Agriculture and Food

AIMS Agriculture and Food, 10(2): 523–542.
DOI: 10.3934/agrfood.2025026
Received: 05 April 2025
Revised: 20 June 2025
Accepted: 25 June 2025
Published: 10 July 2025

http://www.aimspress.com/journal/agriculture

Research article

Small-effect loci that fine-tune heading are responsible for the regional genetic diversity of Hokkaido rice varieties

Md. Imdadul Hoque[1], Shuntaro Sakaguchi[1], Masaki Takatori[1], Hiroshi Shinada[2], Naoya Yamaguchi[3], Tsutomu Nishimura[3], Masafumi Kinoshita[4,*] and Yuji Kishima[1,*]

[1] Graduate School of Agriculture, Hokkaido University, Japan
[2] Kitami Agricultural Experimental Station, Hokkaido Research Organization, Japan
[3] Central Agricultural Experimental Station, Hokkaido Research Organization, Japan
[4] Kamikawa Agricultural Experimental Station, Hokkaido Research Organization, Japan

* **Correspondence:** Email: kishima@agr.hokudai.ac.jp, kinoshita-masafumi@hro.or.jp; Tel: +81117062439, +81166854111.

Abstract: Rice (*Oryza sativa*) is a domesticated crop widely grown from tropical to temperate regions. The complement of polymorphisms in locally adapted varieties (LAVs) of rice helps them acclimate to colder regions such as Hokkaido, a northern, high-latitude region of Japan. Although large-effect genetic variants have been characterized, small phenotypic effects arising from the limited genetic diversity present in LAVs have not been fully elucidated. We used 79 Hokkaido LAVs to investigate days to heading (DTH) over multiple years. Variance in the DTH caused by environmental variation ranged from 2.5 to 11.0 days. As the genome sequences of 70 Hokkaido LAVs are available, we looked for variation in four heading genes: *Ghd7*, *OsPRR37*, *Hd1*, and *DTH8*. Hokkaido LAVs mainly harbored nonfunctional alleles for *Ghd7* and *OsPRR37*, thus suggesting that these alleles influence DTH by hastening heading. *Hd1* and *DTH8* had relatively small effects on the DTH of the Hokkaido varieties, which were comparable to the effects of the environment. Our previous studies that used recombinant inbred lines (RILs) between two Hokkaido varieties, A58 and Kitaake, suggested that another quantitative trait locus (QTL) for the DTH is present on chromosome 4 (*qFDTH4*). We mapped and evaluated the contribution of *qFDTH4* to the DTH using 21 Hokkaido varieties, and detected a small effect compared to the effects of the four other genes. These findings indicate that small-effect QTLs maintain differences among LAVs and finely tune traits within the limited genetic variation of LAVs.

Keywords: cryptic variation; days to heading; locally adapted varieties; photosensitive genes; QTL; rice; small-effect locus

1. Introduction

Locally adapted varieties (LAVs) can be created for crop species by ensuring the presence of genetic variants other than those driving adaptation to common environments [1]. This method is commonly used for crops that are widely grown worldwide, such as rice (*Oryza sativa*) [2], wheat (*Triticum aestivum*) [1], maize (*Zea mays*) [3], and grape (*Vitis vinifera*) [4,5], thus supporting sustainable agriculture that is tailored to the characteristics of each region.

Crop varieties adapted to a specific geographical region are developed by exploiting their standing but sometimes limited genetic variation [6]. While the available genetic resources can be limited, it is important to assess the regional characteristics of crop varieties to identify germplasm that meet the needs of stakeholders such as producers and consumers. When creating new varieties, it is important to determine whether and to what extent genetic variants present in the varieties grown within a given region should be considered. Notably, very few studies have quantitatively investigated the use of region-specific variations.

The contribution of variations related to environmental adaptation among LAVs is small and difficult to distinguish from that related to environmental variations. Moreover, the amplitude of the traits being measured varies as a function of the year and environment, making it difficult to precisely define the genetic landscape responsible for local adaptation [7,8]. However, the differences in quantitative traits among LAVs are much more subtle than those influenced by genes with large effects, thus providing good options for local stakeholders. Locally adapted rice varieties display a more limited range of variation in the flowering time compared to genetically modified or highly bred commercial varieties [9,10]. For example, some rice landraces exhibit adjustments in the flowering time as an adaptation to the local changes in temperature and photoperiod; importantly, the range of variation between very early flowering and very late flowering varieties is fairly modest. Along with single genes with large effects, quantitative trait loci (QTLs) contribute to these variations. Hori et al. [11,12] identified multiple QTLs that regulate the eating quality of rice grains and showed that this trait is fine-tuned through continuous selection by local farmers rather than through abrupt genetic modification.

Rice has been extensively studied for its regional adaptability [9]. Although rice is native to tropical and subtropical regions, it can also grow at high latitudes above 40º north [13,14]. Rice in these regions has acquired early-heading and cold tolerance traits [15]. The genetic architecture of the cold tolerance trait is complex, and the specific mechanisms which underlie adaptation to high latitudes are not fully understood. Therefore, early heading is the trait that is linked to the adaptation of rice varieties to high latitudes [16,17]

The loss of photoperiod responses underlies the early-heading trait related to the adaptation of rice varieties to high latitudes [13]. This loss of photoperiod sensitivity is mainly due to nonfunctional alleles at two genes: *Grain number plant height and heading date 7* (*Ghd7*: *E1/Hd4*) and *PSEUDO-RESPONSE REGULATOR 37* (*OsPRR37*: *Hd2*) [18–22]. In addition, an ortholog of the *Arabidopsis thaliana* floral activator *CONSTANS* named *Heading date 1* (*Hd1*: *Se1*), and *Days to heading 8* (*DTH8*: *Ghd8/Hd5*) are thought to be involved in the adaptation of rice varieties to high latitudes [23,24]. The

adaptation of rice to high latitudes has been studied in detail using a group of varieties adapted to the northernmost limit of the rice cultivation zone in Hokkaido [25,26].

Compared with the genetic diversity among rice varieties grown in Japan as a whole, the genetic diversity of rice varieties adapted to Hokkaido is relatively low [27]. This limited diversity is probably the result of selecting specific traits for adaptation to the high latitudes in Hokkaido [20]. Despite this relatively low genetic diversity, 58 rice varieties have been bred in Hokkaido over the past 50 years according to the rice variety database (https://ineweb.narcc.affrc.go.jp/). These varieties have been developed by combining region-specific genetic variants other than those related to environmentally adapted genes [24].

Early maturation and cold tolerance are primary traits of Hokkaido rice varieties and represent common environmental adaptations. However, these traits likely vary within a certain range of values due to small genetic effects and environmental variation. In this study, we characterize the days to heading (DTH) of rice LAVs from Hokkaido and reveale the contributions of small-effect genes, which are difficult to distinguish from environmental variation, to breeding. The aim of this study is to describe the small-effect variation found in LAVs that is not commonly seen when rice lines with distant genetic relationships are crossed and analyzed.

2. Materials and methods

2.1. Plant materials and experimental design

Days to heading (DTH) was measured in a set of 79 Hokkaido varieties (Supplemental Table 1) over nine years, from 2013 to 2018 and from 2020 to 2023, at Kamikawa Agricultural Experimental (KAE) Station. During the preliminary data analysis of the DTH, the data for 2017 and 2023 were significantly different from the other years based on Grubbs' statistics and were excluded from further evaluations. Therefore, the DTH data for the remaining 7 years was considered. Every variety had DTH data for at least 2 years and as many as 7 years. Another data set used in this study included DTH data for 21 Hokkaido varieties (see Table 4). The DTH data for these varieties were recorded in 2022, and genomic DNA from these varieties were extracted and used for ddRAD-seq genotyping (see below) at the KAE Station. The recombinant inbred lines (RILs) were developed from crosses between the Hokkaido landrace 'A58' and the released variety 'Kitaake'. The 30 materials used for the sequence analysis, DTH quantification, and QTL detection were obtained in a previous study [28]. The 114 other RIL plants (F_6 generation) were used to determine the DTH and for gene mapping in 2022 in the paddy field of Hokkaido University, Sapporo, Japan (43.1° N).

