



# Establishment of adaptability to the northern-limit of rice production

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## Abstract

The domestication of cultivated crops from their wild relatives narrowed down their genetic diversity in a bottleneck effect. Subsequently, the cultivation areas of crops have expanded all over the world into various environmental conditions from the original area along with human migration after domestication. Here, we demonstrated the genetic changes in the adaptation of rice to Hokkaido (41°2–45°3N latitude), Japan, from the tropics of their origin in Asian cultivated rice, *Oryza sativa* L. Although cultivated rice originated from the tropics, Hokkaido is one of the northern-limits of rice cultivation worldwide. Population genomics focusing on the local populations showed the varieties had genetically distinct classes with limited genetic diversity. In addition, some varieties in the class carried unique genotypes for flowering time, exhibiting extremely early flowering time. Certain mutations in unique genotypes can split off the varieties that are able to grow in Hokkaido. Furthermore, the changes in the genotype for flowering time during rice cultivation in Hokkaido demonstrated novel combinations of genes for flowering time owing to the intensive artificial selection on natural variation and rice breeding programs to achieve stable rice production in Hokkaido.

**Keywords** Natural variation · Plant breeding programs · Artificial selection · Adaptability · Flowering time · Rice

## Introduction

Cultivated crop species have been grown all over the world, whereas the wild species of cultivated crops grow in their original areas. The genetic diversity of local gene pools has been narrowed down through various genetic bottlenecks, such as domestication from wild species, adaptability to local environmental conditions including country and latitudinal levels, and human demands in local culture (Tenailon et al. 2004; Hyten et al. 2006; Haudry et al. 2007). Crop domestication has produced dramatic changes in morphological traits of plant architecture to meet human demands (Doebley et al. 2006; Ross-Ibarra et al. 2007; Purugganan

and Fuller 2009). Therefore, it is unclear which genetic mechanisms drive the evolution of the wide adaptability of crops in agriculture.

Asian cultivated rice, *Oryza sativa* L., was domesticated from *O. rufipogon* around 10,000 years ago (Fuller 2011; Huang et al. 2012; Yang et al. 2012; Choi et al. 2017) (Fig. 1a). Rice is the principal staple food responsible for feeding people worldwide. The geographic distribution of *O. rufipogon* is limited to tropical and subtropical Asia, around 10–20°N, (Khush 1997), whereas cultivated rice is distributed across an extremely wide range of climatic conditions, from 53°N to 40°S latitude, due to the extensive efforts of rice breeding programs and through human migration all over the world (Lu and Chang 1980; Agrama et al. 2010). Hokkaido (41°2–45°3N latitude) is the northern-most region of Japan and one of the northern-limits of rice cultivation (Fig. 1a). The climatic conditions of Hokkaido, including a natural daylength of more than 15 h, are severe in terms of rice growth. During the last century, genetic improvements in varieties and the modernization of cultivation systems have enabled the stable production of rice in Hokkaido (Shinada et al. 2014; Fujino et al. 2015, 2017).

Flowering time control may play a role in adaptive responses to changing environmental conditions.

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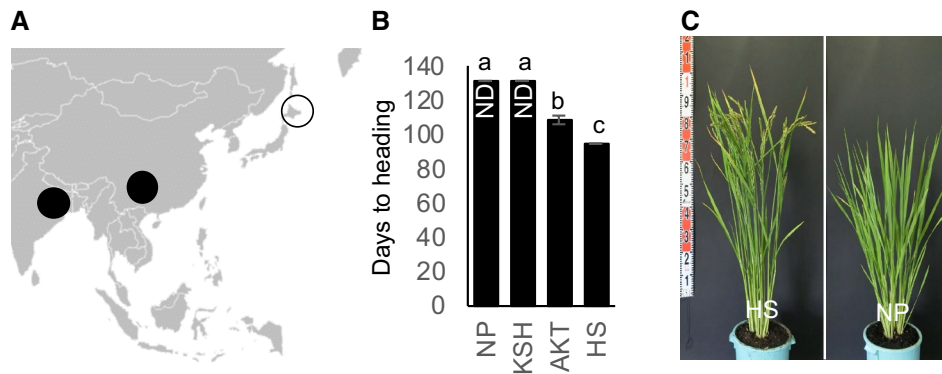
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**Fig. 1** Extremely early flowering time of rice varieties in Hokkaido. **a** Locations of Ganges and Yangtze, where domestication of Asian cultivated rice (closed circle) started, and one of the northern-limits of rice cultivation in the world, Hokkaido (open circle). **b** Days to heading (DTH) of Nipponbare (NP), Koshihikari (KSH), Akitakomachi

