Theor Appl Genet (2013) 126:611–618
DOI 10.1007/s00122-012-2005-5

ORIGINAL PAPER

# Roles of the *Hd5* gene controlling heading date for adaptation to the northern limits of rice cultivation

**Kenji Fujino · Utako Yamanouchi · Masahiro Yano**

Received: 24 August 2012 / Accepted: 6 October 2012 / Published online: 23 October 2012

**Abstract** During the diversification of cultivated rice after domestication, rice was grown in diverse geographic regions using genetic variations attributed to the combination of alleles in loci for adaptability to various environmental conditions. To elucidate the key gene for adaptation in rice cultivars to the northern limit of rice cultivation, we conducted genetic analyses of heading date using extremely early-heading cultivars. The *Hd5* gene controlling heading date (flowering time) generated variations in heading date among cultivars adapted to Hokkaido, where is the northernmost region of Japan and one of the northern limits of rice cultivation in the world. The association of the *Hd5* genotype with heading date and genetical analysis clearly showed that the loss-of-function *Hd5* has an important role in exhibiting earlier heading among a local population in Hokkaido. Distinct distribution of the loss-of-function *Hd5* revealed that this mutation event of the 19-bp deletion occurred in a local landrace Bouzu and that this mutation may have been selected as an early-heading variety in rice breeding programs in Hokkaido in the early 1900s. The loss-of-function *Hd5* was then introduced into the rice variety Fanny from France and contributed to its extremely early heading under the presence of functional *Ghd7*. These results demonstrated that *Hd5* plays roles not only in generating early heading in variations of heading date among a local population in Hokkaido, but also in extremely early heading for adaptation to northern limits of rice cultivation.

## Introduction

Heading date is a major determinant of rice adaptability to regional and environmental conditions and has been a major target in rice breeding programs. Due to its importance, many quantitative trait loci (QTLs) have been identified using various kinds of cross combinations and several genes have been identified by a positional cloning strategy (Izawa 2007a, b; Alonso-Blanco et al. 2009; Yamamoto et al. 2009; Miura et al. 2011; Tsuji et al. 2011). Previously, a series of QTL mapping using 12 diverse rice cultivars from Asia demonstrated that a large portion of the phenotypic variations in heading date could be generated by combinations of different alleles of QTLs for heading date (Ebana et al. 2011). QTL analysis for extremely late heading in an *indica* Nona Bokra revealed that its strong photoperiod sensitivity was generated mainly by the accumulation of additive effects of particular alleles at QTLs (Uga et al. 2007). These studies demonstrated that genetic variations can be attributed to the combination of alleles in loci involved in heading date.

---

Communicated by L. Xiong.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00122-012-2005-5) contains supplementary material, which is available to authorized users.

K. Fujino
Agricultural Research Institute, HOKUREN Federation of Agricultural Cooperatives, Naganuma, Hokkaido 067-1317, Japan

*Present Address:*
K. Fujino (✉)
NARO Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Hitsujigaoka 1, Toyohira, Sapporo, Hokkaido 062-8555, Japan
e-mail: kfujino@affrc.go.jp

U. Yamanouchi · M. Yano
Agrogenomics Research Center, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan

Cultivated rice, *Oryza sativa* L., originating from a tropical region is a short-day plant and an example of adaptation to an extremely wide range of climatic conditions, from 53°N to 40°S latitude. Hokkaido, 41°29′–45°31′N latitude, is the northern-most region of Japan and one of the northern limits of rice cultivation in the world. Intensive selection on heading date by breeding programs has allowed the cultivation of rice in such higher latitudes with longer natural daylength and lower temperatures. Only varieties with extremely low photoperiod sensitivity were grown in such regions with long natural daylength conditions (Tanisaka et al. 1992; Okumoto et al. 1996). Furthermore, this unique heading behavior may contribute to the genetic differentiation of the local population in Hokkaido from those in other regions of Japan (Nagasaki et al. 2010).

To understand the genetic architecture of extremely early heading in rice, several studies have been performed using cultivars adapted to the northern limits of rice cultivation, such as Hokkaido and Europe (Fujino 2003; Fujino and Sekiguchi 2005a, b, 2008; Nonoue et al. 2008; Shibaya et al. 2011; Fujino and Iwata 2011). These results demonstrated that the loss of two photoperiod sensitivity genes, *qDTH7-1/Ghd7* and *qDTH7-2/Hd2*, was significant to this adaptation (Fujino and Sekiguchi 2005a, b; Nonoue et al. 2008; Shibaya et al. 2011). Among the population with such extremely early heading, variations in heading date were observed. This variation is important to generate different maturity types of rice cultivation in a local region. Previously, we identified several QTLs controlling heading date among varieties from Hokkaido (Fujino 2003; Fujino and Sekiguchi 2008; Nonoue et al. 2008). However, their roles on variations in heading date among the local population were unclear.