2.2. Determination of heading date

The heading dates of all Hokkaido varieties were determined at the KAE Station. Meteorological data were obtained from the Automated Meteorological Data Acquisition System (AMeDAS) [29]. The seeds were sown in mid-April in a vinyl house with a natural temperature and light conditions (12.5 h daylength and 12–23 ℃), and the established seedlings were transplanted in the field in mid-May in 2013, 2014, 2016, 2017, and 2018. In 2020, the seeds were sown in early May, and the seedlings were transplanted in late May. In 2021, 2022, and 2023, the seeds were sown in late April, and the seedlings were transplanted in late May. The DTH was recorded from 20 plants per variety when at

least 50% of the effective tillers had flowered. The DTH data for the 21 Hokkaido varieties were collected in 2022, with the seeds being sown in mid-April and the seedlings being transplanted in mid-May. For the RILs, the seeds were sown in late April and the seedlings were transplanted in late May 2022. All seedlings were initially kept in a greenhouse with natural light (12–13 h daylength) and temperature conditions (20–23 ℃); one-month-old seedlings were transplanted into the paddy field. The DTH was recorded as the number of days from sowing to the emergence of the first panicle every day during July and August. The average DTH of each line was calculated from the values of six plants per variety or line; the DTH values of individual plants were also considered during mapping [28].

2.3. Genotyping of known photoperiod-related loci

To determine the genotypes at the four known photoperiod loci in the Hokkaido rice varieties, *Gdh7*, *OsPRR37*, *Hd1*, and *DTH8* were used based on published data from Fujino et al. [20].

2.4. SNP detection by double-digestion RAD-seq (ddRAD-seq)

Genomic DNA for ddRAD-seq were extracted from young leaves of 21-day-old plants (3–5 leaf stage) using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Restriction site–associated DNA (RAD) sequencing libraries were constructed for high-throughput DNA sequencing following the ddRAD-seq method as described [30]. A total of 250 ng of genomic DNA was fragmented by double digestion with *Pst*I and *Msp*I (Thermo Fisher Scientific, Waltham, MA, USA). Nucleotide sequences of the libraries were determined on a DNBSeq-G400 (MGI Tech, Shenzhen, China) in paired-end, 100 bp mode. The *Oryza sativa* reference genome sequence (Os-Nipponbare-Reference-IRGSP-1; https://rapdb.dna.affrc.go.jp/) was used for single nucleotide polymorphism (SNP) mapping. A total of 9,923 genome-wide SNPs were detected by ddRAD-seq in the 79 varieties, and breeding lines after low-quality SNPs (variant missing rate > 50% at a single SNP position) were removed.

2.5. Mapping of qFDTH4

Genomic DNA were extracted from the RILs and their parental varieties using the CTAB (Cetyltrimethylammonium bromide) method using young leaves of 21-day-old seedlings [31]. Based on the RAD-seq data from the KAE station, genotyping was performed with one marker close to the *qFDTH4* QTL [28] located at 31.4 Mb on chromosome 4 as described by [26]. This SNP marker was examined for its association with the DTH of 21 varieties collected at the KAE station. Mapping was performed using three markers (at 29.8 Mb, 31.4 Mb, and 34.1 Mb). The mapping interval was refined using five insertion/deletion (InDel) markers between 31.4 Mb and 34.1 Mb (InDel-1 to InDel-5). After genotyping the RILs, the DTH values were analyzed according to the corresponding genotype. The PCR products were amplified and analyzed using the newly designed InDel primers by conventional PCR and separated on 3% (w/v) agarose gels stained with MIDORI Green Advance (NIPPON Genetics EUROPE, Düren, Germany).

2.6. Statistical analysis

To test the statistical significance of the association between the markers and the DTH, one-way

analysis of variance (ANOVA), paired and unpaired *t*-test, Tukey-Kramer test, and pairwise Dunn tests were performed. The data were analyzed using Microsoft Excel and Prism, version 10.1.1(270).

3. Results

3.1. DTH distribution across Hokkaido varieties

Most rice varieties that are locally adapted to Hokkaido, Japan, exhibit an early-heading trait due to cold temperatures and long daylength in the summer, which results from the high latitude of the island. These varieties flower earlier than rice varieties cultivated at relatively low latitudes, with variation in the DTH observed among varieties. To assess the extent of diversity for the DTH among the Hokkaido varieties, we compiled DTH data available for 79 Hokkaido varieties grown over 9 years (2013 to 2018 and 2020 to 2023) at KAE Station. A Smirnov-Grubbs test indicated a large deviation for the average DTH in 2017 and 2023; therefore, we excluded the DTH values for these two years (Table 1). The number of years with available DTH data varied, ranging from 2 to 7 years (Supplemental Table 1).

Table 1. Average DTH distribution of 79 Hokkaido varieties in nine different years.

Year	2013	2014	2016	2017	2018	2020	2021	2022	2023
No. of varieties	63	63	21	22	17	33	31	33	28
Minimum	56	41	54	61	67	54	46	42	39
25% Percentile	60	48	61	70	69.5	56	50	48.5	46
Median	63	51	62	71.5	70	59	51	54	49.5
75% Percentile	68	56	64	74.25	72.5	60	52	55	51
Maximum	79	71	72	78	74	66	56	63	52
Mean	63.73	52.3	62.43	71.59	70.76	58.58	50.81	52.67	48.39
Std. Deviation	4.988	6.324	3.815	3.608	1.855	3.103	2.104	4.695	3.359
Grubbs' Statistic (G)	1.55	1.79	2.21	2.94	2.03	1.48	2.29	2.27	2.80

Note: Data from 2017 and 2023 were statistically excluded from further analyses due to the large deviations from the average DTH.

3.2. Environmental variation in DTH of the Hokkaido varieties

Among the 79 Hokkaido varieties used in this study, we investigated the variance in the DTH (standard deviation; SD) contributed by the environment for 43 varieties based on DTH data for three or more years. The rice variety that showed the highest environmental variance was 'Akage', with an SD of 11.04, and those with the lowest were 'Kamuimochi' and 'Tannemochi', with an SD of 2.52 (Supplemental Table 2). The mean of the environmental variance for the 43 varieties was 6.34, with an SD of 2.21. Thus, the variety Akage showed the greatest variation from year to year, with a minimum DTH value of 42 days and a maximum value of 72 days, a difference of 30 days (Supplemental Table 2). We conclude that the DTH is easily affected by the environment and is a trait that varies among the varieties (Figure 1, Supplemental Table 2).