(AKT), and Hoshinoyume (HS) in natural field conditions in Hokkaido, 2012. ND; not determined due to lateness, > 130 days. Different letters indicate significant differences ( $P < 0.05$ ; Tukey's significant difference test). **c** Rice varieties HS (left) and NP (right) grown for 130 days in a natural paddy field in Hokkaido

Photoperiodic sensitivity is the most important factor in determining flowering time (expressed as the heading date in crops), which ensures crop adaptation to specific ecological conditions and environmental variations. Mutations generated during the expansion of crop cultivation, including maize, wheat, and barley, may have accelerated the variation in flowering time and driven the spread of such crops all over the world (Turner et al. 2005; Beales et al. 2007; Faure et al. 2012; Hung et al. 2012; Zakhrabekova et al. 2012; Yang et al. 2014; Huang et al. 2018).

Because of its small genome size and importance as a model crop, the molecular and genetic networks of flowering time control in short-day rice have been proposed (Tsuji et al. 2011; Hori et al. 2016). However, it was unclear which genes contribute to the adaptability to local environmental conditions. Varieties cultivated in Hokkaido have unique characteristics, including extremely low photoperiod sensitivity to avoid low-temperature stress at the maturing stage in autumn in Hokkaido (Tanisaka et al. 1992; Okumoto et al. 1996). Previously, we demonstrated the genetic basis of extremely early flowering time involving the loss of two photoperiod sensitivity genes, *qDTH7-1/Ghd7* (Xue et al. 2008) and *qDTH7-2/Hd2/OsPRR37* (Koo et al. 2013; Gao et al. 2014) was significant for the adaptability of rice to cultivation in Hokkaido (Fujino and Sekiguchi 2005a, b; Nonoue et al. 2008; Shibaya et al. 2011; Fujino et al. 2019).

Here, we demonstrated the evolutionary process of the establishment of genotypes for the extremely early flowering time with good fitness for agriculture. Four flowering time genes have played to the extremely early flowering time. First is *Heading date 1 (Hd1)*, which is the rice ortholog of the *Arabidopsis* floral activator *CONSTANS* (Yano et al. 2000). Second is *Grain number, plant height and heading date 7 (Ghd7)* encoding a CCT (CO, CO-LIKE, and

*TIMING OF CAB1*) domain protein (Xue et al. 2008). Third is *Oryza sativa Pseudo-Response Regulator37 (OsPRR37)*, which is an ortholog of the circadian clock genes *PRR3/7* in *Arabidopsis* (Nakamichi et al. 2005; Murakami et al. 2007; Koo et al. 2013; Gao et al. 2014). Finally, fourth is *Days to heading 8 (DTH8)* encoding a putative HAP3 subunit of the CCAAT-box-binding transcription factor (Wei et al. 2010). The extremely early flowering time has a pleiotropic effect on increased panicle number, thereby limiting grain yield. The fitness of the negative correlation between flowering time and panicle number could make rice cultivation possible in Hokkaido. Finally, we discuss the potential of current rice breeding programs for generating the novel genotype for flowering time, and for expanding rice cultivation areas.

## Materials and methods

### Plant material and growth conditions

Three rice populations were used in this study. First, 50 varieties from the Japanese rice core collection (JRC) were used (Table S1), which represented the genetic diversity among the ancestral gene pool of varieties bred in rice breeding programs in Japan (Ebana et al. 2008). Second, 43 varieties were collected from the Genebank, Japan (<http://www.gene.affrc.go.jp/>), as the Hokkaido Landrace Rice Panel (HLP) (Table S2). HLP involved varieties with adaptability to Hokkaido (aHLP) and without adaptability to Hokkaido (nHLP). Because these varieties were stored as landraces in Hokkaido, it was unclear which varieties exhibit the adaptability. The third population was 62 varieties of the Hokkaido Rice Core Panel (HRCP) representing the genetic diversity of the 100-year span of rice breeding programs

