Hybrid Breakdown Caused by Epistasis-Based Recessive Incompatibility in a Cross of Rice (Oryza sativa L.)

KAZUKI MATSUBARA, EJI YAMAMOTO, RITSUKO MIZOBUCHI, JUN-ICHI YONEMARU, TOSHIO YAMAMOTO, HIROSHI KATO, AND MASAHIRO YANO

From the NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan (Matsubara and Kato); and the National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan (Yamamoto, Mizobuchi, Yonemaru, Yamamoto, and Yano). Eiji Yamamoto is now at the NARO Institute of Vegetable and Tea Science, Tsu, Mie 514-2392, Japan. Masahiro Yano is now at the NARO Institute of Crop Science, Tsukuba, Ibaraki 305-8518, Japan. Hiroshi Kato is now at the National Institute of Agrobiological Sciences, Hitachiohmiya, Ibaraki 319-2293, Japan.

Address correspondence to Kazuki Matsubara at the address above, or e-mail: vague@affrc.go.jp; or Hiroshi Kato at the address above, or e-mail: hkato@affrc.go.jp.

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Abstract

Viability and fertility in organisms depend on epistatic interactions between loci maintained in lineages. Here, we describe reduced fitness of segregants (hybrid breakdown, HB) that emerged in an F$_2$ population derived from a cross between 2 rice (Oryza sativa L.) cultivars, “Tachisugata” (TS) and “Hokuriku 193” (H193), despite both parents and F$_2$s showing normal fitness. Quantitative trait locus (QTL) analyses detected 13 QTLs for 4 morphological traits associated with the HB and 6 associated with principal component scores calculated from values of the morphological traits in the F$_2$ population. Two-way analysis of variance of the putative QTLs identified 4 QTL pairs showing significant epistasis; among them, a pair on chromosomes 1 and 12 made the greatest contribution to HB. The finding was supported by genetic experiments using F$_2$ progeny. HB emerged only when a plant was homozygous for the TS allele at the QTL on chromosome 1 and homozygous for the H193 allele at the QTL on chromosome 12, indicating that each allele behaves as recessive to the other. Our results support the idea that epistasis is an essential part of hybrid fitness.

Subject areas: Genomics and gene mapping, Quantitative genetics and Mendelian inheritance

Key words: epistasis, hybrid breakdown, QTL analysis, rice, SNP marker

Deleterious epistatic interaction between alleles at different loci by hybridization and recombination of chromosomes between genetically divergent parents often yields segregants with reduced fitness (inferior viability and fertility) relative to both parents and F$_2$s in the F$_2$ or backcross hybrids, referred to as hybrid breakdown (HB) (Rieseberg and Willis 2007). This type of hybrid incompatibility reflects a physiological and developmental problem in hybrid progeny and has long been commonly known in both plant and animal hybrids (Dobzhansky 1951; Stebbins 1958).

In many cases, the genetic basis for HB is consistent with the Bateson–Dobzhansky–Müller model of incompatibility (Dobzhansky 1951; Coyne and Orr 2004). Let us assume that a population is divided into 2 allopatric populations that start out genetically identical. One population becomes fixed for an allele at one locus (e.g., an $A$ to $a$ mutation), and the other becomes fixed for an allele at another locus (e.g., a $B$ to $b$ mutation). Fitness would be maintained if mutations at both loci were adaptively neutral (or advantageous) within the population in which each arose, but hybrid incompatibility would result when they were brought together in a hybrid.

A key point of this model is that no genotype ever passes through the adaptive valley during the evolution of hybrid incompatibility (Coyne and Orr 2004).

