Genetic basis of transgressive segregation in rice heading phenotypes

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21

22 **ABSTRACT**

23 Transgressive segregation produces hybrid progeny phenotypes that exceed parental

24 phenotypes. Unlike heterosis, extreme phenotypes caused by transgressive segregation

25 are heritably stable. We examined transgressive phenotypes of flowering time in rice.

26 Our previous study examined days to flowering (heading; DTH) in six F2 populations

27 for which the parents had distal DTH, and found very few transgressive phenotypes.

28 Here, we demonstrate that transgressive segregation in F2 populations occurred between

29 parents with proximal DTH. DTH phenotypes of the A58 × Kitaake F2 progenies

30 frequently exceeded those of both parents. Both A58 and Kitaake are *japonica* rice

31 cultivars adapted to Hokkaido, Japan, which is a high-latitude region, and have short

32 DTH. Among the four known loci required for short DTH, three loci had common

alleles in A58 and Kitaake, and only the one locus had different alleles. This result indicates that there is a similar genetic basis for DTH between the two varieties. We identified five new quantitative trait loci (QTLs) associated with transgressive DTH phenotypes by genome-wide single nucleotide polymorphism (SNP) analysis. Each of these QTLs showed different degrees of additive effects on DTH, and two QTLs had epistatic effect on each other. These results demonstrated that genome-wide SNP analysis facilitated detection of genetic loci associated with the extreme phenotypes and revealed that the transgressive phenotypes were produced by exchanging complementary alleles of a few minor QTLs in the similar parental genotypes.

INTRODUCTION

The range of phenotypic variation in a quantitative trait depends on its genetic complexity (ALONSO-BLANCO AND MENDEZ-VIGO 2014; HUANG AND HAN 2014). Cross hybridizations often produce progenies with wider phenotypic variation than their parents, which is referred to as transgressive segregation (RICK AND SMITH 1953; HARLAN 1976; DE VICENTE AND TANKSLEY 1993). Unlike heterosis, the extreme phenotypes that occur as a result of transgressive segregation can be fixed after the second filial generation (F2). Such extreme phenotypes can have important roles in

evolution (RIESEBERG *et al.* 2002; DITTRICH-REED AND FITZPATRICK 2013). From a breeding perspective, this phenomenon has also strongly contributed to crop and animal improvements (VEGA AND FREY 1980; TANKSLEY AND MCCOUCH 1997). However, little is known about the genetic basis of transgressive segregations, which are associated with phenotypic improvement of useful traits in crops.

Days to heading (DTH) determines the regional adaptability of rice (*Oryza sativa* L.), which is cultivated widely in tropical and temperate regions (HORI *et al.* 2016). DTH is an important agronomic trait that controls flowering time in rice. Flowering time is a complicated trait in many crops, and the genetic basis of DTH has been well studied in rice; to date, 14 quantitative trait loci (QTLs) were identified based on natural variation and isolated by map-based cloning strategies (EBANA *et al.* 2011; HORI *et al.* 2016; BRAMBILLA *et al.* 2017). We previously examined DTH in rice using six different F2 populations derived from crosses between Kokusyokuto-2 (a Hokkaido landrace denoted as A58) with a short DTH (81 days) used as the seed parent and six varieties with long DTH (114–126 days) used as the pollen parents (OTA *et al.* 2014). Most F2 plants from all six crosses showed intermediate DTH that fell within the parental ranges. OTA *et al.* (2014) found some plants with shorter DTH than A58 from the A58 ×

69 Kasalath F2 population; only this F2 population had some individuals with shorter DTH
70 relative to those of the parents, and the other five F2 populations did not exhibit such
71 extreme phenotypes. In the plants with shorter DTH, we identified a genetic interaction
72 (*Ghd7* from A58 and *Ehd1* from Kasalath) that contributed to the extreme phenotypes
73 produced by the cross of the distantly related parents (OTA *et al.* 2014).
74
75 Here, we were interested in determining how the range of phenotypic variation is
76 produced and whether extreme phenotypes can be produced when both parents used in
77 the cross have proximal phenotypes. The progenies from parents with the same
78 genotypes would have very narrow phenotypic variation, while certain range of the
79 phenotypic variation like transgressive phenotypes should be expected in the F2
80 generation from parents with different genotypes that could coincidentally cause similar
81 phenotypes. By testing these predictions, we may be able to identify the unknown
82 genetic entities that produce extreme phenotypes.
83
84 We specifically focused on phenotypic variations in DTH of a population derived from
85 a cross between two closely related varieties, A58 and Kitaake (an improved variety),
86 both of which are adapted to Hokkaido, a northernmost rice cultivation area. Compared

with the progenies of distantly related parents with distal DTH phenotypes, more A58 × Kitaake progenies had extreme short or long DTH phenotypes relative to the parents. We evaluated the genetic causes of transgressive segregations of both early and late DTH observed in this segregating population. First, known genes associated with short DTH were evaluated in A58, Kitaake, and their progenies to determine if transgressive phenotypes were produced. Subsequently, we performed genome-wide single nucleotide polymorphism (SNP) analysis to detect unknown QTLs associated with extremely short or long DTH. The results obtained here demonstrated that a relatively small number of minor QTLs and their epistatic interactions produced transgressive segregation in DTH. Important genetic properties of the extreme heading phenotypes caused by transgressive segregation are discussed.

MATERIALS AND METHODS

Genetic stocks

A rice landrace from Hokkaido, A58, and an improved variety of Hokkaido, Kitaake, were used as parents. A58 seeds were obtained from seed stocks at the Plant Breeding Laboratory in Hokkaido University. Kitaake seeds were obtained from the genebank at the Agricultural Research Department of Hokkaido Research Organization. A58 was

105 crossed with Kitaake to obtain F1 seeds. A total of 248 F2 plants were obtained from
106 self-pollination of the F1 plants. From the 248 F2 plants, 132 were randomly selected to
107 obtain F3 populations. These F3 populations were used for genetic analysis of DTH
108 using DNA markers in the *Hd1* locus, which is a major locus that affects DTH in rice
109 (YANO *et al.* 2000). Of the 132 F3 populations, 15 individuals that showed early DTH
110 and had a fixed *Hd1* genotype were selected as early-heading populations. Similarly, 15
111 individuals that showed late DTH and had a fixed *Hd1* genotype were selected as
112 late-heading populations. Plants in these two selected populations were self-pollinated
113 to produce F4 lines. The genotypes of the 15 early and 15 late lines in the F4 generation
114 were determined by genome-wide SNP analysis (Figure S1).
115
116 ***DTH analysis***
117 Plants were grown in an experimental paddy field at Hokkaido University, Sapporo,
118 Japan (43.1 N). For F2 and F3 populations, DTH was measured in 2013 and 2014,
119 respectively. DTH for the F4 generation was measured in 2015 and 2016 as the number
120 of days from sowing to emergence of the first panicle of a plant. Average DTH of the
121 F3 and F4 populations were calculated from the values of five or six plants.
122

