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REVIEW

Developing Isogenic Lines of Japanese Rice Cultivar 'Koshihikari' with Early and Late Heading

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Abstract

Four quantitative trait loci (QTLs) for rice (*Oryza sativa* L.) heading date were previously detected by QTL analysis of progeny derived from crosses between a *japonica* cultivar, Koshihikari, and an *indica* cultivar, Kasalath. To enhance the cropping potential of Koshihikari, a leading cultivar in Japan, our team used marker-assisted selection to develop isogenic lines (ILs) with early and late heading dates. Several types of DNA marker were used to minimize the length of substituted chromosome segments containing target QTLs and to determine the genotype in the target QTL regions and in the background genome of the ILs. We developed four new ILs—Koshihikari Kanto HD1 (*Hd1*), Wakei 370 (*Hd4*), Kanto IL5 (*Hd6*), and Kanto HD2 (*Hd5*)—housing Kasalath chromosome segments of 560, 7960, 170, and 625 kb, respectively. The heading date of Koshihikari Kanto HD1 was 12 days earlier than that of Koshihikari in Ibaraki, and those of Wakei 370, Kanto IL5, and Kanto HD2 were 3, 10, and 11 days later, respectively. Most of the traits, except the heading date of the four ILs, were the same as those of Koshihikari.

Discipline: Plant breeding
Additional key words: heading date, marker-assisted selection, pleiotropic effect, quantitative trait locus

Introduction

The *japonica* rice cultivar Koshihikari is cultivated in western and central Japan. In 2008, its area of cultivation covered 604,000 ha, accounting for 36.4% of the total rice cultivation area in Japan[28]. Koshihikari is characterized by its good eating quality and its cool-temperature tolerance at the booting stage. In the market, it enjoys security of supply, brand recognition by consumers, and advantageous high prices because of the brand. Koshihikari has been a leading cultivar for the last three decades. However, because of its restricted heading and maturity dates, its commercial cultivation is limited to western and central Japan. Modification of its heading date to both earlier and later dates is one of the breeding objectives to enhance its cropping potential. The development of isogenic lines (ILs) of Koshihikari with early and late heading dates would enable the expansion of its cultivation area.

Genetic analysis of heading date

The heading date of rice is a complex quantitative trait that is controlled by several genes and environmental signals, including photoperiod. Many quantitative trait loci (QTLs) for the photoperiod response of heading date have been identified[7,12,13,16,17,19,30,31,35,37]. In particular, 15 QTLs for heading date (*Hd1–Hd3a*, *Hd3b–Hd14*) have been identified in populations derived from crosses between a *japonica* cultivar, Nipponbare, and an *indica* cultivar, Kasalath (Fig. 1)[37]. Ten of these QTLs (*Hd1–Hd3a*, *Hd3b–Hd9*) have been mapped as single Mendelian factors[14,15,20,24,32,33]. Furthermore, five QTLs (*Hd1*, *Hd3a*, *Hd5*, *Hd6*, and *Ehd1*) have been isolated by map-based cloning[2,10,23,36] (Yamanouchi et al., unpublished data). *Hd1*, on the short arm of chromosome 6, promotes heading under short-day conditions and inhibits it under long-day conditions[36]. *Hd5*, on the short arm of chromosome 8, and *Hd6*, on the long arm of chromosome 3, both inhibit heading under long-day conditions[23] (Yamanouchi et al., unpublished data). *Hd3a*, on the short arm of chromosome 6, promotes heading under short-day conditions[10]. *Se5*, on the long arm of chromosome

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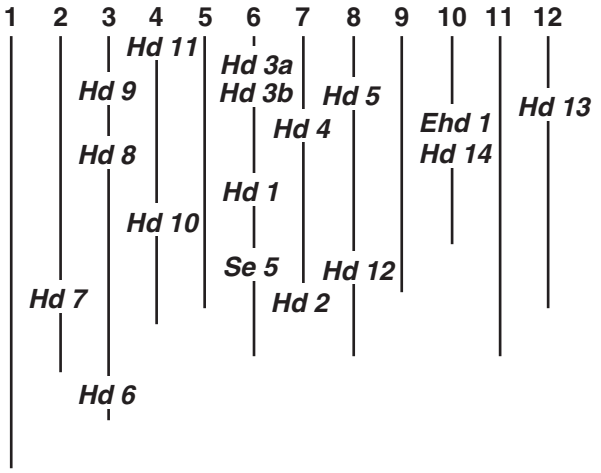

Fig. 1. Chromosomal location of QTLs for heading date
The vertical bars represent the chromosomes, numbered at the top. *Hd1*–*Hd14*, identified in Kasalath; *Se5*, in Norin 8 mutants; *Ehd1*, in *O. glaberrima*[2,8,38].