Among rice hybrids, HB has often been described in inter-subspecific (O. sativa ssp. japonica × ssp. indica) and inter-specific (O. sativa × O. rufipogon) crosses (Wu et al. 1995; Li et al. 1997; Oka 1988; Fukuoka et al. 1998, 2005; Kubo and Yoshimura 2002, 2005; Matsubara et al. 2007; Miura et al. 2008; Yamamoto et al. 2010; Ichitani et al. 2012). In all these cases (except one case in backcross hybrids; Miura et al.
deleterious epistasis between loci led to HB. Although, understanding of the genetic basis of HB is of great importance to evolutionary biology as well as to breeding, little is known about its occurrence in rice, probably because of its low-frequency segregation in later generations, compared with that of $F_1$ hybrid sterility (Ouyang and Zhang 2013).

In some rice crosses, phenotypic differences between normal and inferior segregants could be reliably characterized because of mortality or infertility (Miura et al. 2008; Ichitani et al. 2012). Therefore, the causal genetic factors could be mapped on the basis of the distinguishable phenotype (owing to the discontinuous variation) as a Mendelian trait. But in other crosses, differences could not be conclusively distinguished owing to continuous variation of trait values measured as diagnostic of HB in the segregating populations (Li et al. 1997; Matsubara et al. 2007). In such cases, quantitative trait locus (QTL) analysis using genome-wide DNA markers revealed the genetic basis of HB (Li et al. 1997; Matsubara et al. 2007). This approach has been used in the genome-wide identification of HB in other organisms; for example, *Muq* (Oka et al. 2007), Ceratodon (McDaniel et al. 2008), Iris (Taylor et al. 2009), Arabidopsis (Alcázar et al. 2009), and Avena (Latta et al. 2010). Furthermore, the recent development of high-throughput genotyping tools, such as single nucleotide polymorphism (SNP) and restriction-site–associated DNA markers, has considerably facilitated investigations of the genetic basis and complexity of hybrid incompatibility (e.g., Arabidopsis, Bombolies et al. 2007; Neurospora, Dettman et al. 2010; Muq, White et al. 2011; Jambusé et al. 2012; Naunia, Gibson et al. 2013).

Here, we found $F_2$ segregants inferior to both parents in breeding materials used for biomass improvement, using a cross between 2 rice cultivars, “Tachisugata” and “Hokuriku 193,” selectively bred through inter-subspecific crosses between *O. sativa* ssp. *japonica* and *indica*. To unravel the genetic architecture of the HB, we mapped causative QTL regions by quantitative genetic approaches taking advantage of genome-wide SNP markers. Additionally, we identified the donors of alleles by comparing haplotypes of the QTL regions.

## Materials and Methods

### Plant Materials

We grew the rice cultivars “Tachisugata” (TS) and “Hokuriku 193” (H193), and their $F_1$, $F_2$, and $F_3$ progeny. TS and H193 are high-yielding cultivars with large stems and leaves. Both were bred by inter-subspecific crosses between *O. sativa* ssp. *japonica* and *indica* cultivars (Goto et al. 2009; Ohta et al. 2010; Yonemaru et al. 2014b). The $F_2$ population ($N = 402$) used for QTL analysis was obtained by self-pollination of $F_1$ plants.

To validate the effect of putative QTLs, we selected 3 $F_3$ populations (segregating for one, the other or both of the target QTLs) on the basis of the genotype of the $F_2$ plants. We used 94 plants for analyzing single QTLs and 188 plants for analyzing pairs of QTLs. To evaluate the association between the target QTLs and the HB phenotype as reliably as possible, we selected the $F_3$ populations that were homozygous for the TS-derived region around the 40.3-Mb position on Chr. 1, which showed the greatest additive effect for PH. On the basis of its genomic position, the QTL was most likely identical to the $sdI$ locus (at the 40.1-Mb position; Ashikari et al. 2002).

All plant materials were grown in an experimental paddy field in Tsukubamirai (140°01′E, 35°59′N), Japan. Seeds were sown in late April, and 1-month-old seedlings were then transplanted into the field.

To identify the donors of QTLs, we used DNA samples of another 5 cultivars (“Kanto PL12,” “Takarni,” “Suweon 258,” “Hokuriku 133,” and “Guizhao 2”), which were used as parents in the development of TS and H193.

