Genetic studies on breeding for heading characteristics and its environmental response of photo-insensitive rice

（非感光性イネの出穂特性と環境応答性に関する育種遺伝学的研究）

北海道大学大学院農学院

生産フロンティアコース　博士後期課程

坂口　俊太郎

TABLE OF CONTENTS

ABBREVIATIONS

BVP	basic vegetative phase
Deg_Pre_Hed	Degree of premature heading
DTH	days to heading
E-RIL	Early heading RIL
F-DTH	First days-to-heading
hKO	hemi-knockout
ILE	interval of leaf emergence
KO	knockout
LD	long daylength
L-DTH	Last days-to-heading
L-RIL	Late heading RIL
LT	low temperature
NT	natural temperature
photo-In	photoperiod-insensitive
photo-Se	photoperiod-sensitive
PI	panicle initiation
PSP	photoperiod-sensitive phase
RIL	recombinant inbred lines
RP	reproductive phase
SD	short daylength
TP-ILE	turning point of the interval of leaf emergence
WT	wild-type

LIST OF FIGURES

LIST OF TABLES

Chapter 1. General introduction

Flowering (or heading in crops) is the essential stage of development in the life cycle of plants. Since flowering stage is vulnerable to environmental stresses, appropriate timing of flowering initiation is necessary to ensure seed production (Kazan and Lyons, 2016). To flower in a favorable environment for ensuring reproductive success, plants respond to environmental triggers, such as photoperiod and ambient temperature, and regulate transition from vegetative to reproductive growth (Blümel et al., 2015; Preston and Fjellheim, 2022). The responses of plants to these environmental triggers are genetically controlled and they constitute major factors determining flowering time (Vicentini et al., 2023). Flowering time is the key factor that determines the regional adaptability of plants, and plants have evolved to optimize flowering time in each lineage (Izawa, 2007).

Rice (*Oryza sativa* L.) is one of the main crops in the world, which provides a staple food of nearly half of the human population. Although rice is originally a tropical species and short-day plant, different varieties are grown throughout the world including high-latitude areas above 40°N (Saito et al., 2019). The expansion of the rice cultivation area have been significantly accelerated by decrease in photoperiod sensitivity, which resulted in an earlier heading date (Fujino et al., 2019a, 2019b; Izawa, 2007). One successful example is rice cultivation in Hokkaido, Japan, which is the northern limits of rice cultivation in the world. In Hokkaido, rice can be grown in the field from late May to late September, and the day length are more than 13 h during this period. To adapt these conditions, rice varieties cultivated in Hokkaido have intensively been selected for extremely low photoperiod sensitivity and short days-to-heading (DTH), and equipment of these traits has been achieved through genetic improvement of over 100 years (Fujino et al., 2019a; Saito et al., 2019; Shinada et al., 2014).

Heading date is not only important for regional adaptability but also grain yield. Both too early and too late heading induces lower yield because of inadequate use of light and temperature resources, and incompletion of grain development before cold season, respectively (Fujino, 2020; Hu et al., 2015). Additionally, association has been reported between heading date and development of tiller or panicles, which consist of the yield

component (Chen et al., 2018; Endo-Higashi and Izawa, 2011; Fujino, 2020; Tsuji et al., 2015; Weng et al., 2014; Xue et al., 2008). Since rice has multiple panicles with different heading dates, grain number, and quality, heading dates of each tiller are also important agronomic traits (Takeda, 1986). Understanding and selecting varieties with appropriate heading characteristics are necessary to ensure rice production (Chen et al., 2018).

Heading characteristics important for local adaptability are driven by the genes involved in photoperiod sensitivity (Fjellheim and Preston, 2018). In rice, genes associated with photoperiod sensitivity participate in either of two pathways that induce expression of two florigen genes, *Hd3a* and *RFT1*. *Heading date 1* (*Hd1*) pathway, which is conserved between rice and Arabidopsis, is core photo-periodic pathway where *Hd1* promotes *Hd3a* expression under short-day (SD) condition and inhibits under long-day (LD) condition (Kojima et al., 2002; Yano et al., 2000). The other pathway involves *Early heading date 1* (*Ehd1*), and this pathway is unique to rice (Doi et al., 2004). The expression levels of *Ehd1* affect flowering time (Takahashi et al., 2009), and several flowering genes that regulate *Ehd1* have been identified. *Ghd7* and *Osprr37* were originally identified as a regulator of florigen genes expression through *Ehd1* under LD conditions, and later identified as major floral growth suppressor genes (Doi et al., 2004; Koo et al., 2013; Xue et al., 2008). *Ghd7* and *Osprr37* play very important role in the adaptation of rice to high-latitude areas to confer an extremely low photoperiod sensitivity and short days to heading (DTH) that are required in such areas (Fujino et al., 2013; Fujino and Sekiguchi, 2005a, 2005b; Gao et al., 2014; Ichitani et al., 1997; Koo et al., 2013; Nonoue et al., 2008).

Nowadays, global warming threatens stable rice production. It is estimated that the global increase in temperature of 2℃ will dramatically reduce global rice yield (Chen et al., 2018; Zhao et al., 2017). Altering flowering time is an effective way to avoid heat stress, because it would make possible to adjust growing season by introducing early or late flowering varieties. In addition, such technique would allow extending the cultivation area to northward (Chen et al., 2018; Nemoto et al., 2011). To this end, there are two important research questions; One is what the effect of temperature on heading is and what genes are involved. The other question is whether photo-insensitive (photo-In) rice

grow well and whether there is any defective trait associated with early maturity when photo-In rice is grown in lower latitude area to avoid heat stress. My research conducted to elucidate these questions, and the results are described in Chapters 2 and 3.

Chapter 2

Optimizing heading date under global warming requires an understanding of its temperature sensitivity. In contrast to many studies on the photoperiod sensitivity, relatively few studies had examined temperature sensitivity in rice heading. This is because temperature affects rice growth overall and the method to evaluate temperature-sensitivity of heading is difficult to be established. In this chapter, I divided growth phase based on leaf age (plant age inferred from the number of leaves) kinetics and panicle initiation, allowing identifying the developmental phase critical to photoperiod- and temperature sensitivity. I focused on photo-In varieties and demonstrated that it is possible to study how temperature affects the growth phase without the influence of photoperiod sensitivity.

Chapter 3

Early-heading lines have different characteristics from photosensitive (photo-Se) varieties, such as less heading synchronous, occurrence of premature heading, timing of effective tiller determination, among others. In this chapter, photo-In- and photo-Se varieties were grown at two sites with different latitudes, Sapporo and Iga, and their heading characteristics were evaluated. I found differences between photo-In- and photo-Se varieties, as well as among photo-In varieties. I assumed the observed variation within the photo-In varieties as caused by genetic variation, and I conducted QTL analysis of each trait to search for candidate genes. The relationships between these heading traits and heading earliness were elucidated to understand the reproductive adaptation of early heading rice varieties.

Chapter 2. Early heading in photoperiod-insensitive rice varieties is associated with extremely short photoperiod-sensitive phase and weak temperature sensitivity

2.1 Introduction

Temperature is important factor regulating the transition to flowering. Rice heading is promoted by high temperature and delayed by low temperature (LTs), but the response varies among varieties (Chen et al., 2018; Hosoi, 1979; Vergara and Chang, 1985; Wei et al., 2008). In contrast to the many studies on photoperiod sensitivity, relatively few studies have examined temperature sensitivity in rice or other crops in the flowering study. Evaluating temperature sensitivity in rice flowering is particularly difficult because its temperature response does not express as the readily recognizable traits, such as the requirement for vernalization in other plants (Kovi et al., 2015; Nagalla et al., 2021). The mechanisms how temperature affects the shift from vegetative to reproductive growth are yet to be uncovered in rice.

Vegetative growth in rice consists of two subphases: the basic vegetative phase (BVP) and the photoperiod-sensitive phase (PSP) (Vergara and Chang, 1985). The BVP is insensitive to photoperiod, while the PSP is sensitive; PSP duration is thought to vary depending on the plant's photoperiod sensitivity (Adams et al., 2001). The existence of two growth phases are rationalized by an idea that rice needs to undergo a certain amount of growth before it responds to photoperiod to transit to reproductive growth (Rathnathunga et al., 2019; Vergara and Chang, 1985). Whether temperature sensitivity in flowering can affect BVP- or PSP remains poorly understood.

The shift from vegetative to reproductive growth can be measured by observing panicle initiation (PI) (Yabuta et al., 2010), but the shift from the BVP to PSP is more difficult to determine and is therefore less well understood. Several studies estimated the BVP by comparing the DTH under LD and SD conditions or by transferring plants from LD to SD conditions to see their response (Nishida et al., 2001; Vergara and Chang, 1985;

Yoshitake et al., 2015). Yabuta et al. (2015) identified a certain growth point at which the interval of leaf emergence (ILE) increases, which is known as the turning point of the ILE (TP-ILE). They proposed that the TP-ILE can be used to define the end of the BVP. The TP-ILE is thought to be a sign that the plant begins to shunt the use of photosynthetic products to reproductive growth, instead of leaf growth (Yabuta et al., 2015). As shown in Fig. 2-1, TP-ILE not only defines BVP but also PSP precisely. This approach may be used to evaluate the temperature sensitivity of each growth phase and can be applied to analyses of photo-In varieties.

In this chapter, I measured the duration of BVP and PSP in photo-In varieties under different photoperiod and temperature conditions. The growth phase shift from the PSP to the reproductive phase (RP) was precisely defined by observing PI (Fig.2-2). The results show extremely short PSP in photo-In varieties, and that high temperature reduced the DTH. The effect of *ghd7* loss on PSP and temperature sensitivity was also analyzed. My analysis of photo-In rice varieties demonstrated that it is possible to study how temperature sensitivity regulates the growth phase with little or no influence from photoperiod sensitivity.

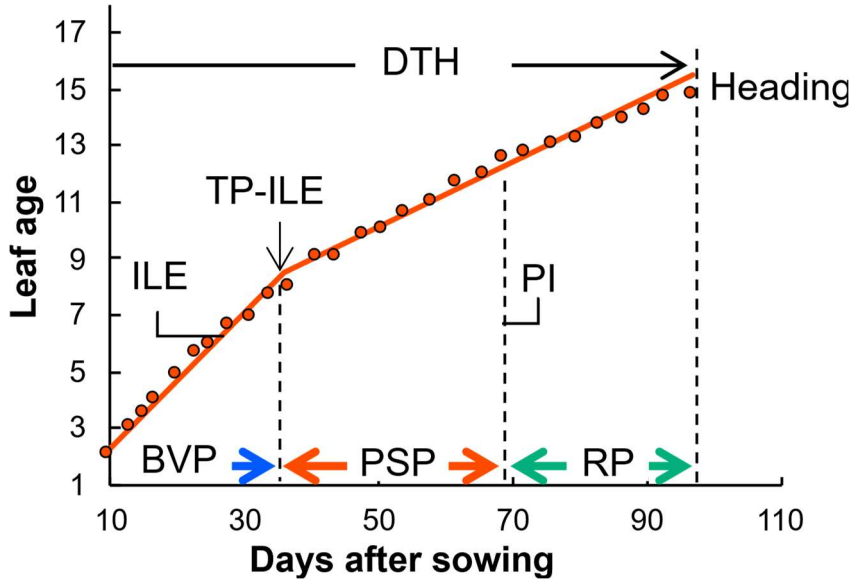


Figure 2-1. A representation for determining the periods of the three growth phases, BVP, PSP and RP, by ILE, TP-ILE and PI.

The plots show the average leaf age every three or four days, and the solid line indicates the interval of the leaf emergence index (ILE). The turning point of the ILE (TP-ILE) is at the intersection of the ILEs where the leaf growth rate changes. The date of panicle initiation (PI) was determined by observations. I defined BVP as the period from germination to TP-ILE, PSP from TP-ILE to PI, and RP from PI to heading. Here is an example of Nipponbare under long daylength (LD) and normal temperature (NT) condition.

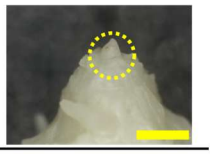
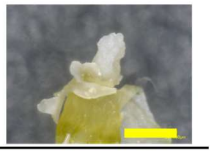
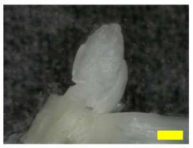
Develop-mental stage	Panicle length (mm)	Days after formed (days)	Image
i	< 0.25	1.5	
ii	0.25 - 0.50	5	
iii	0.50 - 1.00	8.5	
iv	1.00 - 1.50	12	
v	1.50 - 3.50	16	

Figure 2-2. Five stages of panicle development used to estimate the panicle initiation date. Panicle initiation date was estimated by subtracting the predicted number of days after formation that a structure could be observed from the day on which it was first seen. Scale bars indicate 0.5 mm.

2.2 Material and methods

2.2.1 Plant materials and experimental design

Five rice varieties were used to measure the duration of each growth phase under different photoperiod and temperature conditions (Table 2-1). Seeds were obtained from seed stocks at Laboratory of Plant Breeding of Hokkaido University and the gene bank at the Agricultural Research Department of the Hokkaido Research Organization, Japan. Plants were grown in growth chambers under four different controlled environments, which comprised combinations of two photoperiod conditions (LD: 14 h light/10 h dark, SD: 12 h light/12 h dark) and two temperature conditions (normal temperature (NT): 25°C light/20°C dark; low temperature (LT): 22°C light/18°C dark). In addition to the previously mentioned five varieties, Kyodaiodajo, which is a variety of Tohoku area with weak photo-sensitivity, was examined under LD-NT and SD-NT conditions. Twenty plants per variety were grown under each condition. Tillers were removed when they emerged.

To evaluate the effect of *Ghd7* on heading, I produced *Ghd7* knockout (KO) plants in Nipponbare by CRISPR/Cas9. I obtained four *Ghd7* KO plants and one wild-type (WT) plant. These T0 generation plants were obtained at the same time and transplanted to the pods and grown in the growth chamber under LD-NT condition.

2.2.2 DNA analysis

Total DNA of Kyodaiodajo was extracted from young leaves using the CTAB method (Murray and Thompson, 1980). Four heading genes, *Ghd7*, *Osprr37*, and *Hd1* were genotyped by PCR targeting base polymorphisms in their functional region that were reported by Gao et al. (2014), Xue et al. (2008), and Yano et al. (2000), respectively. Primer sequences and restriction enzyme treatments followed the methods described in previous studies (Fujino et al., 2019a, 2019b)

2.2.3 Generation of genome edited plant

A universal Gateway Technology of plasmid construction method was used to construct T-DNA. I used pMR185 as the entry clone, and after successfully inserting the 20mer SgRNA fragment DNA that targets the gene to be edited into pMR185 by ligation reaction, I performed LR reaction using LR clonase to produce the expression vector with pZH_gYSA_PubiMMCas9. This plasmid contains cas9 along with maize ubiquitin promoter to induce mutation according to the SgRNA, gifted by Dr. Anne Britt (UC Davis) and Dr. Masaki Endo (National Agriculture and Food Research Organization, Japan). *E. coli* strain DH5α was used to propagate the plasmid vector. The propagated plasmid was extracted using Nippon gene ISOSPIN plasmid extraction kit, and Agrobacterium-mediated transformation into Nipponbare seed callus was performed. *Ghd7* genomic region was targeted according to the method reported by Endo et al. (2021). I confirmed the knockout sequences of the transformants by Sanger sequencing. These T0 materials were grown concurrently and used continuously for heading analysis.

2.2.4 Growth phase analysis

The leaf number of each plant was counted every three or four days from 10 days after sowing until flag leaf emergence to determine the plant's leaf age. The leaf age at each timepoint was used to calculate the ILE (Fig. 2-1). The TP-ILE of each plant was calculated using two-part linear regression analysis using the R package ‘segmented’ (Muggeo .V, 2008).

The main culms of more than three plants were sampled 30 to 40 days before heading. After peeling away the leaf sheath, the shapes of young panicle were observed under a stereoscopic microscope (Fig. 2-2). The young panicles were classified into five developmental stages by estimating the days after PI, as described by Yabuta et al. (2012) (Fig. 2-2). By counting backward from the panicle stage, the PI date was determined (Fig.

2-2).

DTH was measured as the number of days from sowing to the emergence of the first panicle. The duration of each growth phase was calculated using the following equations (see also Fig. 2-1):

BVP = (days to TP-ILE)

PSP = (days to PI) − (days to TP-ILE)

RP = DTH − (days to PI)

2.2.5 Data analysis

Three-way ANOVA was conducted on the DTH and the lengths of each growth phase using SPSS Statistics (IBM version23) to compare the varieties and evaluate their environmental sensitivities (factors: variety, daylength, and temperature). Three-factor interactions were included in the residual mean square. The simple main effect was tested using Tukey's test when the two-factor interactions were significant at the 5% level. The correlation coefficients between the DTH and the lengths of each growth phase were calculated for each experimental condition.

Table 2-1. List of the materials used for the experiments.

Variety	Origin[a]	Genotypes of three heading date genes[b]
Nipponbare	Honshu	*Ghd7*/*Osprr37*/*Hd1*
A58	Hokkaido (Landrace)	*ghd7*/*osprr37*/*hd1*
Norin 20	Hokkaido (Ⅱ)	*ghd7*/*osprr37*/*hd1*
Hakuchomochi	Hokkaido (Ⅲb)	*ghd7*/*osprr37*/*hd1*
Kitaake	Hokkaido (Ⅴ)	*ghd7*/*osprr37*/*Hd1*
Kyodaiodajo	Tohoku	*Ghd7*/*osprr37*/*hd1*
ghd7 knockout	Nipponbare	*ghd7*/*Osprr37*/*Hd1*

a. Parentheses indicate the classification of Hokkaido rice varieties according to Shinada et al. (2014).

b. Genotypes of known genes involved in photosensitivity (Fujino et al., 2013; Koide et al., 2019; Saito et al., 2019). Kyodaiodajo and *ghd7* knockout were genotyped by PCR and sanger sequence, respectively.

2.3 Results

2.3.1 Environmental effects on DTH are different between photo-Se and photo-In varieties

I subjected five rice varieties to four experimental conditions, comprising combinations of LD and SD photoperiods and NT or LT conditions. All four photo-In varieties showed shorter DTH (60.9 to 99.0 days under the four conditions) than the photo-Se variety Nipponbare (74.4 to 139.0 days under the four conditions) across the experimental conditions (Table 2-2). The photo-In varieties possess two or more nonfunctional alleles of photoperiod genes, such as *Hd1*/*OsPrr37*/*Ghd7*/*DTH8,* whereas all these genes are functional in the photo-Se variety Nipponbare (Fujino et al., 2013; Koide et al., 2019; Saito et al., 2019) (Table 2-1). Among the four genes, nonfunctional alleles of *Ghd7* and *Osprr37*, are strongly associated with the photoperiod insensitivity in the four photo-In varieties (Fujino et al., 2019a; Saito et al., 2019).

In Nipponbare, SD-NT conditions promoted the earliest heading, which occurred after 74.4 days, whereas the longest DTH (139.0 days) was observed under LD-LT conditions (Table 2-2). When the two temperature conditions were combined, the average DTH for Nipponbare was 120.6 days under LD and 95.6 days under SD, indicating that SD promoted heading 25.0 days earlier than LD (Table 2-2). When the two daylength conditions were combined, NT and LT led to average DTHs of 88.3 and 127.9 days, respectively, with NT promoting heading 39.6 days earlier than LT (Table 2-2). Among the photo-In varieties, Kitaake had the shortest DTH (60.9 days), which occurred under SD-NT conditions, while A58 displayed the latest heading (99.0 days under LD-LT conditions) (Table 2-2). When both temperature conditions were combined, the four photo-In varieties began heading only 3.3 days earlier under LD than under SD (Table 2-2). When the two daylengths were combined, the four photo-In varieties began heading 27 days earlier under NT than under LT (Table 2-2). These results indicate that the heading of the photo-In varieties was more affected by temperature but less by daylength, whereas

the DTH of Nipponbare was affected by both temperature and daylength. The statistical analysis will be presented in the later section (see Table 2-2, 2-3, 2-4).

2.3.2 Identification of TP-ILE in photo-In varieties and comparison of BVP with photo-Se

I evaluated the influence of photoperiod and temperature sensitivity on the length of the BVP. Yabuta et al. (2015) observed occurrence of decline in ILE at the end of the BVP, which was designated as TP-ILE. I measured leaf age to see the kinetics of ILE to identify each plant's TP-ILE (Fig. 2-3 and Table S2-2). I was able to detect the TP-ILE for all five varieties under every condition, with the exception of Hakuchomochi under SD-LT conditions (Fig. 2-3 and Table S2-2). The correlation between the number of days from sowing and leaf age was determined for 20 individuals in each experimental plot to approximate the leaf age change. The coefficient of determination (r^2) in each plot was greater than 0.98 (Table S2-2), so the date of change in ILE (TP-ILE) was validated.