6, has been cloned by analysis of *japonica* Norin 8 mutant lines[8]. Loss of function of *Se5* causes early heading and complete loss of photoperiodic response. *Ehd1*, on chromosome 10, has been isolated by analysis using a backcross population between *japonica* Taichung 65 and African rice (*Oryza glaberrima* Steud. acc. no. IRGC104038)[2]. The *O. glaberrima* allele of *Ehd1* confers early heading under short-day conditions. These studies have improved our understanding of the genetic control mechanisms underlying the photoperiodic response of heading date in rice.

Development of Koshihikari ILs with early and late heading

1. Development of ILs

Three QTLs—*qDTH3*, *qDTH6*, and *qDTH8*—were identified on chromosomes 3, 6, and 8, respectively, in one population derived from crosses between Koshihikari and

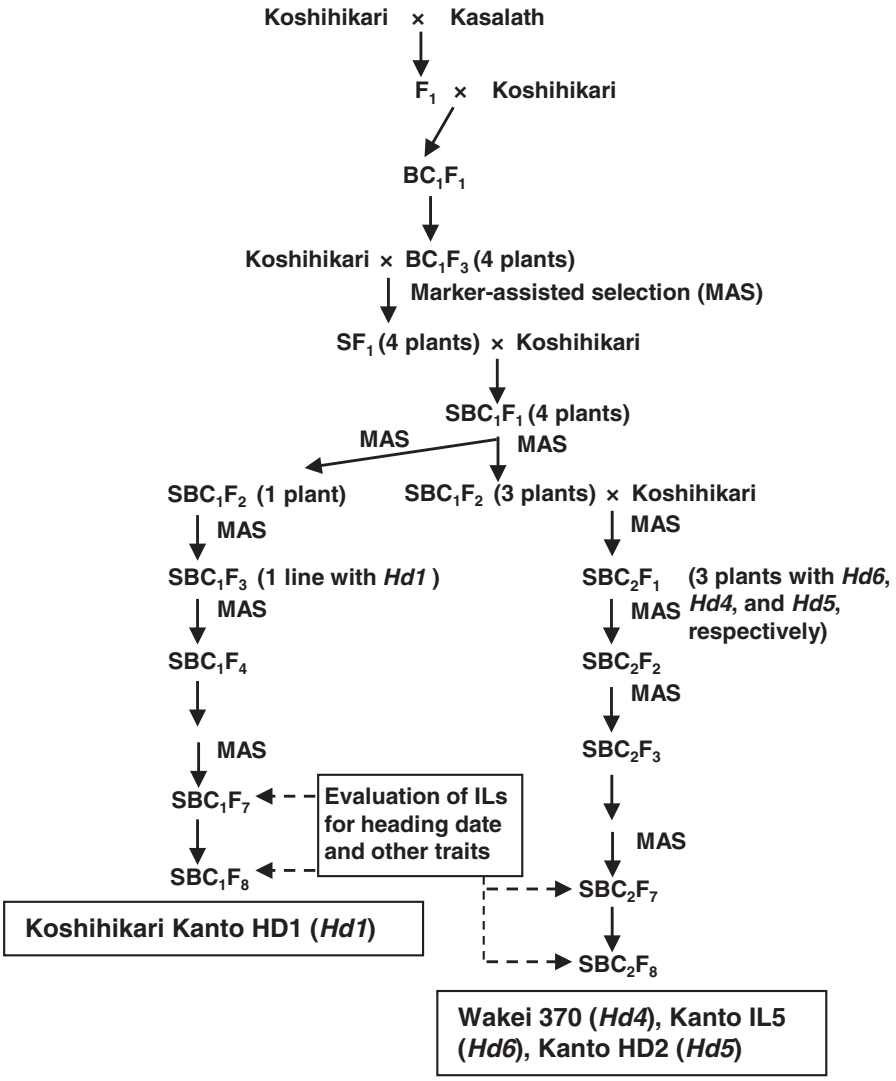

Fig. 2. Scheme for the development of isogenic lines of Koshihikari

Kasalath (Fig. 3A)[34]. An additional putative QTL, tentatively designated *qDTH7*, was identified on chromosome 7 through the use of chromosome segment substitution lines[3]. Their chromosomal locations suggest that the QTLs correspond with *Hd6*, *Hd1*, *Hd5*, and *Hd4*, respectively, which have been detected in populations derived from crosses between Nipponbare and Kasalath[3,34]. Fine-mapping and map-based cloning studies have provided information about the precise position of target QTLs and their tightly linked DNA markers, allowing us to embark on developing ILs of Koshihikari with modified heading dates[15,23,36] (Yamanouchi et al., unpublished data).

The scheme for developing ILs for heading date is shown in Fig. 2. Koshihikari was crossed with Kasalath, and the resulting F_1 plants were backcrossed to Koshihikari to obtain BC_1F_1 plants. These plants were self-pollinated to develop 187 BC_1F_3 lines by the single-seed-descent method[34]. To develop ILs for each QTL, we selected four suitable BC_1F_3 lines in which a recombination had occurred on one side of the target QTL according to genotypes of 116 restriction fragment length polymorphisms (RFLPs). We successively backcrossed these plants with Koshihikari and used marker-assisted selection (MAS) to select desirable plants in which recurrent chromosome segments could be recovered as Koshihikari homozygous (by negative selection) and the chromosomal