123 ***Genotyping and sequencing***

124 Genomic DNA was extracted from leaf samples using Plant DNAzol (Invitrogen,
125 Carlsbad, CA, USA). To genotype the *Hd1* locus, two primers, Hd1L (5′-CGA CGT
126 GCA GGT GTA CTC CG-3′) and Hd1R (5′-AAT CTG TGT AAG CAC TG ACG-3′),
127 were used based on the *Hd1* sequence. Genome-wide SNPs were detected by double
128 digest restriction site-associated DNA sequencing (ddRAD-Seq) (BAIRD *et al.* 2008;
129 PETERSON *et al.* 2012), which began with DNA library preparation using the restriction
130 enzymes *Bgl*II and *Eco*RI. Sequencing was performed with 51 bp single-end reads in
131 one lane of a HiSeq2000 Sequencer (Illumina, San Diego, CA, USA) by Macrogen
132 (Seoul, South Korea). The ddRAD-sequencing reads were trimmed with Trimmomatic
133 ver 0.33 (BOLGER *et al.* 2014) with the following parameters: LEADING:19
134 TRAILING:19 SLIDINGWINDOW:30:20 AVGQUAL:20 MINLEN:51. The trimmed
135 reads were mapped to a RAD reference for the Os-Nipponbare-Reference-IRGSP-1.0
136 using Bowtie 2 (LANGMEAD AND SALZBERG 2012) with a default parameter setting. To
137 build RAD loci, we used the ref_map.pl pipeline in Stacks ver. 1.29 (CATCHEN *et al.*
138 2011). All RAD-Seq procedures were carried out by Clockmics, Inc. (Izumi, Osaka,
139 Japan). A total of 1,402 SNPs between parental varieties were detected by ddRAD-Seq;
140 among these SNPs, 634 were considered reliable after filtering SNPs that appeared in

more than 80% of F4 plants.

PCR amplicons for the four previously identified genes involved in DTH (*Hd1*, *Hd2*/OsPRR37, *E1*/*Hd4*/*Ghd7*, and *Hd5*/*DTH8*) were purified using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). Purified samples were sequenced in both directions using a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI310 automatic sequencer (Applied Biosystems). Sequence alignment was performed using CLUSTAL W 2.1 (THOMPSON *et al.* 1994). The primers used for sequencing the four genes were as follows: *Se1*/*Hd1* [5′-CGA CGT GCA GGT GTA CTC CG-3′ and 5′-AAT CTG TGT AAG CAC TG ACG-3′], *Hd2*/*OsPRR37* [5′-TCT TTC TGA TGG CTG TCT GC-3′ and 5′-GCC ATC GCG TAG GTA GGT AG-3′], *E1*/*Hd4*/*Ghd* [5′-GCT GGC TGG ACT TCA CTA CC-3′ and 5′-CAT GGG CCA CTT CTA AGA TCA-3′], and *Hd5*/*DTH8* [5′-CGG AGT TCA TCA GCT TCG TT-3′ and 5′-TGA CCA TGG TGT GAG TGT GA-3′].

Transgressive index

We defined the transgressive index, which indicates the proportion of phenotypic

differences between both parents and the phenotypic range in the F2 population. The transgressive index was calculated by dividing the width of the distribution of DTH in the F2 population by the parental DTH difference.

Marker genotype value

Allelic effects of each of the six loci that influenced DTH of the A58 × Kitaake hybrid progenies were evaluated as marker genotype values (GODDARD AND HAYES 2007). Average DTH for each allele was calculated based on DTH data collected from all homozygous alleles in the 30 F4 population lines from 2016. Then, the central value was determined based on the two phenotypic averages obtained from each of both alleles at the same locus. The marker genotype value was equivalent to the difference between the central value and either allelic average DTH in the same locus.

Data availability

All genetic stocks and sequence data are available on request. Sequence data for this study were deposited in DDBJ (DDBJ accession number XXXXX).

RESULTS

177 ***Variation and transgressive phenotypes of DTH in A58 × Kitaake progenies***

178 Both A58 and Kitaake are adapted to the high-latitude area between 41.2 N and 45.4 N
179 in Hokkaido, Japan, and consequently have photoperiod-insensitive, short DTH and are
180 cold-resistant (ISHIGURO *et al.* 2014; OTA *et al.* 2014). There was no significant DTH
181 difference between these two varieties (t-test: A58, 81.2 ± 0.38; Kitaake, 80.5 ± 0.66; P
182 = 0.19) (Figure 1A). DTH of these F2 plants were widely distributed from 69 to 87 days
183 (Figure 1A), and the earliest plant DTH was equivalent to that of an extreme
184 early-heading variety (Figure 1A).

185

186 The transgressive index of the A58 × Kitaake progenies was 25.7 (Figure 1A). In the six
187 crosses between A58 and the other varieties that were more genetically distant from
188 A58 than Kitaake (OTA *et al.* 2014), the transgressive indexes ranged from 0.91 to 2.12
189 (Figure 1B). The transgressive index of DTH indicated that the DTH of A58 × Kitaake
190 F2 plants exceeded DTH of either parent, but such a strong transgressive segregation
191 was not observed in the previously published crosses.

192

193 The DTH distribution in the A58 × Kitaake-derived F3 populations was similar to that
194 of the F2 population (Figure S2). For further analysis, we selected 15 early- and

late-heading F3 plants and developed two F4 populations (early and late) by self-pollination. Average DTH of early- and late-heading F4 populations were 63.8 ± 1.32 and 74.6 ± 0.99, respectively, in 2015, and 72.2 ± 1.32 and 80.0 ± 1.00, respectively, in 2016 (Figure 2). These differences between DTH of the early and late populations were significant (t-test, $P < 0.001$) throughout 2015 and 2016, and it was predicted that these two distinct populations were generated by new genetic interactions derived from the A58 × Kitaake cross.

Sequence analysis of genes that control DTH, and the effect of* Hd1 *on DTH

Four loci (*E1*/*Hd4*/*Ghd7*, *Hd2*/*OsPRR37*, *Se1*/*Hd1*, and *Hd5*/*DTH8*) control DTH in varieties from Hokkaido, and their specific alleles facilitated adaptation by producing photoperiod-insensitive varieties with short DTH (ICHITANI *et al.* 1997; FUJINO AND SEKIGUCHI 2005a; FUJINO AND SEKIGUCHI 2005b; NONOUE *et al.* 2008; FUJINO *et al.* 2013; KOO *et al.* 2013). To confirm whether these four loci are related to the DTH differences observed in the A58 × Kitaake F2 population, we compared nucleotide sequences of these loci (Figure 3). Sequence analysis of *Hd1* showed the presence of polymorphisms, including a 312-bp insertion/deletion in A58 and Kitaake. This polymorphism in *Hd1* might have produced the DTH differences observed in the F2