The days from sowing to the TP-ILE, equivalent to BVP length, were between 22 and 41 days for the photo-In varieties and between 30 and 52 days for Nipponbare under the four conditions (Table S2-3), indicating that BVP lengths were shorter in the photo-In varieties than in Nipponbare under each growth condition. When the two temperature conditions were combined, the average BVP lengths for the photo-In varieties were 31 and 29 days under the LD and SD conditions, respectively, while for Nipponbare they were 44 and 39 days, respectively (Table S2-3). The BVP lengths showed an average difference of two and five days between LD and SD conditions in the photo-In varieties and Nipponbare, respectively (Table S2-3). When the two daylengths were combined, the average BVP lengths were 26 and 35 days for the photo-In varieties and 33 and 49 days for Nipponbare under NT and LT conditions, respectively (Table S2-3). The photo-In varieties displayed a nine-day difference in their average BVP lengths between the two temperature

conditions, while Nipponbare had a 16-day difference (Table S2-3). These results indicate that the TP-ILE is controlled by genetic and environmental factors, with temperature playing a larger role than daylength.

2.3.3 Estimating PSP length in photo-In varieties by observing PI

I confirmed that the TP-ILE can be defined as the end of the BVP. According to Yabuta et al. (2015), PI is the switching point between vegetative and reproductive growth; thus, the period between the TP-ILE and PI is the PSP. I observed PI to examine the length of the PSP under the four conditions, as PI determines the length of the PSP. I monitored young panicles in the base of the shoot apical meristem and checked their initiation. Young panicles were collected about 30 to 40 days before heading and categorized into five developmental stages based on Yabuta et al. (2012) (Fig. 2-2). The number of days after PI could be assessed based on panicle sizes according to the five developmental stages (Fig. 2-2). The photo-In varieties displayed an earlier PI (26 to 55 days after sowing) than Nipponbare (41 to 103 days after sowing) across the four conditions (Table S2-3). All five varieties underwent PI after the TP-ILE under the four conditions. PI in Nipponbare occurred 10–50 days after the TP-ILE, whereas the photo-In varieties underwent PI 0–20 days after the TP-ILE (Table S2-3). In Nipponbare, when the two temperature conditions were combined, PI occurred 26 days earlier under SD than under LD (Table S2-3). Similarly, when the two daylength conditions were combined, NT promoted PI 36 days earlier than LT (Table S2-3). This was expected based on the decrease in DTH due to the photoperiod sensitivity of this line (Table S2-3).I calculated PSP for all varieties-condition combinations. In the photo-In varieties, when the two daylength conditions were combined, LT conditions delayed PI about 18 days. On the other hand, when the two temperature conditions were combined, the difference was about 3 days between the two daylength conditions. This result indicated that temperature rather than daylength influenced the timing of the PSP as well as the BVP and RP in

photo-In varieties (Table S2-3).

2.3.4 Effects of photoperiod and temperature on growth phases in the Photo-In varieties

Based on the above definitions of the TP-ILE and PI, the precise durations of the BVP, PSP, and RP could be determined. BVP is defined as the period from germination to the TP-ILE; PSP is the period from the TP-ILE to PI; and RP is the period from PI to heading. The lengths of each of these growth phases and the results of statistical analysis via ANOVA are shown in Fig. 2-4A, Tables 2-3, S2-4, and S2-5. BVP and PSP differed depending on photoperiod and temperature, whereas RP was mostly influenced by temperature and not significantly influenced by photoperiod except for Norin 20 (Table 2-3 and S2-5). The photo-In varieties had shorter BVPs and PSPs than Nipponbare under each of the four conditions, resulting in shorter DTHs for the photo-In varieties. By contrast, although RP was extended by LT, its duration was unaffected by daylength (Fig. 2-4A and Table S2-4). Notably, the PSP of the photo-In varieties was considerably shorter than that of Nipponbare, lasting less than six days under LD-NT conditions (Fig. 2-4A and Table S2-4). Consequently, the end of BVP and the onset of PI seems to occur almost simultaneously in the photo-In varieties.

2.3.5 Differences in growth phase between *ghd7* and *osprr37* genotypes

In the previous section, I found that the PSP in photo-In varieties is shorter than Nipponbare. Among the four photoperiod sensitivity genes, *Ghd7*, *Osprr37*, *Hd1*, and *DTH8*, loss-of-function alleles of *Ghd7* and *Osprr37* strongly involved in photoperiod insensitivity (Fujino et al., 2013; Fujino and Sekiguchi, 2005a, 2005b; Gao et al., 2014; Ichitani et al., 1997; Koo et al., 2013; Nonoue et al., 2008). It is well known that these two genes are defective in most of the photo-In varieties grown in the high latitude region such as Hokkaido (Fujino et al., 2019a; Gao et al., 2014; Saito et al., 2019). The four

photo-In varieties examined in the current study also possess the loss-of-function alleles at the two loci, *Ghd7* and *Osprr37* (Table 2-1). Here, I investigated which of the loss-of-function alleles in *Ghd7* and *Osprr37* is more associated with PSP shortening.

I compared the effect of *ghd7* and *osprr37* on heading and PSP. Kyodaiodajo, which carries the functional *Ghd7* and the nonfunctional *osprr37* alleles, was compared with the other photo-In variety A58, which carries both the non-functional *ghd7* and *osprr37* alleles (Table 2-1). Plants were grown at normal temperature under LD and SD. The DTHs of Kyodaiodajo were 83.3 and 79.0 days under LD and SD conditions, respectively, indicating that in LD Kyodaiodajo headed slightly later than SD (Table 2-4). The DTHs of A58 were 71.5 and 69.0 days under LD and SD conditions, respectively, which reflects ~10 days earlier heading compared to Kyodaiodajo (Table 2-4). For BVP and RP, only a few days of difference were observed between Kyodaiodajo and A58 (Table 2-4). However, the most pronounced difference was observed in PSP: Kyodaiodajo had 12.5 and 11.0 days for LD and SD, while A58 had 6.6 and 0 days for LD and SD, respectively (Table 2-4). Hence, the difference in DTH is due to the PSP length between the two varieties. This can be associated with *ghd7* but not *osprr37* (Table 2-4).

2.3.6 Effect of hemi-knockout *ghd7* plants

To confirm the effect of *ghd7* on PSP length (as well as DTH), I generated four *ghd7* knockout plants of Nipponbare using CRISPER/Cas9 and obtained four hemi-KO plants (hKO-1 to hKO-4) and one WT (same as Nipponbare *Ghd7*) plant. Each of the four hKO plants had a mutation in the first exon of *Ghd7* (Fig. S2-1) but the other three photoperiod sensitive genes (*Osprr37*, *Hd1*, and *DTH8*) remain functional. The plants were grown simultaneously under LD-NT conditions in the growth chamber and their ILEs were measured. The hKO plants had an average of 65.3 days to heading after transplanting, while the WT took 87 days, indicating that the hKO plants headed 21 days earlier than

the WT (Fig. 2-5). According to TP-ILE, BVP extended to an average of 27.2 days for the hKO plants and 34.8 days for the WT plant (Fig. 2-5). Only seven days difference in BVPs between hKO and WT allowed to predict the large difference in DTH occurred after BVP. Although I could not obtain the PI data in each plant, I could assume 33 days for RP from the previous results of Nipponbare in the LD-NT (Table S2-4) because of the consistent duration of RP. Thus, the tentative PSP of each plant was estimated to be 18.9 days for the WT and a range of 0 to 12.7 days for the hKO plants (Fig. 2-5). This circumstantial evidence strongly suggests that hemizygous *ghd7* can shorten PSP.

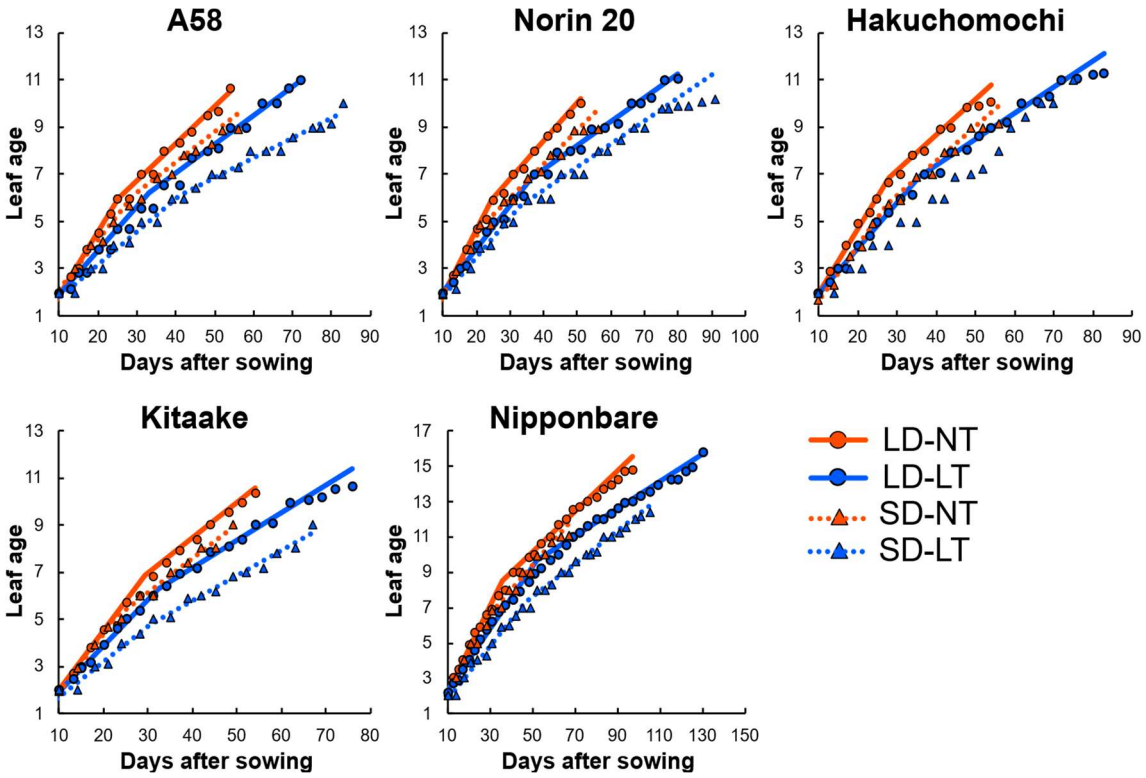


Figure 2-3. Leaf age and interval of leaf emergence index (ILE) of the five varieties under the four growth conditions.

Dots indicate average leaf ages, and lines indicate the average leaf emergence rates calculated using two-part linear regression analysis. Two temperatures were used: NT (normal day/night temperatures: 25/20 ◦C) and LT (low day/night temperatures: 22/18 ◦C). Two different daylength treatments were applied: LD (long-day photoperiod: 14 h light/10 h dark) and SD (short-day photoperiod: 12 h light/12 h dark). The ILE of Hakuchomochi under SD-LT conditions was not calculated.

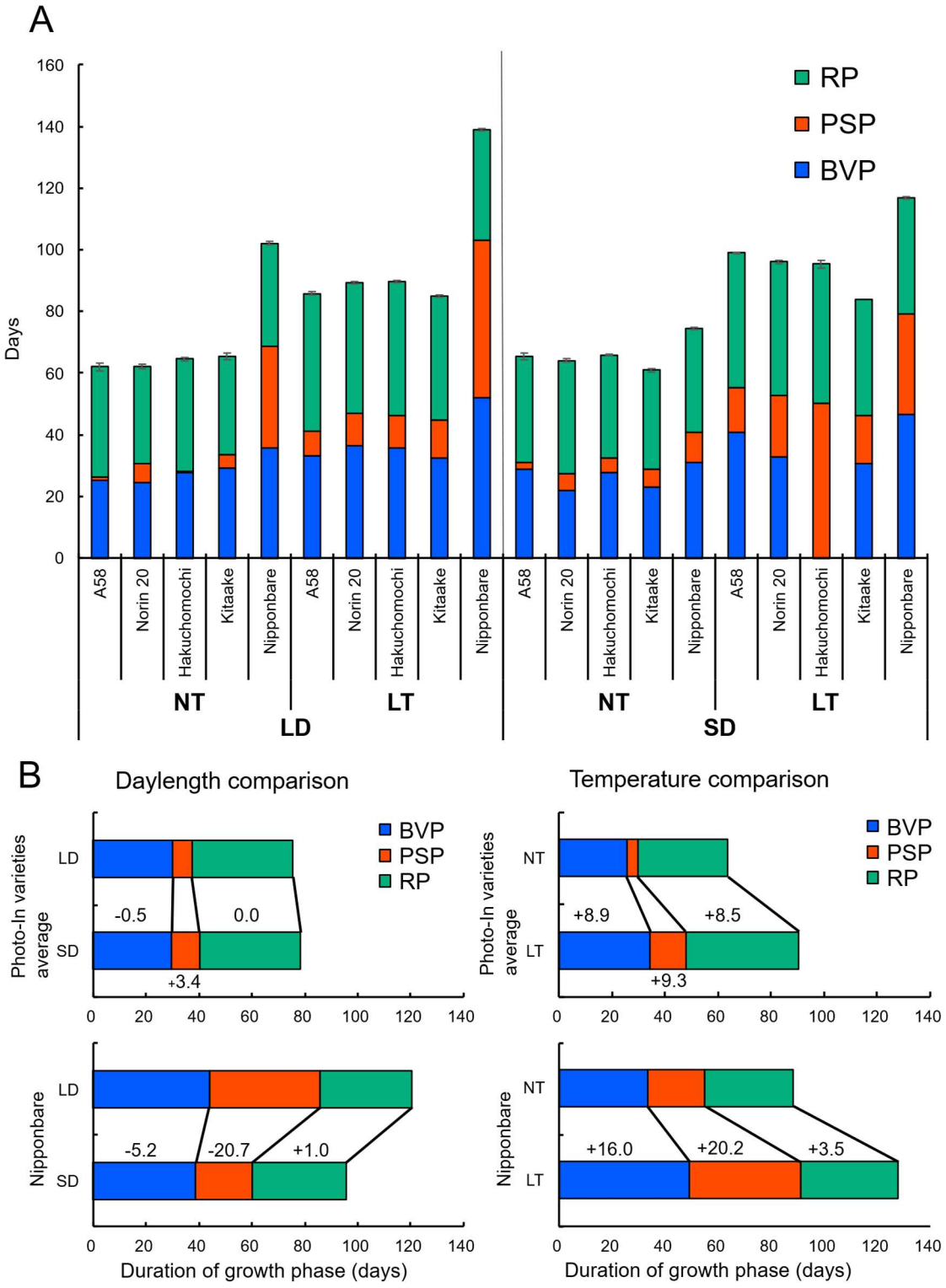


Figure 2-4. The duration of each growth phase and comparison under different daylength and temperature conditions.

(A) The duration of each growth phase of the five varieties under the four growth conditions. The BVP and PSP of Hakuchomochi under SD-LT conditions were not estimated and are shown as a hatched column. Error bars indicate standard error.(B)Comparison of the durations of the growth phases under different daylengths and temperature conditions in the photo-In varieties and Nipponbare. The numbers in the graph show the differences in growth phase durations between the conditions, which were calculated as the values under SD minus LD and LT minus NT. Two temperatures were used: NT (normal day/night temperatures: 25/20 ◦C) and LT (low day/night temperatures: 22/18 ◦C). Two different daylength treatments were applied: LD (long-day photoperiod: 14 h light/10 h dark) and SD (short-day photoperiod: 12 h light/12 h dark).

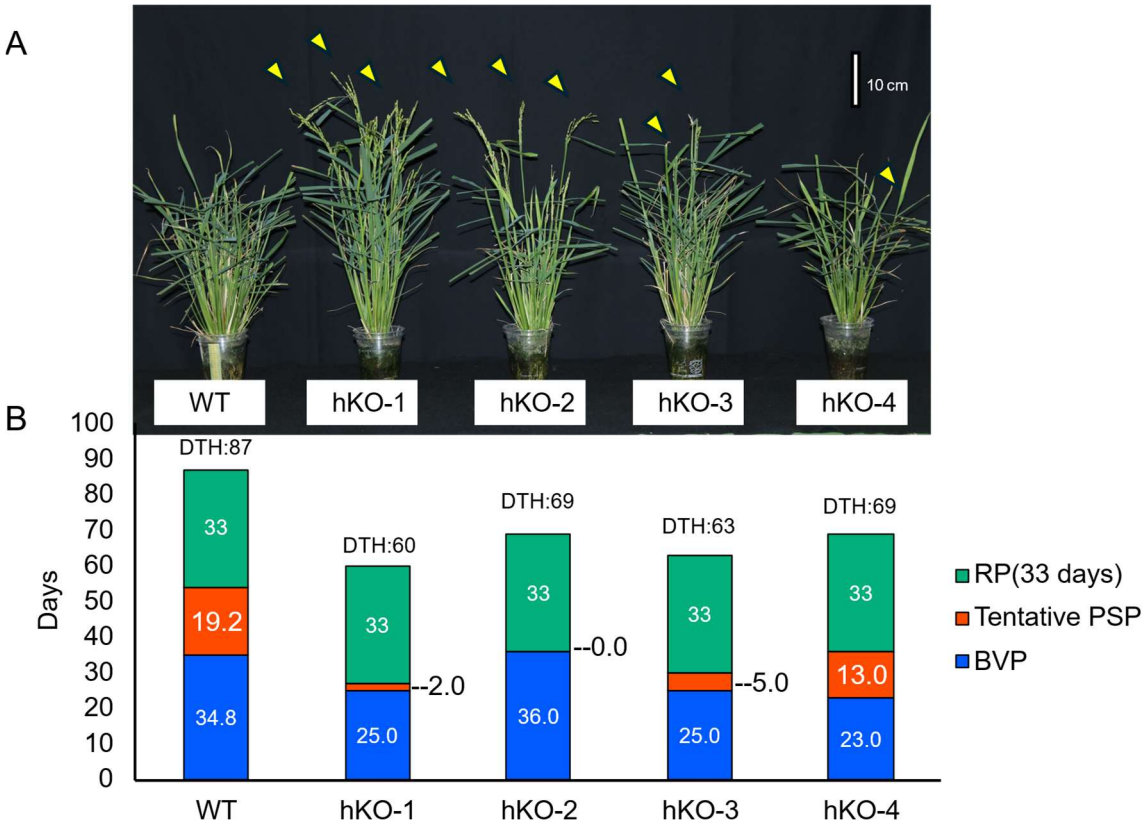


Figure 2-5. *Ghd7*/*ghd7* hemi-knockout promotes DTH.

(A) Flowering phenotypes of WT and *Ghd7*/*ghd7* hKO plants (hKO-1, -2, -3, -4) grown under LD-NT in the growth chamber. Photo of the plants was taken at 78 days after transplanting. Yellow triangles show the heading panicles. (B) The durations of each growth phase of WT and hKO plants. The duration of BVP was determined by TP-ILE. RP was assumed to be 33 days based on the results of the previous experiment (Table 2-3), and tentative PSP was estimated.

Table 2-2. DTHs of each variety under the four conditions.

Varieties	Day length	Temperature	DTH
A58	LD	NT	62.0 ± 1.31
Norin 20	LD	NT	62.2 ± 0.99
Hakuchomochi	LD	NT	64.6 ± 0.53
Kitaake	LD	NT	65.4 ± 0.68
Nipponbare	LD	NT	102.1 ± 0.48
A58	LD	LT	85.9 ± 0.41
Norin 20	LD	LT	89.3 ± 0.39
Hakuchomochi	LD	LT	89.7 ± 0.21
Kitaake	LD	LT	85.1 ± 0.31
Nipponbare	LD	LT	139.0 ± 0.42
A58	SD	NT	65.4 ± 1.06
Norin 20	SD	NT	64.1 ± 0.56
Hakuchomochi	SD	NT	65.9 ± 0.25
Kitaake	SD	NT	60.9 ± 0.53
Nipponbare	SD	NT	74.4 ± 0.40
A58	SD	LT	99.0 ± 0.27
Norin 20	SD	LT	96.1 ± 0.38
Hakuchomochi	SD	LT	95.3 ± 1.20
Kitaake	SD	LT	83.7 ± 0.44
Nipponbare	SD	LT	116.8 ± 0.42

LD: long daylength; SD: short daylength; NT: natural-temperature conditions; LT: low-temperature conditions.

Table 2-3. Effect of photoperiod and temperature on DTH and growth subphases.