region including the target QTL was maintained as heterozygous (by positive selection). Four resulting secondary backcross progeny (SBC_1F_1) were self-pollinated to obtain four SBC_1F_2 plants. MAS was conducted to select appropriate plants for further development of ILs. Self-pollination of one selected SBC_1F_2 plant resulted in Koshihikari Kanto HD1 (*Hd1*). A few chromosomal segments of Kasalath homozygous remained in the non-target regions of the other three SBC_1F_2 plants. We additionally backcrossed the three SBC_1F_2 plants with Koshihikari to recover as Koshihikari homozygous in the non-target chromosomal regions. The additional backcrossing of the other three SBC_1F_2 plants with Koshihikari produced three SBC_2F_1 lines. We selected plants in which recombination occurred in the flanking region of the target QTLs (other side to first recombination). One SBC_1F_2 (with *Hd1*) and three SBC_2F_2 (with *Hd4*, *Hd5*, and *Hd6*) plants with a minimum chromosomal segment containing each target QTL were selected from one SBC_1F_2 (175 SBC_1F_2 plants segregating *Hd1* region) and three SBC_2F_2 (100 SBC_2F_2 plants segregating *Hd4* region, 245 SBC_2F_2 plants segregating *Hd5*, and 100 SBC_2F_2 plants segregating *Hd6* region, respectively) populations, respectively. Additional self-pollinations were required to select ILs. We developed four ILs: Koshihikari Kanto HD1 (*Hd1*), Wakei 370 (*Hd4*), Kanto HD2 (*Hd5*), and Kanto IL5 (*Hd6*).

2. Genotype survey of ILs selected

Through the use of 28 PCR-based DNA markers, comprising sequence-tagged site (STS), cleaved amplified polymorphic sequence (CAPS), derived CAPS (dCAPS), and simple sequence repeat (SSR) markers[25], recombination points were precisely determined. Recombination occurred between markers C10915 and Y4836L and between P0456CT1 and P0456GC1 in Koshihikari Kanto HD1 (Fig. 3B); between E4071 and R46, and between RM6449 and RM5481-1 in Wakei 370 (Fig. 3C); between OS0040CT1 and RM416, and between cnt13I and G1015 in Kanto IL5 (Fig. 3D); and between RM8266 and OS1590CCT1, and between R902 and RM1111 in Kanto HD2 (Fig. 3E). If we assume that recombination occurred in the middle of each marker interval, then the substituted genomic fragments measured approximately 560 kb in Koshihikari Kanto HD1, 7960 kb in Wakei 370, 170 kb in Kanto IL5, and 625 kb in Kanto HD2.