213 population. In contrast to *Hd1*, no polymorphisms were detected in the other three loci
214 (*E1*/*Hd4*/*Ghd7*, *Hd2*/*OsPRR37*, and *Hd5*/*DTH8*) in A58 and Kitaake (Figure 3).
215
216 In terms of the effect of the *Hd1* locus on the A58 × Kitaake F2 population, the average
217 DTH in A58-type homozygous, heterozygous, and Kitaake-homozygous populations
218 were 81.3 ± 0.36, 79.5 ± 0.38, and 78.8 ± 0.46, respectively (Table 1). The results
219 showed that *Hd1* had a significant but small effect on DTH in this population ($P < 0.001$),
220 which revealed that the extremely early phenotype of progenies was not fully
221 explainable by only *Hd1*, and another factor(s) may be involved.
222
223 ***Detection of SNPs associated with extreme DTH phenotypes***
224 If a QTL for DTH was located near an SNP, the SNP alleles tended to be associated
225 with early- or late-heading populations. Genome-wide SNP analysis using ddRAD-Seq
226 provided us a total of 634 reliable SNPs for 15 early and 15 late lines in the F4
227 populations (Figure 4). Among the 634 SNPs, 27 were detected as loci where the
228 frequency of either the A58- or Kitaake-type allele was distorted in early- or
229 late-heading populations (Table 2). Of these possible DTH phenotype-related SNPs, we
230 focused on 19 SNPs that belonged to five clusters on Chs 1, 2, 4, 6, and 10 (Table 2 and

231 Figure 4); these SNP clusters represented the chromosomal regions where QTLs for
232 DTH were present.
233
234 ***Validation of relationships between SNP genotypes and DTH***
235 Table 2 shows average DTH between two alleles of each of the five SNP clusters in the
236 F4 lines examined in 2015 and 2016. Significant differences ($P < 0.001$) in DTH
237 between A58- and Kitaake-type homozygous alleles were observed in SNPs on Ch 4 in
238 both 2015 and 2016 (Table 2 and Figure S3). In the SNP cluster on Ch 10, significant
239 differences in DTH between the two alleles were also observed, although the difference
240 was small in 2016. SNPs on Chs 1 and 6 weakly significantly differed between the two
241 alleles that were only observed in 2015. Among the five clusters, the weakest effect was
242 detected in the Ch 2 cluster, which was not significant ($P = 0.14$), but still clearly
243 discriminated the two alleles by 3 to 4 days. Overall, the order of the five SNP clusters
244 based on additive effects on DTH was: Ch 4 > (*Hd1*) > 10 > 6 > 1 > 2. The
245 Kitaake-derived alleles in the SNPs on Chs 4, 10, and 6 produced shorter DTH than the
246 A58-derived alleles; alternatively, the A58-derived alleles on Chs 1 and 2 produced
247 shorter DTH than the Kitaake-derived alleles.
248

Among the selected chromosomal regions (Chs 1, 4, 6, and 10), genetic interactions were tested using the F2 population (Figure 1). Among several combinations of possible epistatic interactions, a strong genetic interaction was identified in the SNPs on Chs 1 and 10 (Figures 5 and S4). The A58-derived alleles in the SNPs on Ch1 decreased DTH when they were combined with Kitaake-derived alleles in SNPs on Ch 10, but increased DTH when combined with A58-derived alleles in SNPs on Ch 10 (Figure 5). No known genes associated with DTH are located around these two chromosomal regions. These findings revealed that unknown genes from A58 and Kitaake caused epistatic interactions responsible for the transgressive early phenotype.

Loci weighted by marker genotype values based on DTH data from 2016 are shown in Figure 6. Among a total of 30 F4 lines, nine harbored homozygous alleles at all six loci (the five QTLs and *Hd1*). These nine lines were sorted by DTH (Figure 6). Based on marker genotype values, direction of allelic effect, and the numbers of the alleles with an effect, the order based on DTH was reasonable, although it was not identical to the expectation, because of possible genetic interactions or unknown genes. The short DTH lines tended to have more alleles with a short DTH effect, whereas the long DTH lines contained more alleles with the opposite effect. Therefore, the extreme phenotypes

267 produced by transgressive segregation might be defined by allelic composition with
268 different phenotypic effects occurring in either direction.
269

270 **DISCUSSION**

271 Here, we showed that transgressive segregation occurred in the hybrid progenies of two
272 rice varieties, A58 and Kitaake, both of which have short DTH as an adaptation to
273 high-latitude region. Phenotypic variation beyond the parental range was observed in
274 this segregating population and facilitated uncovering of the genetic basis of
275 transgressive segregation and extreme DTH phenotypes. The two parental varieties
276 shared the same genotypes for three known major QTLs (*E1*/*Hd4*/*Ghd7*, *Hd2*/*OsPRR37*,
277 and *Hd5*/*DTH8*) (Figure 3), but different alleles for *Se1*/*Hd1* and several unknown
278 minor QTLs. Such different genotypes in minor QTLs produced new genetic
279 combinations that resulted in transgressive phenotypes of the progenies. QTLs direct
280 either positive or negative actions based on the effect of parental alleles. If negative
281 QTL alleles in either parent are replaced by the positive alleles of the other parent, the
282 progeny could obtain the desired phenotype because of the presence of more positive
283 alleles (DE VICENTE AND TANKSLEY 1993; RIESEBERG *et al.* 1999). Our results appeared
284 to demonstrate this scenario, because we observed allelic complementation at QTLs,

and indicate the importance of such "hidden" genetic variations despite close genetic relationships (HAGIWARA *et al.* 2006).

We employed SNP analysis with deep sequencing to obtain a sufficient number of markers for the closely related varieties. This was a powerful approach that detected more than 600 genome-wide SNPs between both Hokkaido-adapted varieties (Figure 4). In addition, such similar genetic backgrounds of the two varieties, A58 and Kitaake, facilitated identification of the minor QTLs that shape transgressive early heading by genome-wide SNP analysis.

Our analysis detected five SNP clusters that corresponded to QTLs and the *Hd1* locus, which contributed to DTH differences in the A58 × Kitaake progenies (Table 1 and 2). These QTLs were involved in both the additive and epistatic effects on extreme heading phenotypes (Table 2 and Figure 5). Among the SNP clusters, the strongest effect was explained by the Ch 4 cluster, in which the Kitaake-derived allele(s) caused decreased DTH (Table 2). The Ch 4 cluster was located from 29.8 to 32.4 Mb on Ch 4 (Figure 4), where only one gene, *Rice FLO-LFY homolog* (*RFL*) (KYOZUKA *et al.* 1998), is functionally characterized as a flowering-related gene by the QTL Annotation Rice

303 Online (Q-TARO) database (http://qtaro.abr.affrc.go.jp/). Similarly, the Ch 6 cluster
304 (from 24.5 to 25.5 Mb) included a gene for photoperiod sensitivity, *Se5* (IZAWA *et al.*
305 2000). However, no functional polymorphisms in A58 and Kitaake were detected in
306 either *RFL* or *Se5*. In the other QTLs found in the SNP clusters on Chs 1, 2, and 10, no
307 known DTH-related genes were identified. These results demonstrated that some
308 unknown genes present in these SNP clusters affected DTH of the Hokkaido varieties.
309 Interestingly, our analysis also showed possible epistatic interactions between genes in
310 SNP clusters on Chs 1 and 10 that shortened DTH (Figure 5). It was previously thought
311 that epistasis is unlikely to be a major cause of transgressive phenotypes (DE VICENTE
312 AND TANKSLEY 1993; RIESEBERG *et al.* 1999); however, in our study, epistatic
313 interactions explained the transgressive phenotypes observed in the segregating
314 populations (Figure 5).
315
316 To date, four genes (*E1*/*Hd4*/*Ghd7*, *Hd2*/*OsPRR37*, *Se1*/*Hd1*, and *Hd5*/*DTH8*) were
317 reported to control DTH in improved rice varieties in Hokkaido (ICHITANI *et al.* 1997;
318 FUJINO AND SEKIGUCHI 2005a; FUJINO AND SEKIGUCHI 2005b; NONOUE *et al.* 2008;
319 FUJINO *et al.* 2013; KOO *et al.* 2013). Among the four genes, loss of functional alleles in
320 *E1*/*Hd4*/*Ghd7* and *Hd2*/*OsPRR37* are necessary to obtain photoperiod insensitivity in