A single gene involved in photoperiod sensitivity contributes to differences in heading date, early and medium maturity types, among commercial rice cultivars from Hokkaido (Fujino 2003; Nonoue et al. 2008). This gene collocated with a QTL controlling heading date, *Hd5*, which was identified in the population derived from the cross between Nipponbare and Kasalath (Lin et al. 2003). Results of map-based cloning of the genes *DTH8* (QTL for days to heading on chromosome 8) and *Ghd8* (QTL for grain yield, heading date, and plant height), which are allelic to *Hd5* (Shibaya et al. 2011), revealed that this gene encodes the HAP3 subunit of CCAAT-box-binding transcription factor (Wei et al. 2010; Yan et al. 2011).

Here, we report on the roles of *Hd5* on generating variations in heading date among cultivars in the northern limit of rice cultivation. *Hd5* plays a role on generating early heading in variations of heading date among local populations from Hokkaido and the loss-of-function *Hd5* was selected as a spontaneous mutation during rice breeding programs in Hokkaido. In addition, loss-of-function *Hd5* could contribute to extremely early-heading behavior under the presence of functional *Ghd7*.

## Materials and methods

### Plant materials

A total of 204 rice varieties were used for analysis of the distribution of *Hd5* alleles. Core collections for 63 diverse accessions (Kojima et al. 2005) and 37 landrace type varieties from Japan (Ebana et al. 2008) were included for the analysis (Table S1, S2). In addition, 85, 14, and 5 rice varieties were used from Hokkaido (the northern-most region of Japan), Honshu (the main island of Japan), and Europe, respectively (Table S3, S4). These rice varieties included landrace and modern varieties in each region. The 85 varieties from Hokkaido were selected to cover a wide area of cultivation across Hokkaido from the late 1800s to the present. Previously, we determined the population structure among rice varieties from Japan (Nagasaki et al. 2010; Yonemaru et al. 2012). These results demonstrated that varieties from Hokkaido were clearly assigned to a single population, which was genetically distinct from those from other regions of Japan. Eleven $F_2$ populations ($n = 76$–$100$) derived from crosses between the nine varieties were developed for the genetic analysis of heading date. Seeds of rice varieties were provided by the Genebank of the National Institute of Agrobiological Science (Tsukuba, Japan) and Hokkaido Central Agricultural Experiment Station (Takikawa, Japan).

To confirm the effect of *Hd5* on heading date and photoperiod sensitivity, an advanced backcrossed progeny for *Hd5* was developed. First, a *japonica* Kitaibuki was crossed with a *japonica* Hoshinoyume. The resultant $F_1$ plants were backcrossed with Kitaibuki to obtain $BC_5F_1$ seeds. Among the $BC_5F_2$ population derived from the self-pollination of $BC_5F_1$ plants carrying heterozygous of *Hd5*, a plant carrying homozygous for the functional *Hd5* was selected as a near isogenic line (designated as KTm). In each generation, marker assisted selection (MAS) for *Hd5* was conducted using the PCR primer set 19DEL to detect the 19-bp deletion found in Hayamasari; TCACATGAAGAGTAGGAAGAGCT and TGATGAACTCCGACACGCAC.

All rice varieties and $F_2$ populations were cultivated in an experimental paddy field at HOKUREN Agricultural Research Institute (Naganuma, Hokkaido, Japan, 43°03′N latitude) in 2003, 2004, and 2006. Cultivation conditions were described in Fujino and Sekiguchi (2005a, b, 2008). Sowing and transplanting were performed in late April and late May, respectively. Days to heading (DTH) of the earliest heading panicle among individuals was recorded for each plant as the number of days required from sowing to heading. Leaf samples of each plant were collected for DNA extraction.

### Complementation test

To test the function of *Hd5* on heading date, an approximately 4.2-kb genomic fragment of Nipponbare, which was digested by *Hin*dIII and *Eag*I, was transformed into the pPZP2H-lac binary vector (Fuse et al. 2001). The resultant plasmid was then introduced into Hayamasari by means of *Agrobacterium*-mediated transformation (Toki et al. 2006). The Nipponbare and Hayamasari alleles of *Hd5* are function and loss-of-function, respectively (Shibaya et al. 2011). Two $T_2$ homozygous lines were raised under LD conditions (14.5-h light) and scored their DTH.

### Evaluation of photoperiod sensitivity

Kitaibuki, Hoshinoyume, Fanny, and KTm were grown under three different daylength conditions: 16-h day (16D; 16-h light; 27°C for 12 h and 23°C for 12 h), 14-h day (14D; 14-h light; 27°C for 12 h and 23°C for 12 h), and 10-h day (10D; 10-h light; 27°C for 12 h and 23°C for 12 h) in a controlled growth cabinet (Nihonika, Japan). DTH required from sowing to heading was scored for ten plants per lines.

### DNA analysis

Total DNA was extracted from the leaves of each plant individually according to the CTAB method (Murray and Thompson 1980). Genotyping with SSR and STS markers was performed as described in Fujino et al. (2004). For QTL analysis for heading date, SSR and STS markers listed in Table S5 were used, which were linked to the ten known QTLs for heading date (Yano et al. 1997; Lin et al. 1998, 2002; Yamamoto et al. 2000; Yano et al. 2000; Yano 2001; Doi et al. 2004; Xue et al. 2008).