To verify the genotype of the background genome in each candidate IL, we used 116 RFLP markers[5,11]. We detected no introgression of chromosome segments from Kasalath at the marker points.

3. Evaluation of ILs for heading date and other traits with agronomic value

The four ILs were grown in a paddy field at the National Agricultural Research Center, Yawara, Ibaraki, Japan (36°N), in 2003 and 2004 (Fig. 4). They were sown on 18 April, 2003, and 22 April, 2004, and transplanted on 14 and 18 May, respectively. The mean heading date of Koshihikari was 6 August (Table 1). The heading date of Koshihikari Kanto HD1 was 24 July, 12 days earlier than that of Koshihikari; the heading date of Kanto IL5 was 15 August, 10 days later; the heading date of Kanto HD2 was 16 August, 11 days later. The heading date of Wakei 370 was also 8 August, 2 days later than that of Koshihikari, although there was no significant difference to the heading date of Koshihikari. Most of the morphological traits of the four ILs, including ripening period, panicle number, 1000-grain weight, grain quality, and lodging degree were the same as those of Koshihikari (Table 1). However, the culm length and panicle length of Koshihikari Kanto HD1 were shorter, which could result in a slight reduction in brown rice yield. The culm length and panicle length of the other three lines were almost the same as those of Koshihikari (Table 1).

The eating quality of Koshihikari Kanto HD1 was inferior to that of Koshihikari in Ibaraki in 2003, but not in 2004 (Table 1). No clear difference in the other three ILs was found. The eating quality is largely affected by environmental conditions, in particular temperature during ripening[18]. In Ibaraki, the average temperature of the ripening period was notably cooler in 2003 than in 2004, and the