321 rice varieties in northern areas (FUJINO AND SEKIGUCHI 2005a; FUJINO AND SEKIGUCHI
322 2005b; XUE *et al.* 2008; KOO *et al.* 2013). Alternatively, the other two genes (*Se1*/*Hd1*
323 and *Hd5*/*DTH8*) have small effects on photoperiod insensitivity among the varieties in
324 Hokkaido (ICHITANI *et al.* 1997; FUJINO AND SEKIGUCHI 2005b; NONOUE *et al.* 2008). In
325 this study, *Se1*/*Hd1* sequences revealed differences between A58 and Kitaake; A58 has
326 insertion/deletion mutations, whereas Kitaake has the functional allele (Figure 3). Both
327 varieties had the same loss-of-function alleles in the *E1*/*Hd4*/*Ghd7* and *Hd2*/*OsPRR37*
328 loci and had the same functional allele in the *Hd5*/*DTH8* locus (Figure 3). These results
329 indicated that the improved varieties in Hokkaido might have inherited the same
330 *E1*/*Hd4*/*Ghd7*, *Hd2*/*OsPRR37*, and *Hd5*/*DTH8* alleles from a landrace similar to A58,
331 which facilitated adaptation, because photoperiod insensitivity was essential for
332 adaptation. In addition to these four loci, newly identified minor QTLs were identified
333 for DTH on Chs 1, 2, 4, 6 and 10 (Table 2 and Figure 4). These QTLs likely contribute
334 to extreme phenotypes of short and long DTH produced by transgressive segregation
335 based on the composition of their complementary alleles (Figure 6).
336
337 RIESEBERG *et al.* (1999) made several predictions regarding the cause of transgressive
338 segregation, one of which was consistent with our results: transgressive segregation

339 would likely be observed in the F2 population of parents with more proximal
340 phenotypes (Figure 7). Among the known alleles at the four known loci that are
341 necessary for DTH adaptation to Hokkaido, A58 and Kitaake shared the same alleles at
342 three loci, but not *Hd1*, which indicates that these two varieties possess a considerable
343 amount of common alleles that shorten DTH. Our results demonstrate that transgressive
344 segregation mainly occurred as a result of a few unknown QTLs, in which alleles
345 combined in a complementary manner (Figure 7).
346
347 According to RIESEBERG *et al.* (1999), transgressive segregation tends to occur more
348 frequently in intraspecific crosses, inbred populations, and domesticated populations
349 compared with interspecific crosses, outbred populations, and wild populations,
350 respectively. The lack of a strong positive correlation was observed between parental
351 genetic divergence and transgression frequency (RIESEBERG *et al.* 1999; RIESEBERG *et*
352 *al.* 2003). Our previous study (OTA *et al.* 2014) showed that the hybrid progenies of two
353 varieties with distal DTH adapted to different environments exhibited few instances of
354 transgressive DTH phenotypes (Figure 1). Because the parents were adapted to different
355 environments with different genetic backgrounds (e.g., interspecies), a number of the
356 new allelic combinations were generated in the hybrid progenies (Figure 7). Such

357 complex allelic combinations might generate positive and negative genetic interactions,
358 and offset allelic effects. Stochastically, if there is a large number of segregating loci,
359 individuals rarely accumulate only the alleles with positive effects, but most usually
360 contain alleles with negative effects (Figure 7).
361
362 This study showed that a few genes and their combinations expanded variation of the
363 DTH phenotype despite similar genetic backgrounds. Consequently, it might be useful
364 to identify QTLs or allelic interactions associated with the transgressive DTH
365 phenotypes in progenies of other varieties with proximal phenotypes. Similarly, to
366 integrate other transgressive phenotypes into breeding programs, alleles with additive
367 effects of minor QTLs should be targeted in varieties with proximal phenotypes.
368

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378

379 **FIGURE LEGENDS**

380 **Figure 1** Transgressive segregation and genetic relationships between parental varieties.
381 (A) Frequency distribution of DTH from A58 × Kitaake F2 plants. The transgressive
382 index represents the ratio of the of the F2 population DTH distribution to the parental
383 difference. The DTH difference between A58 and Kitaake was 0.7 days, and the DTH
384 range in the F2 population was 18.0 days, which resulted in a transgressive index of
385 25.7. Standard DTH values of 11 varieties in Hokkaido are indicated by gray
386 arrowheads: (a) Kitaibuki, (b) Hakucho-mochi, (c) Daichinohoshi (d) Hatsushizuku, (e)
387 Hoshinoyume, (f) Kuiku180, (g) Hokuiku-mochi, (h) Nanatsuboshi, (i) Kirara397, (j)
388 Hoshimaru, and (k) Gimpu. (B) Transgressive indexes of crosses between A58 and each
389 of five other varieties. The phylogenetic relationships and the associated dendrogram
390 for the five *O. sativa* varieties, Nipponbare (*japonica*), T65 (*japonica*), IR36 (*indica*),
391 #108 (*indica*), and Kasalath (*indica*, Aus), are presented based on information provided
392 in TAKATA *et al.* (2005). To calculate the transgressive index, DTH of parental varieties
393 and F2 plants were calculated based on data from OTA *et al.* (2014).

394

395 **Figure 2** Frequency distribution of DTH in early- and late-heading F4 lines derived
396 from the A58 × Kitaake cross in 2015 and 2016.

397 DTH of the 15 early- and 15 late-heading F4 lines selected in the F3 population was
398 examined in the two years, 2015 and 2016. Early- and late-heading lines are indicated
399 by white and black, respectively. Kitaake and A58 DTH are indicated by white and
400 black arrowheads, respectively, with bars indicating S. E.
401
402 **Figure 3** Comparisons of partial nucleotide sequences from Nipponbare, A58, and
403 Kitaake for the four major loci that affect DTH in Hokkaido.
404 The sequenced positions (based on Nipponbare) were selected using known
405 polymorphisms among varieties in Hokkaido that were observed in previous studies
406 (ICHITANI *et al.* 1997; FUJINO AND SEKIGUCHI 2005a; FUJINO AND SEKIGUCHI 2005b;
407 NONOUE *et al.* 2008; FUJINO *et al.* 2013; KOO *et al.* 2013). *Hd1* showed multiple
408 differences between A58 and Kitaake; Kitaake possesses a functional allele that is also
409 found in Nipponbare. In *DTH8*, a 19-bp segment (indicated by a rectangle) was deleted
410 in most of the other Hokkaido varieties, but not in Nipponbare, A58, and Kitaake, from
411 which we could not detect any polymorphisms. For *Ghd7* and *OsPRR37*, SNPs
412 observed in Nipponbare and the other two varieties are indicated by boxes.
413
414 **Figure 4** Chromosomal positions of five SNP clusters.