Growth phase	Variety	Photoperiod sensitivity			Temperature sensitivity		
		LD	SD	Dif.	NT	LT	Dif.
DTH	A58	73.8 a	81.9 b	-8.1 *	63.3 a	92.4 b	29.1 *
	Norin 20	75.7 ab	80.1 b	-4.4 *	63.2 a	92.7 b	29.5 *
	Hakuchomochi	77.1 b	80.7 b	-3.5 *	65.3 b	92.6 b	27.3 *
	Kitaake	75.3 a	72.4 a	2.9 *	63.1 a	84.4 a	21.3 *
	Nipponbare	120.6 c	95.6 c	25.0 *	88.3 c	127.9 c	39.6 *
BVP	A58	29.2 a	34.9 b	-5.7 *	27.0 a	37.0 b	10.0 *
	Norin 20	30.5 a	27.3 a	3.1 n.s.	23.2 a	34.6 ab	11.4 *
	Hakuchomochi	-	-	- -	-	-	-
	Kitaake	30.7 a	26.8 a	4.0 *	26.1 a	31.4 a	5.3 *
	Nipponbare	43.9 b	38.7 b	5.2 *	33.3 b	49.3 c	16.0 *
PSP	A58	4.6 a	8.2 a	-3.6 *	1.6 a	11.2 a	9.6 *
	Norin 20	8.3 b	12.8 b	-4.4 *	5.9 b	15.2 b	9.3 *
	Hakuchomochi	-	-	-	-	-	-
	Kitaake	8.4 b	10.7 ab	-2.3 *	5.0 b	14.1 ab	9.1 *
	Nipponbare	42.0 c	21.3 c	20.7 *	21.6 c	41.7 c	20.2 *
RP	A58	40.2 bc	39.1 b	1.1 n.s.	35.1 ab	44.2 b	9.1 *
	Norin 20	36.9 ab	40.0 b	-3.1 *	34.0 b	42.9 b	8.9 *
	Hakuchomochi	40.1 c	39.3 b	0.7 n.s.	35.1 ab	44.4 b	9.3 *
	Kitaake	36.1 a	34.8 a	1.2 n.s.	32.0 a	38.9 a	6.8 *
	Nipponbare	34.6 a	35.6 a	-1.0 n.s.	33.4 ab	36.9 a	3.5 *

Phenotypic means are shown for each growth phase. Different letters indicate significant differences between varieties, as determined using ANOVA with Tukey's HSD. Asterisks indicate significant differences between different daylength or temperature conditions.

Table 2-4. Effects of osprr37 difference on DTH and the durations of the three growth phases.

Variety	Day length	Temperature	DTH	BVP	PSP	RP	
A58	LD	NT		71.5	31.4	6.6	33.5 (n=6)
	SD	NT		69.0	34.6	0.0	34.4 (n=5)
Kyodaiodajo	LD	NT		83.3	34.5	12.5	36.3 (n=6)
	SD	NT		81.7	36.4	10.6	34.7 (n=7)

2.4 Discussion

2.4.1 Identification of RSP as the growth phase accounting for short DTH in photo-In varieties

In this study, I subdivided the growth phase in rice into three subphases, which was defined by TP-ILE and the timing of PI (Yabuta et al., 2015). The method used in this study was more accurate than the separation method previously used to determine the PSP of photo-Se varieties (Yabuta et al., 2015) because I estimated the date of PI by visual inspection of young panicles. In each subphases, I evaluated the effects of photoperiod and temperature . I tested the photo-Se variety Nipponbare and four photo-In varieties originating from Hokkaido, Japan, under four different daylength and temperature conditions (Table 2-1). In a comparison of the effects of daylength, the DTH in Nipponbare was accelerated by 25 days under SD conditions, whereas the average difference was only approximately three days in the photo-In varieties (Fig. 2-4B and Table 2-2 and 2-3). The differences in DTH between Nipponbare and the photo-In varieties in response to photoperiod is not unexpected but the question which of the subphases contributes the difference was unanswered. In this study, I identified the principal cause as the differences in the lengths of the PSPs (Fig. 2-4B and Table 2-3). I found that the PSP lengths of the photo-In varieties on average 7 and 11 days under LD and SD conditions, respectively, which are extremely short compared with the 42- and 21-day PSPs observed in Nipponbare (Table S2-4). The effects of daylength on the lengths of the growth phases were within four days in the photo-In varieties while Nipponbare indicated up to 21 days difference in the length of growth phase between two daylength conditions (Fig. 2-4). Altogether, my data consist with the previous finding that photo-In varieties have a short PSP irrespective to daylength (Wei et al., 2008).

2.4.2 Temperature responses in each growth phase

Analysis of the temperature responses of the rice varieties during each growth phase showed that LT extended all growth phases, resulting in delayed heading (Fig. 2-4B). The

DTHs of the photo-In varieties delayed 27 days by LT while that of Nipponbare was 40 days (Fig. 2-4B and Table 2-3). In Nipponbare, the largest LT-mediated delay was observed in the PSP (20 days compared with a 16-day delay in BVP and a 4-day delay in RP); however, this was not the case in the photo-In varieties, in which differences in the degree of delay were comparable at all stages (approximately nine days) (Fig. 2-4B and Table 2-3). These results indicate that LT affects most severely on the PSP in the photo-Se variety whereas such effect is not apparent in the photo-In varieties. Note that overall effect of LT on DTH of photo-In varieties is rather small.

Inappropriate flowering can damage the yield of rice due to cold injury, which is major obstacle for rice cultivation at high latitudes. Delayed heading due to LTs, especially in photo-Se varieties, had been attributed to slower vegetative growth (Andaya, 2003), but my findings suggest that this delay largely arises from the extended PSP (Fig. 2-4B and Table 2-3). On the other hand, I observed that the effect of LT on the heading of photo-In varieties is small, suggesting that heading delay by LT can be genetically controlled to adapt high-latitude regions. In addition, there may be genetic variation in the heading delay by LT in the four photo-In varieties used in this study. Kitaake displayed the smallest LT-mediated delay in heading (Fig. 2-4B and Table 2-3), which was deliberately selected during the breeding process (Fujino et al., 2015). Interestingly, no significant differences were observed in the DTH of these four genotypes under NT. Kitaake was the only variety that showed no significant difference in flag leaf age between NT and LT conditions (Table S2-2). This indicates that Kitaake has a genotype conferring less heading delay by LT. Future study could explore the genetic difference in Kitaake.

2.4.3 Cause and effect of the extremely short PSPs in photo-In varieties

In this study, PSP was defined as the period from the TP-ILE to PI. The TP-ILE, the point at which leaf emergence slows, is thought to occur when the energy previously used for

leaf initiation is instead used for reproductive growth (Kanda et al., 2000; Yabuta et al., 2015). Thus, the PSP is considered to be a period of preparation for the accumulation of tillers and a suitable environment before PI. Under NT conditions, however, the PSPs of the photo-In varieties were extremely short (an average of 3.7 days) compared to Nipponbare (Fig. 2-4B and Table 2-3). I expect this short PSP influence the unique growth characteristics of these varieties, such as premature heading and heading asynchronous in photo-In varieties; the timing of heading differs between tillers whereas most tiller panicles emerge at about the same time in photo-Se varieties (Takada, 1986). This may contribute to avoiding uniform low-temperature damage (Takada, 1986). In the next chapter, I focused on this point and expanded the research to examine the effects the shorten of PSP on heading characteristics.

2.4.4 Effect of *ghd7* and *osprr37* on shortening of PSP

The loss of function of *ghd7* and *osprr37* has been reported in many photo-In varieties in high latitude regions (Fujino et al., 2019a; Gao et al., 2014; Saito et al., 2019). I tested varieties with the four combinations of presence/absence of functional alleles at *Ghd7* and *Osprr37* to find difference in the lengths of PSP (Fig. 2-6). PSP length was shorter in the order of *ghd7*/*osprr37*, *ghd7* (hemizygous)/*Osprr37, Ghd7*/*osprr37*, and *Ghd7*/*Osprr37*. I found that *ghd7* was more effective than *osprr37* at shortening PSP. Loss of function of *osprr37* alone also shortens the PSP when *Ghd7* is functional, but the effect of *osprr37* is obscure when *ghd7* is nonfunctional. (Fig. 2-6). In other words, when *Ghd7* is functional, the onset of PI is inhibited irrespective of *osprr37* allele for a certain period during which PSP is continued, whereas when *ghd7* is non-functional, PI occurs immediately after BVP so that PSP is short. *Osprr37* seems to enhance the function of *Ghd7*, making PSP longer, but its action is insufficient to complement *Ghd7*.

I observed all the four *ghd7* hKO plants headed about 21 days earlier than the WT in T0

generation (Fig. 2-5). Note that these plants are hemizygous and they likely have one copy of functional *Ghd7* allele. Gene dose effect of *Ghd7* has not been reported yet, and it will be necessary to investigate the DTH of their progeny.

PSP lengths were more or less extended by low temperature irrespective to varieties (Fig. 2-4). Therefore, LT probably extend PSP in general. However, the degree of extension was different between Nipponbare and photo-In varieties. PSP of Nipponbare is much longer than those of photo-In in LT. Given that photo-In varieties lack both functional *ghd7* and *osprr37*, it is possible that *Ghd7* and/or *Osprr37* support the extension of PSP, which can be accomplished by the suppression of PI. Further study is necessary to clarify the mechanism of PSP extension in LT.

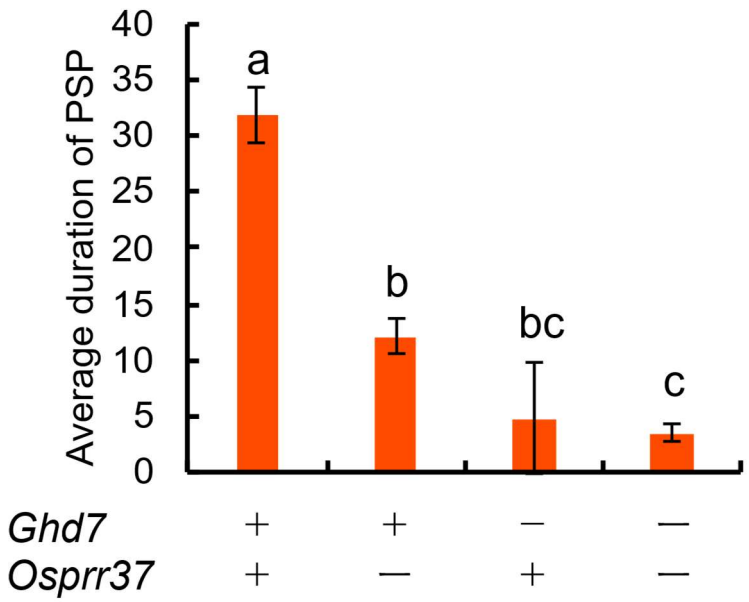


Figure 2-6. PSP variation by *Ghd7* and *Osprr37* genotype.

The durations of PSP periods for each genotype set were examined under LD (long-day photoperiod: 14 h light/10 h dark) and NT (normal day/night temperatures: 25/20 ◦C). Each genotype set corresponds as follows, *ghd7*/*osprr37* (A58, Norin 20, Hakuchomochi, Kitaake), *ghd7*/*Osprr37* (four *Ghd7*/*ghd7* hKO plants), *Ghd7*/*osprr37* (Kyodaiodajo), and *Ghd7*/*Osprr37* (Nipponbare and the WT plant), respectively.+ and below bars indicate functional and nonfunctional alleles of *Ghd7* and *Osprr37*. Alphabets above bars show the significant differences between varieties under each daylength condition, as determined using ANOVA with Tukey's HSD.

Chapter 3. Genetic and environmental factors that regulate early heading in photo-insensitive rice varieties influence heading synchrony, premature heading, and lag phase associated with effective tiller production.

3.1 Introduction

Many studies define the time of flowering in cereals as the stage when 50% of the tillers have headed or when the first panicle begins to emerge (Lukac et al., 2012; Ma et al., 2009). However, rice has multiple tillers that appear sequentially, and it takes a considerable number of days from the appearance of first panicle to the last panicle heading. The length of this period is called heading synchrony and is an agronomic trait that influences rice yield and grain quality (Takeda 1986; Fig. 3-1). Earlier-heading panicles are superior to later-heading panicles in terms of panicle weight, filled grain rate, and quality (Counce et al., 1996; Zhang et al., 1998). Short-term completion of panicle heading leads synchronous maturation, which is desirable for efficient harvest. On the other hand, the simultaneous development of panicles increases vulnerability to environmental challenges such as cold and heat stress (Takeda, 1986). Heading synchrony is known to correlate with heading earliness, and early-heading varieties generally show less heading synchrony, leading to a longer period of heading (Ma et al., 2009; Takeda, 1986). As seen in Chapter 2, photo-In varieties head earlier than Nipponbare (a photo-Se variety). The photo-In varieties would show less heading synchrony than photo-Se varieties, but this has not been clarified yet..

Premature heading is a characteristic of rice plants that head extremely-early at the same time as development of the tiller and it does not allow fully mature panicles to form. Occurrence of premature heading is seen on the main stem, which means that the first panicle does not fully mature. Therefore, premature heading reduces the rice yield (Hu et al., 2015; Osada et al., 1975). Photo-In varieties are more prone to premature heading when exposed to high temperatures during the seedling stage, whereas photo-Se varieties

are less (Hu et al., 2015; Osada et al., 1975; Sakata et al., 2004). However, the mechanism why photo-In varieties prone to premature heading but not photo-Se ones is unknown.

Tillers that produce panicles are called effective tillers and tillers that do not produce panicles are called non-effective tillers. In the early-heading varieties, each panicle develops simultaneously with tiller development, and effective tillers are produced one after another as tillers form. In contrast, in the middle- and late-heading varieties, panicle development is likely to occur after the tillers are developed, and most effective tillers initiate panicle development at similar times. The period from the day when the effective tiller number is determined to the day of panicle initiation is called the lag phase and an important trait related to heading synchrony (Takeda 1986) (Fig. 3-1). Early-heading varieties have negative values for the lag phase because panicle initiation precedes the determination of the number of effective tillers. For middle- and late-heading varieties, the lag phase usually shows positive values (Takeda 1986). Occurrence of premature heading accompanies the lag phase with a large negative value, which means that panicle initiation of the main stem occurs much earlier than the day when the effective tillering is completed.

In chapter 2, photo-In varieties are characterized as early-heading varieties under several environmental conditions including altered photoperiod and temperature. As has been described above, heading is not the only determinant of the yield but other characters associated with panicle and tiller should be considered. However, there is the question whether such characters express constitutively or they are affected by environmental factors. Use of photo-In varieties as early heading varieties in various regions needs these information. It is also important to investigate the genetic variation of these characters because presence of the variation suggests the room for genetic improvement. In this chapter, I compared heading traits such as heading synchrony, premature heading, and effective tillering in the photo-Se and photo-In varieties at different latitudes, Sapporo and Iga, Mie, Japan (34° N). I attempted to detect quantitative trait loci (QTL) for these

traits in the recombinant inbred lines (RILs) using the genome-wide SNP markers (Koide et al., 2019). These results not only contribute to the understanding of the genetic basis of heading traits, but also to the application of the photo-In varieties to the cultivation in lower latitude area.

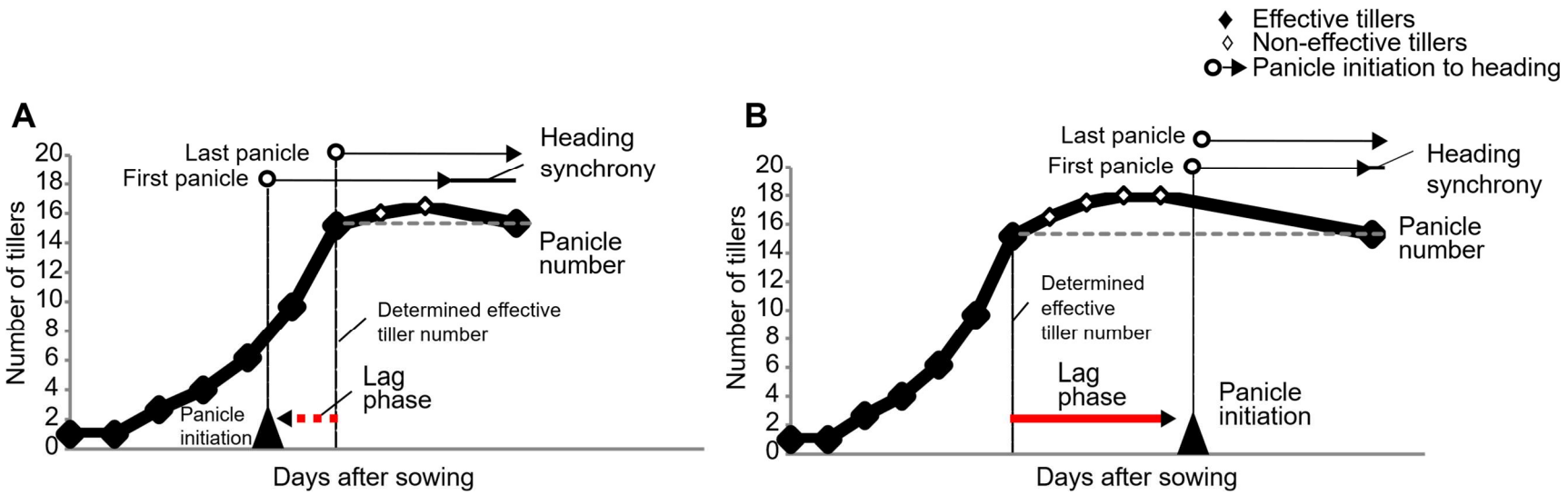


Figure 3-1. Lag phase pattern based on panicle initiation and day of the determined effective tiller number.

Lag phase was calculated as the days to panicle initiation minus day to the effective tiller number determination. Lag phase shows negative values when panicle initiation occurs before the day of the effective tiller number determination (A) and positive values when panicle initiation occurs after the day of the effective tiller number determination (B). In general, early heading lines indicate negative values of lag phase (A) and mid- and late- heading lines indicate positive values of lag phase (B). Black fills indicate effective tillers and white fills indicate non-effective tillers. Early heading varieties show a higher ratio of effective tillers producing panicles to the total number of tillers than the middle and late heading varieties (Takeda 1986).

3.2 Material and methods

3.2.1 Plant materials and growth conditions

The genotypes of four photoperiod sensitivity genes in plant materials used in this study are shown in Table S3-1. *Ghd7* and *Osprr37* are fixed for non-functional alleles and *Hd1* and *DTH8* genotypes varied among materials (Fujino et al., 2019a). A58/Kitaake RILs have either non-functional (A58 type) or functional (Kitaake type) *Hd1* allele (Koide et al., 2019).

The experiments were conducted in two consecutive years (2018 and 2019) in two different paddy fields: one at Hokkaido University, Sapporo, Japan (43.1° N) and the other at Mie Prefectural Agricultural Research Institute, Iga, Japan (34.4° N). Seeds were sown in greenhouses in early May and late April in Sapporo and Iga, respectively, and three- to four-week-old plants were transplanted into paddy fields (Table 3-1). Six plants of each variety were examined. Growth chamber was set as described in chapter 2.

In 2018, I used two photo-In varieties adapted to Hokkaido, A58 and Kitaake, and 30 recombinant inbred lines (RILs) derived from a cross between A58 and Kitaake. The thirty RILs consisted of 15 each of early- and late-heading lines, which were selected from 132 lines in the F3 generation (Koide et al., 2019). I selected two of the earliest heading RILs (E-RIL-1 and -2) and two of the latest heading RILs (L-RIL-1 and -2) in 2018. In 2019, I evaluated these four RILs as well as nine photo-In varieties (A58, Kitaake, Akage, Norin 20, Hakuchomochi, Kirara 397, Hoshinoyume, Nanatsuboshi, and Daichinohoshi) . As photo-Se varieties, I used Koshihikari in all the test plots, and Mie 33 in Iga for two years and in Sapporo in 2019.

3.2.2 Heading properties analysis

Days of heading were recorded for all tillers of single plant. Heading status of each plant was designated as follows: the first day of heading, which was used to calculate days from sowing to first panicle emergence (F-DTH); the last day of heading, which was used to calculate days from sowing to last panicle emergence (L-DTH). Panicle number per plant was defined as the total number of panicles headed. Index of heading synchrony and the degree of premature heading (Deg_Pre_Hed) were calculated using the following equations:

Heading synchrony = (L-DTH) – (F-DTH)

Deg_Pre_Hed = (days from sowing to second panicle heading) – (F-DTH)

I calculated the lag phases of A58, Kitaake, four RILs (E-RIL-1, -2 and L-RIL-1, -2), and Koshihikari. Lag phase was calculated based on the days to PI (see below) and the day when the effective tiller number was determined.

The number of tillers was recorded every week, starting from three weeks after transplanting, and the average tiller number for each variety was calculated. The day of the effective tiller number determination for each plant was assessed as the number of days from sowing until the number of tillers reached the panicle number (Fig. 3-1).