in Hokkaido (Shinada et al. 2014; Fujino et al. 2015a, b, 2017) (Table S3). In addition, six near isogenic lines (NILs) for genes controlling flowering time were used as a series with Hoshinoyume (HS) as a genetic background. HSGhd7, HShd1, and HShd5 have already been described (Fujino et al. 2013, 2019). *Hd5* is an allelic to *DTH8* (Wei et al. 2010; Fujino et al. 2013). HSOsPRR37 was derived from repeated backcrossing between HS and Arroz Da Terra with marker-assisted selection (MAS) using RM1306 linked to *OsPRR37* (Fujino and Sekiguchi 2005). Three rice varieties, Akitakomachi (AKT) from Akita, Koshihikari (KSH) from Niigata, and Nipponbare (NP) from Aichi, which are popular in Japan, were used as controls (Fig. S1).

All rice materials were cultivated in experimental paddy fields at Hokkaido Agricultural Research Center, Sapporo, Japan (43°00'N latitude), in accordance with Fujino et al. (2015a, b). Sowing and transplanting were performed in late April and late May, respectively. All materials were planted with a 15.0-cm spacing between plants within each row and 30.0-cm spacing between rows. Cultivation management followed the standard procedures used at Hokkaido Agricultural Research Center.

## Phenotype evaluation

Seven agronomic traits (Table S4), days to heading (DTH), culm length (CL), panicle length (PL), panicle number (PN), 1000-grain weight (TGW), low-temperature germination (LTG), and seeds per panicle (SP), were measured in accordance with Fujino et al. (2017).

## DNA analysis

Total DNA was isolated from young leaves using the CTAB method (Murray and Thompson 1980). A total of 36 Indel markers (Fujino et al. 2015) were used for phylogenomic analysis with the JRC and HLP cultivars (Table S5). The genotype of three genes, *Hd1*, *OsPRR37*, and *Ghd7* controlling flowering time, were determined by PCR, STS, and CAPS, targeted for functional nucleotide polymorphism

(Tables 1, S6). PCR and sequencing were performed as described by Fujino et al. (2004, 2005).

## Data analysis

Phylogenetic analysis with the PHYLIP software suite (Felsenstein 1989) and STRUCTURE program ver. 2.3.4 (Pritchard et al. 2000; Evanno et al. 2005) was performed as described by Shinada et al. (2014). The polymorphism information content (PIC) was calculated for each marker in accordance with Nei (1973):  $PIC = 1 - \sum h_k^2$ , where  $h_k$  is the frequency of  $k$ th allele.

## Results

### Extremely early flowering time in rice varieties adapted to Hokkaido

To characterize the flowering time of varieties from different local breeding programs, four rice varieties, HS, AKT, NP, and KSH, were grown in natural environmental conditions in Hokkaido. In the paddy field in Tsukuba, Japan (36.3°N), the flowering time of HS and NP were approx. 80 and 110 days, respectively (Nonoue et al. 2008). Whereas only HS could exhibit flowering time to mature rice seeds in agriculture, 95.0 of DTH (Fig. 1b, c). Flowering time of AKT was 108.6 days, and the flowering times of KSH and NP were more than 130 days (Fig. 1b, c).

### Genetic population structure of rice varieties adapted to Hokkaido

Phylogenetic analysis was conducted to understand the genetic relationship of two different populations, the JRC and HLP collections. A UPGM dendrogram using Nei's distance showed that there were three groups, I, II, and III (Fig. 2). The JRC was divided into two major groups, groups II and III, which corresponded to upland and lowland habitats, respectively (Kojima et al. 2005).