415 Physical map positions of each SNP detected by ddRAD-Seq are shown by horizontal
416 bars in each chromosome. Positions of SNP clusters that showed significant differences
417 in allele frequency between early- and late-heading populations are indicated by vertical
418 bars on the right side of each chromosome.
419
420 **Figure 5** Epistatic interaction between SNPs on chromosomes 1 and 10 on DTH
421 observed in 2015 and 2016.
422 Average DTH values for the four combinations of genotypes with central SNPs
423 (C1_2301 and C10_43613) in the clusters on Chs 1 and 10, which are indicated by
424 squares. The case of Chs 1 and 10 were selected from all the combinations with Chs 1, 4,
425 6 and 10 (Figure S4). When the A58 SNP on Ch 10 (black line) and Kitaake SNP on Ch
426 10 (gray line) were respectively coupled with the different parental SNPs, epistatic
427 (allelic) interactions occurred; in particular, the combination of the A58 allele on Ch 1
428 and Kitaake allele on Ch 10 resulted in the shortest DTH.
429
430 **Figure 6** Phenotypic relationships with combinations of six marker genotype values.
431 Among the 30 F4 lines, nine retained the homozygous alleles in the six loci that
432 corresponded to the SNP clusters with QTLs for DTH and *Hd1*. The effect of each locus

433 on DTH was weighted according to marker genotype values (see Materials and
434 Methods) based on DTH in 2016. Larger values indicate a stronger effect on DTH.
435 Empty squares indicate shorter DTH effects relative to black squares. Kitaake contained
436 four shorter DTH alleles in Ch 4, *Hd1,* Ch 10, and Ch 6, whereas A58 possessed two
437 shorter DTH alleles in Ch 2 and Ch 1. The two parental cultivars, Kitaake and A58, had
438 DTH of 74.1 and 75.1 days, respectively. DTH in the selected F4 lines ranged from 60.6
439 to 79.0 days. Each marker name indicates the central SNP in the cluster.
440
441 **Figure 7** Model of different segregation patterns that occurred in the F2 populations
442 derived from two parental combinations of proximal and distal DTH phenotypes.
443 The left panel represents the segregation pattern of the F2 population between parent-a
444 and -b with proximal DTH phenotypes due to the similar genotypes with a few
445 differences. Because of differences in a few alleles with minor effects on DTH, the F2
446 progenies produced transgressive phenotypes. The right panel represents the F2
447 population produced by parents with distal phenotypes and opposite genotypes shows
448 intermediate segregation between both parents. Most of the F2 progenies with mixed
449 genotypes of the parental alleles did not have DTH phenotypes that exceeded those of
450 the parental phenotypes. There are seven loci involved in DTH, and their effects on

451 DTH are ordered as 1 >>> 7. S and L indicate the effect of an allele at each locus that
452 makes DTH shorter or longer, respectively.
453
454 **Figure S1** Experimental scheme of this study. ddRAD-Seq was carried out to detect
455 differences in allele frequency of genome-wide SNPs between early- and late-heading
456 F4 lines.
457
458 **Figure S2** Frequency distribution of DTH in the F3 population derived from the A58 ×
459 Kitaake cross.
460
461 **Figure S3** Effect of the Ch 4 SNP cluster on DTH in 2015 and 2016.
462 Average DTH values of F4 lines with homozygous A58- and Kitaake-type alleles of the
463 SNP cluster on Ch 4, which is represented by the central SNP in the cluster, C4_19396.
464 Significant differences ($P < 0.01$) between average DTH values were observed in both
465 2015 and 2016.
466
467 **Figure S4** Genetic interactions between SNP clusters on Chs 1, 4, 6, and 10 relative to
468 DTH in 2015.

469 Average DTH values (y-axis) of F4 lines with each genotype are shown in squares. Six
470 combinations of any two loci among the SNP clusters on Chs 1, 4, 6 and 10 are depicted.
471 A58-type alleles (black line) and Kitaake-type alleles (gray line) are paired with another
472 locus corresponding to A58-type alleles (the left side) or Kitaake-type alleles (the right
473 side) in x-axis, respectively. An allelic interaction (non-additive interaction) was
474 detectable where the black line crossed the gray line.
475
476
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577

Table 1 The effect of Hd1 locus on days to heading in F2 population derived from A58 x Kitaake cross

Marker			No. of plants			Average DTH			
name	Chr.	Position	A58-type	Heterozygous	Kitaake-type	A58-type	Heterozygous	Kitaake-type	*P*
Hd1	6		56	103	73	81.3	79.5	78.8	4×10^{-4}

Table 2 The effect of SNP clusters on days to heading in F4 lines derived from the cross between A58 and Kitaake

			DTH (2015)				DTH (2016)			
Marker name	Chr.	Position	A58-type	Kitaake-type	*P*		A58-type	Kitaake-type	*P*	
C1_2292	1	25,250,821	68.5±2.02	73.6±1.78	0.07856		75.9±1.72	79.3±1.72	0.1745	
C1_2301	1	25,270,205	68.5±2.02	73.6±1.78	0.07856		75.9±1.72	79.3±1.72	0.1745	
C1_2745	1	28,608,975	68.1±1.85	73.4±1.54	0.03627	*	75.3±1.59	79.0±1.52	0.1095	
C1_2968	1	30,128,355	66.9±1.96	72.7±1.66	0.03616	*	74.1±1.94	78.1±1.46	0.1195	
C2_10782	2	790,734	68.1±1.83	71.8±1.59	0.139		74.8±1.65	78.1±1.47	0.1461	
C2_10784	2	790,866	68.1±1.83	71.8±1.59	0.139		74.8±1.65	78.1±1.47	0.1461	
C4_19210	4	29,823,605	74.8±1.14	66.6±1.61	0.0003583	***	81.9±0.65	73.7±1.34	0.000012	***
C4_19304	4	30,511,751	76.3±1.03	66.1±1.61	0.0000237	***	82.1±0.72	73.2±1.43	0.0000162	***
C4_19390	4	31,264,195	76.9±1.08	65.1±1.39	1.13E-06	***	82.5±0.74	72.5±1.3	1.07E-06	***
C4_19586	4	32,438,249	75.5±1.21	65.7±1.43	0.0000955	***	81.6±0.68	72.9±1.31	9.34E-06	***
C6_27238	6	24,515,347	66.5±1.72	70.3±1.91	0.2326		73.5±1.78	76.6±1.62	0.1031	
C6_27270	6	24,574,372	71.9±1.64	65.7±2.03	0.02257	*	77.2±1.78	73.8±1.51	0.1675	
C6_27338	6	24,978,329	71.1±1.78	66.6±2.16	0.09858		76.5±1.67	75.1±1.92	0.5787	
C6_27340	6	24,985,031	71.3±1.57	65.2±2.24	0.04015	*	76.9±1.53	73.3±1.74	0.178	
C6_27365	6	25,230,125	71.7±1.67	66.0±2.21	0.0532		77.6±1.59	74.5±1.67	0.1907	
C6_27415	6	25,515,786	71.5±1.67	66.9±2.03	0.1137		77.3±1.59	75.1±1.51	0.3546	
C10_43613	10	593,246	71.9±1.68	64.4±1.93	0.008726	***	77.3±1.42	72.1±1.9	0.04205	*
C10_43829	10	709,525	72.5±1.4	65.7±2.28	0.022	*	78.1±1.22	73.3±2.42	0.09918	
C10_43830	10	709,622	72.8±1.47	65.7±2.06	0.01105	*	78.2±1.3	73.5±2.2	0.08329	