Because the day of PI is difficult to determine in paddy field, I examine PI of the same materials in a greenhouse, and the data were used as proxies. The periods from the PI to heading (i.e., RP, see chapter 2) were examined, which were used to estimate the day of PI from F-DTH. Note that RP is rather stable irrespective with environment (see chapter 2). The method of determination of panicle initiation date was described in Chapter 2.

3.2.3 Correlation analysis

The correlation coefficients among F-DTH, L-DTH, heading synchrony, Deg_Pre_Hed, and panicle number for each experimental condition were calculated using Excel 2019 (Microsoft). The correlation coefficients between years and locations were estimated using Excel 2019.

3.2.4 QTL analysis

QTL analysis of heading traits and panicle number were performed for the 30 RILs. Of 634 markers detected using ddRAD-seq from 30 RILs (Baird et al., 2008; Koide et al., 2019; Peterson et al., 2012), I selected 224 polymorphisms that were confirmed by the A58 whole-genome resequencing data and the Kitaake sequence from the DDBJ database (accession numbers DRA007777 and SRA054074, respectively). A linkage map was constructed using version 3.0 of MAPMAKER/EXP (Lander et al., 1987). QTL analysis was performed by composite interval mapping using Windows QTL Cartographer v.2.5 software (Wang et al., 2012). The analysis parameters were standard model 6, window size of 10 cM, walking speed of 1 cM, and forward and backward regression models. A significance threshold value ($\alpha = 0.05$) was determined with 1000 permutations for each trait. Multiple-trait composite interval mapping was also performed to test pleiotropic and environmental effects of QTLs.

3.2.5 Photoperiod and temperature sensitivity analysis

A58, Kitaake, E-RIL-1, -2, L-RIL-1, and -2 were examined for F-DTH. Each line consisted of four plants. Plants were grown in growth chambers under four different controlled environments, which comprised combinations of two photoperiod conditions (14 h light/10 h dark and 12 h light/12 h dark) and two temperature conditions (25 °C light/20 °C dark and 22 °C light/18 °C dark). Tillers were removed when they emerged.

Table 3-1. Test environments and materials used in this study

Year	Place	Date of sowing	Date of transplanting	Materials
2018	Iga	19-Apr	10-May	A58, Kitaake, Koshihikari, 30 RILs[a]
	Sapporo	6-May	7-Jun	A58, Kitaake, Koshihikari, 30 RILs
2019	Iga	24-Apr	15-May	9 Photo-insensitive varieties[b], Koshihikari, Mie 33, 4 RILs
	Sapporo	9-May	4-Jun	9 Photo-insensitive varieties, Koshihikari, Mie 33, 4 RILs

[a] RILs were derived from the cross between A58 and Kittake.

[b] Nine photo-insensitive varieties were A58, Kitaake, Akage, Norin 20, Hakuchomochi, Kirara 397, Hoshinoyume, Nanatsuboshi, and Daichinohoshi.

3.3 Results

3.3.1 Environmental differences between Sapporo and Iga

To investigate the environmental effects on heading characteristics, experiments were conducted in two years in two different paddy fields in Sapporo (43° N) and Iga (34° N). The sowing and transplanting dates at each site were set according to the local growing season (Table 3-1). The seedling periods were 32 and 26 days in Sapporo in 2018 and 2019, respectively, and 21 days in Iga in both years. In both 2018 and 2019, the temperatures were generally lower in Sapporo than in Iga (Fig. 3-2A). However, due to the longer seedling periods in Sapporo, the accumulated temperature until transplanting was higher in Sapporo (two-year average: 476 ℃) than in Iga (two-year average: 389 ℃). The difference between the two latitudes resulted in longer daylight hours at Sapporo than at Iga throughout the growing season (Fig. 3-2B).

3.3.2 Variations of F-DTH, L-DTH, heading synchrony, and Deg_Pre_Hed in Sapporo and Iga

The two-year averages of F-DTH for photo-In varieties were 79 and 72 days in Sapporo and Iga, respectively, whereas the F-DTH averages for photo-Se varieties were 121 and 98 days, respectively (Fig. 3-3A). The difference in F-DTH between the photo-Se and -In lines was greater in Sapporo than in Iga (Fig. 3-3A), indicating that heading of photo-Se varieties was suppressed by longer day length in Sapporo. On the other hand, the variation in L-DTH in Iga was smaller than that in Sapporo, and while L-DTH took about 100 to 140 days in Sapporo, it took only about 80 to 110 days in Iga (Fig. 3-3A). Comparing the two-year averages, the differences in F-DTH between photo-In lines and photo-Se varieties were 42 days in Sapporo and 25 days in Iga, while the differences in L-DTH were 26 days in Sapporo and 19 days in Iga. Based on average, the difference in F-DTH was larger than that in L-DTH (Fig. 3-3A). Taking the above data together, the heading synchrony of the photo-In varieties (26 days in Sapporo and 12 days in Iga on a

two-year average) was longer than that of the photo-Se varieties (10 days in Sapporo and five days in Iga on a two-year average) (t-test, $P < 0.01$) (Fig. 3-3A). Premature heading also exhibited a trend similar to that of heading synchrony. In Sapporo, Deg_Pre_Hed of the photo-Se varieties were less than three days in two years, whereas the photo-In varieties showed Deg_Pre_Hed ranging from 1 to 11 days (Fig. 3-3A). Although some of the photo-In varieties had Deg_Pre_Hed similar to that of the photo-Se varieties, the photo-In varieties tended to have a longer Deg_Pre_Hed than the photo-Se varieties (Fig. 3-3A). In Iga, all varieties had two days or less Deg_Pre_Hed in both years, and there were no differences between the photo-Se and -In varieties (Fig. 3-3A) (t-test, $P > 0.1$). There were no clear differences in panicle numbers between the photo-Se and photo-In lines (Fig. 3-3A) (t-test, $P>0.1$).

3.3.3 Variations in panicle initiation, effective tiller, and lag phase in Sapporo and Iga

I compared the lag phase of two photo-In varieties (A58 and Kitaake), four RILs (E-RIL-1, -2, and L-RIL-1, -2), and Koshihikari in 2019 in Sapporo and Iga. I observed the tiller number transition at seven-day intervals starting 21 days after transplanting. Tiller number increase is slower in Sapporo than in Iga for all lines (Fig. 3-4). The slower increase in tiller number in Sapporo means that the day when the effective tiller number determined is later than Iga: it takes between 84 and 96 days in Sapporo whereas it takes between 63 and 66 days in Iga (Table 3-2). A good correlation was observed between F-DTH and the days to PI irrespective to line or study site ($r = 0.99$ at Sapporo and $r = 0.96$ at Iga) (Fig. 3-5A). On the other hand, F-DTH did not correlate with the day when the effective tiller number is determined (Fig. 3-5B). In both study sites, PI occurred earlier in the photo-In varieties (A58, Kitaake, and the four RILs) than in the photo-Se variety, Koshihikari (Table 3-2). In Sapporo, there was a large difference in PI among the photo-In varieties, ranging from 25 to 51 days, whereas in Iga, the difference was small, ranging from 30 to 40 days (Table 3-2). All lines took much time for PI in Sapporo than in Iga

except for E-RIL-1 (Table 3-2). The differences in days for PI between Sapporo and Iga were less than four days for E-RIL-1 and E-RIL -2, whereas the differences were more than nine days for L-RIL-1 and L-RIL -2, and 31 days for Koshihikari (Table 3-2). These indicated that PI in Sapporo tended to be delayed in the late heading lines than in the early heading lines. The lag phases for the photo-In varieties were ranged from -32 to -58 days in Sapporo and from -22 to -33 days in Iga (Table 3-2). For the photo-Se variety Koshihikari, the lag phase was -2 days in Sapporo (PI, 85 days; days of the determined effective tiller number, 87 days) and -10 days in Iga (PI, 54 days; days of the determined effective tiller number, 64 days) (Table 3-2). As shown in Fig. 3-5C, the length of lag phase exhibited correlation with F-DTH in both Sapporo (r=0.95) and Iga (r=0.97).

3.3.4 Correlation analyses for the traits and areas

Pairwise Pearson's correlation analysis was performed for the photo-In varieties and RILs to determine the relationships between each combination of F-DTH, L-DTH, heading synchrony, Deg_Pre_Hed, and panicle number. The correlation coefficients for the five traits are listed in Table 3-3. All experimental plots showed negative correlations with F-DTH and heading synchrony, and F-DTH and Deg_Pre_Hed (Table 3-3). In addition, positive correlations were observed between the heading synchrony and Deg_Pre_Hed at both locations (Table 3-3). Although heading synchrony was positively correlated with L-DTH in Iga in 2018, the correlations were less significant and not as consistent as other traits. Although there were some exceptions, panicle number weakly correlated to F-DTH and heading synchrony ($|r| < 0.65$), suggesting that panicle number plays minor role in heading characteristics. In 2019, I examined eight traits in Table 3-4, and sought whether they showed any relationship between days to PI, days to the effective tiller number determination, and lag phase (Table 3-4). At first glance, figures in tables of Sapporo and Iga are similar to each other (Table 3-4). Among these, F-DTH and L-DTH showed high correlation coefficients with days to PI and lag phase ($r > 0.84$) (Table 3-4). In addition,

correlation coefficients were high between days to PI and lag phase in both Sapporo and Iga (Table 3-4). On the other hand, days to the effective tiller number determination did not show high correlation coefficient with any of the traits examined (Table 3-4).

To confirm how environmental differences between Sapporo and Iga affected heading traits, I compared five heading traits. F-DTH, L-DTH, heading synchrony and Deg_Pre_Hed showed wider variation in Sapporo than in Iga (Fig. 3-3). The coefficients of determination (r^2) between Sapporo and Iga for F-DTH, L-DTH, and heading synchrony were greater than 0.53 in the two years (Fig. 3-6A to C), and the orders of lines according to phenotypic values were mostly consistent between the two study sites for all the traits. The regression lines for F-DTH between Sapporo and Iga exhibited slopes of 0.24 and 0.49 in 2018 and 2019, respectively (Fig. 3-6A), and the late heading line in 2019 tended to have a greater difference between the two study sites. Indices for heading synchrony (i.e., L-DTH - H-DTH) are greater in Sapporo than Iga, suggesting less synchronous heading in Sapporo(Fig. 3-6C). As the index of heading synchrony was inversely correlated with F-DTH (Table 3-3), large F-DTH (i.e., the first heading occurs earlier) resulted in large index value of heading synchrony in Sapporo than in Iga (Fig. 3-6D). Both F-DTH and the index of heading synchrony showed large differences among the lines in Sapporo, but the differences were limited to Iga. As shown in Figs. 3-3 and 3-6, variation in heading traits tended to occur at high latitudes.

3.3.5 Day-length and temperature affects F-DTH in photo-In lines

As shown in Fig. 3-3, F-DTH of photo-In varieties differs between Sapporo and Iga. In chapter 2, I observed the effects of day length and temperature on DTH (=F-DTH) of A58 and Kitaake but not E-RIL-1, E-RIL-2, L-RIL-1, and L-RIL-2. I tested the heading changes of E-RIL-1, E-RIL-2, L-RIL-1, and L-RIL-2, as well as A58, Kitaake under different day length and temperature conditions: four different environmental conditions

were created in growth chambers, consisting of two photoperiod conditions (long day length: 14 h light/10 h dark, short day length:12 h light/12 h dark) and two temperature conditions (25 °C light/20 °C dark, 22 °C light/18 °C dark). Regardless of temperature conditions, F-DTH was longer under long day lengths than under short day lengths for all lines examined (Fig. 3-7). A58, Kitaake, L-RIL-1, and L-RIL -2 had F-DTHs of 60 days or more under short day lengths, but their F-DTHs extended by 20 to 40 days by long day length or low temperatures. Although E-RIL-1 and E-RIL -2 had F-DTHs of 60 days or less under short day lengths, their F-DTHs were extended by only approximately 10 days in the condition of long day length or low temperatures (Fig. 3-7). Altogether, long day length and low temperature extends F-DTH although the magnitude of the impacts differs among genotypes. The observed differences appear to be consistent with those in Sapporo and Iga.

3.3.6 Genetic variations of heading traits among RILs

To understand heading variation and genetic regulation among photo-In lines, I tested 30 RILs derived from a cross between two photo-In varieties, A58 and Kitaake. These photo-In varieties possess non-functional alleles for *Ghd7* and *Osprr37*, which contributed to adaptation to Hokkaido through extremely early heading (Fujino et al., 2013; Fujino and Sekiguchi, 2005b, 2005a; Nonoue et al., 2008; Ota et al., 2014), while the functional *Hd1* and *DTH8* alleles remain in some Hokkaido varieties, reducing their importance than *Ghd7* and *Osprr37* (Table S3-1). For further analysis, I evaluated nine photo-In varieties in addition to four RILs of two of the earliest and latest heading lines (E-RIL-1, E-RIL-2, L-RIL-1, and L-RIL-2). Compared to A58 and Kitaake, these varieties exhibited similar heading patterns, and no significant differences were observed in F-DTH, L-DTH, heading synchrony, or Deg_Pre_Hed in Sapporo either of the year (*t*-test, $P>0.05$) (Figs. 3-3 and S3-1). In Iga, heading synchrony and Deg_Pre_Hed were not significantly different from those of A58 and Kitaake. The F-DTH and L-DTH of Kitaake occurred 2–

3 days earlier than those of A58 in Iga (Fig S3-1).

The 30 RILs in 2018 segregated phenotypic values in large extent, although their mean values were close to those of their parents (Fig. 3-3, S3-1). In 2018, the F-DTH for the 30 RILs ranged from 69 to 98 days and 79 to 90 days in Sapporo and Iga, respectively, whereas the L-DTH ranged from 112 to 126 days and 95 to 100 days, respectively (Fig. 3-3). L-DTH exhibited a smaller variation than F-DTH (F-test; $P<0.01$ in both Sapporo and Iga). As I showed in Table 3-3, L-DTH showed the weaker correlations with heading synchrony or Deg_Pre_Hed than F-DTH. Together with the small variation of L-DTH, role of L-DTH in heading synchrony and Deg_Pre_Hed seems to be small. As the index of heading synchrony and Deg_Pre_Hed are composite values with F-DTH as a component, it means that L-DTH (i.e., the end of heading) is largely independent of the start of heading.

The indices of heading synchrony of RILs ranged from 18 to 47 days and 18 to 37 days in 2018 and 2019, respectively, in Sapporo, whereas those in Iga ranged from 8 to 18 days in 2018 and 11 to 18 days in 2019 (Fig. 3-3). The Deg_Pre_Hed of RILs also showed a very similar distribution pattern to that of heading synchrony; in Sapporo, it was distributed over a wide range from 3 to 19 days in both years, whereas in Iga, it was within 3 days. In addition, I observed that premature heading rarely occurred in Iga (Deg_Pre_Hed in Fig. 3-3). As shown in Fig. S3-1, the variations in the indices of heading synchrony and Deg_Pre_Hed of the RILs were mostly greater than those for the photo-In varieties. Hence, it was obvious that the RILs possessed different genetic factors regulating early heading, heading synchrony, and Deg_Pre_Hed. The expression of these traits are more exaggerated at higher latitudes than at lower latitudes.

3.3.7 QTL analysis for heading synchrony and F-DTH

The RILs seemed to segregate genes for heading traits and panicle number. To analyze these genes, I performed a QTL analysis at the two study sites. I used thirty RILs including E-RIL-1, E-RIL-2, L-RIL-1, and L-RIL-2, which were produced using A58 and Kitaake as the parents. The two parental lines lack the functional *Ghd7* and *Osprr37* alleles. As I showed previously, the RILs headed under long days but, for example, their F-DTH varied by more than 30 days (Sapporo, 2018). This difference suggests the segregation of genetic factors affecting F-DTH and other heading traits.

Of 634 SNP markers between A58 and Kitaake that were available (Koide et al., 2019), I selected 224 SNP markers. I detected five QTLs for F-DTH, seven for L-DTH, three for heading synchrony, five for Deg_Pre_Hed, and five for panicle number (Fig. S3-2, Table 3-5). Among the four QTLs for F-DTH, only *qFDTH4* was detected in both Sapporo and Iga, whereas the other three were obtained from Iga. The *qFDTH4* had the largest effect, accounting for 64% and 41% of the total phenotypic variation in Sapporo and Iga, respectively (Fig. S3-2, Table 3-5). I detected seven QTLs for L-DTH; however, none were common in Sapporo and Iga. One of the seven QTLs named *qLDTH5* explained 40% of the total phenotypic variation in L-DTH (Table 3-5). I also identified three QTLs for heading synchrony: *qHS1* from Sapporo, *qHS4* from Sapporo and Iga, and *qHS8* from Iga (Fig. S3-2, Table 3-5). Among these, *qHS4* showed the largest effect accounted for 63% of the total phenotypic variation in Iga, and the total phenotypic variation in *qHS4* in Sapporo was 27% (Fig. S3-2, Table 3-5). Five QTLs for Deg_Pre_Hed were detected; *qPH4* was detected in both Sapporo and Iga, and others were detected in either Sapporo or Iga. *qPH4* showed the largest effect in Sapporo, and *qPH8* indicated the largest effect in Iga. The QTLs, *qFDTH4, qHS4,* and *qPH4* were mapped to similar positions on chromosome 4 (Table 3-5). Similarly, *qFDTH8*, *qLDTH8*, *qHS8*, and *qPH8* were located close to each other on chromosome 8 (Table 3-5). Multiple trait analysis did not show any epistatic interaction among the QTLs, implying additive effects of these QTLs.

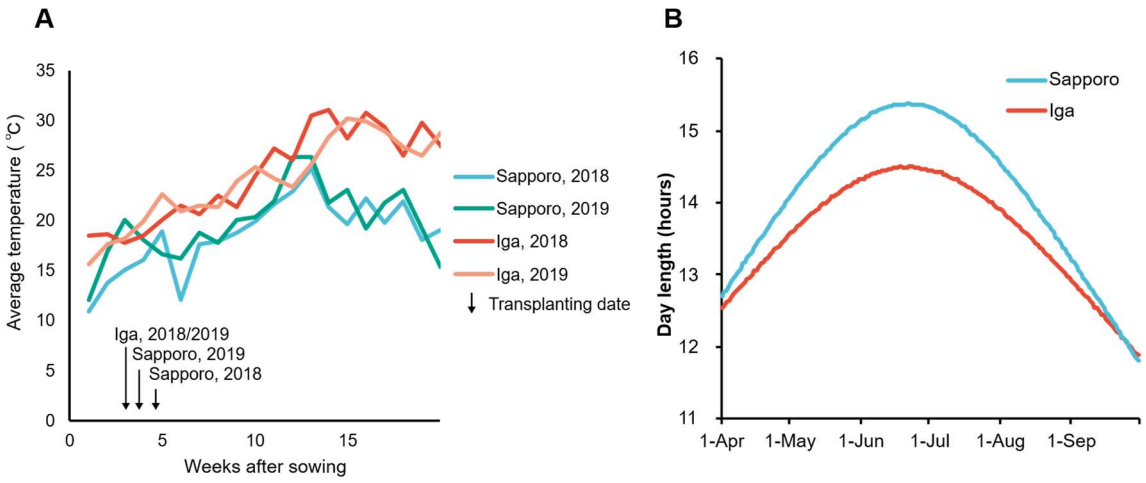


Figure 3-2. Environmental conditions in Sapporo and Iga

(A) Average weekly temperature obtained from the stations of AMEDAS. Arrows indicate the transplanting date of each experimental plot. (B) Day length condition from April to the end of September.