### Genotyping

Genomic DNA was extracted from 2-month-old seedlings by the cetyltrimethylammonium bromide method as described by Murray and Thompson (1980) with a slight modification for mini-scale extraction. From a previously published data set of SNPs (Yonemaru et al. 2012; Yonemaru et al. 2014a, 2014b; Q-TARO database, http://qaro.abr.affrc.go.jp/; Supplementary Table S1), a subset of 344 SNPs chosen for their polymorphism between TS and H193 were used for genotyping. Another 22 SNPs were used for genotyping target regions on chromosomes (Chrs.) 1 and 12 of TS, H193 and their parents (Supplementary Table S1). The $F_2$ plants were genotyped on the Illumina GoldenGate BeadArray platform (Illumina Inc., San Diego, CA). The $F_3$ populations were genotyped with simple sequence repeat (SSR) markers RM5496 and RM2529 (International Rice Genome Sequencing Project 2005), which were located within 1-logarithm of odds (LOD) confidence intervals (CIs) of the 2 target QTLs, according to Matsubara et al. (2007).

### Statistical Analysis

To evaluate HB as a trait, we conducted principal component analysis (PCA) of 4 traits: plant height (PH), panicle number per plant (PN), days to heading (DTH), and seed fertility (SF) in the primary $F_2$ population (TS × H193) at maturity. Principal components (PCs) were calculated from coefficients of the correlations between phenotypic values of the 4 traits (PH, PN, DTH, and SF). DTH was the number of days from germination to heading. SF was defined as seed set percentage (number of fertile seeds/number of spikelets per panicle × 100). In $F_3$ populations, we measured PH and leaf number at the seeding stage (1 month after germination), as well as PH, PN, DTH, and SF at maturity.

A linkage map of the 344 SNP markers was made using the $F_2$ population from the TS × H193 cross. Linkage order and genetic distances of marker loci were calculated using MAPMAKER/Exp 3.0 software (Lander et al. 1987). Genetic distances were calculated by Kosambi function, and linkage groups were identified with the “group” command at min LOD 3.0 and max distance 37.2.

Where the Shapiro–Wilk test rejected normality, trait values were Box–Cox-transformed (PH, PN, and DTH) and arcsine-transformed (SF) before analysis.
In the F₂ population, QTL analyses were performed using the interval mapping procedure as implemented by the Zmapqtl program of QTL Cartographer v. 2.5 software (Wang et al. 2012). In the analyses, the cross type was set in SF(n), and the walk speed (cM) was set at 1.0. Genome-wide threshold values (a = 0.05) for declaring the presence of QTLs were estimated from 1000 permutations. We defined 1-LOD CIs for the positions of QTLs on the basis of the interval mapping results.

To test whether epistatic interaction between QTLs was involved in HB in the F₂ population, we conducted 2-way analysis of variance (ANOVA) of the 4 traits as well as PCs. Significance levels were corrected on the basis of the false discovery rate for multiple testing according to the number of interaction tests (Benjamini and Hochberg 1995). Adjusted R² values (coefficient of determination) were calculated as a measure of goodness-of-fit to 2 multiple linear regression models: \( y = \mu + \beta_1 q_1 + \beta_2 q_2 + \varepsilon \) (additive model) and \( y = \mu + \beta_1 q_1 + \beta_2 q_2 + \gamma(q_1 \times q_2) + \varepsilon \) (full model with interaction term), where \( y \) is the phenotypic value (dependent variable), \( \mu \) is the overall mean, \( \beta_1, \beta_2, \gamma \) are the partial regression coefficients, \( q_1 \) and \( q_2 \) are the QTL genotypes (independent variables), and \( \varepsilon \) is the residual.

In F₂ populations, the significance of associations between the putative QTLs and HB was assessed by 1-way ANOVA. The QTL genotypes were estimated from SSR markers as above. R² was calculated as a measure of goodness-of-fit to a simple linear regression model \( y = \mu + \beta q + \varepsilon \) (single QTL model). Additionally, 2-way ANOVA was performed to test whether or not epistasis underlies the HB between the QTLs.