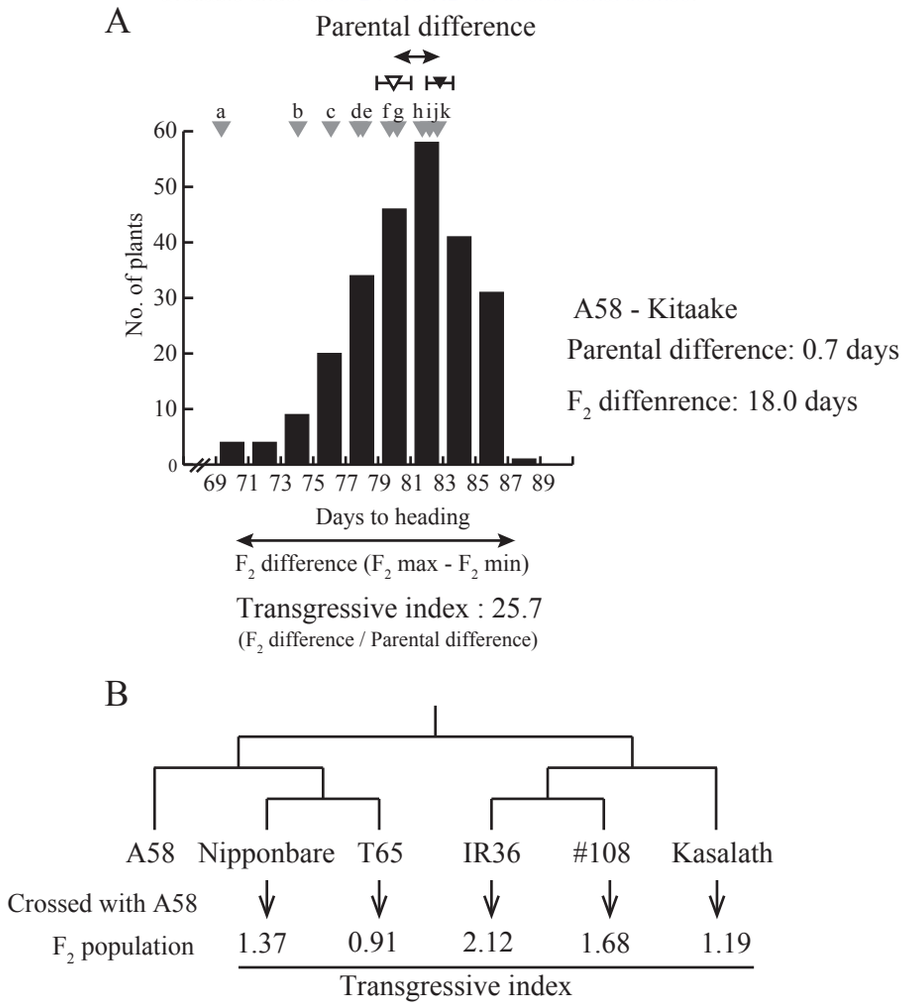


Figure 1 Transgressive segregation and genetic relationships between parental varieties. (A) Frequency distribution of DTH from A58 × Kitaake F2 plants. The transgressive index represents the ratio of the of the F2 population DTH distribution to the parental difference. The DTH difference between A58 and Kitaake was 0.7 days, and the DTH range in the F2 population was 18.0 days, which resulted in a transgressive index of 25.7. Standard DTH values of 11 varieties in Hokkaido are indicated by gray arrowheads: (a) Kitaibuki, (b) Hakucho-mochi, (c) Daichinohoshi (d) Hatsushizuku, (e) Hoshinoyume, (f) Kuiku180, (g) Hokuiku-mochi, (h) Nanatsuboshi, (i) Kirara397, (j) Hoshimaru, and (k) Gimpu. (B) Transgressive indexes of crosses between A58 and each of five other varieties. The phylogenetic relationships and the associated dendrogram for the five O. sativa varieties, Nipponbare (japonica), T65 (japonica), IR36 (indica), #108 (indica), and Kasalath (indica, Aus), are presented based on information provided in TAKATA et al. (2005). To calculate the transgressive index, DTH of parental varieties and F2 plants were calculated based on data from OTA et al. (2014).

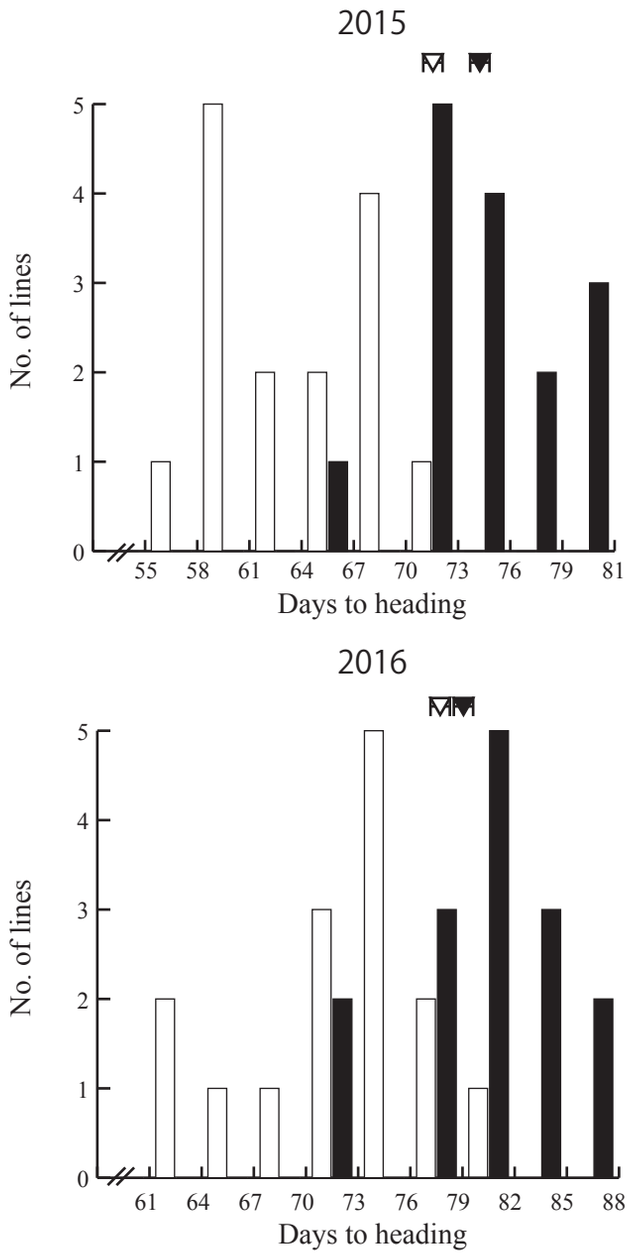


Figure 2 Frequency distribution of DTH in early- and late-heading F4 lines derived from the A58 × Kitaake cross in 2015 and 2016.