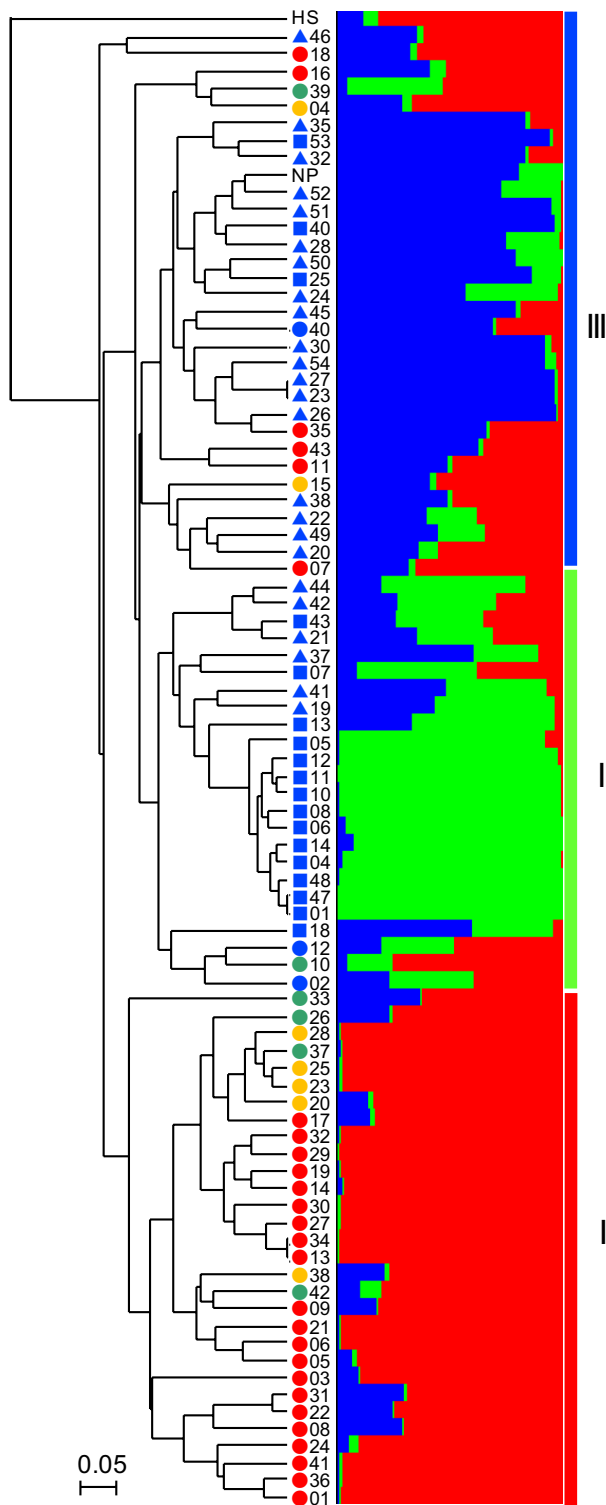
**Table 1** Definition of *Hd1* allele

Allele	Function	Origin of variety	Polymorphism		Size (bp)	References
			Region	Insertion/deletion		
G	F	Ginbouzu	–	–	–	Yano et al. (2000)
NP	F	Nipponbare	First exon	Deletion	36	Yano et al. (2000)
H43	f	HS66	First exon	Insertion/deletion	43/36	Yano et al. (2000)
HW	f	Hokkaiwase	First exon	Insertion	156	AB300056
AK	f	Akage	First exon	Insertion	312	AB300058

The sequence of Ginbouzu is used as the control

Accession no. in the DDBJ is shown in the reference

F and f indicate functional (*Hd1*) and loss-of-function (*hd1*) alleles in *Hd1*, respectively



**Fig. 2** UPGMA dendrogram and population structures of varieties in the JRC and the HLP with controls, Nipponbare (NP) and Hoshinoyume (HS). The varieties were divided into three groups, I–III. Triangles and squares indicate the growth habits, lowland and upland, of varieties in the JRC, respectively. Circles indicate varieties in the HLP. Colors indicated the genotype of the two genes, *Ghd7* and *OsPRR37*; *ghd7ospr37* (red), *Ghd7ospr37* (orange), *ghd7osPRR37* (green) and *Ghd7osPRR37* (blue). Numbers following the figure indicate the serial number of each population. (Color figure online)

HLP varieties were clearly distinguished from those in the JRC. Most varieties in the HLP, 30 of 43, were classified into group I. Three and nine varieties were classified into groups II and III, respectively. The three populations with  $K=3$  in the STRAUCURE analysis (Fig. S2) corresponded well with the three groups obtained by phylogenetic analysis. Degree on admixtures of genetic structures between each group, I/II and II/III, were observed, showing genetic relationships among each group. Genetic diversity represented as PIC varied (Table S7). The PIC value of group I, 0.16, was lower than those of group II, 0.25, and group III, 0.32, suggesting that there may have been genetic bottlenecks caused by intensive artificial selection on natural variation adapted to Hokkaido.