All calculations other than linkage and QTL analyses were performed in JMP v. 9 software (SAS Institute Inc., Cary, NC).

Results

HB Found in a Rice Cross

In an F₂ population (\( N = 402 \)) derived from TS × H193, 12 segregants were inferior to the parents and the others, all with a shorter PH, fewer panicles, longer DTH and reduced SF (Figure 1).

PC1 (Figure 1) accounted for 47.8% of the variation of the 4 traits in the F₂ population (Supplementary Figure S1a), and showed significant correlations with all 4 traits (Supplementary Figure S1b). Thus, PC1 was able to distinguish between normal and inferior segregants.

QTL Mapping of HB

Interval mapping identified 13 significant QTLs for the 4 traits in the F₂ population: 4 QTLs for PH on Chrs. 1, 5, and 6; 2 QTLs for PN on Chrs. 5 and 12; 3 QTLs for SF on Chrs. 1, 11, and 12, and 4 QTLs for DTH on Chrs. 2, 6, 10, and 11 (Table 1, Supplementary Figure S2). No significant QTLs were found on Chrs. 3, 4, 7, 8 or 9.

The 1-LOD CIs of QTLs for some traits overlapped: PH and PN on Chr. 5, DTH and SF on Chr. 11, and PN and SF on Chr. 12 (Figure 2a). On the other hand, a QTL for PH on Chr. 1 and 3 QTLs for DTH on Chrs. 2, 6, and 10 were independent.

QTL analysis on PC1 detected 6 QTLs on Chrs. 1, 5, 6, 11, and 12. From the comparison of 1-LOD CIs among the detected QTLs, the QTLs for PC1 were likely to co-localize with those for the phenotypic traits (Figure 2a).

Identification of Epistatic QTLs

Two-way ANOVA detected 8 significant QTL × QTL interactions for PN, SF, and PC1 in the F₂ population, but not for PH or DTH (Table 2). We found no significant 3-way interactions among the QTLs.

Comparison of the differences in R² between additive and full models showed a larger contribution of epistasis between QTLs for PC1 on Chrs. 1 and 12 to HB than of the other epistatic QTL pairs (Table 2).

Classification of each QTL genotype in the F₂ population revealed that HB occurred when plants were homozygous for the TS allele at the QTL on Chr. 1 and homozygous for the H193 allele at the QTL on Chr. 12, suggesting that each of the causative alleles behaved as recessive to the other (Figure 2b, Supplementary Figure S3).

Validation of Epistatic QTLs by Using F₃ Progeny

Genetic analysis in the F₂ population suggested that epistasis between the QTLs on Chrs. 1 and 12 accounted for most of the HB. To validate this, we conducted progeny tests using 3 F₃ populations: 1) segregating for the QTL region on Chr. 1 but homozygous for the H193-derived region on Chr. 12 (\( N = 94 \)) (Figure 3a); 2) homozygous for the TS-derived QTL region on Chr. 1 but segregating on Chr. 12 (\( N = 94 \)) (Figure 3b); and 3) segregating for both regions (\( N = 188 \)) (Figure 3c).