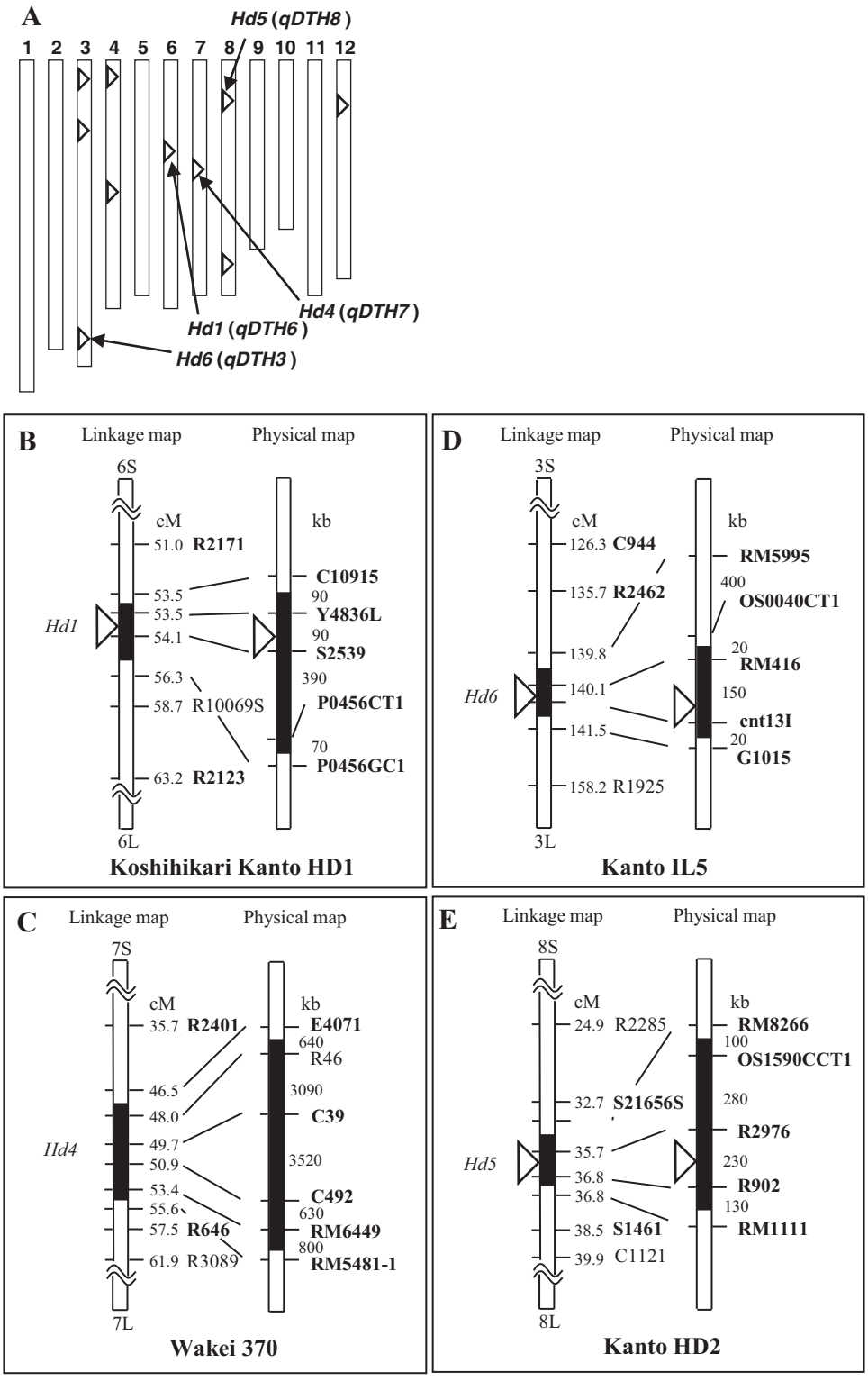

Fig. 3. Graphical representation of the genotypes of Koshihikari Kanto HD1, Wakei 370, Kanto IL5, and Kanto HD2

A: QTLs for heading date detected in previous studies[3,34] are indicated by triangles. B–E: Graphical representations of each genotype. White blocks, chromosomal regions derived from Koshihikari; black blocks, Kasalath. RFLP, STS, CAPS, dCAPS, and SSR markers are shown on the right. PCR-based STS, CAPS, dCAPS, and SSR markers are indicated in bold. The positions of markers are derived from a high-density RFLP linkage map[5].

temperature was abnormally low from the end of July to the middle of August 2003. This low temperature might have caused the lower eating quality of Koshihikari Kanto HD1.

Koshihikari Kanto HD1 was grown in a paddy field at Miyazaki Agricultural Research Institute, Sadowara, Miyazaki, Japan (32°N), in 2003; it was sown on 21

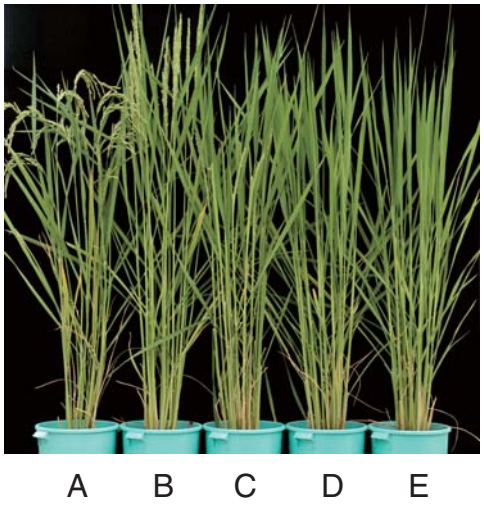

Fig. 4. Four ILs and their isogenic control, Koshihikari
A: Koshihikari Kanto HD1 (*Hd1*); B: Koshihikari; C: Wakei 370 (*Hd4*); D: Kanto IL5 (*Hd6*); E: Kanto HD2 (*Hd5*).

February and transplanted on 25 March. The heading date of Koshihikari was 18 June, and that of Koshihikari Kanto HD1 was 17 June, only 1 day earlier (Table 1). All other traits, including culm length, panicle length, panicle number, 1000-grain weight, grain quality, degree of lodging, yield of brown rice, and eating quality, were also the same or nearly the same as those of Koshihikari.