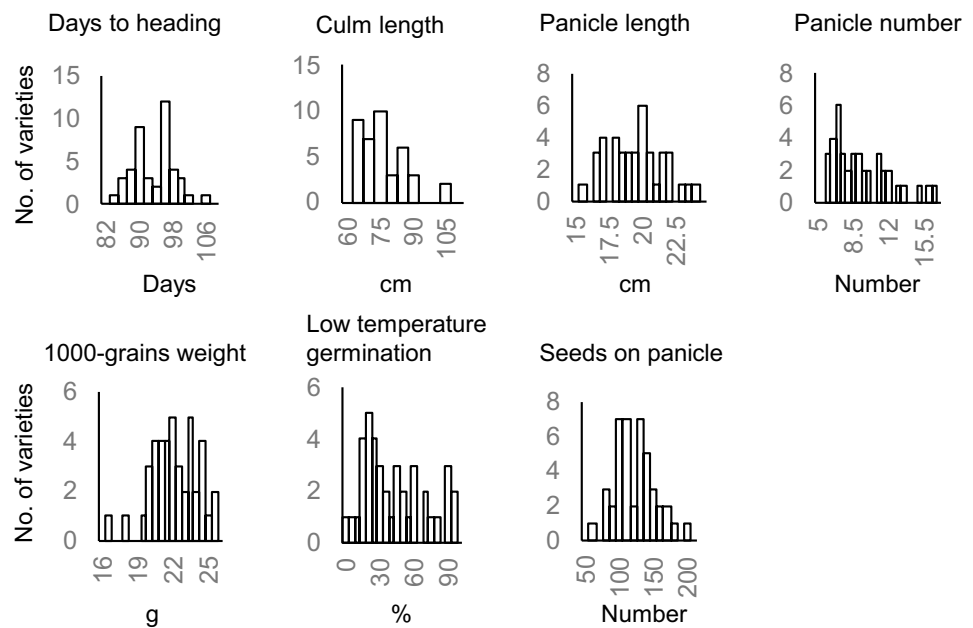
### Phenotypic variation among HLP varieties

Genetic differences between ancestral and selected individuals (Fig. 2) may contribute to phenotypic yield differences in agriculture. To elucidate which traits were targeted by artificial selection, seven traits were evaluated among the HLP varieties (Table S8, Fig. 3). A wide range of phenotypic variation was observed, with continuous distributions in five traits, whereas relatively bimodal distributions were observed in DTH and CL, which are pleiotropic and interact in each other.

Next, we determined the genotype for flowering time, *Ghd7* and *OsPRR37*, which are known to be involved in the expression of extremely early flowering time (Fujino and Sekiguchi 2005a, b; Fujino et al. 2019). The genotype was clearly correlated with phenotypic variation in DTH among HLP varieties and phylogenetic classification (Fig. 2, Table S9). Most varieties in group I, 21/30, had *ghd7ospr37*. Whereas *ghd7osPRR37* and *Ghd7ospr37* were only present in four and five varieties in group I. *Ghd7osPRR37* was not found in group I.

To investigate whether variation has the potential to confer a selectable fitness advantage, all seven agronomic traits were compared between varieties with/without *ghd7ospr37*. We found that significant differences were detected in only DTH and CL (Table S10). Agronomic traits are results of interactions between QTLs or between QTLs and the particular genetic and environmental background (Li et al. 2003; Ando et al. 2008). In addition, genes for flowering time control the traits for yield (Xue et al. 2008; Endo-Higashi and Izawa 2011; Koo et al. 2013; Gao et al. 2014; Tsuji et al. 2015). We found relatively low coefficient values between DTH/CL and other traits,  $-0.37 < r < 0.47$  (Table S11) among aHLP. We could not find a significant correlation of DTH with agronomic traits among varieties with *ghd7ospr37*, indicating that extremely early flowering time had no drastic changes in phenotype.

**Fig. 3** Frequency distributions of seven agronomical traits for plant architecture among HLP varieties



### Phenotype of NILs for flowering time

To elucidate the role of genes for flowering time on agronomic traits, we compared six agronomic traits among six NILs (Tables 2, S12). The DTH of each line was significantly different depending on the genotype for flowering time. The NILs did not exhibit drastic changes in plant architecture along with genotype or DTH. In addition, each NIL exhibited significant differences in the agronomic traits compared with HS of the genetic background (Table 2).