We measured the 4 traits (PH, PN, DTH, and SF) in each F₃ population and performed PCA. Then we assessed the significance of the association between marker genotype and PC1 using 2 SSR markers, RM5496 (at the 7.6-Mb position on Chr. 1) and RM2529 (at the 8.1-Mb position on Chr. 12), which are located within the 1-LOD CIs of each QTL. In the first population, 81 plants were scored as normal and 13 as inferior (shorter PH, fewer panicles, longer DTH and reduced SF in comparison with the parents and the others; Supplementary Figure S4a). There was a significant association between the RM5496 genotype and the PC1 score (1-way ANOVA, \( R^2 = 0.576, P < 0.0001; \) Figure 3a). Inferior segregants were homozygous for the TS-derived allele at RM5496 on Chr. 1. In the second population, 73 plants were scored as normal and 21 as inferior (Supplementary Figure S4b). There was a significant association between the RM2529 genotype and the PC1 score (1-way ANOVA, \( R^2 = 0.580, P < 0.0001; \) Figure 3b). Inferior segregants were homozygous for the H193-derived allele at RM2529 on Chr. 12. In the third population, 182 plants were scored as normal and 6 as inferior (Supplementary Figure S4c). The variation in the
PC1 score in the F\textsubscript{3} population was well explained by significant epistasis between the RM5496 and RM2529 genotypes (2-way ANOVA, \(R^2 = 0.653, P < 0.0001\); Figure 3c). HB emerged when plants carried the genotypes \(TS/TS\) at RM5496 on Chr. 1 and \(H193/H193\) at RM2529 on Chr. 12.

In all F\textsubscript{3} populations, the QTLs significantly accounted for variations in PH and leaf number at the seedling stage, except for leaf number in the first population (Supplementary Figure S5). On the other hand, the PH and leaf number of many segregants carrying the HB genotype (black boxes) was normal (or indiscernible from that of the other genotypes) at that stage (Supplementary Figure S5).

Taken together, these results are consistent with the results of QTL analysis in the F\textsubscript{2} population, indicating that the HB was explained by epistasis-based recessive incompatibility. Thus, we tentatively designate the TS allele at the QTL on Chr. 1 as \textit{hybrid breakdown 4} (\textit{hbd4}) and the H193 allele at the QTL on Chr. 12 as \textit{hybrid breakdown 5} (\textit{hbd5}).

\textbf{Figure 1.} (a) Parents and their F\textsubscript{2} progeny. (b) Panicles of an inferior segregant. Arrow shows a panicle that is still erect at the maturity stage of normal segregants. (c) Frequency distributions of plant height, panicle number, days to heading, seed fertility and PC1 in the F\textsubscript{2} population. T, mean of TS; H, mean of H193. Black boxes show inferior segregants.
Table 1  Summary of QTLs detected in an F$_2$ population derived from a cross between “Tachisugata” and “Hokuriku 193”