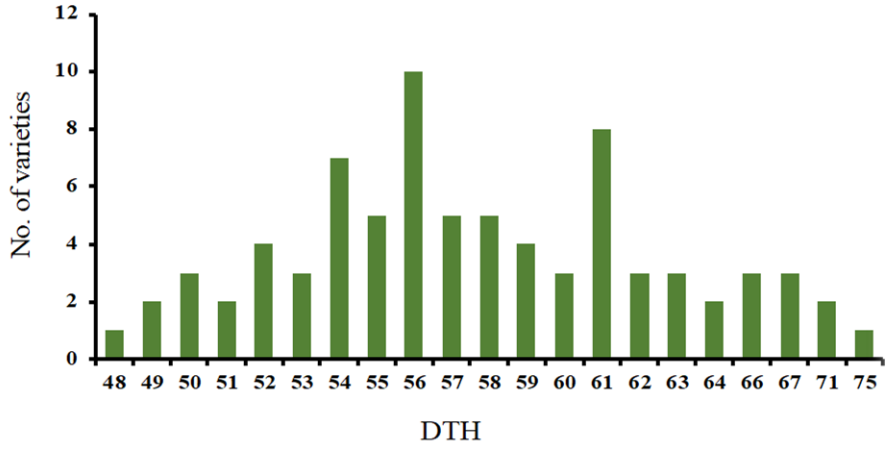

Figure 1. Frequency distribution of days to heading for 79 rice varieties over seven years.

Note: Each bar indicates the total number of varieties with the respective mean days to the DTH value. Mean DTH values were calculated using 2–7 years of data. The DTH was calculated from the day of seedling transplantation in the paddy field to the emergence of 50% effective panicles per plant.

Table 2. Functional and non-functional effects of major flowering genes on DTH.

Note: During single gene effect calculation, only the targeted gene effect on the DTH was calculated without considering other genes, whether they are functional or nonfunctional.

Gene Type	*GHD7*	*ghd7*	*OsPRR37*	*osprr37*	*HD1*	*hd1*	*DTH8*	*dth8*
No. of Varieties	10	60	5	65	26	44	52	18
Minimum	57	48.33	55.5	48.33	50	48.33	48.33	50
25% Percentile	62	54.08	60.5	54.33	53.67	54.75	54.33	55.23
Median	64.5	56.86	67	57.29	55.83	60	57.54	57.11
75% Percentile	71	60.44	71	60.54	59.16	63	60.89	62.31
Maximum	75	67	71	75	66.5	75	75	66.67
Mean	65.57	56.94	66	57.57	56.28	59.29	58.1	58.37
Std. Deviation	5.463	4.48	6.354	4.998	3.835	6.05	5.767	4.809
Std.E. Mean	1.727	0.5783	2.842	0.62	0.752	0.9121	0.7998	1.133

3.3. Genotypes at four known photoperiod genes in the Hokkaido varieties

The early-heading characteristics of rice varieties in Hokkaido are likely largely due to four genes with large effects on the flowering time: *Ghd7*, *OsPRR37*, *Hd1,* and *DTH8*. Of the 79 varieties used in this study, genome sequences were available for 70, which allowed us to assess the functionality of the alleles present at each locus (Supplemental Table 2). Of the 70 Hokkaido varieties, 10 harbor a functional allele at *Ghd7*, with the remaining 60 carry a nonfunctional allele of *Ghd7*. Five varieties

have a functional allele at *OsPRR37* (Table 2). Therefore, 60 and 65 of the Hokkaido varieties harbor nonfunctional alleles at *Ghd7* and *OsPRR37*, respectively (Table 2). Twenty-six of the varieties carry a functional allele at *Hd1*, and 52 varieties have a functional allele at *DTH8*; therefore, functional alleles at *Hd1* and *DTH8* are more frequent than functional alleles at *Ghd7* and *OsPRR37* (Table 2).

3.4. Effects of the four known DTH genes on flowering time

To explore the individual contribution of each major photoperiod gene to the DTH, we sorted all 70 varieties with available genotype information into a group harboring a functional allele and another group with a nonfunctional allele. Varieties with a nonfunctional allele at *Ghd7* or *OsPRR37* flowered 8.55 and 9.7 days earlier, respectively, than those with a functional allele (Figure 2). Varieties with a functional allele at *Hd1* or *DTH8* reached heading 3.0 and 0.3 days earlier, respectively, than those with a nonfunctional allele, thus suggesting that the effects of *Hd1* and *DTH8* on early heading are more limited than those of *Ghd7* and *OsPRR37* (Figure 2). These results indicate that early heading in the Hokkaido varieties is mainly due to nonfunctional alleles at *Ghd7* and *OsPRR37*. When we classified the 70 varieties as a function of their genotypes at all four major-effect loci, we defined nine groups, with very close DTH values when the varieties had nonfunctional alleles at both *Ghd7* and *OsPRR37*, ranging from 54.5 to 57.0 days (Table 3). The 13 varieties from the five relevant genotype groups had a functional allele at either *Ghd7* or *OsPRR37*. The average DTH values of these varieties were higher than those with both nonfunctional alleles (65.23 ± 3.59 vs. 56.31 ± 1.21: mean ± SD); this difference was significant (8.92 days, *t*-test = 0.0022) (Table 3). Therefore, when *Ghd7* and *OsPRR37* are nonfunctional, the effects imposed by functional alleles at *Hd1* and *DTH8* are partially masked by environmental variations (Figure 3).

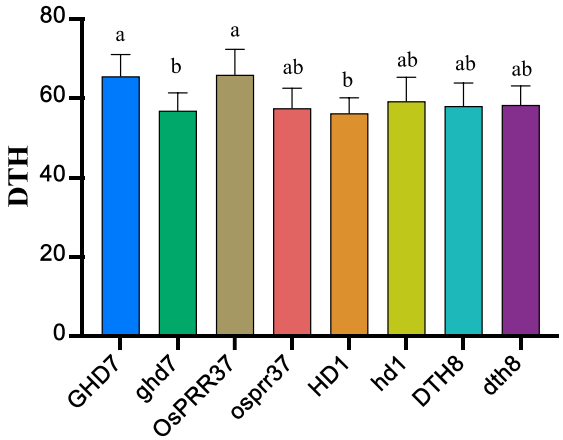

Figure 2. Effect of the genotypes at individual large-effect photoperiod genes on DTH.

Note: Each of the large-effect photoperiod genes was considered individually without considering other genes. Genotyping of major flowering-time genes was performed as described by Fujino et al. [20]. The effect of each gene was calculated using the average DTH values for all varieties harboring the indicated genotype. Values are means ± SD. Different lowercase letters indicate significant differences, as determined by Anova ($p < 0.0001$).

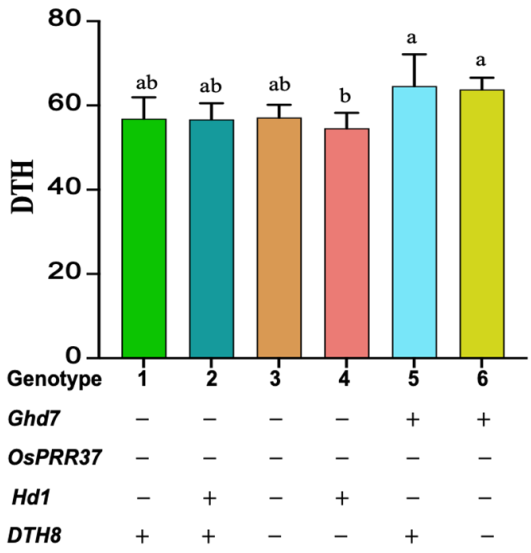

Figure 3. Effect of the combined genotypes at *Hd1*, *Ghd7*, *OsPRR37*, and *DTH8* on the DTH.

Note: Functional and nonfunctional alleles were as described by Fujino et al. [20]. Varieties were grouped based on their genotypes at the four major photoperiod genes: *Ghd7*, *OsPRR37*, *Hd1*, and *DTH8*. "Genotype-1 to 6" were ranked by the number of varieties with the given genotype, as listed in Table 3. The DTH values were calculated using the average data for each constituent variety. Values are means ± SD. Different lowercase letters indicate significant differences, as determined by Anova (p = 0.0020).

Table 3. Genotype groups showing different functional and nonfunctional allele combinations with DTH difference.