The sequence of the *Hd5* region including 1.3 kb of the 5′ upstream, 0.9 kb of the coding, and 1.0 kb of the 3′ downstream regions were determined in Kitaibuki and Hoshinoyume from Hokkaido, Italica Livorno from Italy, Arroz Da Terra from Portugal, and Dunghung Shali from Hungary. Amplified DNA fragments from genomic DNA with specific primers were purified and sequenced with the Sanger dideoxy terminator method on capillary sequencers (Applied Biosystems, USA). The sequences of *Hd5* in these varieties were deposited in GenBank as Accession Nos. AB693200–AB693204.

In addition to the functional nucleotide polymorphism (FNP) of the 19-bp deletion, the genotype of three FNPs, the 1, 8, and 1,116-bp deletions (Wei et al. 2010; Yan et al. 2011), were determined in 45 varieties from Hokkaido and 32 varieties in the Japanese landrace core collection. To detect these FNPs, specific primer sets were designed for 1DEL (F: AAGGAGACGGTGCAGGAGT and R: TTGATGGTCTTCCGCTTCTC), 8DEL (F: CCGCTCAAGTCCTACCTCAA and R: TGACCATGGTGTGAGTGTGA), and 1116DEL (F: GTCAGGGAACAAGGCGTACT and R: AGGGCAGTACAACAGCATCC).

### Statistical analysis

The association of the genotype of *Hd5* with heading date among 67 varieties from Hokkaido was calculated by ANOVA. The genotype of *Hd5* was determined using the PCR primer set 19DEL to detect the 19-bp deletion, deletion (Del) or non-deletion (Nd). For QTL analysis of heading date, the mean value of DTH in $F_2$ populations was compared between each homozygous genotype class of parental type by ANOVA according to the procedure described in Fujino and Sekiguchi (2005a, b, 2008). The detection threshold for QTLs in this study was $P < 0.001$.

## Results

### *Hd5* controls heading date

Previously, we mapped a gene controlling heading date on the chromosomal region near *Hd5* on the short arm of chromosome 8 using the $F_2$ population derived from the cross between Hayamasari and Hoshinoyume (Nonoue et al. 2008). It was also reported that the difference in DTH between Kitaibuki and Hoshinoyume was controlled by a single gene (Fujino 2003). Sequence analysis clearly revealed that Kitaibuki carries the loss-of-function *Hd5* with the 19-bp deletion (GenBank accession number AB693201), the same as that of Hayamasari, while Hoshinoyume carries the functional *Hd5* (GenBank accession number AB693200). In addition, the frequency distribution of DTH in the $F_2$ population derived from the cross between Kitaibuki and Hoshinoyume showed continuous variation that could be explained by the genotype of *Hd5* (Fig. 1).

Differences in heading date and photoperiod sensitivity were observed between KTm and its recurrent parent Kitaibuki (Fig. 2). DTH of KTm (110.3 and 91.9) was larger than that of Kitaibuki (99.2 and 86.8) in both years (2003 and 2004, respectively). Kitaibuki showed no clear delay in heading due to increased daylength, while KTm showed delayed heading depending on daylength conditions, similar to Hoshinoyume (Fig. 2).

To confirm that this gene is identical to *Hd5*, the functional *Hd5* was introduced into Hayamasari by means of

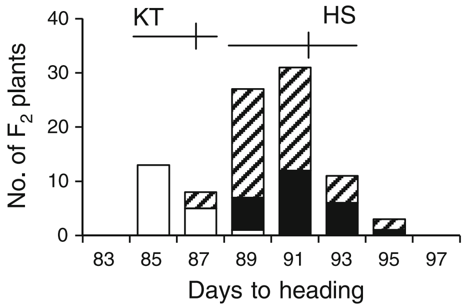


**Fig. 1** Frequency distribution of days to heading in the $F_2$ population ($n = 95$) derived from a cross between Kitaibuki (KT) and Hoshinoyume (HS). *Horizontal and vertical lines* represent ranges and mean values for the parents, respectively. Three classified genotypes assessed by the marker 19DEL for the *Hd5* gene were indicated: *white*, *black*, and *hatched* were homozygous for Kitaibuki and Hoshinoyume, and heterozygous

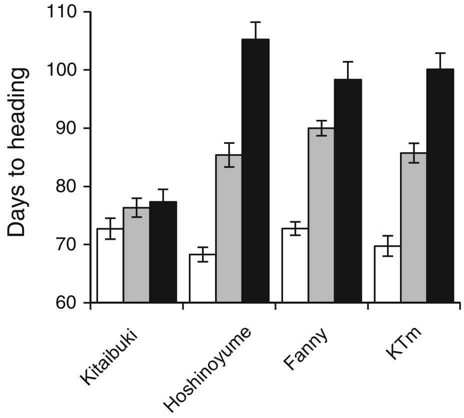


**Fig. 2** Days to heading of rice varieties under three different daylength conditions, 10D (*white*), 14D (*gray*), and 16D (*black*) in a growth chamber. Data are mean values and SD ($n = 10$). KTm is the advanced backcrossed progeny of Kitaibuki with the functional *Hd5*