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chr</th>
<th>Nearest marker</th>
<th>Position$^a$ (Mb)</th>
<th>LOD score</th>
<th>Additive effect</th>
<th>Dominance effect</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>1</td>
<td>A8</td>
<td>8.3</td>
<td>5.6</td>
<td>-4.53</td>
<td>-4.61</td>
<td>0.063</td>
</tr>
<tr>
<td>PH</td>
<td>1</td>
<td>A27</td>
<td>40.3</td>
<td>59.5</td>
<td>15.08</td>
<td>-4.10</td>
<td>0.514</td>
</tr>
<tr>
<td>PH</td>
<td>5</td>
<td>E25</td>
<td>28.2</td>
<td>6.9</td>
<td>-4.64</td>
<td>5.12</td>
<td>0.076</td>
</tr>
<tr>
<td>PH</td>
<td>6</td>
<td>F40</td>
<td>29.4</td>
<td>9.2</td>
<td>-5.43</td>
<td>-6.54</td>
<td>0.109</td>
</tr>
<tr>
<td>PN</td>
<td>5</td>
<td>E25</td>
<td>28.2</td>
<td>3.8</td>
<td>-1.00</td>
<td>-0.51</td>
<td>0.043</td>
</tr>
<tr>
<td>PN</td>
<td>12</td>
<td>L9</td>
<td>10.5</td>
<td>4.3</td>
<td>0.96</td>
<td>-1.19</td>
<td>0.048</td>
</tr>
<tr>
<td>DTH</td>
<td>2</td>
<td>B17</td>
<td>10.6</td>
<td>6.6</td>
<td>-0.95</td>
<td>0.53</td>
<td>0.076</td>
</tr>
<tr>
<td>DTH</td>
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<td>F27</td>
<td>21.7</td>
<td>4.3</td>
<td>-0.81</td>
<td>0.19</td>
<td>0.048</td>
</tr>
<tr>
<td>DTH</td>
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<td>J34</td>
<td>23.7</td>
<td>16.4</td>
<td>1.58</td>
<td>0.19</td>
<td>0.171</td>
</tr>
<tr>
<td>DTH</td>
<td>11</td>
<td>K2</td>
<td>3.0</td>
<td>7.0</td>
<td>1.10</td>
<td>0.99</td>
<td>0.106</td>
</tr>
<tr>
<td>SF</td>
<td>1</td>
<td>A4</td>
<td>6.7</td>
<td>9.4</td>
<td>-5.64</td>
<td>-3.96</td>
<td>0.102</td>
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<tr>
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<td>11</td>
<td>K1</td>
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<td>-3.40</td>
<td>-4.91</td>
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</tr>
<tr>
<td>SF</td>
<td>12</td>
<td>L12</td>
<td>14.2</td>
<td>13.5</td>
<td>6.94</td>
<td>-4.52</td>
<td>0.143</td>
</tr>
<tr>
<td>PCI</td>
<td>1</td>
<td>A5</td>
<td>7.0</td>
<td>4.8</td>
<td>-0.39</td>
<td>-0.42</td>
<td>0.053</td>
</tr>
<tr>
<td>PCI</td>
<td>1</td>
<td>A27</td>
<td>40.3</td>
<td>10.3</td>
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<td>-0.27</td>
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<td>E16</td>
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</tr>
<tr>
<td>PCI</td>
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<td>F40</td>
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<td>3.9</td>
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<td>-0.45</td>
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</tr>
<tr>
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<td>K1</td>
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<td>4.9</td>
<td>-0.44</td>
<td>-0.52</td>
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</tr>
<tr>
<td>PCI</td>
<td>12</td>
<td>L3</td>
<td>5.7</td>
<td>9.8</td>
<td>0.57</td>
<td>-0.58</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Chr, chromosome; PH, plant height; PN, panicle number; DTH, days to heading; SF, seed fertility; PCI, principal component 1. The sign of the additive effect corresponds to the direction of the effect of the “Tachisugata” allele.

$^a$Positions are based on the physical position within the rice genome sequence (Build 4, [http://rgp.dna.affrc.go.jp/](http://rgp.dna.affrc.go.jp/)) of the cultivar “Nipponbare” (O. sativa ssp. japonica).

Linkage Mapping of hbd4 and hbd5

To narrow down the genomic regions of the QTLs for HB, we selected 29 plants with hbd4 and 40 plants with hbd5, which showed inferior phenotype in the F$_2$ and F$_3$ populations, and performed linkage analyses using the SNP markers. We mapped hbd4 to the interval between A5 and A7 on Chr. 1 (0.8 Mb), and we found no recombination between hbd4 and A6 (Figure 4a); and we mapped hbd5 to the interval between L9 and L14 on Chr. 12 (9.5 Mb), and we found no recombination between hbd5 and L10–L13 (Figure 4b).

Donors of hbd4 and hbd5

To determine donors of hbd4 (the TS allele) and hbd5 (the H193 allele), we compared haplotypes of the hbd4 and hbd5 regions among TS, H193 and their progenitors (Figure 5). The TS haplotypes for both regions were the same as those of “Kanto PL12” but not “Takanari.” The H193 haplotypes for both regions were the same as those of “Suweon 258” and “Hokuriku 133,” and that for hbd4 was the same as that of “Guizhao 2.” Thus, hbd4 was inherited from “Kanto PL12” to TS, and hbd5 was inherited from “Suweon 258” or “Hokuriku 133” through “Jou 344” (unfortunately its DNA sample was not available) to H193.