DTH of the 15 early- and 15 late-heading F4 lines selected in the F3 population was examined in the two years, 2015 and 2016. Early- and late-heading lines are indicated by white and black, respectively. Kitaake and A58 DTH are indicated by white and black arrowheads, respectively, with bars indicating S. E.

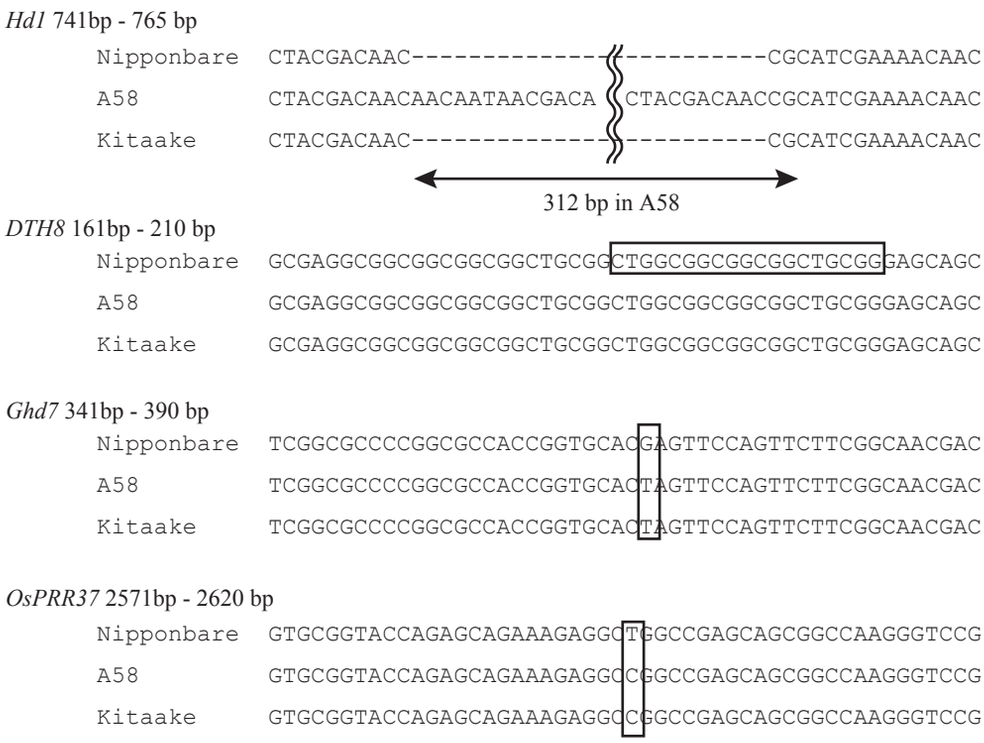


Figure 3 Comparisons of partial nucleotide sequences from Nipponbare, A58, and Kitaake for the four major loci that affect DTH in Hokkaido.

The sequenced positions (based on Nipponbare) were selected using known polymorphisms among varieties in Hokkaido that were observed in previous studies (ICHITANI et al. 1997; FUJINO AND SEKIGUCHI 2005a; FUJINO AND SEKIGUCHI 2005b; NONOUE et al. 2008; FUJINO et al. 2013; KOO et al. 2013). Hd1 showed multiple differences between A58 and Kitaake; Kitaake possesses a functional allele that is also found in Nipponbare. In DTH8, a 19-bp segment (indicated by a rectangle) was deleted in most of the other Hokkaido varieties, but not in Nipponbare, A58, and Kitaake, from which we could not detect any polymorphisms. For Ghd7 and OsPRR37, SNPs observed in Nipponbare and the other two varieties are indicated by boxes.

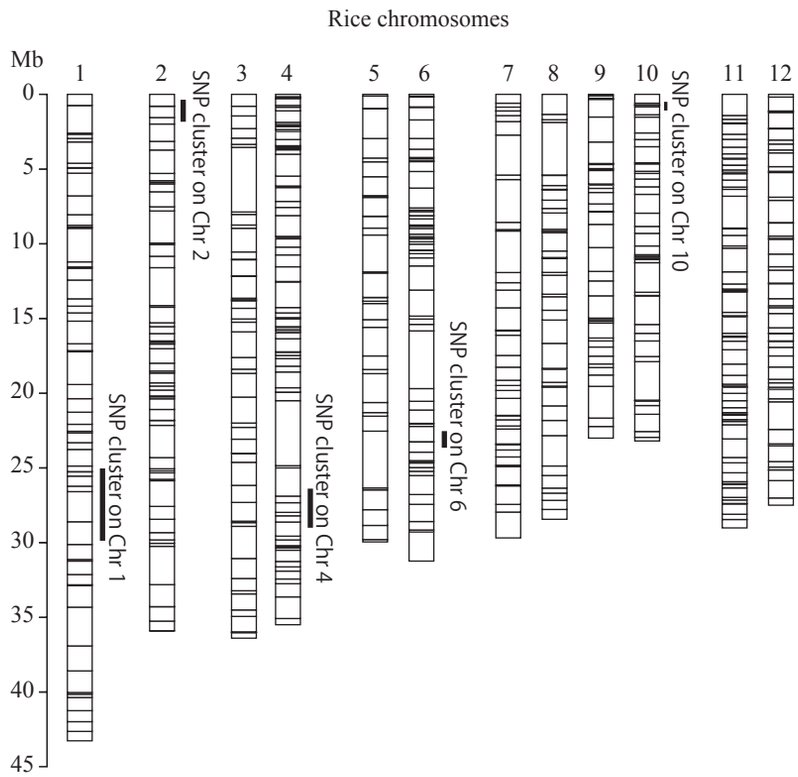


Figure 4 Chromosomal positions of five SNP clusters.

Physical map positions of each SNP detected by ddRAD-Seq are shown by horizontal bars in each chromosome. Positions of SNP clusters that showed significant differences in allele frequency between early- and late-heading populations are indicated by vertical bars on the right side of each chromosome.

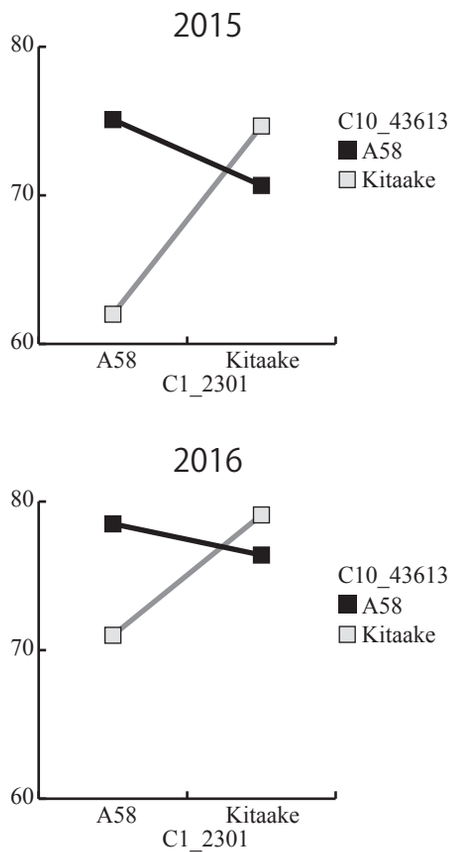


Figure 5 Epistatic interaction between SNPs on chromosomes 1 and 10 on DTH observed in 2015 and 2016.