*Agrobacterium*-mediated transformation. Hayamasari is one of the parents for Kitaibuki and carries the 19-bp deletion in *Hd5* (Shibaya et al. 2011). Transgenic $T_2$ plants exhibited more delayed DTH than that of Hayamasari under LD conditions (Table S6). These results clearly show that allelic variations in *Hd5* such as functional and loss-of-function are major determinants of differences in heading date in Hoshinoyume with Kitaibuki and Hayamasari.

## Distribution of the 19-bp deletion in *Hd5*

To elucidate the distribution of the 19-bp deletion causing loss-of-function of *Hd5*, a total of 204 rice varieties from different populations with wide diversity were surveyed by PCR analysis to detect the deletion (Table 1). Among 85 varieties from Hokkaido, 15 carried the loss-of-function *Hd5* (Table S3), while it was rare in other populations including varieties from Honshu and Japanese landraces (Table S2, S4). The pedigrees of these varieties from Hokkaido were well characterized because these were breeding materials. In the late 1800s, rice cultivation begun in Hokkaido using the variety Akage carrying the functional *Hd5* (Fig. S1). Then, the variety Bouzu was selected by a pure-line selection from the Akage population as a spontaneous awnless mutant. From the Bouzu population, several pure lines were selected as varieties adapting to local environmental conditions in the early 1900s. These varieties carry the functional *Hd5*, while only Bouzu No. 6 carries the loss-of-function *Hd5*, which was known to be an early-heading type at that time (Fig. S1A). These results indicated that the 19-bp deletion in *Hd5* was generated as a spontaneous mutant during rice breeding programs in Hokkaido and was selected as an earlier heading variety.

**Table 1** Summary of the distribution of the loss-of-function *Hd5* among different populations

| Population | No. of varieties | Genotype | |
|---|---|---|---|
| | | Del | Nd |
| Hokkaido, Japan | 85 | 15 | 70 |
| Honshu, Japan | 14 | 0 | 14 |
| Europe | 5 | 1 | 4 |
| Japanese landrace core collection | 37 | 0 | 37 |
| Rice core collection | 63 | 1 | 62 |
| Total | 204 | 17 | 187 |

Genotypes of Del and Nd indicate 19-bp deletion and non-deletion alleles, respectively

The loss-of-function *Hd5* was found in only two varieties other than a local population in Hokkaido. Dianyu1 from China carries the loss-of-function *Hd5* in the rice core collection over the world (Table S1). Dianyu1 is the progenitor of Jianjing6 carrying the loss-of-function *Hd5* (Wei et al. 2010). Norin No. 34 is the progenitor of Dianyu1 and carries the loss-of-function *Hd5*, indicating that the loss-of-function *Hd5* was introgressed into these varieties in China from Norin No. 34 in Hokkaido (Fig. S1B, C). Fanny from France carries the loss-of-function *Hd5* among varieties from Europe (Table S4), in whom the pedigree was unknown. Because the same mutation rarely occurs several times within 10,000 years, we can consider this mutation to be a single event in rice evolution. These results strongly suggested that the loss-of-function *Hd5* generated in Hokkaido was introduced into varieties from China and France by rice breeding programs.

## Role of *Hd5* on variations in heading date among rice varieties from Hokkaido

To elucidate the role of *Hd5* on variations in heading date among rice varieties from Hokkaido, the association of the

**Table 2** Relationships between the genotypes of *Hd5* and heading date among rice varieties from Hokkaido

| Year | Genotype | No. of varieties | Days to heading | | Probability |
|---|---|---|---|---|---|
| | | | Mean | Range | |
| 2003 | Del | 10 | 99.9 | 84.6–102.2 | 0.0100 |
| | Nd | 15 | 108.9 | 95.1–122.9 | |
| 2004 | Del | 13 | 87.5 | 77.9–93.6 | 0.0039 |
| | Nd | 54 | 91.0 | 82.8–104.7 | |