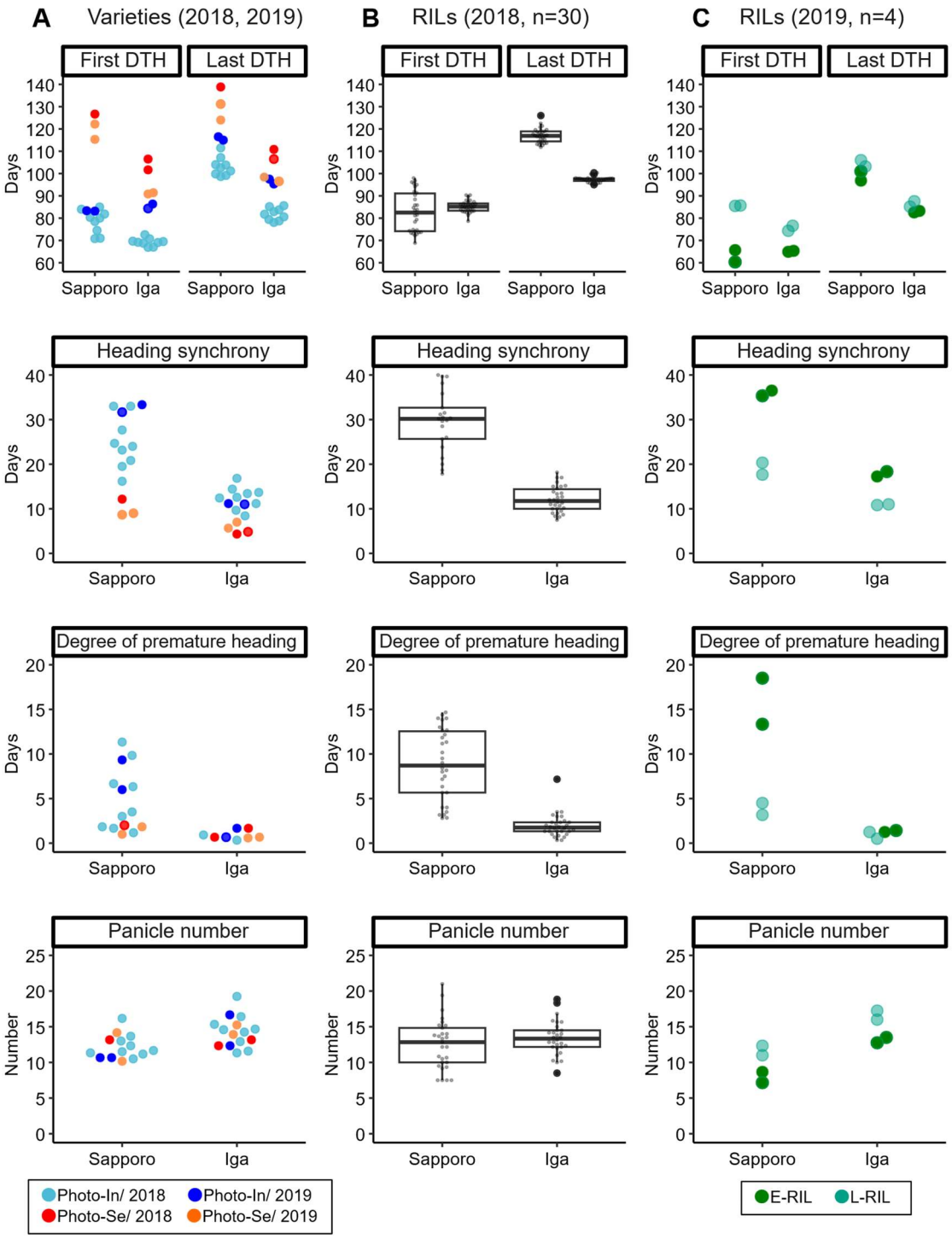


Figure 3-3. Variations of four heading traits and panicle number in Sapporo and Iga.

(A) The materials used in 2018 and 2019 from the photo-In and -Se varieties were common to Sapporo and Iga, listed in Table 1. (B) In 2018, the materials were derived from the 30 RILs were

used in both Sapporo and Iga. (C) In 2019, the four materials of two early and late RILs (E-RIL-1, -2 and L-RIL-1, -2) were tested in both Sapporo and Iga. The first DTH, last DTH, heading synchrony, degree of premature heading, and panicle number in Sapporo and Iga over the two years are shown. Each dot represents the average of each variety.

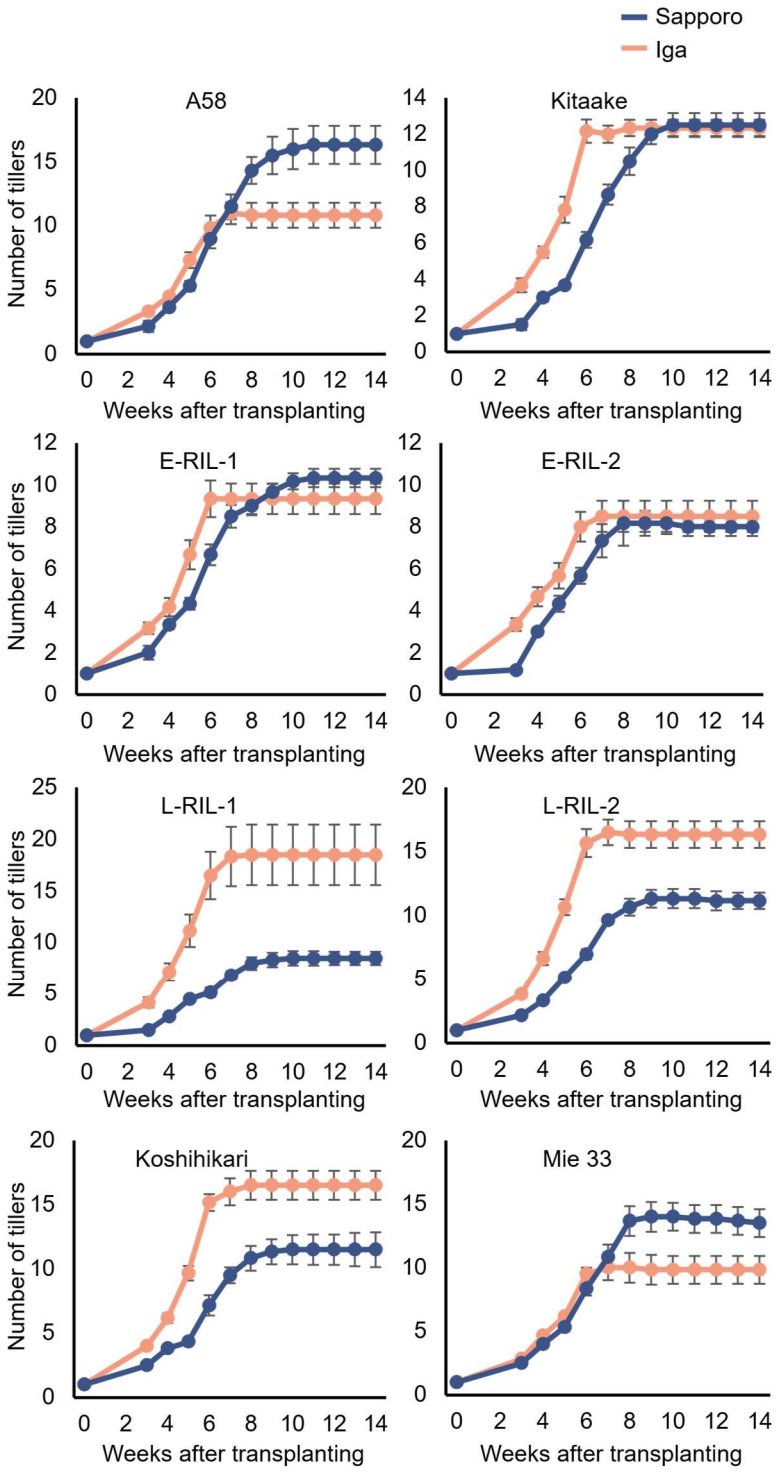


Figure 3-4. Tiller number transitions of the eight lines in Sapporo and Iga in 2019.

Each dot indicates the average number of tillers in Sapporo (blue) and Iga (red). Tiller numbers were recorded every week from three weeks after transplanting.

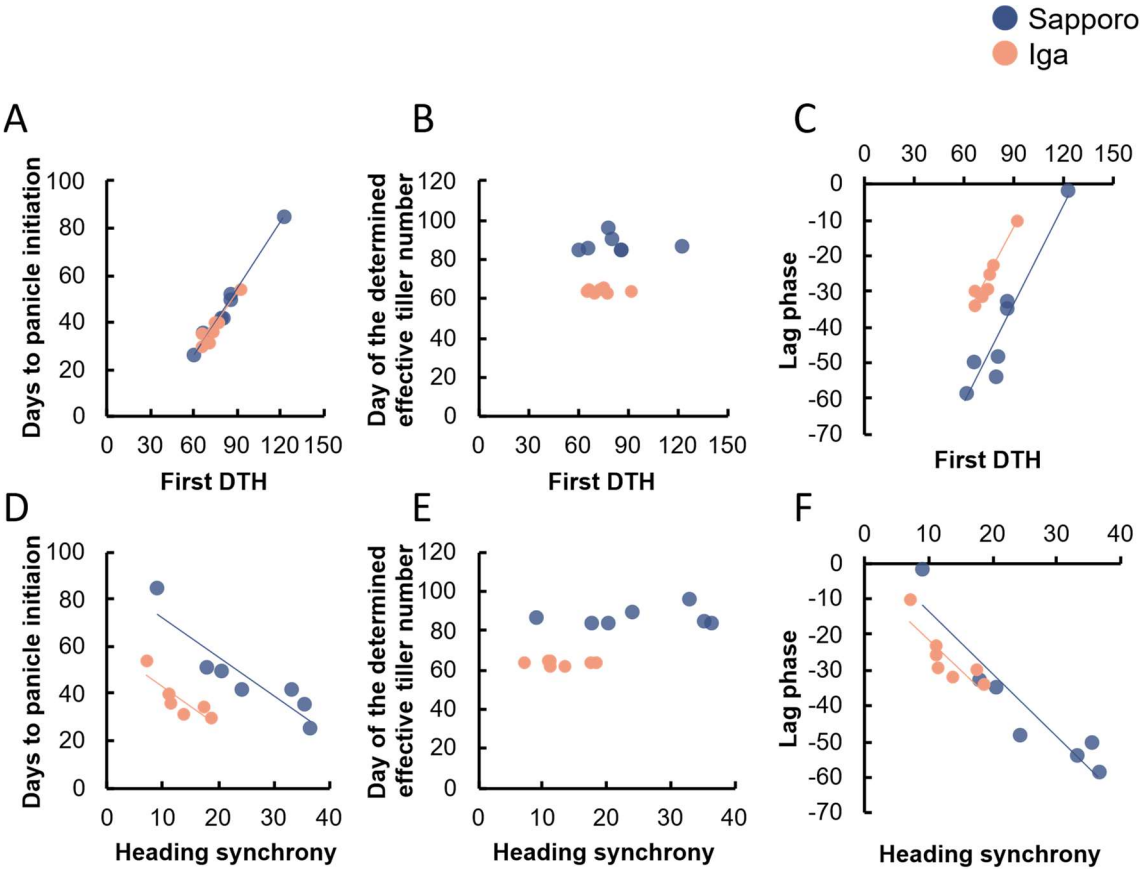


Figure 3-5. The relationships of first DTH or heading synchrony to the three-lag phase related traits.

(A), (B), and (C) show the relationships between first DTH and day to panicle initiation, day of the determined effective tiller number, and lag phase, respectively. (D), (E), and (F) show the relationships between heading synchrony and day to panicle initiation, day of the determined effective tiller number, and lag phase, respectively. Each value represents the average of A58, Kitaake, the four RILs (E-RIL-1, -2 and L-RIL-1, -2), and Koshihikari.

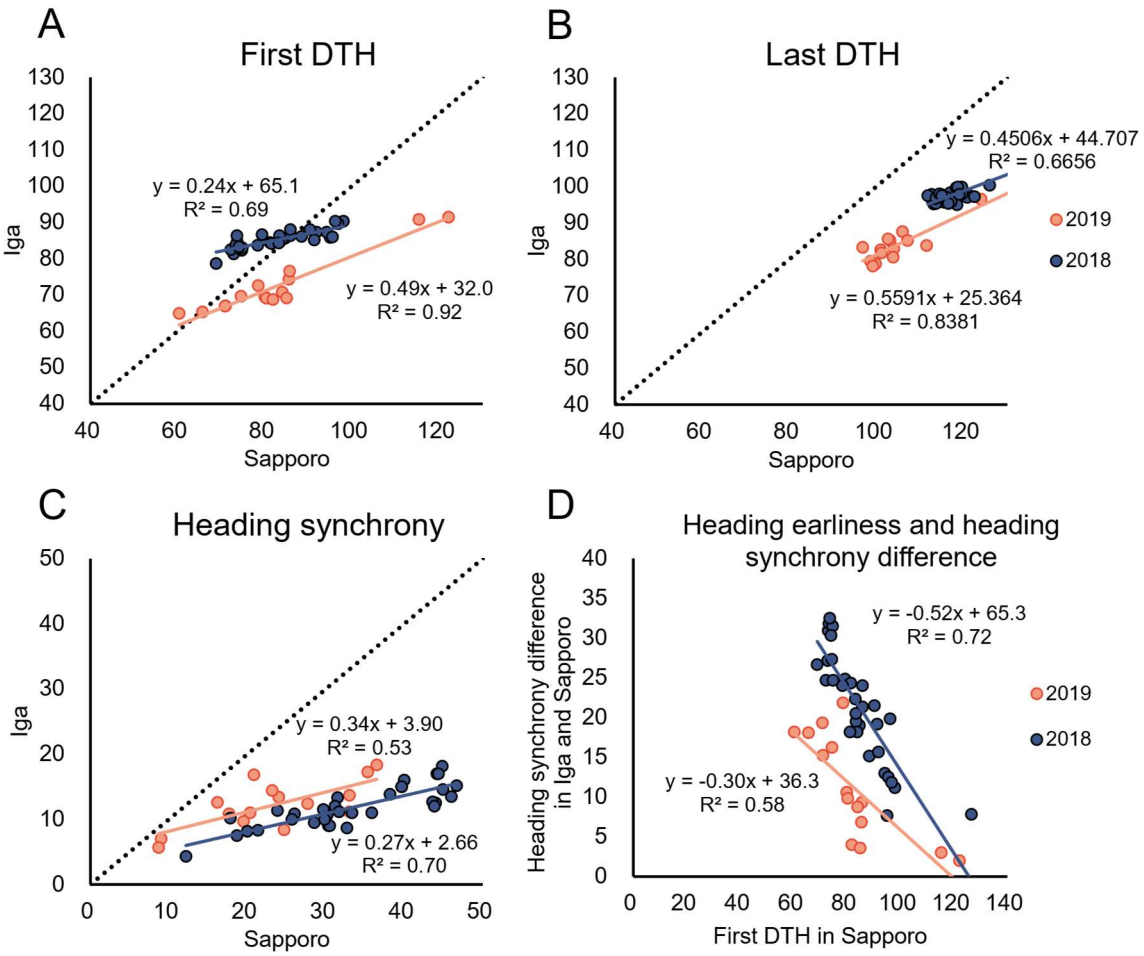


Figure 3-6. Difference in heading traits between Sapporo and Iga.

(A), (B), and (C) show the first DTH, last DTH, and heading synchrony difference between Sapporo and Iga among the lines over two years, respectively. The dotted line represents y=x. (D) Relationship between the heading synchrony difference in the two places and the heading earliness.

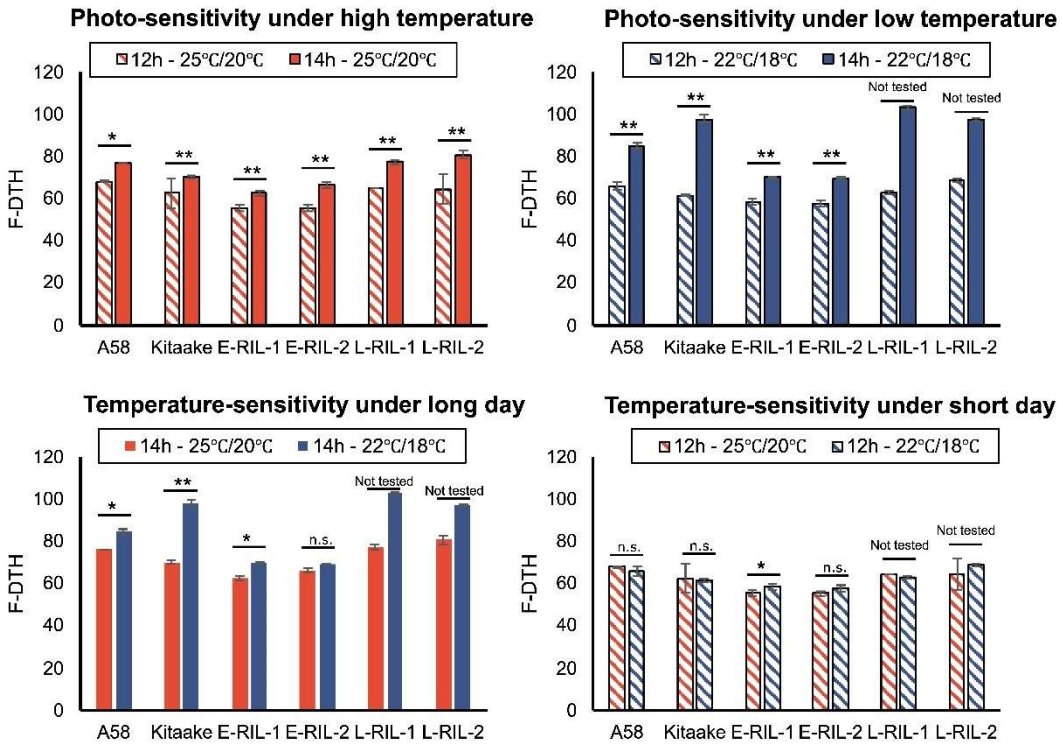


Figure 3-7. F-DTH of A58, Kitaake, and RILs under four different photoperiod and temperature conditions.

Each bar plot indicates the average F-DTH of four plants under four different controlled environments, which comprised combinations of two photoperiod conditions (long day length: 14 h light/10 h dark, short day length:12 h light/12 h dark) and two temperature conditions (25 °C light/20 °C dark, 22 °C light/18 °C dark). Statistical analysis was not performed on some plots because some individuals died.

Table 3-2. The difference in days to panicle initiation, day of the determined effective tiller number, and lag phase in Sapporo and Iga.

	Days to panicle initiation (A)			Day of the determined effective tiller number (B)			Lag phase (A-B)[a]	
	Sapporo	Iga	Difference[b]	Sapporo	Iga	Difference	Sapporo	Iga
A58	42.0 ± 2.3	36.1 ± 0.2	5.9 **	96 ± 2.3	65 ± 2.1	30.7 **	-54.0	-29.3
Kitaake	42.0 ± 1.2	31.5 ± 0.3	10.5 **	90 ± 2.6	63 ± 0.0	27.2 **	-48.2	-31.5
Early RIL-1	25.7 ± 0.6	30.3 ± 0.2	-4.6 **	84 ± 4.3	64 ± 1.1	20.2 **	-58.7	-33.9
Early RIL-2	35.3 ± 0.7	35.0 ± 0.3	0.3 n.s.	86 ± 2.7	65 ± 1.5	20.8 **	-50.2	-29.8
Late RIL-1	51.5 ± 1.6	40.4 ± 0.3	11.2 **	84 ± 1.3	66 ± 1.5	18.5 **	-32.8	-25.4
Late RIL-2	49.4 ± 1.0	40.3 ± 0.4	9.1 **	84 ± 2.7	63 ± 0.0	21.3 **	-35.0	-22.7
Koshihikari	84.9 ± 0.4	54.2 ± 0.1	30.8 **	87 ± 3.2	64 ± 1.1	22.5 **	-1.8	-10.0

[a] Lag phase was calculated by days to panicle initiation minus day of the determined effective tiller number.

[b] Difference was calculated by the values in Sapporo minus in Iga.

Table 3-3. Pairwise Pearson correlation coefficients among heading characteristics.

Year	Place	Traits	First DTH	Last DTH	Heading synchrony	Deg_Pre_Hed	Panicle number
2018	Sapporo	First DTH	1.00				
		Last DTH	0.18	1.00			
		Heading synchrony	-0.93 **	0.18	1.00		
		Deg_Pre_Hed	-0.90 **	-0.10	0.87 **	1.00	
		Panicle number	0.49 **	0.34	-0.37 *	-0.33	1.00
	Iga	First DTH	1.00				
		Last DTH	0.07	1.00			
		Heading synchrony	-0.87 **	0.42 *	1.00		
		Deg_Pre_Hed	-0.75 **	0.26	0.81 **	1.00	
		Panicle number	0.43 *	0.07	-0.35 *	-0.29	1.00
2019	Sapporo	First DTH	1.00				
		Last DTH	0.52	1.00			
		Heading synchrony	-0.87 **	-0.04	1.00		
		Deg_Pre_Hed	-0.95 **	-0.41	0.88 **	1.00	
		Panicle number	0.65 *	0.77 **	-0.31	-0.61 *	1.00
	Iga	First DTH	1.00				
		Last DTH	0.55	1.00			
		Heading synchrony	-0.60 *	0.34	1.00		
		Deg_Pre_Hed	-0.66 *	-0.44	0.69 **	1.00	
		Panicle number	0.44	0.72 **	0.19	-0.44	1.00

In 2018, two photo-In varieties and 30 RILs were tested. In 2019, nine photo-In varieteis and four RILs were tested.

Table 3-4. Pairwise Pearson correlation coefficients between traits related to lag phase.