GHD7	OsPRR37	HD1	DTH8	Genotype	No. of Varieties	Minimum	Maximum	Median	Mean	SD	SEM
f	**f**	**f**	**F**	Gen-1	23	48.33	65.5	57.29	56.86	5.069	1.057
f	**f**	**F**	**F**	Gen-2	21	50.67	66.5	57	56.69	3.844	0.8388
f	**f**	**f**	**f**	Gen-3	8	54	62.25	56.36	57.17	2.992	1.058
f	**f**	**F**	**f**	Gen-4	5	50	59.2	55.67	54.53	3.648	1.631
F	**f**	**f**	**F**	Gen-5	4	57	75	63.25	64.63	7.521	3.76
F	**f**	**f**	**f**	Gen-6	4	60.5	66.67	64	63.79	2.81	1.405
F	**F**	**f**	**f**	Gen-7	2	-	-	-	71	-	-
f	**F**	**f**	**F**	Gen-8	2	55.5	67	61.25	61.25	8.13	5.75
f	**F**	**f**	**f**	Gen-9	1	-	-	-	65.5	-	-

Note: Several data include (-), which means the minimum, maximum, median, SD & SEM are not available or the same because of the same DTH average value of two varieties or only one DTH average value of a single variety. F and f indicate the functional and loss-of-function alleles in the locus, respectively.

3.5. Contributions of the QTL qFDTH4 to DTH in the Hokkaido varieties

In a previous study [28], we detected QTLs for the DTH using RILs produced by crossing the Hokkaido landrace A58 with the Hokkaido variety Kitaake. These QTLs mapped to chromosomes 1, 2, 4, 6, and 10, which are different positions from those reported for previously established heading-related genes in rice. Here, we focused our investigation on the QTL that mapped to chromosome 4, *qFDTH4*, which had the largest effect on the DTH among the five QTLs. We subjected the genomic DNA of 21 Hokkaido varieties to RAD-seq analysis to detect their complement of polymorphisms at this locus. We used an SNP marker located near the position of *qFDTH4* in the 29.8–32.4 Mb interval on chromosome 4 [28]. We identified a SNP marker at 31.4 Mb (Position: 31,409,997 bp) and used it to genotype the 21 Hokkaido varieties into two groups according to their genotype: the A58 (A) type and the Kitaake (K) type, representing 8 and 13 varieties, respectively.

The average DTH values of the varieties were 62.6 ± 4.1 (A-type) and 60.5 ± 3.4 (K-type), a difference that is significant (*t*-test = 0.022). Thus, by using the genotype at this SNP as a proxy for the allele at *qFDTH4*, we conclude that *qFDTH4* across the 21 Hokkaido varieties displays a potential effect on the DTH of about 2.1 days, which is independent of the genotypes at *Ghd7*, *OsPRR37*, *Hd1*, and *DTH8*. We suggest that other gene(s) in addition to the four photoperiod genes control the DTH in the Hokkaido LAVs (Table 4).

Table 4. Hokkaido varieties genotyping of *qFDTH4* using 31.4Mb SNP marker based on Rad-seq data.

Varities	Genotype	*qFDTH4*	DTH
Emimaru	Gen-1	A	54
Kazenokomochi	Gen-1	A	64
Kitayukimochi	Gen-1	A	61
Sorayutaka	Gen-1	K	59
Kitafukumochi	Gen-1	K	58
Kitaake	Gen-2	K	62
Kirara 397	Gen-2	K	64
Hoshinoyume	Gen-2	K	61
Daichinohoshi	Gen-2	K	54
Oborozoki	Gen-2	K	62
Yumeperika	Gen-2	K	62
Nanatsuboshi	Gen-2	A	62
Fukurinko	Gen-2	A	66
Shimahikari	Gen-3	A	68
Hakutomochi	Gen-3	A	63
Hoshimaru	Gen-4	K	54
Sorayuki	Gen-4	K	62
Kitakurin	Gen-4	A	63
Ginpu	-	K	64
Suisei	-	K	63
Kitashijoku	-	K	62

Note: Ginpu, Suisei, and Kitashijoku, these three varieties genotype group (Gen-n) are not available, but they can contribute independently to the DTH effect of *qFDTH4* without considering the genotype group. Same as other genotype groups. A and K indicate the A58-type and Kitaake-type alleles in the locus, respectively.

3.6. Mapping of qFDTH4

To narrow down the location of *qFDTH4* on chromosome 4, we genotyped the *qFDTH4* region using a population of F_6 RILs derived from a single F_5 individual that was heterozygous at this region and derived from the parental varieties A58 and Kitaake. We scored the DTH in 114 F_6 plants and the two parental varieties. A58 flowered 3–5 days later than Kitaake in the paddy field in 2022. The range of the DTH values for the 114 F_6 plants was 63–85 days, with a mean DTH of 71.2 ± 6.4 days.

We genotyped all 114 plants with insertion/deletion (InDel) markers near *qFDTH4*. Accordingly, we designed five InDel markers (InDel-1–5) based on the polymorphisms between A58 and Kitaake in the 29- to 35-Mb interval on chromosome 4 (Figure 4). We identified eight plants that had recombinations in this region. Among these eight plants, four had a recombination event between InDel-2 and InDel-3: two of these plants were late heading, whereas the other two were early heading (Figure 4). Therefore, the *qFDTH4* locus that affects the DTH is located between InDel-2 and InDel-3 within a 2.21-Mb region (Figure 4).

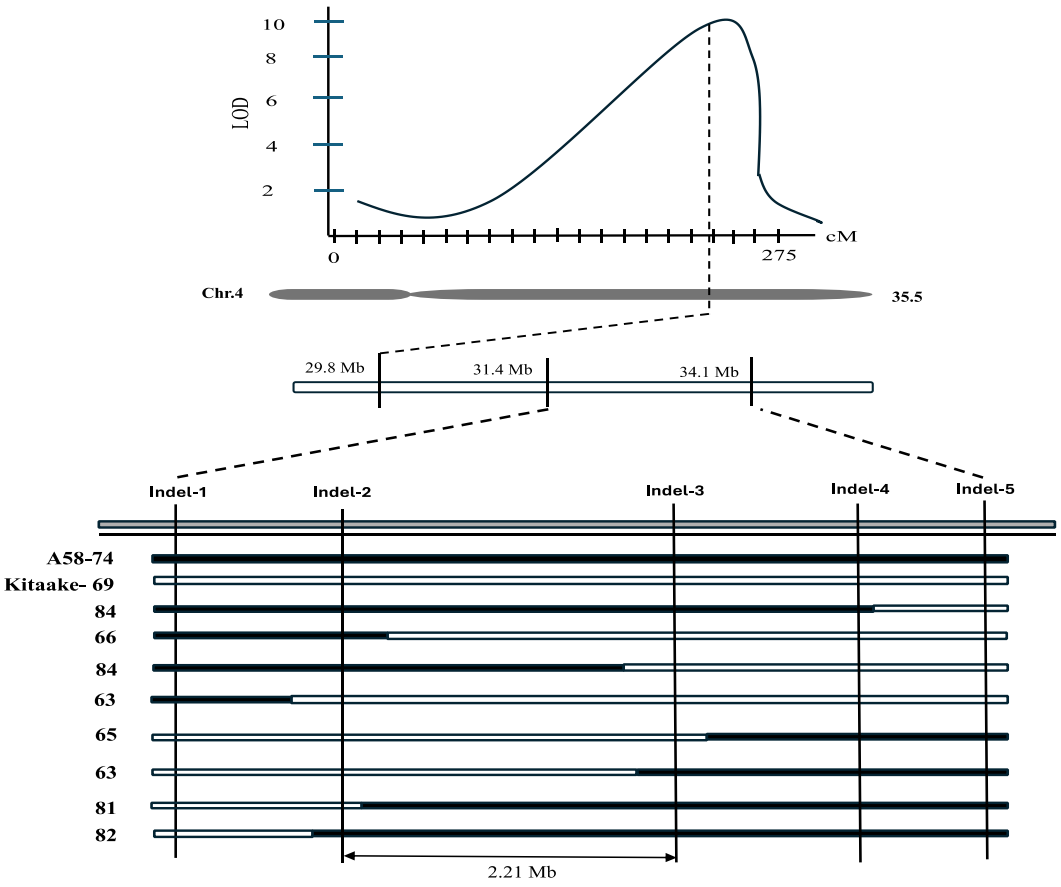

Figure 4. Molecular mapping of *qFDH4*.