The phenotypic performances of the three ILs, Wakei 370, Kanto IL5, and Kanto HD2, were almost the same as those of Koshihikari except heading date. However, the culm length and panicle length of Koshihikari Kanto HD1 in Ibaraki were also modified (Tables 1). A shorter culm length and panicle length may improve lodging resistance, but may reduce yield potential. We propose that the Kasalath *Hd1* allele has pleiotropic effects on culm length and panicle length. Kawai and Sato[9] reported that most early-heading mutant strains had short culms. Li et al.[12] and Yu et al.[38] found a significant positive correlation between heading date and plant height. Using progeny of the same cross-combination as used in this study, Yamamoto et al.[34] identified a QTL controlling internode length in the same region as *Hd1*. In addition, in our trial in Miyazaki, where the heading date of Koshihikari Kanto HD1 was similar to that of Koshihikari, culm length, panicle length, and yield were also nearly the same as those of Koshihikari. Thus, it is likely that the *Hd1* gene has pleiotropic effects on culm length and panicle length.

Field resistance to leaf blast was assessed in an upland field at the National Agricultural Research Center, Tsukuba, Ibaraki, in 2003 and 2004. In all four ILs it was almost the same as that of Koshihikari (data not shown). Therefore, the ILs appears to possess no major gene for resistance to leaf blast.

Cool-temperature tolerance of ILs at the booting stage was also tested in running cold-water paddy fields at the Miyagi Prefectural Furukawa Agricultural Experiment Station, Furukawa, Miyagi, Japan (38°N), and the Nagano Agricultural Experiment Station Haramura Branch, Suwa, Nagano, Japan (35°N), in 2003. The cool-temperature tolerance of Koshihikari Kanto HD1 at the booting stage was also tested in a glass house at the Miyazaki Agricultural Research Institute, Sadowara, Miyazaki, Japan (32°N), in 2005. The ILs and five reference cultivars—Chubo 35 (highly tolerant), Koshihikari (highly tolerant), Akitakomachi (tolerant), Hourei (tolerant), and Mutsunishiki (moderately tolerant)—were irrigated with cool water (about 19°C). The degree of cool-temperature tolerance at booting was measured as floret sterility at maturity. The cool-temperature tolerance of the three ILs, Wakei 370, Kanto IL5, and Kanto HD2, was similar to that of Hourei or Koshihikari (Table 2). The tolerance of Koshihikari Kanto HD1 was similar to that of Mutsunishiki and inferior to that of Chubo 35 in Miyagi and Nagano. On the other hand, the cool-temperature tolerance of Koshihikari Kanto HD1 at the booting stage was similar to that of Akitakomachi in Miyazaki in 2005. In general, the degree of cool-temperature tolerance of particular cultivars or lines should be evaluated by comparison with reference cultivars with the same heading date. In Miyagi and Nagano, where heading dates were different, we could not directly compare the cool-temperature tolerance of Koshihikari with that of early-heading Koshihikari Kanto HD1, which was inferior. On the other hand, in Miyazaki, where heading date was the same, the cool-temperature tolerance of Koshihikari Kanto HD1 was either the same as that of Koshihikari or only slightly less than that of Koshihikari. These results suggest that the cool-temperature tolerance of Koshihikari Kanto HD1 may be the same as that of Koshihikari (pleiotropic effect). However, it is difficult to exclude two other possibilities: (1) the effect of another gene for cool-temperature tolerance linked to *Hd1* (linkage drag), and (2) the effect of other genes in the background genome. (1) Even though we tried to minimize the length of the introgressed chromosome segment from the donor parent in the target QTL region, the 560-kb region in Koshihikari Kanto HD1 contains about 40 genes[4]. Thus, it is difficult to rule out the possibility of two tightly linked genes. (2) A graphical genotype of each IL determined by using 116 RFLP markers covering the 12 rice chromosomes showed that only one heading QTL region was substituted from Kasalath in the genetic background of Koshihikari. However, it is difficult to exclude the possibility that tiny chromosome segments of Kasalath were substituted in non-target regions. Further analysis, such as QTL analysis and further fine mapping using progeny of a cross between Koshihikari Kanto HD1 and Koshihikari, and mutant lines

Table 1. Agronomic characteristics of Koshihikari Kanto HD1, Wakei 370, Kanto IL5, Kanto HD2, and their isogenic control, Koshihikari