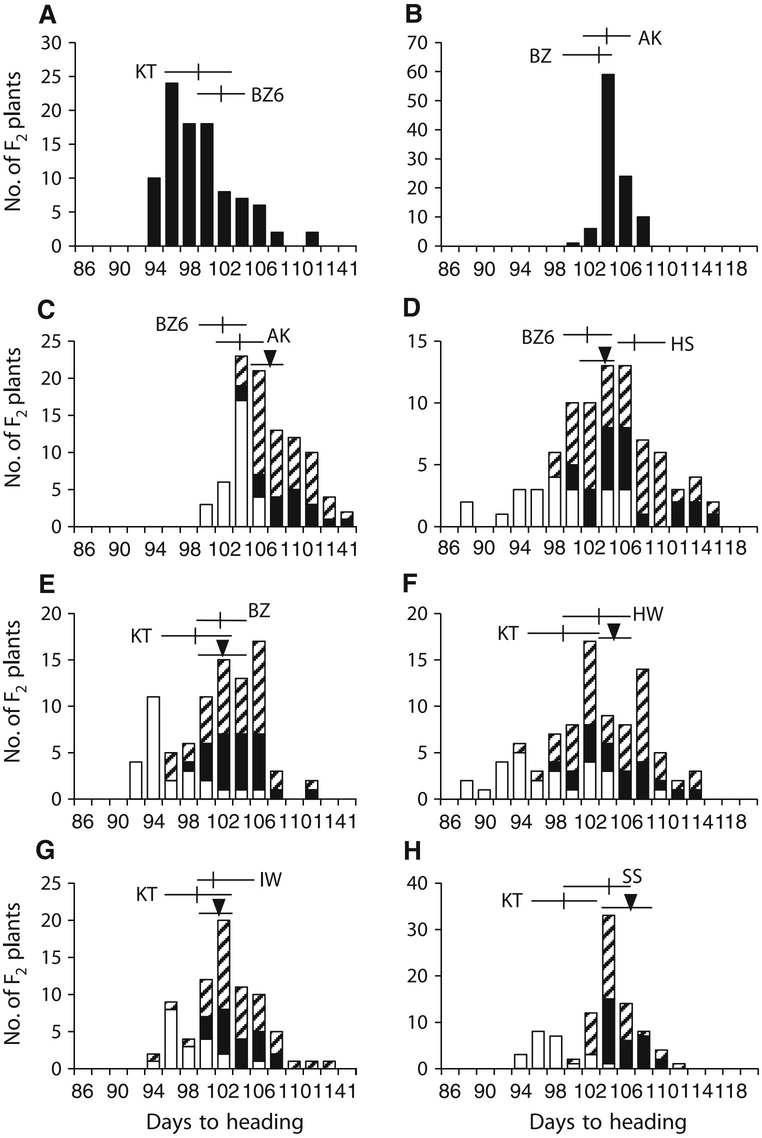
Genotypes of Del and Nd indicate 19-bp deletion and non-deletion alleles, respectively

Probability indicates the difference between the two genotypes calculated by ANOVA

*Hd5* genotype, the 19-bp deletion and non-deletion, with heading date was evaluated. This study was replicated in 2 years: 25 varieties in 2003 and 67 varieties in 2004. A wide variation in DTH was observed among the 67 varieties from Hokkaido, ranging from 77.9 of Norin No. 11 to 104.7 of Kudoumochi in 2004 (Table S3). A significant difference in DTH was detected between the two *Hd5* groups of the 19-bp deletion and non-deletion, 87.5 and 91.0, respectively ($P = 0.0039$) (Table 2). In addition to the FNPs of the 19-bp deletion, three FNPs have been identified in *Hd5/DTH8/GHD8* (Wei et al. 2010; Yan et al. 2011). Only the 1-bp deletion was found in four varieties among the Japanese landrace core collection (Table S2), while no FNP was found in a local population in Hokkaido other than the 19-bp deletion. Results strongly suggested that the loss-of-function *Hd5* has a major role on generating early heading in variations of heading date among this local population.

Results of genetical analysis also indicated that the loss-of-function *Hd5* is a major determinant of early heading

**Fig. 3** Frequency distributions of days to heading in eight $F_2$ populations. **a** Bouzu No. 6 (BZ6)/Kitaibuki (KT), $n = 96$. **b** Bouzu (BZ)/Akage (AK), $n = 100$. **c** Akage (AK)/Bouzu No. 6 (BZ6), $n = 95$. **d** Bouzu No. 6 (BZ6)/Hoshinoyume (HS), $n = 83$. **e** Bouzu (BZ)/Kitaibuki (KT), $n = 87$. **f** Kitaibuki (KT)/Hokkaiwase (HW), $n = 89$. **g** Kitaibuki (KT)/Iburiwase (IW), $n = 76$. **h** Sasahonami (SS)/Kitaibuki (KT), $n = 92$. *Horizontal and vertical lines* represent ranges and mean values for the parents, respectively. Three classified genotypes assessed by the marker for the *Hd5* gene were indicated: *white*, *black*, and *hatched* were homozygous for the deletion and non-deletion, and heterozygous. *Arrowhead* indicates the mean values of $F_1$ plants ($n = 4$–$7$)