Note: Molecular mapping of *qFDTH4* after confirming the position of the QTL using the DTH values for Hokkaido using the nearest marker (31.4 Mb) by RAD-seq (Table 4). Five InDel markers were developed between 31.4 Mb and 34.1 Mb to refine the mapping interval. The location of the *qFDTH4* QTL was narrowed down to between InDel-2 and InDel-3 based on the DTH values and the genotype. Black bars, A58 genotype; white bars, Kitaake genotype. The DTH values for the individual RILs are shown on the left.

4. Discussion

Rice cultivation in Hokkaido is unique due to the high latitude of this region, which is characterized by a long daylength, relatively low temperatures, and a short growing season [32,33]. To complete their life cycle in this environment, locally adapted varieties must flower early; however, beyond this general adaptation, substantial variations in the DTH can accumulate, as evidenced here among the Hokkaido LAVs (Table 1). In addition to genetic factors, the flowering time in rice is strongly affected by environmental conditions. Annual variations in temperature and other climatic factors introduce inconsistencies in DTH data collected across different years (Table 1) [19]. As demonstrated in our study, the DTH is influenced by environmental factors, with considerable differences in sensitivity among the varieties (Supplemental Table 2). Genetic variation can sometimes be masked by environmental variations. While varieties such as Akage exhibited high variance in response to the environment, the two other varieties, Kamuimochi and Tannemochi, had more stable DTH values (Supplemental Table 2). Understanding the reasons for this variation is crucial for breeding locally adapted rice varieties, as even small changes in the DTH can have a large effect on local preference. In Hokkaido, the varieties destined for human consumption are generally more valuable and in higher demand. These varieties are selected for earlier maturation, even if their overall yield might be lower. Conversely, fodder rice varieties offer higher yields, even though they mature later. Despite being cultivated in the same region, the critical differences in their heading times are largely controlled by the cumulative influence of multiple genetic loci, including the small-effect loci. Differences in the timing and duration of the heading stage observed in rice varieties growing in Hokkaido highlight the genetic control and environmental interactions that influence the DTH [34].

The early-heading characteristics of Hokkaido LAVs are known to be influenced by four major genes: *Ghd7*, *OsPRR37*, *Hd1*, and *DTH8* [23,25]. These genes regulate the flowering time through a combination of functional and nonfunctional alleles in response to the daylength and temperature, which are two important factors in the adaptability of rice to high latitudes. As functional alleles at *Ghd7* and *OsPRR37* delay flowering under long-day conditions [35,36], their loss-of-function alleles are important for the early-heading varieties of Hokkaido in the prevailing short growing season. Among the 70 varieties analyzed here, 60 had a nonfunctional allele at *Ghd7* and 65 had a nonfunctional allele at *OsPRR37* (Supplemental Table 2), thus suggesting the importance of these genotypes in varieties locally adapted to Hokkaido. The predominance of nonfunctional alleles at *Ghd7* and *OsPRR37* suggests that a diminished photoperiod sensitivity is a critical factor in the early heading trait among Hokkaido LAVs (Table 3). Such a genetic landscape for LAVs may not easily be replaced by alleles at other loci. Of the 70 Hokkaido LAVs, 26 and 52 harbor a functional allele at *Hd1* and *DTH8*, respectively (Table 3). The presence of a functional allele at *Hd1* and *DTH8* maintains early heading and yield stability in these varieties. These findings are valuable for the development of rice varieties with improved adaptation to and productivity in high-latitude environments. Therefore, in Hokkaido LAVs, the selection of genotypes at *Hd1* and *DTH8* is less strict than at *Ghd7*; additionally, *OsPRR37* is not as essential for regional adaptation. While the alleles at *Hd1* and *DTH8* do not display strong effects, they help fine-tune the DTH when combined with other genetic factors [37–40]. Genes involved in adaptation to harsh environments have been the target of selection in crop breeding, whereas those with small genetic effects have helped optimize the plant growth and yield [35].

Crosses between varieties with a similar phenotype reveal the underlying genetic architecture of a given trait, as evidenced by the transgressive segregation of phenotypes of interest in the

progeny [28]. To harness transgressive segregation, we previously crossed A58 to Kitaake, which has a similar heading date. The progeny had heading dates that were either earlier or later than those of the two parents. A QTL analysis detected a previously unreported moderate-effect locus on chromosome 4, *qFDTH4*, which mapped to a location distinct from that of other known genetic factors which influence heading in Hokkaido varieties, such as *Ghd7*, *OsPRR37*, *Hd1*, and *DTH8*. In our study, we attempted to evaluate the effect of *qFDTH4* using Hokkaido LAVs by separating them into two groups based on their genotype at a nearby SNP marker (Figure 4). We observed a two-day difference in the DTH between the Hokkaido LAVs with an A58-type or Kitaake-type allele (Table 4). The effect of *qFDTH4* might be hidden by the environmental effect or some major-effect gene(s) when varieties with different genetic backgrounds are used for crossing. We detected an effect for *qFDTH4* under the similar genetic backgrounds of Hokkaido LAVs (Figure 4). *qFDTH4* may fine-tune the DTH in Hokkaido LAVs. The small but significant effect of 2.1 days independent of other photoperiod genes suggests that *qFDTH4* is likely a small-effect modifier that interacts with other heading-time genes. The polygenic nature of heading-time regulation emphasizes the need for fine-mapping of *qFDTH4* to identify the causal gene. A small-scale fine-mapping experiment using 114 F_6 plants with polymorphic InDel markers between 31.4 Mb and 34.1 Mb on chromosome 4 delineated *qFDTH4* to between InDel-2 and InDel-3 (Figure 4).

In this study, we characterized a QTL related to an early DTH. This small-effect QTL could explain the adaptation of Hokkaido LAVs to local conditions. The LAVs adapt to their environment through genes with strong effects, but diversity among the LAVs is maintained through genes with small effects [41,42]. Small-effect genes facilitate the variation of locally adapted rice varieties to specific environmental conditions and provide more options for the stakeholders [43,44].

5. Conclusions

In varieties that have adapted to environments far away from their regions of origin, genetic effects that have a significant influence on local-scale environmental adaptation are essential. Our study highlights the presence of cryptic variations in the flowering times of locally adapted rice varieties in Hokkaido. Major-effect heading-related genes such as *Ghd7* and *OsPRR37* play key roles in determining the DTH in Hokkaido LAVs, while *Hd1* and *DTH8* have relatively small effects, which are equivalent to environmental effects. We characterized a small-effect locus, *qFDTH4*, that contributes to the cryptic variation among LAVs. We mapped *qFDTH4* to a 2.1-Mb interval on chromosome 4 and demonstrated that small-effect QTLs maintain genetic diversity in rice populations with limited genetic backgrounds adapted to specific environments. Future studies which focus on *qFDTH4* may provide insights into the genetic makeup of the flowering-time regulation in high-latitude environments.