Place	Traits	Days to panicle initiation	Day of the determined effective tiller number	Lag phase
Sapporo	First DTH	0.99 **	0.02	0.95 **
	Last DTH	0.93 **	0.24	0.84 *
	Heading synchrony	-0.91 **	0.22	-0.93 **
	Degree of premature heading	0.55 *	0.67	0.38
	Panicle number	-0.77	-0.22	-0.69
	Days to panicle initiation	1.00	-0.04	0.97 **
	Day of the determined effective tiller number		1.00	-0.26
	Lag phase			1.00
Iga	First DTH	0.96 **	-0.06	0.97 **
	Last DTH	0.95 **	-0.12	0.97 **
	Heading synchrony	-0.84 *	-0.04	-0.83 *
	Degree of premature heading	0.18	-0.23	0.21
	Panicle number	-0.45	0.21	-0.48
	Days to panicle initiation	1.00	0.09	0.99 **
	Day of the determined effective tiller number		1.00	-0.05
	Lag phase			1.00

Table 3-5. Overview of QTLa identified for all traits in Sapporo and Iga.

Trait	QTL		Place	Chromosome	Peak position (cM)	LOD score	Variance explained of total (%)	Additive effect[b]	LOD threshold
First DTH	*qFDTH4*	*	Sapporo	4	121	12.4	64	7.8	3.7
		*	Iga	4	121	8.4	41	1.8	2.9
	qFDTH8		Iga	8	55	5.0	20	-1.3	2.9
	qFDTH11		Iga	11	0	4.5	14	1.0	2.9
	qFDTH12		Iga	12	17	4.0	13	-1.1	2.9
Last DTH	*qLDTH2*		Iga	2	76	2.9	16	-0.7	2.8
	qLDTH3		Sapporo	3	2	3.6	36	2.1	2.9
	qLDTH4		Iga	4	56	5.0	28	-0.8	2.8
	qLDTH5	*	Sapporo	5	82	4.7	40	-2.3	2.9
	qLDTH6		Sapporo	6	2	4.0	26	-1.8	2.9
	qLDTH8		Iga	8	62	5.1	35	0.9	2.8
	qLDTH9		Iga	9	196	5.1	31	0.9	2.8
Heading synchrony	*qHS1*		Sapporo	1	112	5.6	18	4.0	3.4
	qHS4		Sapporo	4	121	7.4	27	-5.3	3.4
		*	Iga	4	121	11.5	63	-2.6	3.3
	qHS8		Iga	8	62	7.7	23	1.4	3.3
Degree of premature heading	*qPH4*		Sapporo	4	122	7.5	39	-2.7	3.7
			Iga	4	123	3.7	19	-0.6	2.2
	qPH5		Sapporo	5	19	4.9	24	1.9	3.7
	qPH8	*	Iga	8	64	7.0	45	0.9	2.2
	qPH12-1		Iga	12	69	3.8	23	1.0	2.2
	qPH12-2		Iga	12	82	2.5	12	-0.7	2.2
Panicle number	*qPN1*		Sapporo	1	58	3.2	19	-1.9	2.7
	qPN2		Sapporo	2	35	2.8	18	1.8	2.7
	qPN9		Iga	9	28	3.4	23	-1.4	2.9
	qPN11		Sapporo	11	56	3.6	24	-2.2	2.7
			Iga	11	43	4.3	25	-1.3	2.9
	qPN12		Iga	12	39	3.9	30	-1.6	2.9

[a] QTLs that explain more than 40% of the total variance are marked with an asterisk.

[b] Additive effects of A58 allele.

3.4 Discussion

3.4.1 F-DTH is the principal component of other heading properties

Early heading is known to inversely correlate with heading synchrony, and early heading lines grown in high-latitude regions show lesser level of heading synchrony (Ma et al., 2009; Takeda, 1986). This is consistent with this study, in which F-DTH of photo-In varieties inversely correlated with heading synchrony very well (F-DTH and the index of heading synchrony correlated inversely). In addition to this, I could examine the relationships among heading characteristics in detail. I found significant correlations among four traits, F-DTH, heading synchrony, Deg_Pre_Hed, and panicle number (Table 3-3). The photo-In varieties/lines had larger index of heading synchrony (i.e., heading of the tillers is less synchronous) and larger Deg_Pre_Hed than the photo-Se varieties (Fig. 3-3). An interesting observation is the correlation between F-DTH and days to PI (Fig. 3-5). This correlation produces following byproducts: lag phase is a composite value determined by PI and the days to the effective tiller number determination. In this study, the latter is longer in the photo-In varieties/lines than photo-Se and the value is rather stable irrespective to photosensitivity. Hence, the lag phase in the photo-In is composed of smaller PI (=smaller F-DTH) and larger days to the effective tiller number determination. Consequently, the absolute value of the lag phase becomes larger in photo-In than photo-Se. The value is negative because PI precedes the determination of effective tiller number in photo-In varieties, and F-DTH and lag phase shows positive correlation, as shown in Fig. 3-5. Unlike F-DTH, L-DTHs were mostly constant among the lines, and their variations were not large enough to explain the correlation with the variations in heading synchrony, Deg_Pre_Hed, and lag phase (Fig. 3-3, Table 3-3, 3-4). Based on these results, I propose that early heading of photo-In is a complex trait affecting heading characteristics as well as tillering, and all such phenotypes stem from early PI. Given their less synchrony in heading, I might assume that photo-In varieties express apical dominance to head main stem while perturbating the others (Fig. 3-3). Some studies have reported relationships between heading and tillering, such as negative relationship

between DTH and panicle number in photo-In lines (Fujino, 2020) or *Hd3a* promotion of branch formation (Tsuji et al., 2015). However, few reports have focused on the heading synchrony. A detailed understanding of the balance between panicle development and tiller development may contribute to the understanding of F-DTH-related heading characteristics.

3.4.2 The difference between Sapporo and Iga

Focusing on the differences between Sapporo and Iga in terms of the timing of panicle initiation and F-DTH, two characteristics were observed. First, the differences in F-DTH among lines were larger in Sapporo than in Iga, and second, the difference in F-DTH between the two areas was smaller for the photo-In lines than the photo-Se lines. The panicle initiation stage is primarily controlled by day length and temperature (Blümel et al., 2015; Preston and Fjellheim, 2022). In both years, growing season temperatures were higher in Iga and lower in Sapporo, and day length was shorter in Iga and longer in Sapporo (Fig. 3-2). To elucidate how environmental differences affect PI, I evaluated the environmental responses of photo-In lines and four RILs under four different environments (combination of two day length conditions and two temperatures conditions in growth chambers). I observed that F-DTHs were extended by low temperatures; however, the differences between F-DTH caused by temperature became larger at longer day lengths (Fig. 3-7). This result indicates that the effect of temperature on heading was more pronounced in Sapporo than in Iga, owing to the long day length and low temperature.

I found that the photo-In lines exhibited premature heading in Sapporo but not in Iga, which can be seen as increased Deg_Pre_Hed in Sapporo. As the Photo-Se lines showed little or no premature heading in the two study sites, the observed premature heading is specific to photo-In lines grown in Sapporo, hence gene x environmental effect is the

likely explanation. Premature heading has been reported to be induced by a long seedling period and high temperatures during the seedling stage (Hu et al., 2015; Osada et al., 1975; Sakata et al., 2004). In the present study, the seedling growth period was longer in Sapporo in both years (Table 3-1). Although the temperatures during seedling growth remained low, a longer seedling growth period resulted in higher accumulated temperatures until transplanting (Fig. 3-2). This could be examined if photo-In lines were grown under various environmental conditions.

The increase in tillers at Sapporo was slower than that at Iga, which resulted in an increase in the days of the determined effective tiller number (Fig. 3-4, Table 3-2). The growth of tillers is regulated by several factors, such as solar radiation, field water, and nitrogen supply (Yuan et al., 2024). They are potential causes of the increased days to effective tiller number termination but one of the most likely causes may be temperature. the temperature in Sapporo was relatively low compared to that in Iga, particularly during the early stages of transplanting (Fig. 3-2). This could have resulted in the delayed tiller growth. Given that there was no correlation between F-DTH and tillering ability (tiller growth rate or day of the determined effective tiller number), it can be inferred that this tillering delay in Sapporo was attributed to extended heading synchrony and the lag phase, particularly for the early heading lines. I believe that the key to improving heading synchrony is to improve tillering ability and to have a stable heading even under various temperature conditions.

3.4.3 Genetic analysis

In this study, I characterized photo-In lines as short F-DTH, low level of heading synchrony, and occurrence of premature heading compared to photo-Se. The phenotypic values of these traits vary among the photo-In lines, suggesting genetic variation in photo-In lines. Hence, I analyzed the RILs derived from a cross between the two photo-In

varieties, A58 and Kitaake (Table 3-3). I detected five QTLs involved in F-DTH, seven involved in L-DTH, three involved in heading synchrony, five involved in Deg_Pre_Hed, and five involved in panicle number (Table 3-5). Koide et al. (2019) had previously analyzed these RILs to report five QTLs with different effects on DTH (equal to F-DTH of this study). Among the five QTLs one located in chromosomes 4 could be close to *qFDTH4*, which had the largest effect on F-DTH. I identified another QTLs associated with heading synchrony, and degree of premature heading, *qHS4* and *qPH4*, respectively, which were closely mapped to *qDTH4*. The three QTLs showed the largest effects among the QTLs for F-DTH, heading synchrony, and degree of premature heading in Sapporo, suggesting that the QTLs had effects not only on F-DTH, but also on heading synchrony and effective tillering. Note that the indices of heading synchrony and the degree of premature heading are composite values with a component of F-DTH or days to PI, two variables showing good correlation (Fig. 3-5A). Therefore, the result of my QTL mapping seems to be reasonable (see 3.4.1). Similarly, *qFDTH8*, *qLDTH8*, and *qHS8* were closely mapped to each other on chromosome 8. As no heading-associated genes had been located on QTLs on chromosomes 4 and 8, I may expect unknown genes on these QTL. Because of the lack of detection of epistatic interactions between the QTLs located on chromosomes 4 and 8, these QTLs had additive effects on F-DTH, heading synchrony, and effective tillers. However, details of other QTLs associated with either F-DTH, L-DTH, or heading synchrony are still unclear, and further study of their genetic action will be necessary.

Some QTLs associated with heading synchrony were reported by Ishimaru and Kashiwagi (2004) and Ma et al. (2009) using *japonica* and *indica* varieties. But neither of the QTLs reported in these studies seem to match the QTLs of this study. As I found in this study, heading synchrony is not a stand-alone trait but is in a complex interaction among other heading- and tillering characteristics as well as environmental factors. Hence, a number of QTLs are likely associated with heading synchrony, and suite of QTLs detected in a study would be content dependent.

Chapter 4. General discussion

4.1 Shorten of PSP characterize heading characteristics of photo-In varieties

Rice heading is strongly affected by daylength and temperature. Differences exist in the photoperiod responses among rice varieties (Collinson et al., 1992; Vergara and Chang, 1985), and the length of the PSP is generally used as an indicator of photosensitivity (Adams et al., 2001; Yin et al., 1997). I found that the PSP was the most sensitive phase not only to photoperiod but also to temperature, and the effects of temperature were not constant through plant development (Fig. 2-4). Another important finding is that photo-In varieties mostly lost PSP (Fig 2-4). This short PSP characterized the heading traits of photo-In varieties from two perspectives; One is that stable early heading under low temperatures, and another is that association with F-DTH and other heading traits.

Delayed heading due to LTs, especially in photo-Se varieties, has conventionally been attributed to slower vegetative growth (Andaya, 2003), but our findings suggest that this delay largely arises from the extended PSP (Fig. 2-4B and Table 2-3). The smaller delay due to LT in the photo-In varieties than photo-Se varieties observed in chapter 2 might represent a growth characteristic that could be targeted for adaptation to high-latitude regions. Among the photo-In varieties, Kitaake displayed the smallest LT-mediated delay in heading in the artificial conditions examined in chapter 2 (Fig. 2-4B and Table 2-3). In paddy field experiment in Sapporo and Iga described in chapter 3, the differences in F-DTH among A58/Kitaake RILs were larger in Sapporo than in Iga, and the difference in F-DTH between the two areas was smaller for the photo-In lines than the photo-Se lines. These results suggested that there are the variations of temperature sensitivity among the photo-In varieties, and Kitaake may be particularly insensitive to LT in terms of heading.

In chapter 3, I evaluated heading traits such as heading synchrony, L-DTH, premature heading. These traits have been recognized in rice breeding but have not been well studied. I found that heading earliness influenced on Deg_Pre_Hed, the timing of PI, and the lag

phase (Fig. 3-5). The photo-In lines were less heading synchronous and showed more premature heading than photo-Se lines (Fig. 3-3). As discussed in chapter 3, these characters correlated well with days to PI, one the two parameters defining PSP. Hence, I expect these heading phenotypes associated with early heading of photo-In lines could be explained by short PSP. PSP is considered to be a period of preparation for the accumulation of tillers and waiting for a suitable environment before PI (Yabuta et al., 2015). During PSP, PI should be suppressed. In the case of photo-In lines, PSP is very short or almost none, and the suppression (i.e., brake of heading) is less efficient. This situation leads simultaneous occurrence of PI with tiller development. However, since the number of prepared tiller is inadequate, the plant continues to form tillers after the PI, making apparently gradual production of effective tillers. It is interesting to note that vegetative and reproductive growth overlap within each tiller. For further study, measuring the growth phase of each tiller in field cultivation will lead to a more detailed understanding of heading characteristics and temperature sensitivity.

4.2 Heading genes associated with photo- and temperature- sensitivity

A significant number of photosensitive genes have been discovered and their networks are becoming clearer. *Ghd7* and *Osprr37* are reported to be loss of function in photo-In varieties (Fujino et al., 2013; Fujino and Sekiguchi, 2005a, 2005b; Gao et al., 2014; Ichitani et al., 1997; Koo et al., 2013; Nonoue et al., 2008). My present study demonstrated that the contribution of *Ghd7* loss to PSP shortening is greater than that of *Osprr37* loss (Fig. 2-6). *Ghd7* has been reported as a temperature-sensitive gene in rice, whose expression is known to be decreased under high temperature conditions, leading to increased expression level of the florigen gene, *RFT1* (Nagalla et al., 2021). Loss of function of Ghd7 may contribute stable early heading in high latitude area. Further analyses are needed to clarify how *Ghd7* is involved in temperature sensitivity in photo-In varieties, in which *Ghd7* is non-functional.

The PSP shortening is associated with short daylength- and low temperature-insensitivities. In As heading characteristics may be under the influence of PSP (see above), they should be affected by day length and temperature. Accordingly, in chapter 3, I detected several QTLs associated with heading characteristics, and the detected QTLs exhibited temperature sensitivity. Note that, as shown in Fig. 3-7, some characters such as F-DTH exhibited temperature sensitivity in day length-dependent manner. It seems that there might be another photosensitive gene that governs heading. As the expression of such gene is under the influence of temperature, careful genetic experiments will be necessary to fully understand the mechanism of early heading in rice.

4.3 The use of photo-In varieties cope with climate change

Avoiding stress by changing the heading period is considered an effective way to cope with the extreme climate especially intense heat in summer (Chen et al., 2018; Nemoto et al., 2011). This has become a serious problem in the Southern part of Japan (Yoshida et al., 2015). Photo-In varieties have potential to achieve the early heading but studies with this viewpoint had been scarce. I confirm that the photo-In varieties showed early heading in low-latitude region. By comparing photo-In varieties with photo-Se varieties, I characterize the heading traits of photo-In varieties grown in low-latitude region, and verified their potential to practical use. My notable findings include amelioration of heading synchrony in photo-In varieties grown in low-latitude region, the level of which is close to that observed in photo-Se varieties at high latitudes. I also found a low frequency of premature heading in photo-In varieties grown in low-latitude region (Fig. 3-3). My findings suggest it being promising that photo-In varieties cultivated in low-latitude areas may contribute to preventing damage to rice growth, which is achieved by sliding rice cultivation season more earlier to avoid high temperatures. Note that effective tiller number of photo-In varieties grown in low-latitude region is comparable to photo-

Se varieties (Fig. 3-4), suggesting that yield penalty would be minimized. In Japan and temperate regions of the Northern Hemisphere, the use of photo-In varieties permits the commencement of sowing and planting from the end of February to early March, and the completion of harvesting processes before July. My findings of QTLs can contribute to elucidate the genetic basis of the relationship between early heading and heading characteristics in high-latitude varieties, leading to effectively control of these traits.

Chapter 5. Summary

Heading date is the important trait that determines regional adaptability of rice. Photoperiod and temperature are the major trigger that induce the transitions of the growth phase from vegetative to reproductive. Although many studies are carried out on photoperiod sensitivity, relatively few studies have examined temperature sensitivity in rice. I focused on photo-In rice varieties cultivated in Hokkaido to understand temperature sensitivity because these varieties can flower independent of photoperiod and expected to be appropriated to understand the effects of temperature. In addition, the natural conditions in the high-latitude region to which the early-heading varieties have adapted may influence not only the heading date but also the heading characteristics. I studied the unique heading characteristics of photo-in varieties, such as heading synchrony, early heading, and effective tiller production, which elucidate the reproductive adaptation of early-heading rice varieties and the genetic basis of heading traits.

The transitions of the growth phases in photo-In rice varieties have not been precisely defined using conventional methods. In chapter 2, I observed leaf emergence and the initiation of the panicle in photo-In rice varieties, which allowed us to determine the duration of the growth phases BVP, PSP, and RP. I found that the low photosensitivity of the photo-In varieties led to extremely short PSPs compared with the photo-Se varieties, which in turn contributed to their low DTH values. In addition, the photo-In varieties showed limited LT-mediated delays in DTH. The photo-Se varieties, which carried either of the functional *Ghd7* or *Osprr37* alleles, were also temperature sensitive and had a prolonged PSP under both LT and LD condition. Compared with *osprr37*, I found that *ghd7* is a major contributor to early heading. Evaluation of the hemi-*ghd7* knockout plants confirmed early heading through shortening of PSP. Consequently, shortened PSP occurs in the photo-In varieties carrying the non-functional *ghd7* allele, which may lead to a stable early heading.

In chapter 3, I examined photo-In varieties both in Sapporo and Iga, and evaluated the heading traits such as L-DTH, heading synchrony, premature heading and tiller developments. These traits vary more in photo-In lines than in day-length-sensitive lines. We used photo-in lines to study the associations of these traits with heading earliness and the influence of the earliness genes and the different latitudes. The results of this study showed these traits were significantly correlated with the earliness of F-DTH among the photo-In lines. I also found that photo-In lines had a wider range of F-DTH tested in Sapporo than in Iga; LT and LD in Sapporo delayed heading, whereas high temperatures and short days in Iga caused it to occur earlier, which was confirmed under artificial conditions. I identified five significant QTLs for F-DTH among the Photo-In lines. A QTL with a major effect associated with F-DTH, heading synchrony, and premature heading was detected on chromosome 4.

Taken together, this study proposed the understandings of heading characteristics of photo-In varieties and their environmental response on heading. I hope this study provides the insight to ensure stable rice production.

Supplemental Figures

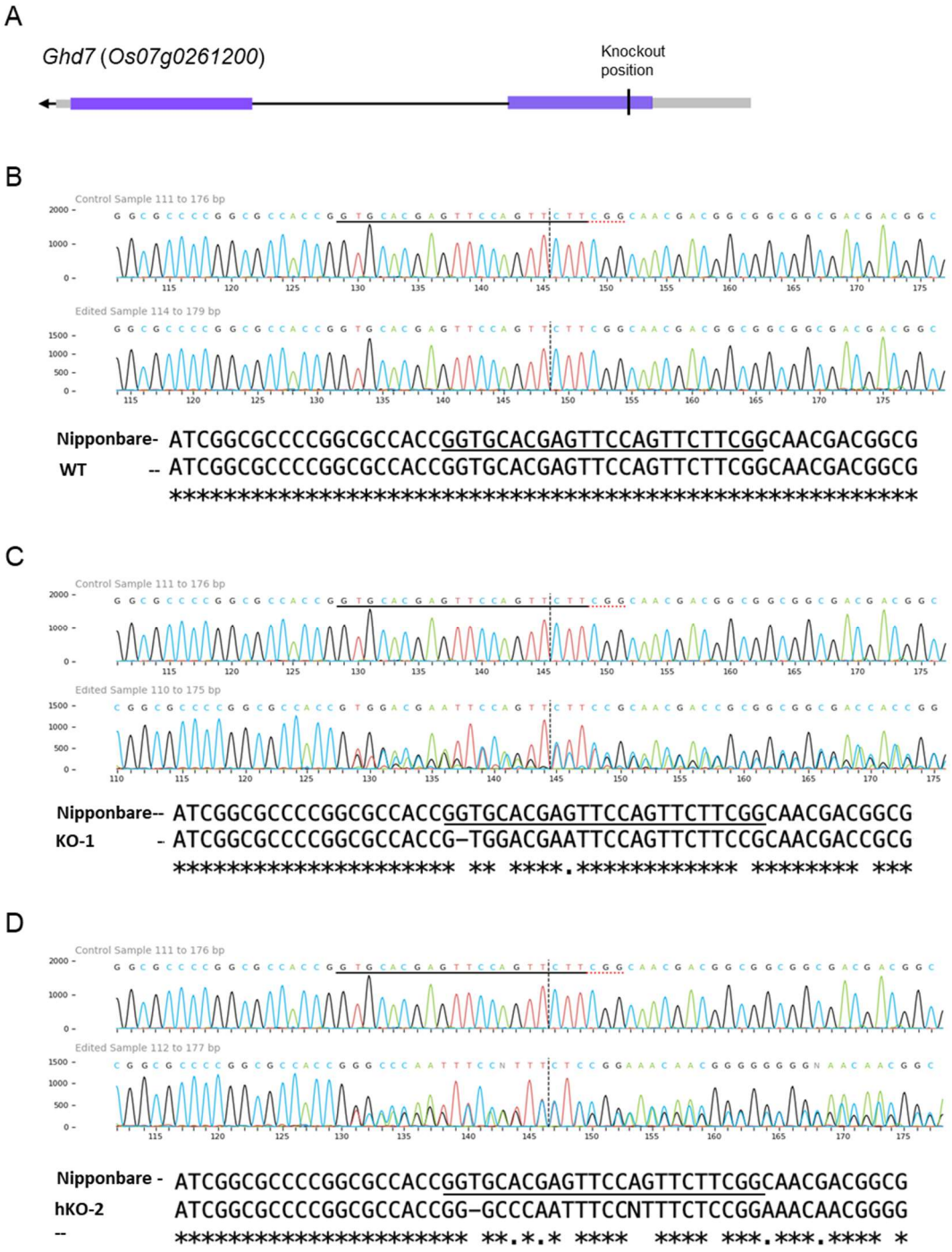


Figure S2-1. Genome editing of Ghd7.