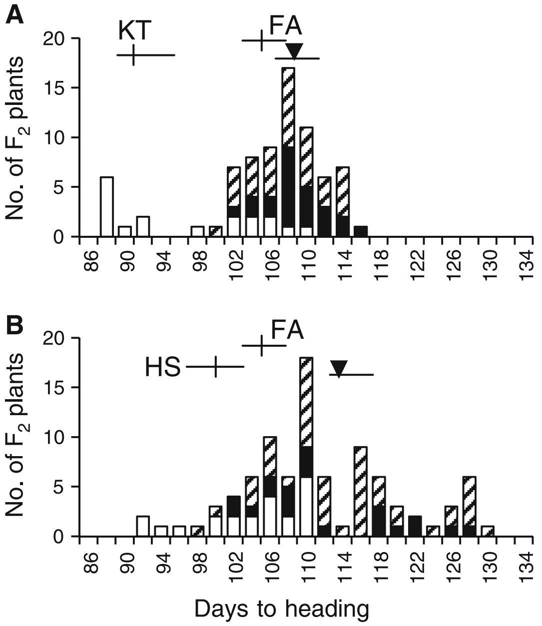


**Fig. 4** Frequency distributions of days to heading in $F_2$ populations of Fanny. **a** Kitaibuki (KT)/Fanny (FA), $n = 77$. **b** Hoshinoyume (HS)/Fanny (FA), $n = 90$. *Horizontal and vertical lines* represent ranges and mean values for the parents, respectively. Three classified genotypes assessed by the markers RM3859 (**a**) and 19DEL (**b**) for *Ghd7* and *Hd5* genes, respectively, were indicated: *white*, *black*, and *hatched* were homozygous for KT/HS and FA, and heterozygous. *Arrowhead* indicates the mean values of $F_1$ plants ($n = 6$)

among this local population. The frequency distribution of DTH was narrow and within the ranges of the parents in the $F_2$ population of Bouzu/Akage, both of whose *Hd5* is non-deletion, (Fig. 3b). Although a late transgressive segregation was observed in the $F_2$ population of Akage/Bouzu No. 6, three genotype classes of *Hd5* were clearly correlated with DTH; the homozygous of the loss-of-function and the functional *Hd5* showed early and late heading, respectively (Fig. 3c). Only a single QTL corresponding to *Hd5* was detected in this $F_2$ population (Table S5, S6). These results strongly supported that the loss-of-function *Hd5* occurred in Bouzu and had been selected as the variety Bouzu No. 6 with earlier heading (Fig. S1A).

In an $F_2$ population of Bouzu No. 6/Kitaibuki, both with the 19-bp deletion of *Hd5*, no early transgressive segregation was observed, indicating that the loss-of-function *Hd5* contributed to early heading in both varieties (Fig. 3a). The same results were also obtained in four $F_2$ populations derived from crosses between landrace and modern varieties (Fig. 3d–g). QTL analysis revealed that only two loci corresponding to *Hd1* and *Hd5* contributed to variations in heading date (Table S5, S7). The loss-of-function *Hd5* decreased DTH in these four populations (Table S7), and almost all of the early transgressive segregants carried the loss-of-function *Hd5*. Furthermore, bimodal distribution of DTH was clearly correlated with *Hd5* genotypes in the $F_2$ population of Sasahonami/Kitaibuki (Fig. 3h).

### Role of *Hd5* in the rice variety Fanny from France

Although Fanny carries the loss-of-function *Hd5*, Fanny showed a similar photoperiod sensitivity to Hoshinoyume (Fig. 2) and DTH of Fanny (104.4) was later than that of Hoshinoyume (99.8) in natural field conditions. To elucidate the role of the loss-of-function *Hd5* in the heading date of Fanny, QTL analysis of DTH was conducted. The frequency distribution of DTH in the $F_2$ population of Kitaibuki/Fanny was bimodal (Fig. 4a). Only a single QTL corresponding to *Ghd7* was detected (Table S5, S8). The Fanny allele of QTL increased DTH. In contrast, a wide variation in DTH including early and extremely late heading transgressive segregations was observed in the $F_2$ population of Hoshinoyume/Fanny (Fig. 4b). Three QTLs corresponding to *Hd1*, *Hd5*, and *Ghd7* were detected (Table S5, S8). The Fanny alleles at QTLs for *Ghd7* increased DTH, while the Hoshinoyume alleles at QTLs for *Hd1* and *Hd5* increased DTH.

Based on the QTL analysis with the sequence of *Hd1*, *Hd5*, and *Ghd7* or FNPs typing for *Hd5* (the 19-bp deletion) and *Ghd7* (a premature stop codon), the genotypes of these genes in Kitaibuki (*Hd1hd5ghd7*), Hoshinoyume (*Hd1Hd5ghd7*), and Fanny (*hd1hd5Ghd7*) were identified. Due to the low resolution of QTL analysis including population size and epistatic interactions of QTLs, QTL could not be detected on the *Hd1* locus in the $F_2$ population of Kitaibuki (*Hd1*) and Fanny (*hd1*) (Table S8). Extremely late heading transgressive segregation was observed in the $F_2$ population of Fanny with Hoshinoyume, but not in that with Kitaibuki (Fig. 4). These results revealed that extremely early heading in Fanny was dependent on the loss-of-function *Hd5* under the presence of functional *Ghd7*.