Discussion

By using genome-wide SNP markers, we demonstrated that the HB found here was explained largely by the complementary effect of 2 recessive alleles: hbd4, carried by “Tachisugata” on Chr. 1, and hbd5, carried by “Hokuriku 193” on Chr. 12. In other words, the HB was caused by a deleterious heterospecific interaction between the cultivar “Nipponbare” (O. sativa ssp. japonica).
Table 2  Significant epistatic QTLs detected in an F2 population derived from a cross between “Tachisugata” and “Hokuriku 193”

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL1</th>
<th>Chr</th>
<th>Position (Mb)</th>
<th>QTL2</th>
<th>Chr</th>
<th>Position (Mb)</th>
<th>P</th>
<th>R²c</th>
<th>Additive model</th>
<th>Full model</th>
</tr>
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<tr>
<td>PN</td>
<td>E25</td>
<td>5</td>
<td>28.2</td>
<td>I.9</td>
<td>12</td>
<td>10.5</td>
<td>1.03E-02**</td>
<td>0.076</td>
<td>0.098</td>
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</tr>
<tr>
<td>SF</td>
<td>A4</td>
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<td>6.7</td>
<td>K.1</td>
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<td>1.44E-04**</td>
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<tr>
<td>SF</td>
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<td>6.7</td>
<td>L.12</td>
<td>12</td>
<td>14.2</td>
<td>7.56E-46**</td>
<td>0.156</td>
<td>0.509</td>
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</tr>
<tr>
<td>SF</td>
<td>K1</td>
<td>11</td>
<td>0.2</td>
<td>L.12</td>
<td>12</td>
<td>14.2</td>
<td>7.30E-04**</td>
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<td>0.202</td>
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<tr>
<td>PC1</td>
<td>A5</td>
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<td>7.0</td>
<td>K.1</td>
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<td>0.2</td>
<td>1.57E-06**</td>
<td>0.135</td>
<td>0.196</td>
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</tr>
<tr>
<td>PC1</td>
<td>A5</td>
<td>1</td>
<td>7.0</td>
<td>L.3</td>
<td>12</td>
<td>5.7</td>
<td>7.56E-46**</td>
<td>0.156</td>
<td>0.509</td>
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<tr>
<td>PC1</td>
<td>K1</td>
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<td>L.3</td>
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<td>0.135</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>E16</td>
<td>5</td>
<td>24.1</td>
<td>L.3</td>
<td>12</td>
<td>5.7</td>
<td>5.13E-06**</td>
<td>0.135</td>
<td>0.166</td>
<td></td>
</tr>
</tbody>
</table>

Chr, chromosome; PN, panicle number; SF, seed fertility; PC1, principal component 1. The sign of the additive effect corresponds to the direction of the effect of the “Tachisugata” allele.

aPositions are based on the physical position within the rice genome sequence (Build 4, http://rgpdna.affrc.go.jp/) of the cultivar “Nipponbare” (O. sativa ssp. japonica).

bP values of the interaction term; *α = 0.05, **α = 0.01.

R² values as a measure of goodness-of-fit to the multiple linear regression models (see Materials and Methods for details).

Figure 2. (a) Graphical representation of significant QTLs and epistasis for HB in the F2 population. Blue dashed lines connect pairs of markers (QTLs) with significant epistasis for PC1 score. Vertical bars to the right of chromosomes denote 1-LOD confidence interval; horizontal bars denote the position of the LOD peak at each QTL. Broken lines on Chr. 1, 5, and 6 show linkage gaps. (b) Average PC1 of each genotype class at the QTLs on Chr. 1 and 12. The number of segregants is shown on each column.
In the model, one population loses function at one locus and retains it at the other, whereas the other population experiences the opposite. Consequently, \( \frac{1}{4} \) of the F\(_1\) zygotes do not have functional genes (Lynch and Conery 2000). In rice, 2 examples of reciprocal silencing have been demonstrated in F\(_1\) hybrid sterility (Mizuta et al. 2010; Yamagata et al. 2010). As rice has experienced both whole genome and segmental duplication (Yu et al. 2005; Wang et al. 2007), reciprocal silencing could be prevalent as a cause of hybrid incompatibility. As yet, there is no example of HB caused by reciprocal silencing of duplicated genes in rice, but HB described by Ichitani et al. (2012) may be an example, because Chrs. 11 and 12, on which the causative recessive genes lies, share ancestral chromosomal segments (Rice Chromosomes 11 and 12 Sequencing Consortia 2005; Yu et al. 2005). According to the scenario, the HB found here could result from the reciprocal silencing of duplicated genes, because \( hbd4 \) and \( hbd5 \) behave as nearly completely recessive alleles (Figure 2b), implying that they are loss-of-function alleles. However, as yet, we do not have evidence for segmental or gene duplication between the genomic regions where \( hbd4 \) and \( hbd5 \) are mapped. To test this hypothesis, molecular cloning of \( hbd4 \) and \( hbd5 \) is needed.