The selection of the best suitable lines for local environmental conditions could have pleiotropic effects on not only flowering time but also grain yield in agriculture. Compared with HS, functional *Ghd7* elongated PL and reduced PN and functional *OsPRR37* and loss-of-function *hd1* reduced PN significantly (Table 2). Whereas

loss-of-function *hd5* increased PN and SP and reduced PL and CL, HShd5 exhibited the earliest flowering time among the NILs (Table 2). There were trends for alterations in agronomic traits along with DTH (Table S13). Depending on DTH, three traits and PN were positively and negatively correlated with higher coefficient values (Table S13). Rice panicle phenotypes including PN limit yield and grain quality and are under selection in rice breeding programs. NILs for earlier flowering time tend to have greater PN.

Genes for flowering time may coordinately regulate DTH and PN as yield potential (Wang and Li 2008; Liang et al. 2014; Wang et al. 2015a, b). Variations in flowering time and yield may be intensively selected to maximize grain production for local environmental conditions. Pleiotropic effects of genes for flowering time on yield impacted on adaptation to new local environmental conditions.

**Table 2** Phenotypic values of six agronomic traits in the NILs

Variety	DTH	TN	PN	PL	CL	SP
HS	95.2 ± 1.0b	26.0 ± 2.9	23.2 ± 1.6	15.5 ± 0.5	73.0 ± 2.5	79.2 ± 7.2
HSGhd7	131.0 ± 0.0e	28.2 ± 1.3 <sup>ns</sup>	19.0 ± 2.7*	17.1 ± 1.1*	73.2 ± 1.7 <sup>ns</sup>	90.0 ± 9.7 <sup>ns</sup>
HShd5	80.1 ± 0.5a	31.6 ± 5.0 <sup>ns</sup>	32.4 ± 4.5**	12.4 ± 0.9***	53.1 ± 1.2***	38.6 ± 5.2***
HSGhd7hd5	101.3 ± 1.0c	22.6 ± 1.5*	18.6 ± 1.1***	16.7 ± 0.5**	74.0 ± 1.8 <sup>ns</sup>	92.4 ± 15.0 <sup>ns</sup>
HSOsPRR37	111.5 ± 0.9d	28.6 ± 0.9 <sup>ns</sup>	20.4 ± 1.3*	16.1 ± 0.7 <sup>ns</sup>	76.2 ± 3.5 <sup>ns</sup>	82.8 ± 10.0 <sup>ns</sup>
HShd1	100.4 ± 1.4c	23.0 ± 2.5 <sup>ns</sup>	19.0 ± 2.7*	16.1 ± 0.8 <sup>ns</sup>	70.7 ± 3.1 <sup>ns</sup>	86.0 ± 10.2 <sup>ns</sup>

Variety names are based on Table S12

Value indicates mean ± SD

Different letters in DTH indicate significant differences at  $p < 0.05$

<sup>ns</sup> no significance

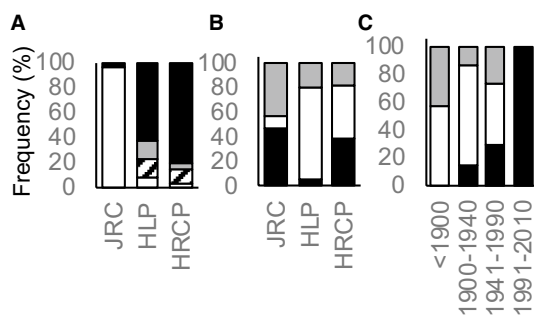
\* \*\* \*\*\* Significant differences at 0.05, 0.01, and 0.001 levels, respectively, with HS.

## Dramatic changes in genotype for flowering time during rice cultivation in Hokkaido

Only two varieties from the JRC representing genetic diversity across Japan, 48/50, carried *ghd7osprrr37*, which were collected from Hokkaido. Whereas among 43 varieties in the HLP, 27 (62.8%) carried *ghd7osprrr37* (Fig. 4a, Table S14). In addition, most varieties from the HRCP, 49/62 (80.3%) carried *ghd7osprrr37*. Given the need for meeting the demands of society, extremely early flowering time expressed by *ghd7osprrr37* was selected for adaptability to Hokkaido.