## Discussion

Agronomic traits have been the targets of selection during crop domestication and diversification processes and breeding programs. Identification of the genes that control the range of these trait variations could present opportunities to study phenotypic diversification among cultivated crops. This study can give us some insights into the diversification of the traits in breeding programs for shaping adaptability to local regions. This study demonstrated that *Hd5* has a role in generating early heading in variations of heading date among local populations from Hokkaido and loss-of-function *Hd5* could contribute to extremely early-heading behavior under the presence of functional *Ghd7*.

QTLs for heading date including *Hd5* have been identified using several populations between landrace and modern varieties from Hokkaido (Fujino and Sekiguchi 2008; Nonoue et al. 2008). However, it was difficult to understand how these QTLs contributed to variations of heading date among the local population due to the limited number of populations examined. QTL analysis can identify genes by differences between parental varieties. Therefore, once the corresponding gene to QTL has been identified at the molecular level, it would be possible to elucidate the role of the gene in the variation of traits among populations. A series of genetic analyses and a survey of the *Hd5* genotype in this study clearly demonstrated that *Hd5* has a role in generating early heading in variations of heading date among this local population.

Distribution of the loss-of-function *Hd5* revealed that the mutation event of the 19-bp deletion occurred in the landrace Bouzu in the early 1900s. This spontaneous mutation exhibiting earlier heading has been selected as the variety Bouzu No. 6 (Fig. S1A). Cultivation areas of Bouzu were limited within south and south-western regions of Hokkaido, while Bouzu No. 6 could be grown in northern and eastern regions of Hokkaido, where rice never grew at that time. Human demands that enlarge rice cultivation areas had selection pressure for such mutations exhibiting earlier heading. In the process of shaping the adaptability to unique environmental conditions, desirable genes may have accumulated into the local population using standing alleles and newly occurred alleles. These varieties with unique adaptability would be useful in identifying novel genes for adaptability.

This study also demonstrated that the utility of the loss-of-function *Hd5* selected in Hokkaido takes a new genotype for heading date under a different local population, France. Previously, we identified genes for extremely early heading and for variations in heading date among a local population in Hokkaido. Loss-of-function of *qDTH7-1*/*Ghd7* has been shown to be important for extremely early heading in varieties from both Hokkaido and Europe (Fujino and Sekiguchi 2005b; Nonoue et al. 2008; Xue et al. 2008; Shibaya et al. 2011). Allelic variations in *Hd5* are important for variations in heading date among a local population in Hokkaido, which carries the loss-of-function *Ghd7*, *ghd7* (Fujino 2003; Nonoue et al. 2008). In this study, we identified a new genotype exhibiting extremely early heading other than *ghd7*. Under the presence of functional *Ghd7*, Fanny showed extremely early heading due to the loss-of-function *Hd5*. Because *Hd5* exhibited epistatic interactions with *Hd1* and *Ghd7*, *Hd5* showed these different effects on heading date (Lin et al. 2003; Gu and Foley 2007; Nonoue et al. 2008; Xue et al. 2008; Yan et al. 2011).

For the adaptation of rice to the northern limits of rice cultivation, *Hd5* has these roles in different genetic backgrounds. The understanding of genetic bases for adaptation to the local region and for controlling heading date in rice can design new genotypes with desirable heading dates. On the other hand, genes for heading date regulate agronomic traits including plant height and yield potentials (Xue et al. 2008; Wei et al. 2010; Yan et al. 2011; Endo-Higashi and Izawa 2011). Different genotypes for extremely early heading have the possibility to develop varieties with different agronomic traits and heading habits.

**Acknowledgments** We are grateful to Kazuo Ise (Japan International Research Center for Agricultural Sciences, Japan) and Professor Dai Luyuan (Yunnan Academy of Agricultural Sciences, China) for information on the pedigree of rice varieties from China.

## References

Alonso-Blanco C, Aarts MG, Bentsink L, Keurentjes JJ, Reymond M et al (2009) What has natural variation taught us about plant development, physiology, and adaptation? Plant Cell 21:1877–1896

Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T et al (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls *FT-like* gene expression independently of *Hd1*. Genes Dev 18:926–936

Ebana K, Kojima Y, Fukuoka S, Nagamine T, Kawase M (2008) Development of mini core collection of Japanese rice landrace. Breed Sci 58:281–291

Ebana K, Shibaya T, Wu J, Matsubara K, Kanamori H et al (2011) Uncovering of major genetic factors generating naturally occurring variation in heading date among Asian rice cultivars. Theor Appl Genet 122:1199–1210

Endo-Higashi N, Izawa T (2011) Flowering time genes *Heading date 1* and *Early heading date 1* together control panicle development in rice. Plant Cell Physiol 52:1083–1094

Fujino K (2003) Photoperiod sensitivity gene controlling heading date in rice cultivars in the northernmost region of Japan. Euphytica 131:97–103

Fujino K, Iwata N (2011) Identification of QTLs controlling heading date on the short arm of chromosome 3 in rice (*Oryza sativa* L.). Breed Sci 61:294–300