Average DTH values for the four combinations of genotypes with central SNPs (C1_2301 and C10_43613) in the clusters on Chs 1 and 10, which are indicated by squares. The case of Chs 1 and 10 were selected from all the combinations with Chs 1, 4, 6 and 10 (Figure S5). When the A58 SNP on Ch 10 (black line) and Kitaake SNP on Ch 10 (gray line) were respectively coupled with the different parental SNPs, epistatic (allelic) interactions occurred; in particular, the combination of the A58 allele on Ch 1 and Kitaake allele on Ch 10 resulted in the shortest DTH.

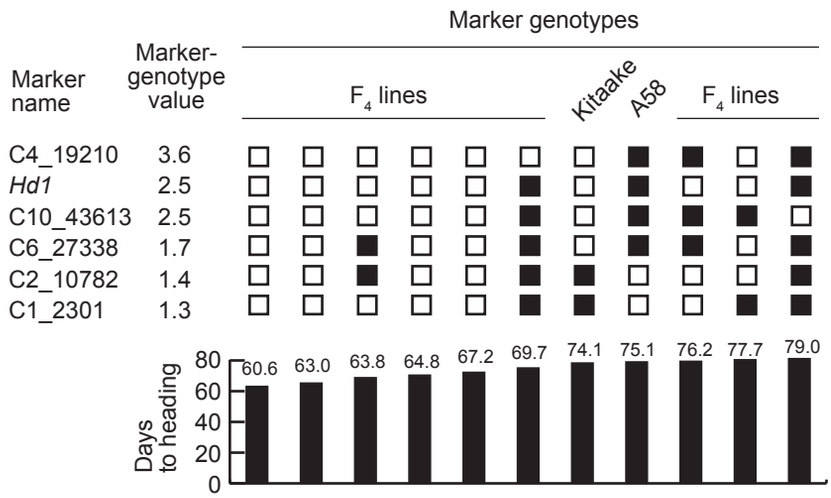
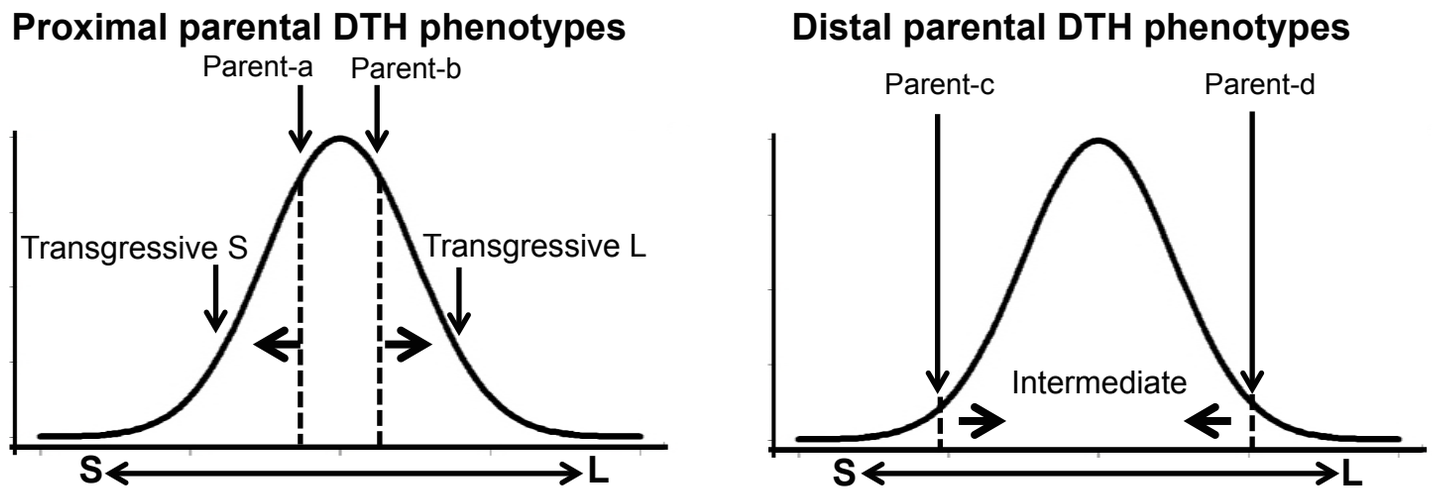


Figure 6 Phenotypic relationships with combinations of six marker genotype values.

Among the 30 F4 lines, nine retained the homozygous alleles in the six loci that corresponded to the SNP clusters with QTLs for DTH and Hd1. The effect of each locus on DTH was weighted according to marker genotype values (see Materials and Methods) based on DTH in 2016. Larger values indicate a stronger effect on DTH. Empty squares indicate shorter DTH effects relative to black squares. Kitaake contained four shorter DTH alleles in Ch 4, Hd1, Ch 10, and Ch 6, whereas A58 possessed two shorter DTH alleles in Ch 2 and Ch 1. The two parental cultivars, Kitaake and A58, had DTH of 74.1 and 75.1 days, respectively. DTH in the selected F4 lines ranged from 60.6 to 79.0 days. Each marker name indicates the central SNP in the cluster.



QTLs	1	2	3	4	5	6	7
Parent-a	S	S	S	S	S	L	L
Parent-b	S	S	S	S	L	S	S
Transgressive short DTH							
Line-A	S	S	S	S	S	S	S
Line-B	S	S	S	S	S	S	L
Line-C	S	S	S	S	S	L	S
Transgressive long DTH							
Line-D	S	S	S	S	L	S	L
Line-E	S	S	S	S	L	L	S
Line-F	S	S	S	S	L	L	L

QTLs	1	2	3	4	5	6	7
Parent-c	S	S	S	S	S	S	S
Parent-d	L	L	L	L	L	L	L
Intermediate DTH							
Line-G	S	S	S	S	S	S	L
Line-H	S	S	S	S	S	L	L
Line-I	S	S	S	S	L	L	L
Line-J	S	S	S	L	L	L	L
▼							
▼							
▼							
Line-Z	L	L	L	L	L	L	S

Effect of QTL 1>2>3>4>5>6>7

Figure 7 Model of different segregation patterns that occurred in the F2 populations derived from two parental combinations of proximal and distal DTH phenotypes.

The left panel represents the segregation pattern of the F2 population between parent-a and -b with proximal DTH phenotypes due to the similar genotypes with a few differences. Because of differences in a few alleles with minor effects on DTH, the F2 progenies produced transgressive phenotypes. The right panel represents the F2 population produced by parents with distal phenotypes and opposite genotypes shows intermediate segregation between both parents. Most of the F2 progenies with mixed genotypes of the parental alleles did not have DTH phenotypes that exceeded those of the parental phenotypes. There are seven loci involved in DTH, and their effects on DTH are ordered as 1 >>> 7. S and L indicate the effect of an allele at each locus that makes DTH shorter or longer, respectively.