Line	Year	Heading date (m.d)	Days-to-heading (days)	Maturing date (m.d)	Ripening period (days)	Culm length (cm)	Panicle length (cm)	No. of panicles (No./m^2)	Yield of brown rice (kg /a)	1,000-grain weight (g)	Grain quality[1)]	Lodging degree[2)]	Eating quality[3)]
National Institute of Crop Science, Ibaraki, Japan[4)]													
Koshihikari Kanto HD1	2003	7.28	101 **	8.31	34	74.4 **	16.7	348	47.8	20.6	4.0	1.0	-1.26 **
	2004	7.20	89 **	8.25	36	88.8 **	17.0 **	484	60.4 **	21.6	5.7	9.0	-0.09
	Average	7.24	95	8.28	35	81.6	16.8	416	54.1	21.1	4.9	5.0	-0.68
Wakei 370	2003	8.12	116	9.23	42	87.0	18.1	350	52.4	21.0	4.0	2.0	-0.17
	2004	8.04	104	9.15	42	95.4	19.7	426	62.3	21.4 **	6.0	9.0	0.48 **
	Average	8.08	110	9.19	42	91.2	18.9	388	57.3	21.2	5.0	5.5	0.16
Kanto IL5	2003	8.18	122 **	9.29	43	93.9	18.4	349	54.0 **	21.0 **	4.4	2.5	0.09
	2004	8.12	112 **	9.23	43	99.9	19.5	430	60.7	20.5	6.1 **	8.0	0.30
	Average	8.15	117	9.26	43	96.9	18.9	389	57.3	20.8	5.3	5.3	0.20
Kanto HD2	2003	8.21	125 **	10.02	42	89.4	18.6	367 **	54.1	20.8 **	3.6	3.0	0.04
	2004	8.11	111 **	9.22	42	102.0	18.9	432	60.8	21.4	5.3	9.0	0.22
	Average	8.16	118	9.27	42	95.7	18.8	399	57.4	21.1	4.5	6.0	0.13
Koshihikari	2003	8.10	114	9.18	40	88.7	18.5	328	48.8	20.1	4.1	2.0	-0.17
	2004	8.01	101	9.09	39	100.8	18.7	498	64.3	21.0	4.9	9.0	0.09
	Average	8.06	107	9.14	40	94.8	18.6	413	56.6	20.6	4.5	5.5	-0.04
Akitakomachi	2003	7.31	104 **	9.07	38	78.5 **	17.5	367 **	47.3	20.0	3.3 **	0.0 **	-0.91 **
	2004	7.25	94	9.08	46	90.3	19.2	408 **	59.6	21.4	4.3	8.0	0.09
	Average	7.28	99	9.08	42	84.2	18.4	388	53.5	20.7	3.8	4.0	-0.41
Asanohikari	2003	8.13	117	9.20	39	71.8 **	18.7	367 **	44.6	21.2 **	3.0 **	0.0 **	-1.48 **
Nipponbare	2004	8.12	112 **	9.22	41	82.1	17.8	420	59.0	22.4	3.7	0.0 **	-0.35
Miyazaki Agricultural Research Institute, Miyazaki, Japan[5)]													
Koshihikari Kanto HD1	2003	6.17	116	7.22	35	76.2	15.7	442	46.6	21.6	3.7	0.0	0.17
Koshihikari	2003	6.18	117	7.23	35	74.7	16.2	463	46.2	20.6	4.0	0.0	0.00

[1)] Grain quality was estimated in comparison with ordinary rice varieties and classified into nine grades (1: excellent to 9: especially bad) based on appearance.
[2)] Lodging degree was classified into ten degrees (0: standing to 9: lodged).
[3)] Eating quality shows aggregate evaluation and was classified into eleven degrees (5: excellent to -5: especially bad). Koshihikari was used as the standard variety. ** showed significant differences at the 1% level.
[4)] Sowing dates: 18 April, 2003, and 22 April, 2004; Transplanting dates: 14 May, 2003, and 18 May, 2004, respectively.
[5)] Sowing dates: 21 February, 2003; Transplanting date: 25 March, 2003.

development and the mutant analysis will be required to test these possibilities.