(A) Knockout position of Ghd7.The position of the guide RNA position is after 118bp from the start codon of the first exon. (B)~(F) Sanger sequencing results at the corresponding knockout position for each edited plant.

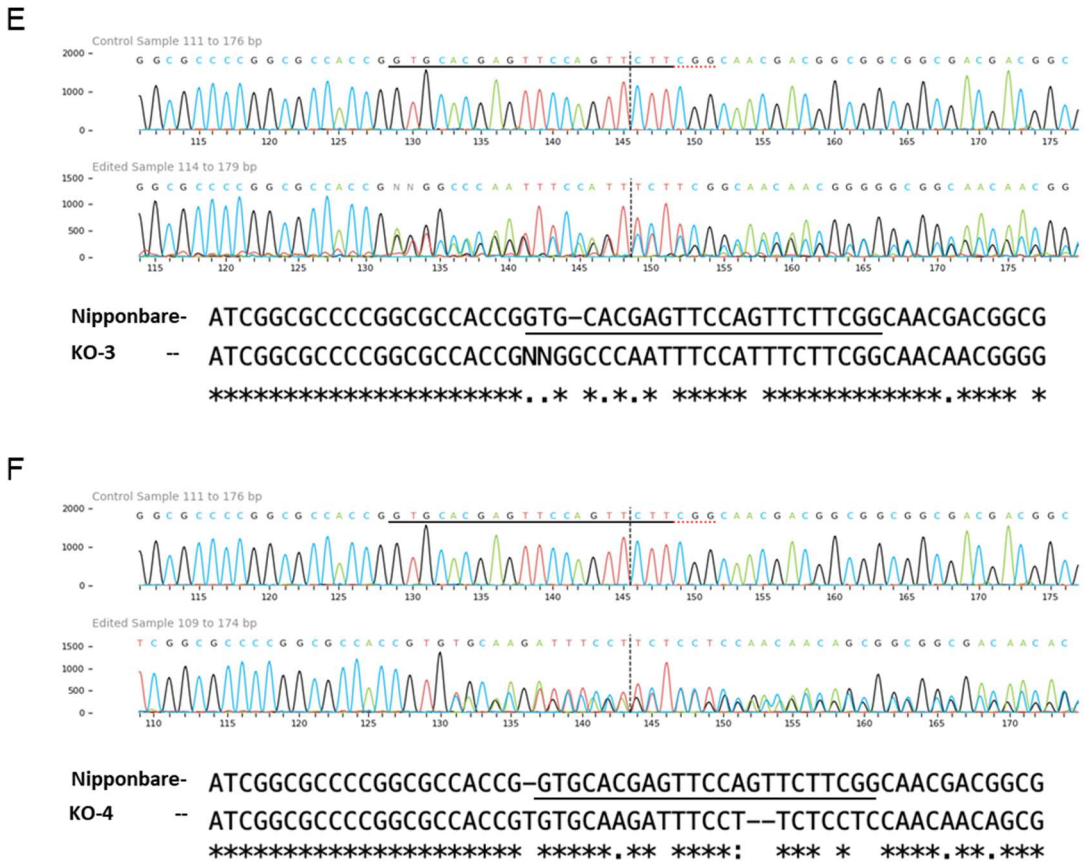


Figure S2-1. Continued.

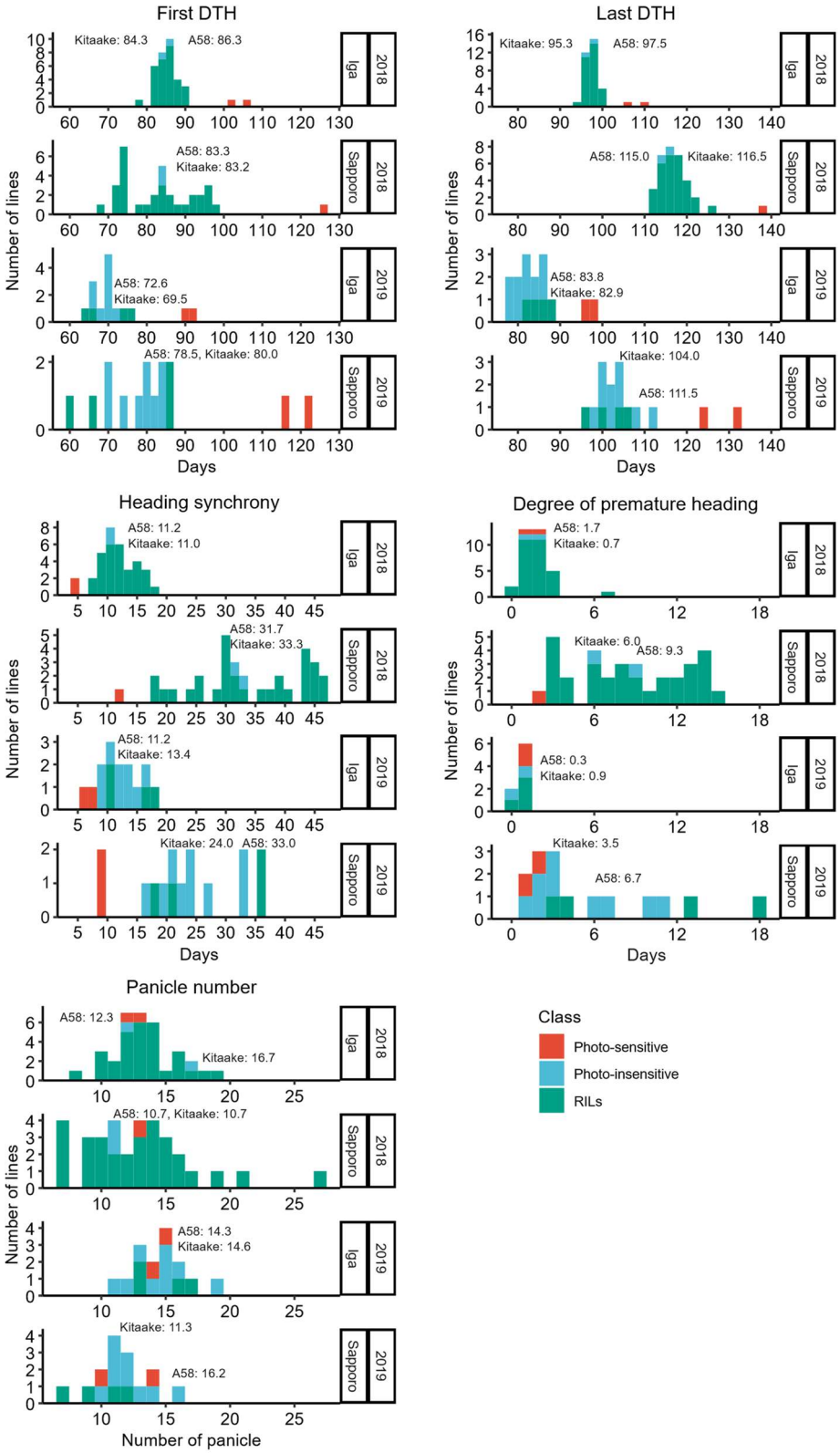


Figure S3-1. Frequency distribution of heading traits and panicle number.

Photo-Se varieties, photo-In varieties, and RIL distribution are shown in different colors. In 2018,

two photo-In varieties, one photo-Se variety, and 30 RILs were used. In 2019, nine photo-In varieties, two photo-Se varieties, and four RILs (E-RIL-1, -2 and L-RIL-1, -2) were used. In Iga in 2019, degree of premature heading was measured for two out of nine photo-In varieties.

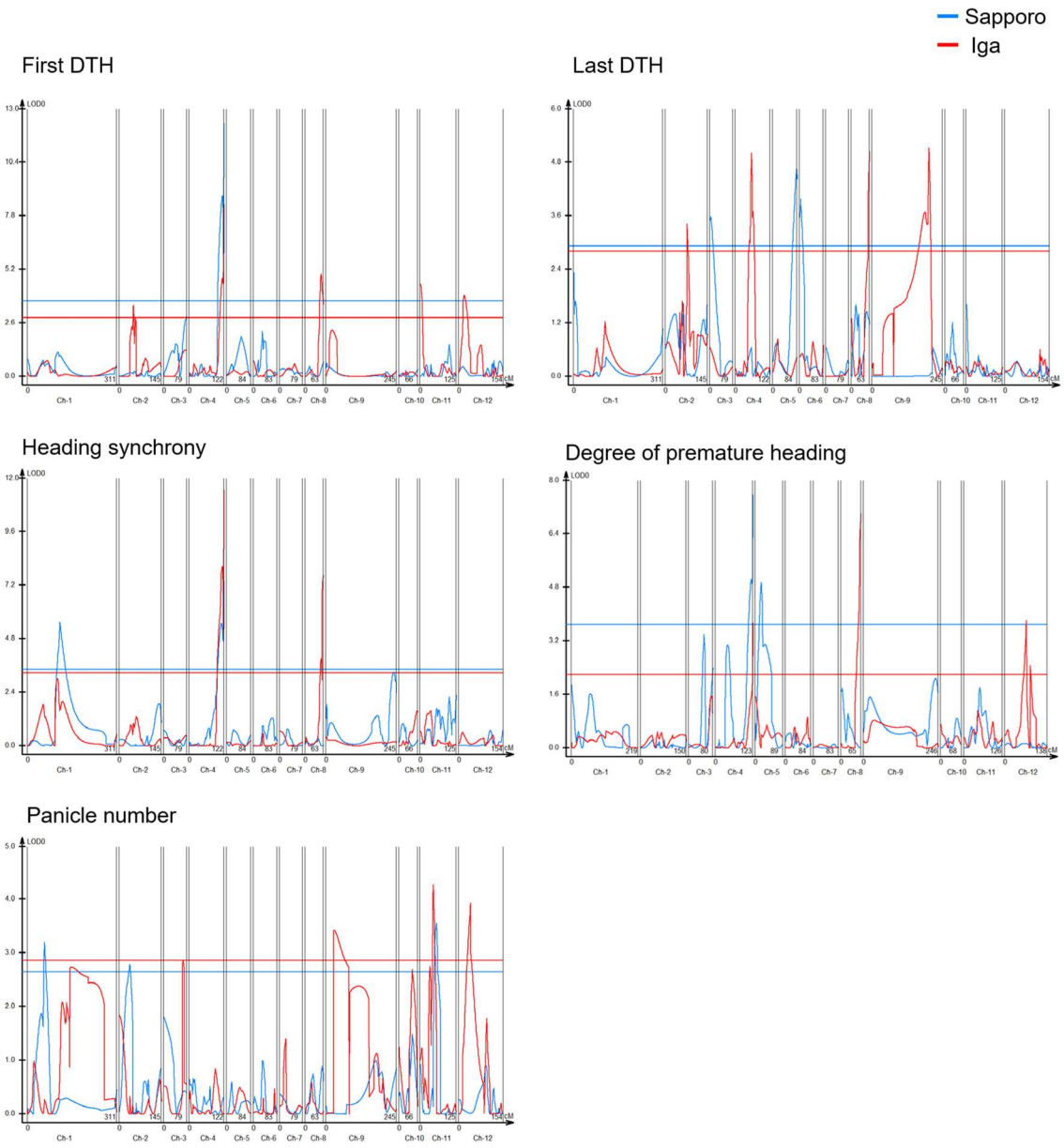


Figure S3-2. Likelihood odds ratio (LOD) score of qualitative trait loci (QTL)s of F-DTH, L-DTH, heading synchrony, degree of premature heading, and panicle number.

Five QTLs for the first DTH, seven for the last DTH, three for heading synchrony, five for degree of premature heading, and five for panicle number were detected in 30 RILs derived from A58 and Kitaake, with 224 SNP markers. The LOD scores for Sapporo and Iga are shown in different colors. The horizontal line shows the threshold determined by 1000 permutation tests.

Supplemental Tables

Table S2-1. Flag leaf age and leaf emergence index for each variety.

Variety	Day length	Temperature	Flag leaf age	Leaf emergence index		
				Before TP-ILE	After TP-ILE	R-square value
A58	LD	NT	10.0 ± 0.33	0.271 ± 0.014	0.156 ± 0.193	0.983
Norin 20	LD	NT	10.0 ± 0.00	0.276 ± 0.007	0.162 ± 0.150	0.987
Hakuchomochi	LD	NT	10.1 ± 0.08	0.272 ± 0.009	0.151 ± 0.167	0.983
Kitaake	LD	NT	10.4 ± 0.15	0.251 ± 0.009	0.150 ± 0.140	0.989
Nipponbare	LD	NT	14.8 ± 0.19	0.243 ± 0.009	0.115 ± 0.146	0.989
A58	LD	LT	10.9 ± 0.13	0.187 ± 0.003	0.122 ± 0.086	0.990
Norin 20	LD	LT	11.1 ± 0.08	0.183 ± 0.003	0.101 ± 0.088	0.991
Hakuchomochi	LD	LT	11.3 ± 0.13	0.191 ± 0.005	0.110 ± 0.095	0.988
Kitaake	LD	LT	10.6 ± 0.17	0.196 ± 0.004	0.116 ± 0.135	0.988
Nipponbare	LD	LT	15.8 ± 0.13	0.174 ± 0.008	0.078 ± 0.159	0.992
A58	SD	NT	8.9 ± 0.13	0.211 ± 0.009	0.127 ± 0.154	0.985
Norin 20	SD	NT	8.9 ± 0.07	0.249 ± 0.006	0.143 ± 0.114	0.982
Hakuchomochi	SD	NT	9.1 ± 0.09	0.230 ± 0.008	0.147 ± 0.108	0.988
Kitaake	SD	NT	9.0 ± 0.00	0.247 ± 0.007	0.145 ± 0.153	0.984
Nipponbare	SD	NT	11.1 ± 0.08	0.221 ± 0.004	0.137 ± 0.077	0.984
A58	SD	LT	10.0 ± 0.00	0.141 ± 0.001	0.083 ± 0.144	0.989
Norin 20	SD	LT	10.2 ± 0.17	0.167 ± 0.006	0.097 ± 0.081	0.989
Hakuchomochi	SD	LT	11.0 ± 0.00	Not determined	Not determined	Not determined
Kitaake	SD	LT	9.0 ± 0.00	0.150 ± 0.004	0.106 ± 0.170	0.988
Nipponbare	SD	LT	12.4 ± 0.17	0.150 ± 0.004	0.093 ± 0.094	0.990

LD: long daylength; SD: short daylength; NT: natural temperature; LT: low temperature; TP-ILE: turning point of the interval of leaf emergence.

Table S2-2. Days to the turning point of the interval of leaf emergence and panicle initiation. (BVP length) and panicle initiation (BVP+PSP length).

Varieties	Day length	Temperature	BVP length	BVP+PSP length
A58	LD	NT	25.2 ± 1.67	26.3 ± 0.27
Norin 20	LD	NT	24.6 ± 1.70	30.7 ± 0.83
Hakuchomochi	LD	NT	27.9 ± 1.22	28.0 ± 0.90
Kitaake	LD	NT	29.2 ± 1.84	33.4 ± 0.85
Nipponbare	LD	NT	35.7 ± 2.28	68.8 ± 0.68
A58	LD	LT	33.2 ± 1.09	41.2 ± 1.42
Norin 20	LD	LT	36.3 ± 0.66	46.9 ± 1.10
Hakuchomochi	LD	LT	35.9 ± 1.71	46.1 ± 1.35
Kitaake	LD	LT	32.3 ± 0.98	44.9 ± 1.14
Nipponbare	LD	LT	52.1 ± 2.60	103.0 ± 0.00
A58	SD	NT	28.9 ± 1.63	30.9 ± 1.27
Norin 20	SD	NT	21.8 ± 0.74	27.5 ± 0.89
Hakuchomochi	SD	NT	27.7 ± 0.89	32.4 ± 0.64
Kitaake	SD	NT	23.0 ± 0.85	28.8 ± 0.76
Nipponbare	SD	NT	30.9 ± 1.44	40.9 ± 1.28
A58	SD	LT	40.9 ± 1.06	55.3 ± 0.82
Norin 20	SD	LT	32.9 ± 3.22	52.7 ± 0.64
Hakuchomochi	SD	LT	Not determined	50.2 ± 0.44
Kitaake	SD	LT	30.5 ± 0.56	46.2 ± 1.23
Nipponbare	SD	LT	46.4 ± 2.76	79.0 ± 0.00

BVP length: days to the turning point of leaf emergence from sowing; BVP+PSP length: days to panicle initiation from sowing.

Table S2-3. The durations of the three growth phases under each growth condition.

Varieties	Day length	Temperature	BVP	PSP	RP
A58	LD	NT	25.2	1.2	35.7
Norin 20	LD	NT	24.6	6.1	31.5
Hakuchomochi	LD	NT	27.9	0.1	36.6
Kitaake	LD	NT	29.2	4.2	31.9
Nipponbare	LD	NT	35.7	33.1	33.3
A58	LD	LT	33.2	8.0	44.7
Norin 20	LD	LT	36.3	10.6	42.4
Hakuchomochi	LD	LT	35.9	10.3	43.6
Kitaake	LD	LT	32.3	12.6	40.2
Nipponbare	LD	LT	52.1	50.9	36.0
A58	SD	NT	28.9	2.0	34.5
Norin 20	SD	NT	21.8	5.7	36.6
Hakuchomochi	SD	NT	27.7	4.7	33.5
Kitaake	SD	NT	23.0	5.8	32.1
Nipponbare	SD	NT	30.9	10.0	33.5
A58	SD	LT	40.9	14.4	43.8
Norin 20	SD	LT	32.9	19.8	43.4
Hakuchomochi	SD	LT	Not determined	Not determined	45.2
Kitaake	SD	LT	30.5	15.6	37.6
Nipponbare	SD	LT	46.4	32.6	37.8

BVP: basic vegetative growth; PSP: photoperiod-sensitive phase; RP: reproductive phase.

Table S2-4. Results of ANOVA for DTH and the duration of each growth phase.

Growth phase	Source	Degrees of freedom	Mean square	F value	P-value	
DTH	Variety	4	7935.1	1620.5	0.000	**
	Day length	1	289.4	59.1	0.000	**
	Temperature	1	44400.9	9067.4	0.000	**
	Variety * Day length	4	1708.9	349.0	0.000	**
	Variety * Temperature	4	464.0	94.8	0.000	**
	Day length * Temperature	1	350.8	71.6	0.000	**
	Residuals	200	4.9			
BVP	Variety	3	1426.1	43.5	0.000	**
	Day length	1	104.4	3.2	0.076	n.s.
	Temperature	1	4288.1	130.9	0.000	**
	Variety * Day length	3	186.5	5.7	0.001	**
	Variety * Temperature	3	203.2	6.2	0.001	**
	Day length * Temperature	1	28.3	0.9	0.354	n.s.
	Residuals	148	52.8			
PSP	Variety	3	1876.8	322.8	0.000	**
	Day length	1	111.9	19.2	0.000	**
	Temperature	1	2417.1	415.7	0.000	**
	Variety * Day length	3	526.6	90.6	0.000	**
	Variety * Temperature	3	106.3	18.3	0.000	**
	Day length * Temperature	1	119.2	20.5	0.000	**
	Residuals	59	20.2			
RP	Variety	4	87.3	15.7	0.000	**
	Day length	1	1.0	0.2	0.678	n.s.
	Temperature	1	1230.9	221.2	0.000	**
	Variety * Day length	4	16.2	2.9	0.027	*
	Variety * Temperature	4	22.8	4.1	0.005	**
	Day length * Temperature	1	0.1	0.0	0.923	n.s.
	Residuals	78	21.0			

* and ** indicate significant differences at the 5% and 1% levels, respectively.