Author contributions

Md. Imdadul Hoque: Designed the research plans, obtained heading data for the RIL population at Sapporo and performed the QTL analysis, prepared and wrote the manuscript; Shuntaro Sakaguchi: Obtained heading data for the RIL population at Sapporo and performed the QTL analysis; Masaki Takatori: Obtained heading data for the RIL population at Sapporo and performed the QTL analysis; Hiroshi Shinada: Obtained heading data for Hokkaido varieties at KAE Station, performed the ddRAD-

seq analysis; Naoya Yamaguchi: Performed the ddRAD-seq analysis; Tsutomu Nishimura: Obtained heading data for Hokkaido varieties at KAE Station; Masafumi Kinoshita: Designed the research plans, obtained heading data for Hokkaido varieties at KAE Station; Yuji Kishima: Designed the research plans, prepared, wrote the manuscript and supervised. All authors have read and agreed to the published version of the manuscript.

Use of Generative-AI tools declaration

The authors declare that they have not used artificial intelligence tools in the creation of this article.

Acknowledgments

We gratefully acknowledge Dr. Y. Koide (Research Faculty of Agriculture, Hokkaido University, Hokkaido, Japan) and Mr. K. Konno (Former Master student in the Laboratory of Plant Breeding) for their valuable suggestions concerning this study. We also acknowledge the Science and Technology Fellowship Trust, Ministry of Science and Technology, Government of the People's Republic of Bangladesh for providing scholarship to Mr. Md. Imdadul Hoque, during his study period at Hokkaido University, Japan. This study was carried out under a collaboration agreement between the Agricultural Research Department of the Hokkaido Research Organization and the Research Faculty of Agriculture, Hokkaido University. This work was supported by JSPS KAKENHI grants (19H00937 and 23H02180) and RiceBACS in Science and Technology Research Partnership for Sustainable Development (SATREPS).

Data availability

The data that support the findings of this study are available upon request from the corresponding authors (M.K. and Y.K.).

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

References

1. Sthapit K, Jinita MH, Zhiwu Z, et al. (2018) Genetic diversity of clinal freezing tolerance variation in winter wheat landraces. *Agronomy* 8: 95. https://doi.org/10.3390/agronomy8060095
2. Reig-Valiente JL, Viruel J, Sales E, et al. (2016) Genetic diversity and population structure of rice varieties cultivated in temperate regions. *Rice* 9: 58. https://doi.org/10.1186/s12284-016-0130-5
3. Aguirre-Liguori JA, Ramirez-Barahona S, Tiffin P, et al. (2019) Climate change is predicted to disrupt patterns of local adaptation in wild and cultivated maize. *Proc R Soc B* 286: 20190486. https://doi.org/10.1098/rspb.2019.0486
4. Zhou Y, Minio A, Massonnet M, et al. (2019) The population genetics of structural variants in grapevine domestication. *Nat Plants* 5: 965–979. https://doi.org/10.1038/s41477-019-0507-8

5. Long Q, Cao S, Huang G, et al. (2024) Population comparative genomics discovers gene gain and loss during grapevine domestication. *Plant Physiol* 195: 1401–1413. https://doi.org/10.1093/plphys/kiae039
6. Fujino K, Kawahara Y, Shirasawa K (2022) Genetic diversity among the varieties exhibiting early heading date in rice. *Euphytica* 218: 18. https://doi.org/10.1007/s10681-021-02965-y
7. Malosetti M, Ribaut JM, van Eeuwijk FA (2013) The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. *Front Physiol* 4: 44. https://doi.org/10.3389/fphys.2013.00044
8. Walter GM, Monro K, Terranova D, et al. (2024) Environmental effects on genetic variance are likely to constrain adaptation in novel environments. *Evol Lett* 8: 374–386. https://doi.org/10.1093/evlett/qrad065
9. Hori K, Matsubara K, Yano M (2016) Genetic control of flowering time in rice: integration of Mendelian genetics and genomics. *Theor Appl Genet* 129: 2241–2252. https://doi.org/10.1007/s00122-016-2773-4
10. Liu C, Tu Y, Liao S, et al. (2021) Genome-wide association study of flowering time reveals complex genetic heterogeneity and epistatic interactions in rice. *Gene* 770: 145353. https://doi.org/10.1016/j.gene.2020.145353
11. Hori K, Suzuki K, Iijima K, et al. (2016) Variation in cooking and eating quality traits in Japanese rice germplasm accessions. *Breed Sci* 66: 309–318. https://doi.org/10.1270/jsbbs.66.309
12. Hori K, Suzuki K, Ishikawa H, et al. (2021) genomic regions involved in differences in eating and cooking quality other than Wx and Alk Genes between *indica* and *japonica* rice cultivars. *Rice* 14: 8. https://doi.org/10.1186/s12284-020-00447-8
13. Saito H, Okumoto Y, Tsukiyama T, et al. (2018) Allelic differentiation at the *E1/Ghd7* locus has allowed expansion of rice cultivation area. *Plants* 8: 550. https://doi.org/10.3390/plants8120550
14. Cui Y, Wang J, Feng L, et al. (2020) A combination of long-day suppressor genes contributes to the northward expansion of rice. *Front Plant Sci* 11: 864. https://doi.org/10.3389/fpls.2020.00864
15. Tanaka N, Shenton M, Kawahara Y, et al. (2021) Investigation of the genetic diversity of a rice core collection of Japanese landraces using whole-genome sequencing. *Plant Cell Physiol* 61: 2087–2096. https://doi.org/10.1093/pcp/pcaa125
16. Sakaguchi S, Hoque MI, Kishima Y (2024) Stable early heading in photoperiod-insensitive rice varieties results from an extremely short photoperiod-sensitive phase and weak temperature sensitivity. *Plant Stress* 13: 100561. https://doi.org/10.1016/j.stress.2024.100561
17. Li Z, Gui R, Yu X, et al. (2022) Genetic basis of the early heading of high-latitude weedy rice. *Front Plant Sci* 13: 1059197. https://doi.org/10.3389/fpls.2022.1059197
18. Fujino K, Sekiguchi H (2005) Mapping of QTLs conferring extremely early heading in rice (*Oryza sativa* L.). *Theor Appl Genet* 111: 393–398. https://doi.org/10.1007/s00122-005-2035-3
19. Fujino K, Sekiguchi H (2005) Identification of QTLs conferring genetic variation for heading date among rice varieties at the northern-limit of rice cultivation. *Breeding Sci* 55: 141–146. https://doi.org/10.1270/jsbbs.55.141
20. Fujino K, Obara M, Ikegaya T (2019) Establishment of adaptability to the northern-limit of rice production. *Mol Genet Genomics* 294: 729–737. https://doi.org/10.1007/s00438-019-01542-2
21. Nonoue Y, Fujino K, Hirayama Y, et al. (2008) Detection of quantitative trait loci controlling extremely early heading in rice. *Theor Appl Genet* 116: 715–722. https://doi.org/10.1007/s00122-007-0704-0