Loss-of-function allele *hd1* of the AK allele was infrequent in JRC varieties, 4 of 37, whereas most the HLP population carried the AK allele, 32 of 43 varieties (Fig. 4b, Table S15). In addition, a high frequency of *hd1*, five among seven varieties, was observed in the early phase of rice breeding programs in Hokkaido during 1900–1940 (Fig. 4c, Table S16). Whereas all varieties, 11/11, released during 1991–2010 carried functional *Hd1*. Previously, we showed that functional *Hd1* promotes flowering time in the genotype of *ghd7osprrr37* in long-day conditions (Fujino et al. 2019). Once functional *Hd1*, the G allele, was introduced into the gene pool in to Hokkaido varieties from Honshu varieties carrying the *Hd1*, it was immediately dispersed within the population.

The increase in the genotype of *ghd7osprrr37* during rice cultivation in Hokkaido and the functional allele *Hd1* during rice breeding programs in Hokkaido suggested an effect of direct selection for flowering earliness on the genes, which are the main drivers of adaptation to northern regions with naturally long daylength.



**Fig. 4** Dramatic changes in allele frequencies in the genotypes of genes for flowering time among the populations and between generations. **a** Frequency (%) of the combination of *Ghd7* and *OsPRR37*, among the three populations. Boxes indicate the four genotypes; *Ghd7OsPRR37* (white), *Ghd7osprrr37* (hatched), *ghd7OsPRR37* (gray), and *ghd7osprrr37* (black). **b** Frequency (%) of alleles in *Hd1* among the three populations. **c** Change in allele frequency (%) in *Hd1* among HRCP between four generations. White, black, and gray boxes indicate Akage (AK), Ginbouzu (G), and other alleles of *Hd1*, respectively

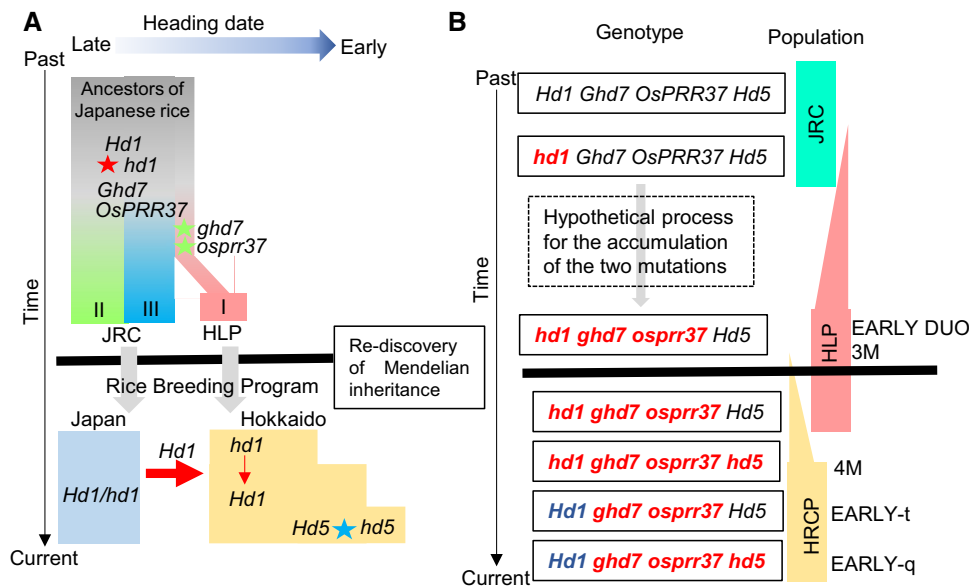
## Discussion

The middle area of the Pearl River, China, was the first place where rice cultivation was developed (Huang et al. 2012). Cultivated rice has since spread over diverse geographic regions. Here, we demonstrated the genetic and phenotypic bases of the establishment of local rice varieties from Hokkaido, a northern-limit of rice cultivation. Artificial selection on natural variation and reconstruction of genotypes for flowering time by plant breeding programs may optimize flowering time for better fitness for agriculture. Genetic variations among local populations are limited due to the lack of recombination from the inbreeding of cultivated rice, which can generate adaptability rapidly. Once desirable traits/genes are introduced into populations in new environments, they immediately spread within the population.

