genet 97:446–449

Fukuoka S, Newingham MCV, Ishtaq M, Nagamine T, Kawase M, Okuno K (2005) Identification and mapping of two new loci for hybrid breakdown in cultivated rice. Rice Genet Newsl 22:29

Guyot R, Keller B (2004) Ancestral genome duplication in rice. Genome 47:610–614

Jennings PR (1966) Evaluation of partial sterility in *indica* × *japonica* rice hybrids. IRRI Tech Bull 5:1–63

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Kubo T, Yoshimura A (2002) Genetic basis of hybrid breakdown in a *japonica*/*indica* cross of rice, *Oryza sativa* L. Theor Appl Genet 105:906–911

Li Z, Pinson SRM, Paterson AH, Park WD, Stancel JW (1997) Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. Genetics 145:1139–1148

Mayr E. (1942) Systematics and the origin of species. Columbia University Press, New York

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D,

Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Muller HJ (1940) Bearing of the *Drosophila* work on systematics. In: Huxley JS (ed) The new systematics. Clarendon Press, Oxford, pp 185–268

Oka HI (1957) Phylogenetic differentiation of cultivated rice. XV. Complementary lethal genes in rice. Jpn J Genet 32:83–87

Oka HI (1958) Intervarietal variation and classification of cultivated rice. Indian J Genet Plant Breed 18:79–89

Oka HI (1974) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetics 77:521–534

Oka HI (1978) Phylogenetic differentiation of cultivated rice. XXI. The sporophytic pollen sterility: its genetic basis and interval relationship as shown by F_2 sterility. Jpn J Genet 53:397–410

Oka HI, Doida Y (1962) Phylogenetic differentiation of cultivated rice. XX. Analysis of the genetic basis of hybrid breakdown in rice. Jpn J Genet 37:24–35

Okuno K (1986) Geographical distribution of complementary genes controlling hybrid breakdown in cultivated rice. Jpn J Breed 36(Suppl 1):372–373

Presgraves DC, Balagopalan L, Abmayr SM, Orr HA (2003) Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. Nature 423:715–719

Sato YI, Morishima H (1987) Distribution of complementary genes causing F_1 weakness in Asian native cultivars and its wild relatives. II. Distribution of Hwc-1 and Hwc-2 in native cultivars and wild relatives in Southeast Asia. Euphytica 36:425–431

Sato YI, Morishima H (1988) Distribution of the genes causing F_2 chlorosis in rice cultivars of the *indica* and *japonica* types. Theor Appl Genet 75:723–724

Scannell DR, Byrne KP, Gordon JL, Wong S, Wolfe KH (2006) Multiple rounds of speciation associated with reciprocal gene loss in polyploidy yeasts. Nature 440:341–345

Smith JM (1989) Macroevolution. In: Smith JM (ed) Evolutionary genetics. Oxford University Press, Oxford pp 272–304

Stebbins GL Jr (1950) Isolation and the origin of species. In: Stebbins GL Jr (ed) Variation and evolution in plants. Columbia University Press, New York, pp 189–250

Ting CT, Tsaur SC, Wu ML, Wu CI (1998) A rapidly evolving homeobox at the site of a hybrid sterility gene. Science 282:1501–1504

Ware DH, Jaiswal P, Ni J, Yap IV, Pan X, Clark KY, Teytelman L, Schmidt SC, Zhao W, Chang K, Cartinhour S, Stein LD, McCouch SR (2002) Gramene, a tool for grass genomics. Plant Physiol 130:1606–1613

Wittbrodt J, Adam D, Malitschek B, Maueler W, Raulf F, Telling A, Robertson SM, Schartl M (1989) Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*. Nature 341:415–421

Wu P, Zhang G, Huang N, Ladha JK (1995) Non-allelic interaction conditioning spikelet sterility in an F_2 population of *indica/japonica* cross in rice. Theor Appl Genet 91:825–829

Yano M (2001) Genetic and molecular dissection of naturally occurring variation. Curr Opin Plant Biol 4:130–135

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35:145–153

Yokoo M (1984) Female sterility in an *indica–japonica* cross of rice. Jpn J Breed 34:219–227

Yu J, Wang J, Lin W, Li S, Li H, Zhou J, Ni P, Dong W, Hu S, Zeng C, Zhang J, Zhang Y, Li R, Xu Z, Li S, Li X, Zheng H, Cong L, Lin L, Yin J, Geng J, Li G, Shi J, Liu J, Lv H, Li J, Wang J, Deng Y, Ran L, Shi X, Wang X, Wu Q, Li C, Ren X, Wang J, Wang X, Li D, Liu D, Zhang X, Ji Z, Zhao W, Sun Y, Zhang Z, Bao J, Han Y, Dong L, Ji J, Chen P, Wu S, Liu J, Xiao Y, Bu D, Tan J, Yang L, Ye C, Zhang J, Xu J, Zhou Y, Yu Y, Zhang B, Zhuang S, Wei H, Liu B, Lei M, Yu H, Li Y, Xu H, Wei S, He X, Fang L, Zhang Z, Zhang Y, Huang X, Su Z, Tong W, Li J, Tong Z, Li S, Ye J, Wang L, Fang L, Lei T, Chen C, Chen H, Xu Z, Li H, Huang H, Zhang F, Xu H, Li N, Zhao C, Li S, Dong L, Huang Y, Li L, Xi Y, Qi Q, Li W, Zhang B, Hu W, Zhang Y, Tian X, Jiao Y, Liang X, Jin J, Gao L, Zheng W, Hao B, Liu S, Wang W, Yuan L, Cao M, McDermott J, Samudrala R, Wang J, Wong GK, Yang H (2005) The genomes of *Oryza sativa*:a history of duplications. PLoS Biol 3:266–281