A complementary effect of \( hbd4 \) and \( hbd5 \) explains most of the HB, yet we cannot rule out the contribution of other QTLs, as found in Arabidopsis hybrids (Alcázar et al. 2009). Three other significant epistatic QTL pairs were detected, although their contributions to phenotypic variation were relatively small. Of them, a QTL near marker K1 on Chr. 11 appeared to show epistatic interactions with both \( hbd4 \) and \( hbd5 \) regions (Figure 2a, Table 2). Nevertheless, no 3-way interaction was detected among the QTLs. Additionally, other QTLs with additive effects also yielded continuous distributions in trait variations in the F\(_2\) population (Figure 1c). In particular, a QTL for PH, which was mapped around 40.3 Mb on Chr. 1, had a very large effect (\( R^2 = 0.514 \)) on the trait variation (Table 1).

We also observed pleiotropic consequences of the HB, which affected multiple traits, PH, PN, DTH, and SF, but in spite of the fact that these traits should be developmentally independent of each other (Figure 1c). These consequences imply that the causative genes lie upstream of gene cascades or at the center of gene regulatory networks, as discussed by Burton et al. (2006), thereby affecting multiple physiological and developmental processes. If so, this may provide an explanation for differences in growth rate (from inferior to normal) observed in the HB segregants at the seedling stage (Supplementary Figure S5). By using a cDNA microarray, Renaut and Bernatchez (2011) identified the transcriptome-wide signature of HB in lake whitefish hybrids (Coregonus). Such an analysis, as well as the cloning of the causative genes, might provide evidence for the implication.

To improve resistance to biotic and abiotic stresses and yield potential, cultivars derived from crosses between subspecies, as used here, are often bred. The publication of reference genome sequences and the development of next-generation sequencing technologies have greatly accelerated the progress in molecular genomics and breeding of crops (Morrell et al. 2011). In this advancement, genome-based selection on the basis of genotypes of genome-wide DNA markers has been used in plant and animal breeding (such as...
genomic selection, Crossa et al. 2014; Druet et al. 2014). In crop breeding, genome-based selection often uses advanced-generation populations such as recombinant inbred lines as reference populations from which genome-wide genotype and phenotype data are obtained. In such populations, inferior progeny should have been eliminated in the early generations after crossing. Thus, selection based only on marker genotypes is performed in the progeny of early generations (e.g., F2). In this selection method, if crosses causing HB were carried out, we would have to abandon some important selection candidates. Alternatively, we might select undesirable candidates in which HB becomes apparent in later generations. Therefore, knowledge of the distribution of HB-related alleles among crop cultivars is a prerequisite for genome-based selection.

In summary, the HB found here was accounted for mostly by epistasis-based recessive incompatibility, with a simple genetic basis. We mapped the genes responsible to specific genomic regions by using genome-wide SNP markers. Our results support the idea that epistasis plays an important role in hybrid fitness (Demuth and Wade 2005; Corbett-Detig et al. 2013), and it reinforces the need for caution in genome-based selection in rice breeding. The molecular cloning of hbd4 and hbd5 will provide better understanding of the genetic basis of HB.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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References