The genetic structure and phenotypic shifts (Figs. 2, 3, Table S10) in the population from Hokkaido clearly provide insights into the significant contributions of genetically induced mutations for phenotypic variation. Our results clearly proposed a hypothetical model on the establishment of early flowering time (Figs. 5, S3, Table S17). In the initial step in the direction to early flowering time following intensive artificial selection along with human migration all over the world, loss-of-function *hd1* generated varieties with early flowering time. Then, the Southern–Northern distribution among Japanese rice was regulated by *Hd1* versus *hd1* (Yokoo and Kikuchi 1977; Yokoo et al. 1980). Subsequently, the Northern distribution with *hd1*, which showed extremely early flowering time, was generated by loss-of-function *ghd7* and *osprrr37* selected together.

Adaptability can be defined by various kinds of phenotype. Optimization of flowering time to maximize yield has been shown to adaptively differentiate plant populations from different environments. Flowering time variation correlated with fitness played as indicators of adaptation to different environments. Earlier flowering time, short vegetative phase, fewer seeds, and small reproductive phase. The degree of adaptation differed across measures of fitness components. The early flowering time with greater PN might be a break-through in fitness for agriculture.

During the process for earlier flowering time (Fig. 4), functional *Hd1*, which was lost in the initial phase of selection for earlier flowering (Ebana et al. 2011; Hori et al. 2015), was introgressed into the local gene pool with *hd1*. Furthermore, intensive selection focusing on *Hd1* for earlier flowering time exhibited not only early flowering but also greater tillering, tuning the fitness of yield (Table 2). *Hd1*, a ortholog of *CONSTANS* in *Arabidopsis*, plays a central role in flowering time control (Yano et al.



**Fig. 5** Model of the establishment process in rice varieties with early flowering time. **a** Relationships with the mutations for population structure in Japan. Both functional and loss-of-function alleles in *Hd1* were distributed across Japan. Two mutations for loss-of-function in *Ghd7* and *OsPRR37* generated group I for early flowering time. The mutation for loss-of-function in *Hd5* happened during the initial phase of rice breeding programs in Hokkaido (Fujino et al. 2013). Red, green, orange, and blue stars indicate mutation events

2000). Previously, we demonstrated that the introgression around *Hd1* of *japonica* into the proto-*aus* group might generate *aus* subspecies, which is an ecotype with early flowering time (Fujino et al. 2010). This is generally consistent with differentiation among cultivated rice *O. sativa*, L. by global population genomics (Huang et al. 2012). The *aus* subspecies has a small geographic distribution derived from a cross between *japonica* and local wild rice, proto-*aus* (Huang et al. 2012). In addition, loss-of-function *hd1* alleles played a role in the adaptation to northern areas (Ebana et al. 2011; Hori et al. 2015). Furthermore, a functional *Hd1* allele might achieve stable rice production at the northern-limit of rice cultivation. Taken together, *Hd1* might have been an evolutionary force for the further expansion of cultivated rice worldwide.

Understanding of the genetic mechanisms found in the adaptability to a northern-limit to cultivation can facilitate adaptive divergence within genetically improved local populations, thereby expanding the species range. The loss-of-function alleles in *Ghd7* and *OsPRR37* could split off a variety group from the JRC. Intensive selection on natural variation in flowering time might drive this adaptability. The introduction of functional *Hd1*, which was lost during the process for earlier flowering time, could achieve a stable rice production with *ghd7osprr37*. The genotype of the combination of *Hd1* with *ghd7osprr37* may be rare

of flowering time genes. Red arrows indicated the introgression of functional *Hd1* into rice breeding programs in Hokkaido using exotic germplasm. **b** Changes of the genotype for early flowering time in three different populations. 3M; triple mutant, 4M; quadruple mutant, EARLY-t; EARLY TRIO, EARLY-q; EARLY QUARTETTE. Red and blue bold figures indicated the loss-of-function mutation allele and the functional allele transferred by rice breeding programs, respectively. (Color figure online)

or impossible in natural variation among cultivated rice with inbreeding, achieving stable rice production. Plant breeding programs could generate novel genotypes other than those in natural populations, exploring phenotypic variation.

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**Author contributions** Conceived and designed the experiments and wrote the manuscript: KF. Performed the experiments, analyzed the data, and approved the final manuscript: TI, MO, KF.

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## Compliance with ethical standards

**Conflict of interest** All author declares that he/she has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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