22. Shibaya T, Nonoue Y, Ono N, et al. (2011) Genetic interactions involved in the inhibition of heading by heading date QTL, *Hd2* in rice under long-day conditions. *Theor Appl Genet* 123: 1133–1143. https://doi.org/10.1007/s00122-011-1654-0
23. Fujino K (2020) Days to heading, controlled by the heading date genes, *Hd1* and *DTH8*, limits rice yield-related traits in Hokkaido, Japan. *Breed Sci* 70: 277–282. https://doi.org/10.1270/jsbbs.19151
24. Fujino K, Kawahara Y, Koyanagi KO, et al. (2021) Translation of continuous artificial selection on phenotype into genotype during rice breeding programs. *Breed Sci* 71: 125–133. https://doi.org/10.1270/jsbbs.20089
25. Fujino K, Yamanouchi U, Nonoue Y, et al. (2019) Switching genetic effects of the flowering time gene *Hd1* in LD conditions by *Ghd7* and *OsPRR37* in rice. *Breed Sci* 69: 127–132. https://doi.org/10.1270/jsbbs.18060
26. Shinada H, Yamamoto T, Yamamoto E, et al. (2014) Historical changes in population structure during rice breeding programs in the northern limits of rice cultivation. *Theor Appl Genet* 127: 995–1004. https://doi.org/10.1007/s00122-014-2274-2
27. Fujino K, Shirasawa K (2022) Fine-scale genetic structure of the rice landrace population in Japan. *Mol Genet Genomics* 297: 711–718. https://doi.org/10.1007/s00438-022-01880-8
28. Koide Y, Sakaguchi S, Uchiyama T, et al. (2019) Genetic properties responsible for the transgressive segregation of days to heading in rice. *G3 (Bethesda)* 9: 1655–1662. https://doi.org/10.1534/g3.119.201011
29. Japan Meteorological Agency (2023) Meteorological daily element data of Kamikawa (pippu town). Available from: https://www.data.jma.go.jp/stats/etrn/index.php.
30. Shirasawa K, Hirakawa H, Isobe S (2016) Analytical workflow of double-digest restriction site-associated DNA sequencing based on empirical and *in silico* optimization in tomato. *DNA Res* 23: 145–153. https://doi.org/10.1093/dnares/dsw004
31. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8: 4321–4325. https://doi.org/10.1093/nar/8.19.4321
32. Ebana K, Yonemaru J-I, Fukuoka S, et al. (2010) Genetic structure revealed by a whole-genome single-nucleotide polymorphism survey of diverse accessions of cultivated Asian rice (*Oryza sativa* L.). *Breeding Sci* 60: 390–397. https://doi.org/10.1270/jsbbs.60.390
33. Kou Y, Liao Y, Toivainen T, et al. (2020) Evolutionary Genomics of Structural Variation in Asian Rice (*Oryza sativa*) Domestication. *Mol Biol Evol* 37: 3507–3524. https://doi.org/10.1093/molbev/msaa185
34. Itoh Y (2018) Characterization of heading times and duration of heading time of an individual using a wide range of variety of rice (*Oryza sativa* L.) in one of the northern-limit regions of rice cultivation, Hokkaido islands. *J Agric Ecol Res Int* 8: 8. https://doi.org/10.9734/JAERI/2016/27210
35. Liu C, Song G, Zhou Y, et al. (2015) *OsPRR37* and *Ghd7* are the major genes for general combining ability of DTH, PH and SPP in rice. *Sci Rep* 5: 12803. https://doi.org/10.1038/srep12803
36. Hu Y, Zhou X, Zhang B, et al. (2021) *OsPRR37* alternatively promotes heading date through suppressing the expression of *Ghd7* in the Japonica variety Zhonghua 11 under natural long-day conditions. *Rice* 14: 20. https://doi.org/10.1186/s12284-021-00464-1

37. Hori K, Ogiso-Tanaka E, Matsubara K, et al. (2013) *Hd16*, a gene for casein kinase I, is involved in the control of rice flowering time by modulating the day-length response. *Plant J* 76: 36–46. https://doi.org/10.1111/tpj.12268
38. Zhang J, Zhou X, Yan W, et al. (2015) Combinations of the *Ghd7*, *Ghd8* and *Hd1* genes largely define the ecogeographical adaptation and yield potential of cultivated rice. *New Phytol* 208: 1056–1066. https://doi.org/10.1111/nph.13538
39. Zhang B, Liu H, Qi F, et al. (2019) Genetic interactions among *Ghd7*, *Ghd8*, *OsPRR37* and *Hd1* contribute to large variation in heading date in rice. *Rice* 12: 48. https://doi.org/10.1186/s12284-019-0314-x
40. Sun C, He C, Zhong C, et al. (2022) Bifunctional regulators of photoperiodic flowering in short day plant rice. *Front Plant Sci* 13: 1044790. https://doi.org/10.3389/fpls.2022.1044790
41. Jin L, Lu Y, Xiao P, et al. (2010) Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. *Theor Appl Genet* 121: 475–487. https://doi.org/10.1007/s00122-010-1324-7
42. Hang Y, Yue L, Bingrui S, et al. (2023) genetic diversity and breeding signatures for regional *Indica* rice improvement in Guangdong of Southern China. *Rice* 16: 25. https://doi.org/10.1186/s12284-023-00642-3
43. Melandri G, Sikirou M, Arbelaez JD, et al. (2020) Multiple small-effect alleles of Indica origin enhance high iron-associated stress tolerance in rice under field conditions in West Africa. *Front Plant Sci* 11: 604938. https://doi.org/10.3389/fpls.2020.604938
44. Xie W, Wang G, Yuan M, et al. (2015) Breeding signatures of rice improvement revealed by a genomic variation map from a large germplasm collection. *Proc Natl Acad Sci U S A* 112: E5411–5419. https://doi.org/10.1073/pnas.1515919112

AIMS Press

Supplementary

Supplemental Table 1. Heading days of 79 Hokkaido varieties in 9 different years observed in Kamikawa Agricultural Experimental Station.

Name/Year	2013	2014	2016	2017	2018	2020	2021	2022	2023
Akage	68	51	61	75	72	60	47	42	39
Bozu	68	54	64	75	74	59	52	53	51
Bozu 6	64	48							
Hashiribozu	66	53	62	74	73				
Wasebozu	58	41							
Iburiwase	62	49	61	71	67				
Fukoku	64	55				63	51	52	51
Wasefukoku	61	50	62	71	69				
Norin 9	61	46							
Norin11	57	41							
Tomoenishiki	73	69							
Ounochuutou	79	71							
Ishikarishiroge	63	51							
Eicho	65	54	70	76					
Kyowa	68	56							
Hakuchomochi	65	49				56	50	52	50
Norin15	57	41	54	61					
Norin19	60	46							
Norin20	61	47	57	72	70	55	52	55	42
Minamisakae	70	61							
Shinsakae	70	61							
Hokkai112	57	47	59	68					
Shinsetsu	69	57							
Tomoemasari	75	67							
Norin34	62	49							
Hokuto	62	47							
Yukara	69	58							
Mimasari	66	55							
Sasahonami	60	48	62	70	70				
Fukuyuki	60	49							
Shiokari	67	53				61	52	47	52
Matsumae	72	61							
Kitakogane	61	47							
Ishikari	59	48	61	70	73	57	51	54	50
Sorachi	71	57	72	78					
Hayayuki	64	47	61	70	69				
Kitahikari	67	58				61	56	63	
Tomoyutaka	59	50							