Potential utility of ILs

ILs are developed to introduce a small chromosomal segment containing a favorable gene from a donor cultivar into an elite cultivar. Since the development of DNA markers as tools for mapping major genes, such as those for disease and insect resistance, and QTLs, MAS has been used to develop new cultivars with particular traits[27]. However, few examples of MAS have been reported in rice so far, being limited to bacterial blight resistance, blast resistance, plant height, and seed number per panicle[1,6,21,22,29].

To enhance the utility of Koshihikari by modifying its heading date so as to expand its cultivation area, we used MAS to develop ILs with early and late heading dates[25,26]. We developed three ILs—Koshihikari Kanto HD1, Kanto IL5, and Kanto HD2—with a very small Kasalath chromosome segment (170–625 kb) including a heading-date QTL in the genetic background of Koshihikari by MAS. Wakei 370 had a larger integrated chromosome segment of Kasalath than the other ILs. Because *Hd4* has been only roughly mapped on chromosome 7, we could not design any DNA marker that was tightly linked to the QTL. This prevented us from selecting plants with recombination near *Hd4*. We demonstrated that DNA markers tightly linked to the QTLs for heading date were very effective tools for

Table 2. Cool-temperature tolerance of Koshihikari Kanto HD1, Wakei 370, Kanto IL5, Kanto HD2, and their isogenic control, Koshihikari

Line	Miyagi Pref. Furukawa Agr. Exp. Sta.[1)]		Nagano Agr. Exp. Sta. Haramura Branch[2)]		Miyazaki Agr. Res. Inst.[3)]	
	Heading date (m.d)	Floret sterility (%)	Heading date (m.d)	Floret sterility (%)	Heading date (m.d)	Floret sterility (%)
Koshihikari Kanto HD1	8.10	89.2	8.21	84.5	6.20	56.4
Wakei 370	9.06	60.2	9.01	55.3	–	–
Kanto IL5	9.09	46.1	8.30	53.7	–	–
Kanto HD2	9.15	69.9	9.09	32.0	–	–
Chubo 35	8.05	61.8	8.21	44.8	–	–
Mutsunishiki	8.07	94.7	–	–	–	–
Koshihikari	9.02	52.3	8.30	64.7	6.20	44.5
Hourei	9.07	56.2	–	–	–	–
Akitakomachi	–	–	–	–	6.19	55.9

[1)] Sowing date: 17 April 2003; Transplanting date: 15 May 2003.
[2)] Sowing date: 16 April 2003; Transplanting date: 27 May 2003.
[3)] Sowing date: 21 February 2005; Transplanting date: 25 March 2005.

minimizing the length of substituted chromosome segments.

It is very important to maintain the same phenotypic performance of the ILs as that of Koshihikari. Independent of the size of introgressed chromosome segments, Kanto IL5, Wakei 370, and Kanto HD2 showed almost the same phenotypic performance as Koshihikari. These ILs will be very important materials for breeding new cultivars to achieve several practical objectives, such as expanding the northern limit of cultivation of Koshihikari, avoiding labor concentration during harvest time, and avoiding high-temperature damage during the ripening period. On the other hand, Koshihikari Kanto HD1 exhibited a slightly lower cool-temperature tolerance. Cool-temperature damage should be considered a potential risk if Koshihikari Kanto HD1 is introduced into northern Japan.

Once we develop ILs for each QTL, it becomes easier to pyramid the QTLs. We are developing new ILs in which two QTLs are combined by crossing our ILs and using MAS. Combining two or more late-heading genes may enable us to develop extremely late-heading ILs. This pyramiding strategy will allow us to perform further fine-tuning of heading date. Furthermore, it might be used to shift the heading date of a cultivar and so avoid periods of environmental stress, such as flood and drought.

Recently, a semi-dwarfing gene, *sd1*, from *indica* cultivar IR24 was introduced into Koshihikari by MAS[29]. *Grain number 1* (*Gn1a*), involving the number of spikelets per panicle, was cloned by a map-based strategy and has been introduced into Koshihikari[1]. Once further Koshihikari ILs with single QTLs are developed, it will be possible to combine several target genes by simple crossing and MAS. Koshihikari ILs will be useful resources for the improvement of Koshihikari.

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