Fujino K, Sekiguchi H (2005a) Identification of QTLs conferring genetic variation for heading date among rice varieties at the northern-limit of rice cultivation. Breed Sci 55:141–146

Fujino K, Sekiguchi H (2005b) Mapping of QTLs conferring extremely early heading in rice (*Oryza sativa* L.). Theor Appl Genet 111:393–398

Fujino K, Sekiguchi H (2008) Mapping of quantitative trait loci controlling heading date among rice cultivars in the northernmost region of Japan. Breed Sci 58:367–373

Fujino K, Sekiguchi H, Sato T, Kiuchi H, Nonoue Y et al (2004) Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). Theor Appl Genet 108:794–799

Fuse T, Sasaki T, Yano M (2001) Ti-plasmid vectors useful for functional analysis of rice genes. Plant Biotechnol 18:219–222

Gu XY, Foley ME (2007) Epistatic interactions of three loci regulate flowering time under short and long daylengths in a backcross population of rice. Theor Appl Genet 114:745–754

Izawa T (2007a) Adaptation of flowering-time by natural and artificial selection in Arabidopsis and rice. J Exp Bot 58:3091–3097

Izawa T (2007b) Daylength measurements by rice plants in photoperiodic short-day flowering. Int Rev Cytol 256:191–222

Kojima Y, Ebana K, Fukuoka S, Nagamine T, Kawase M (2005) Development of an RFLP-based rice diversity research set of germplasm. Breed Sci 55:431–440

Lin SY, Sasaki T, Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. Theor Appl Genet 96:997–1003

Lin HX, Ashikari M, Yamanouchi U, Sasaki T, Yano M (2002) Identification and characterization of a quantitative trait locus, *Hd9*, controlling heading date in rice. Breed Sci 52:35–41

Lin HX, Liang Z-W, Sasaki T, Yano M (2003) Fine mapping and characterization of quantitative trait loci *Hd4* and *Hd5* controlling heading date in rice. Breed Sci 53:51–59

Miura K, Ashikari M, Matsuoka M (2011) The role of QTLs in the breeding of high-yielding rice. Trends Plant Sci 16:319–326

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl Acid Res 8:4321–4325

Nagasaki H, Ebana K, Shibaya T, Yonemaru J, Yano M (2010) Core single-nucleotide polymorphisms—a tool for genetic analysis of the Japanese rice population. Breed Sci 60:648–655

Nonoue Y, Fujino K, Hirayama Y, Yamanouchi U, Lin S, Yano M (2008) Detection of quantitative trait loci controlling extremely early heading in rice. Theor Appl Genet 116:715–722

Okumoto Y, Ichitani K, Inoue H, Tanisaka T (1996) Photoperiod insensitivity gene essential to the varieties grown in the northern limit region of paddy rice (*Oryza sativa* L.) cultivation. Euphytica 92:63–66

Shibaya T, Nonoue Y, Ono N, Yamanouchi U, Hori K, Yano M (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

Tanisaka T, Inoue H, Uozu S, Yamagata H (1992) Basic vegetative growth and photoperiod sensitivity of heading-time mutants induced in rice. Jpn J Breed 42:657–668

Toki S, Hara N, Ono K, Onodera H, Tagiri A, Oka S et al (2006) Early infection of scutellum tissue with *Agrobacterium* allows high-speed transformation of rice. Plant J 47:969–976

Tsuji H, Taoka K, Shimamoto K (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. Curr Opin Plant Biol 14:45–52

Uga Y, Nonoue Y, Liang ZW, Lin HX, Yamamoto S, Yamanouchi U, Yano M (2007) Accumulation of additive effects generates a strong photoperiod sensitivity in the extremely late-heading rice cultivar 'Nona Bokra'. Theor Appl Genet 114:1457–1466

Wei X, Xu J, Guo H, Jiang L, Chen S et al (2010) *DTH8* suppresses flowering in rice, influencing plant height and yield potential simultaneously. Plant Physiol 153:1747–1758

Xue WY, Xing YZ, Weng XY, Zhao Y, Tang WJ et al (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nat Genet 40:761–767

Yamamoto T, Lin HX, Sasaki T, Yano M (2000) Identification of heading date quantitative trait locus *Hd6* and characterization of its epistatic interactions with *Hd2* in rice. Genetics 154:885–891

Yamamoto T, Yonemaru J, Yano M (2009) Towards the understanding of complex traits in rice: substantially or superficially? DNA Res 16:141–154

Yan WH, Wang P, Chen HX, Zhou HJ, Li QP et al (2011) A Major QTL, *Ghd8*, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. Mol Plant 4:319–330

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

Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T (1997) Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. Theor Appl Genet 95:1025–1032

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L et al (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12:2473–2483

Yonemaru J, Yamamoto T, Ebana K, Yamamoto E, Nagasaki H et al (2012) Genome-wide haplotype changes produced by artificial selection during modern rice breeding in Japan. PLoS ONE 7:e32982