Continued on next page

Name/Year	2013	2014	2016	2017	2018	2020	2021	2022	2023
Hayakogane	57	47							
Shimahikari	63	49	64	72	73				
Kitaake	59	49	63	71	71				
Michikogane	66	55							
Tomohikari	65	50							
Yukihikari	67	53	64	75	70	58		54	50
Aya	68	57				66	55	61	
Hayakaze	56	47							
Hayamasari	58	42							
Kirara397	61	53	65	74	71	60	51	55	51
Honoka224	68	57							
Kitaibuki	63	49							
Hoshimaru	60	48				56	49	46	46
Akiho	59	50							
Hoshinoyume	61	53	64	72	70	61	51	47	49
Nantsuboshi	61	53	63	71	72	59	51	55	50
Fukkurinko	64	56				63	52	58	
Minakuchiine	71	63							
Kuroge	57	51							
Yumeperika	61	53				59	50	55	49
Oborozuki	62	54							
Hokkaiwase	62	49							
Toyohikari	68	63							
Daichinohoshi	57	49	61	66		54	49	46	46
Hokkai 287	61	53							
Sorayuki			61	72	70	59	52	54	52
Sorayutaka				71	69	57	51	53	48
Kitakurin						60	51	56	52
Emimaru						54	46	45	43
Ayahime						59	51	54	49
Ginpu						60	52	56	52
Suisei						60	52	55	51
Kitashijuku						57	51	55	51
Yukimochi						65		58	
Kamuimochi						59	54	56	
Annemochi						58	50	52	49
Tannemochi						55	50	52	45
Kazenokomochi						59	51	53	50
Shirokumochi						54	47	47	45
Kitayukimochi						55	49	47	46
Kitafukumochi						54	49	50	46

Note: Meteorological data (temperature and total rainfall) are provided as reference [28] in Kamikawa (pippu town) from April to September in 2013–2023 obtained from the stations of AMeDAS.

Supplemental Table 2. Average heading dates, standard deviation and standard error of the mean of 79 Hokkaido varieties along with allele combination of *Ghd7*, *OsPRR37*, *Hd1* and *DTH8* heading genes in 70 Hokkaido varieties [20].

Variety	Year of breeding	*Ghd7*	*OsPRR37*	*Hd1*	*DTH8*	AVG. DTH	SD.	SEM
Akage	ND	f	f	f	F	57.29	11.04	4.173
Bozu	1895	f	f	f	F	60.57	8.40	3.176
Bozu 6	1919	f	f	f	f	56	11.31	8
Hashiribozu	1932	f	f	f	F	63.5	8.35	4.173
Wasebozu	1936	f	f	f	F	49.5	12.02	8.5
Iburiwase	ND	f	f	f	F	59.75	7.63	3.816
Fukoku	1935	F	f	f	F	57	6.12	2.739
Wasefukoku	1936	f	f	f	F	60.5	7.85	3.926
Norin 9	1937	f	f	f	F	53.5	10.61	7.5
Norin11	1937	f	f	f	F	49	11.31	8
Tomoenishiki	1941	F	F	f	F	71	2.83	2
Ounochuutou	1941	F	f	f	F	75	5.66	4
Ishikarishiroge	1941	f	f	f	F	57	8.49	6
Eicho	1942	F	f	f	F	63	8.19	4.726
Kyowa	1942	f	f	f	F	62	8.49	6
Hakuchomochi	1989	f	f	f	f	54.4	6.50	2.909
Norin15	1940	f	f	F	F	50.67	8.50	4.91
Norin19	1941	f	f	f	F	53	9.90	7
Norin20	1941	f	f	f	f	56.71	7.27	2.749
Minamisakae	1951	f	F	f	f	65.5	6.36	4.5
Shinsakae	1951	f	f	f	F	65.5	6.36	4.5
Hokkai112	1951	f	f	F	F	54.33	6.43	3.712
Shinsetsu	1954	f	f	f	F	63	8.49	6
Tomoemasari	1951	F	F	f	F	71	5.66	4
Norin34	1948	f	f	f	f	55.5	9.19	6.5
Hokuto	1953	f	f	f	F	54.5	10.61	7.5
Yukara	1962	F	f	f	F	63.5	7.78	5.5
Mimasari	1959	F	f	f	f	60.5	7.78	5.5
Sasahonami	1961	f	f	F	F	60	9.09	4.546
Fukuyuki	1958	f	f	F	F	54.5	7.78	5.5
Shiokari	1963	f	f	f	F	56	7.94	3.55
Matsumae	1970	f	f	F	F	66.5	7.78	5.5
Kitakogane	1973	f	f	f	f	54	9.90	7
Ishikari	1971	f	f	F	F	57.57	8.16	3.085
Sorachi	1967	F	f	f	f	66.67	8.39	4.842
Hayayuki	1969	f	f	f	F	60.25	9.43	4.715
Kitahikari	1975	f	f	f	F	61	4.30	1.924
Tomoyutaka	1977	f	f	F	F	54.5	6.36	4.5

Continued on next page

Variety	Year of breeding	*Ghd7*	*OsPRR37*	*Hd1*	*DTH8*	AVG. DTH	SD.	SEM
Hayakogane	1977	f	f	F	F	52	7.07	5
Shimahikari	1980	f	f	f	f	62.25	9.91	4.956
Kitaake	1983	f	f	F	F	60.5	9.15	4.573
Michikogane	1982	f	f	f	F	60.5	7.78	5.5
Tomohikari	1983	f	f	f	f	57.5	10.61	7.5
Yukihikari	1984	f	f	f	f	61	7.04	2.875
Aya	1991	f	f	F	F	61.4	5.59	2.502
Hayakaze	1990	f	f	F	F	51.5	6.36	4.5
Hayamasari	1988	f	f	F	f	50	11.31	8
Kirara397	1988	f	f	F	F	59.43	7.07	2.671
Honoka224	1990	F	f	f	f	62.5	7.78	5.5
Kitaibuki	1993	f	f	F	f	56	9.90	7
Hoshimaru	2006	f	f	F	f	51.8	5.93	2.653
Akiho	1996	f	f	F	F	54.5	6.36	4.5
Hoshinoyume	1996	f	f	F	F	58.14	8.09	3.058
Nantsuboshi	2001	f	f	F	F	59.14	7.13	2.694
Fukkurinko	2003	f	f	F	F	58.6	4.98	2.227
Minakuchiine	ND	f	F	f	F	67	5.66	4
Kuroge	ND	f	f	F	F	54	4.24	3
Yumeperika	2008	f	f	F	F	55.6	4.45	1.99
Oborozuki	2003	f	f	F	F	58	5.66	4
Hokkaiwase	ND	f	F	f	F	55.5	9.19	6.5
Toyohikari	1953	F	f	f	f	65.5	3.54	2.5
Daichinohoshi	2003	f	f	F	F	52.67	5.68	2.319
Hokkai 287	1998	f	f	F	F	57	5.66	4
Sorayuki	2014	f	f	F	f	59.2	7.05	3.153
Sorayutaka	2016	f	f	f	F	57.5	8.06	4.031
Kitakurin	2012	f	f	F	f	55.67	4.51	2.603
Emimaru	2018	f	f	f	F	48.33	4.93	2.848
Ayahime	2001	-	-	-	-	54.67	4.041	2.333
Ginpu	1999	-	-	-	-	56	4	2.309
Suisei	2004	-	-	-	-	55.67	4.041	2.333
Kitashijuku	2010	-	-	-	-	54.33	3.055	1.764
Yukimochi	1951	-	-	-	-	61.5	4.95	3.5
Kamuimochi	1965	-	-	-	-	56.33	2.517	1.453
Annemochi	1970	-	-	-	-	53.33	4.163	2.404
Tannemochi	1983	-	-	-	-	52.33	2.517	1.453
Kazenokomochi	1995	f	f	f	F	54.33	4.16	2.404
Shirokumochi	2007	-	-	-	-	49.33	4.041	2.333
Kitayukimochi	2009	f	f	f	F	50.33	4.16	2.404
Kitafukumochi	2013	f	f	f	F	51	2.65	1.528

Note: ND: Not Detected (These includes landraces, usually the landrace don't have specific breeding years); F and f indicate the functional and loss-of-function alleles in the locus, respectively; The year of breeding obtain from the NARO database (https://ineweb.narcc.affrc.go.jp/), and Shinada et al. 2014.