Table S3-1. List of varieties used in this study and genotype of four genes.

Variety[a]	Genotype[b]				Reference
	Hd1[c]	*Ghd7*	*Osprr37*	*DTH8*	
A58	f	f	f	F	Koide et al. (2019)
Kitaake	F	f	f	F	Fujino et al. (2019a)
Akage	f	f	f	F	Fujino et al. (2019a)
Norin 20	f	f	f	f	Fujino et al. (2019a)
Hakuchomochi	f	f	f	f	Fujino et al. (2019a)
Kirara397	F	f	f	F	Fujino et al. (2019a)
Hoshinoyume	F	f	f	F	Fujino et al. (2019a)
Nanatsuboshi	F	f	f	F	Fujino et al. (2019a)
Daichinohoshi	F	f	f	F	Fujino et al. (2019a)
Koshihikari	-	-	-	-	-
Mie 33	-	-	-	-	-
RILs No. 1 (E-RIL-1)	F	f	f	F	Koide et al. (2019)
RILs No. 2 (E-RIL-2)	F	f	f	F	Koide et al. (2019)
RILs No. 3 (L-RIL-1)	f	f	f	F	Koide et al. (2019)
RILs No. 4 (L-RIL-2)	-	f	f	F	Koide et al. (2019)
RILs No. 5	F	f	f	F	Koide et al. (2019)
RILs No. 6	f	f	f	F	Koide et al. (2019)
RILs No. 7	F	f	f	F	Koide et al. (2019)
RILs No. 8	F	f	f	F	Koide et al. (2019)
RILs No. 9	-	f	f	F	Koide et al. (2019)
RILs No. 10	F	f	f	F	Koide et al. (2019)
RILs No. 11	F	f	f	F	Koide et al. (2019)
RILs No. 12	F	f	f	F	Koide et al. (2019)
RILs No. 13	F	f	f	F	Koide et al. (2019)
RILs No. 14	F	f	f	F	Koide et al. (2019)
RILs No. 15	f	f	f	F	Koide et al. (2019)
RILs No. 16	F	f	f	F	Koide et al. (2019)
RILs No. 17	F	f	f	F	Koide et al. (2019)
RILs No. 18	F	f	f	F	Koide et al. (2019)
RILs No. 19	F	f	f	F	Koide et al. (2019)
RILs No. 20	F	f	f	F	Koide et al. (2019)
RILs No. 21	F	f	f	F	Koide et al. (2019)
RILs No. 22	-	f	f	F	Koide et al. (2019)
RILs No. 23	F	f	f	F	Koide et al. (2019)
RILs No. 24	f	f	f	F	Koide et al. (2019)
RILs No. 25	F	f	f	F	Koide et al. (2019)
RILs No. 26	F	f	f	F	Koide et al. (2019)
RILs No. 27	F	f	f	F	Koide et al. (2019)
RILs No. 28	f	f	f	F	Koide et al. (2019)
RILs No. 29	-	f	f	F	Koide et al. (2019)
RILs No. 30	f	f	f	F	Koide et al. (2019)

[a] Genotypes of Koshihikari and Mie 33 are not tested.

[b] F and f indicate functional type and non-functional type, respectively.

[c] A58/Kitaake RILs have either non-functional (A58 type) or functional (Kitaake type) alleles. The hyphen indicates that the *Hd1* genotype was heterozygous in the F4 generation.

References

Adams, S.R., Pearson, S., Hadley, P., 2001. Improving quantitative flowering models through a better understanding of the phases of photoperiod sensitivity. J. Exp. Bot. 52, 655–662. https://doi.org/10.1093/jexbot/52.357.655

Andaya, V.C., 2003. Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J. Exp. Bot. 54, 2579–2585. https://doi.org/10.1093/jxb/erg243

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3, 1–7. https://doi.org/10.1371/journal.pone.0003376

Blümel, M., Dally, N., Jung, C., 2015. Flowering time regulation in crops — what did we learn from Arabidopsis? Curr. Opin. Biotechnol. 32, 121–129. https://doi.org/10.1016/j.copbio.2014.11.023

Chen, J.Y., Zhang, H.W., Zhang, H.L., Ying, J.Z., Ma, L.Y., Zhuang, J.Y., 2018. Natural variation at qHd1 affects heading date acceleration at high temperatures with pleiotropism for yield traits in rice. BMC Plant Biol. 18, 1–11. https://doi.org/10.1186/s12870-018-1330-5

Collinson, S.T., Ellis, R.H., Summerfield, R.J., Roberts, E.H., 1992. Durations of the photoperiod-sensitive and photoperiod-insensitive phases of development to flowering in four cultivars of rice (Oryza sativa L.). Ann. Bot. 70, 339–346. https://doi.org/10.1093/oxfordjournals.aob.a088483

Counce, P.A., Siebenmorgen, T.J., Poag, M.A., Holloway, G.E., Kocher, M.F., Lu, R., 1996. Panicle emergence of tiller types and grain yield of tiller order for direct-seeded rice cultivars. F. Crop. Res. 47, 235–242. https://doi.org/10.1016/0378-4290(96)00011-1

Doi, K., Izawa, T., Fuse, T., Yamanouchi, U., Kubo, T., Shimatani, Z., Yano, M., Yoshimura, A., 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. https://doi.org/10.1101/gad.1189604

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. https://doi.org/10.1093/pcp/pcr059

Endo, M., Iwakami, S., Toki, S., 2021. Precision genome editing in plants via gene targeting and subsequent break-induced single-strand annealing. Plant Biotechnol. J. 19, 563–574. https://doi.org/10.1111/pbi.13485

Fjellheim, S., Preston, J.C., 2018. Shedding light on the role of seasonal flowering in plant niche transitions. Am. J. Bot. 105, 136–138. https://doi.org/10.1002/ajb2.1037

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

Fujino, K., Obara, M., Ikegaya, T., 2019a. 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

Fujino, K., Obara, M., Ikegaya, T., Tamura, K., 2015. Genetic shift in local rice populations during rice breeding programs in the northern limit of rice cultivation in the world. Theor. Appl. Genet. 128, 1739–1746. https://doi.org/10.1007/s00122-015-2543-8

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. https://doi.org/10.1270/jsbbs.55.141

Fujino, K., Sekiguchi, H., 2005b. 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

Fujino, K., Yamanouchi, U., Nonoue, Y., Obara, M., Yano, M., 2019b. 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

Fujino, K., Yamanouchi, U., Yano, M., 2013. Roles of the Hd5 gene controlling heading date for adaptation to the northern limits of rice cultivation. Theor. Appl. Genet. 126, 611–618. https://doi.org/10.1007/s00122-012-2005-5

Gao, H., Jin, M., Zheng, X.M., Chen, J., Yuan, D., Xin, Y., Wang, M., Huang, D., Zhang, Z., Zhou, K., Sheng, P., Ma, J., Ma, W., Deng, H., Jiang, L., Liu, S., Wang, H., Wu, C., Yuan, L., Wan, J., 2014. Days to heading 7, a major quantitative locus determining photoperiod sensitivity and regional adaptation in rice. Proc. Natl. Acad. Sci. U. S. A. 111, 16337–16342. https://doi.org/10.1073/pnas.1418204111

Hosoi, N., 1979. Studies on meteorological fiuctuation in the growth of rice plants. III: Relation between the heading response of rice varieties to temperature under natural daylength and the thermo-sensitivity, photo-sensitivity, basic vegetative growth under controlled. Japanese J. Breed. 29, 294–304. https://doi.org/10.1270/jsbbs1951.29.294

Hu, Z., Liu, Y., Huang, L., Peng, S., Nie, L., Cui, K., Huang, J., Wang, F., 2015. Premature heading and yield losses caused by prolonged seedling age in double cropping rice. F. Crop. Res. 183, 147–155. https://doi.org/10.1016/j.fcr.2015.08.002

Ichitani, K., Okumoto, Y., Tanisaka, T., 1997. Photoperiod sensitivity gene of Se-1 locus found in photoperiod insensitive rice cultivars of the northern limit region of rice cultivation. Japanese J. Breed. 47, 145–152. https://doi.org/10.1270/jsbbs1951.47.145

Ishimaru, K., Kashiwagi, T., 2004. Identification of a locus for asynchronous heading in rice, Oryza sativa L. Euphytica 139, 141–145. https://doi.org/10.1007/s10681-004-2559-7

Izawa, T., 2007. Adaptation of flowering-time by natural and artificial selection in Arabidopsis and rice. J. Exp. Bot. 58, 3091–3097. https://doi.org/10.1093/jxb/erm159

Kanda, E., Torigoe, Y., Kobayashi, T., 2000. A model to estimate the increase of leaf number on the main

culm of the rice plant. Japanese J. Crop Sci. 69, 540–546. https://doi.org/10.1626/jcs.69.540

Kazan, K., Lyons, R., 2016. The link between flowering time and stress tolerance. J. Exp. Bot. 67, 47–60. https://doi.org/10.1093/jxb/erv441

Koide, Y., Sakaguchi, S., Uchiyama, T., Ota, Y., Tezuka, A., Nagano, A.J., Ishiguro, S., Takamure, I., Kishima, Y., 2019. Genetic properties responsible for the transgressive segregation of days to heading in rice. G3 Genes, Genomes, Genet. 9, 1655–1662. https://doi.org/10.1534/g3.119.201011

Kojima, S., Takahashi, Y., Kobayashi, Y., Monna, L., Sasaki, T., Araki, T., Yano, M., 2002. Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant Cell Physiol. 43, 1096–1105. https://doi.org/10.1093/pcp/pcf156

Koo, B.H., Yoo, S.C., Park, J.W., Kwon, C.T., Lee, B.D., An, G., Zhang, Z., Li, J., Li, Z., Paek, N.C., 2013. Natural variation in OsPRR37 regulates heading date and contributes to rice cultivation at a wide range of latitudes. Mol. Plant 6, 1877–1888. https://doi.org/10.1093/mp/sst088

Kovi, M.R., Hu, Y., Bai, X., Xing, Y., 2015. QTL mapping for thermo-sensitive heading date in rice. Euphytica 205, 51–62. https://doi.org/10.1007/s10681-015-1383-6

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., Newburg, L., 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174–181. https://doi.org/10.1016/0888-7543(87)90010-3

Lukac, M., Gooding, M.J., Griffiths, S., Jones, H.E., 2012. Asynchronous flowering and within-plant flowering diversity in wheat and the implications for crop resilience to heat. Ann. Bot. 109, 843–850. https://doi.org/10.1093/aob/mcr308

Ma, L., Yang, C., Zeng, D., Cai, J., Li, X., Ji, Z., Xia, Y., Qian, Q., Bao, J., 2009. Mapping QTLs for heading synchrony in a doubled haploid population of rice in two environments. J. Genet. Genomics 36, 297–304. https://doi.org/10.1016/S1673-8527(08)60118-6

Muggeo .V, 2008. segmented: an R Package to Fit Regression Models with Broken-Line Relationships. R news 8, 20–25.

Murray, M.G., Thompson, W.F., 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8, 4321–4326. https://doi.org/10.1093/nar/8.19.4321

Nagalla, A.D., Nishide, N., Hibara, K.I., Izawa, T., 2021. High ambient temperatures inhibit Ghd7-mediated flowering repression in rice. Plant Cell Physiol. 62, 1745–1759. https://doi.org/10.1093/pcp/pcab129

Nemoto, M., Hamasaki, T., Shimono, H., 2011. Extraordinary hot summer in Hokkaido decrease rice yield and satisfy growing of cultivar in Tohoku region “Hitomebore.” J. Agric. Meteorol. 67, 269–274. https://doi.org/10.2480/agrmet.67.4.1

Nishida, H., Okumoto, Y., Nakagawa, H., Ichitani, K., Inoue, H., Tanisaka, T., 2001. Analysis of tester lines for rice (Oryza sativa L.) Heading-time genes using reciprocal photoperiodic transfer

treatments. Ann. Bot. 88, 527–536. https://doi.org/10.1006/anbo.2001.1490

Nonoue, Y., Fujino, K., Hirayama, Y., Yamanouchi, U., Lin, S.Y., Yano, M., 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

Osada, A., Dhammanuvong, S., Rahong, M., Chawanayotin, W., 1975. Premature heading of non-photosensitive indica rice varieties by long nursery duration. Jap. J. Trop. Agr 19, 15–20.

Ota, Y., Ishiguro, S., Aoyama, E., Aiba, R., Iwashiro, R., Tanabata, T., Takamure, I., Fujino, K., Kishima, Y., 2014. Isolation of a major genetic interaction associated with an extreme phenotype using assorted F2 populations in rice. Mol. Breed. 33, 997–1003. https://doi.org/10.1007/s11032-013-9998-6

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7. https://doi.org/10.1371/journal.pone.0037135

Preston, J.C., Fjellheim, S., 2022. Flowering time runs hot and cold. Plant Physiol. 190, 5–18. https://doi.org/10.1093/plphys/kiac111

Rathnathunga, E.U.U., Senanayake, G., Seneweera, S., Geekiyanage, S., 2019. Varietal diversity of Sri Lankan traditional rice based on sensitivity to temperature and photoperiod at vegetative stage. J. Natl. Sci. Found. Sri Lanka 47, 51. https://doi.org/10.4038/jnsfsr.v47i1.8928

Saito, H., Okumoto, Y., Tsukiyama, T., Xu, C., Teraishi, M., Tanisaka, T., 2019. Allelic differentiation at the E1/Ghd7 locus has allowed expansion of rice cultivation area. Plants 8, 550. https://doi.org/10.3390/plants8120550

Sakata, M., Suzuki, K., Yamamoto, Y., Miyazaki, A., 2004. Effects of premature heading on growth, yield and brown rice quality in extremely early rice cultivar 'Tosapika.' Japanese J. Crop Sci. 73, 443–449. https://doi.org/https://doi.org/10.1626/jcs.73.443

Shinada, H., Yamamoto, T., Yamamoto, E., Hori, K., Yonemaru, J., Matsuba, S., Fujino, K., 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

Takada, K., 1986. Synchrony of heading in relation to earliness of rice varieties. Japanese J. Breed. 36, 291–303. https://doi.org/10.1270/jsbbs1951.36.291

Takahashi, Y., Teshima, K.M., Yokoi, S., Innan, H., Shimamoto, K., 2009. Variations in Hd1 proteins, Hd3a promoters, and Ehd1 expression levels contribute to diversity of flowering time in cultivated rice. Proc. Natl. Acad. Sci. U. S. A. 106, 4555–4560. https://doi.org/10.1073/pnas.0812092106

Takeda, K., 1986. Synchrony of heading in relation to earliness of rice varieties. Japanese J. Breed. 36, 291–303. https://doi.org/10.1270/jsbbs1951.36.291

Tsuji, H., Tachibana, C., Tamaki, S., Taoka, K.I., Kyozuka, J., Shimamoto, K., 2015. Hd3a promotes lateral branching in rice. Plant J. 82, 256–266. https://doi.org/10.1111/tpj.12811

Vergara, B., Chang, T., 1985. The flowering response of the rice plant to photoperiod: A review of the literature, International Rice Research Institute (IRRI).

Vicentini, G., Biancucci, M., Mineri, L., Chirivì, D., Giaume, F., Miao, Y., Kyozuka, J., Brambilla, V., Betti, C., Fornara, F., 2023. Environmental control of rice flowering time. Plant Commun. 4. https://doi.org/10.1016/j.xplc.2023.100610

Wang, S., Basten, C.J., Zeng, Z.-B., 2012. Windows QTL cartographer 2.5.

Wei, X., Jiang, L., Xu, J., Zhang, W., Lu, G., Zhang, Y., Wan, J., 2008. Genetic analyses of heading date of Japonica rice cultivars from Northeast China. F. Crop. Res. 107, 147–154. https://doi.org/10.1016/j.fcr.2008.01.008

Weng, X., Wang, L., Wang, J., Hu, Y., Du, H., Xu, C., Xing, Y., Li, X., Xiao, J., Zhang, Q., 2014. Grain Number, Plant Height, and Heading Date7 is a central regulator of growth, development, and stress response. Plant Physiol. 164, 735–747. https://doi.org/10.1104/pp.113.231308

Xue, W., Xing, Y., Weng, X., Zhao, Y., Tang, W., Wang, L., Zhou, H., Yu, S., Xu, C., Li, X., Zhang, Q., 2008. Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. Nat. Genet. 40, 761–767. https://doi.org/10.1038/ng.143

Yabuta, S., Hakoyama, S., Inafuku, S., Fukuzawa, Y., Kawamitsu, Y., 2015. The photoperiodic sensitivity estimation at three growth phases of rice fractionated by turning point of leaf emergence and panicle initiation. Japanese J. Crop Sci. 84, 64–68. https://doi.org/10.1626/jcs.84.64

Yabuta, S., Hakoyama, S., Inafuku, S., Fukuzawa, Y., Kawamitsu, Y., 2012. Effects of short-day treatment on leaf emergence rate and its turning point in several cultivars of rice. Japanese J. Crop Sci. 81, 299–308. https://doi.org/10.1626/jcs.81.299

Yabuta, S., Minami, S., Hakoyama, S., Kawamitsu, Y., 2010. Varietal difference in the duration of reproductive phase in Oryza sativa L. Japanese J. Crop Sci. 79, 327–335. https://doi.org/10.1626/jcs.79.327

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., Nagamura, Y., Sasaki, T., 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. https://doi.org/10.1105/tpc.12.12.2473

Yin, X.Y., Kropff, M.J., Ynalvez, M.A., 1997. Photoperiodically sensitive and insensitive phases of preflowering development in rice. Crop Sci. 37, 182–190. https://doi.org/10.2135/cropsci1997.0011183X003700010031x

Yoshida, R., Fukui, S., Shimada, T., Hasegawa, T., Ishigooka, Y., Takayabu, I., Iwasaki, T., 2015. Adaptation of rice to climate change through a cultivar-based simulation: A possible cultivar shift in eastern Japan. Clim. Res. 64, 275–290. https://doi.org/10.3354/cr01320

Yoshitake, Y., Yokoo, T., Saito, H., Tsukiyama, T., Quan, X., Zikihara, K., Katsura, H., Tokutomi, S., Aboshi, T., Mori, N., Inoue, H., Nishida, H., Kohchi, T., Teraishi, M., Okumoto, Y., Tanisaka, T.,

2015. The effects of phytochrome-mediated light signals on the developmental acquisition of photoperiod sensitivity in rice. Sci. Rep. 5, 16–18. https://doi.org/10.1038/srep07709

Yuan, R., Mao, Y., Zhang, D., Wang, S., Zhang, H., Wu, M., Ye, M., Zhang, Z., 2024. The formation of rice tillers and factors influencing it. Agronomy 14, 2904. https://doi.org/10.3390/agronomy14122904

Zhang, J.G., Li, C., Zhang, S.Y., Shi, Y.H., Yang, G.L., Zhao, J.S., 1998. The comparison analysis for the uniformity of the population in different round grained rice varieties (I): the difference among different varieties of uniformity and the influence to the yield. J Jilin Agric Sci 23, 23–27.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D.B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S., Peñuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D., Liu, Z., Zhu, Y., Zhu, Z., Asseng, S., 2017. Temperature increase reduces global yields of major crops in four independent estimates. Proc. Natl. Acad. Sci. U. S. A. 114, 9326–9331. https://doi.org/10.1073/pnas.1701762114

Acknowledgements

本博士論文を完成させるにあたり、多くの方々から温かいご支援とご指導を賜りました。この場をお借りして、心より感謝申し上げます。

まず、指導教官であられる植物育種学分野教授 貴島祐治博士には、研究のあらゆる側面において懇切丁寧なご指導を賜りました。特に論文執筆に際しては、私の拙い文章を何度も校閲いただき、研究者としての成長を促してくださりました。そのご助言と励ましが、私にとってかけがえのない経験となりました。心より深謝申し上げます。同分野講師でおられました高牟禮逸朗博士には、植物材料の管理や栽培に関する貴重なご指導をいただきました。同分野准教授 小出陽平博士には、研究のご指導はもちろん、研究者としてのあり方をご教授いただき、私の研究に対する意欲を支えてくださいました。厚く御礼申し上げます。

研究室の皆様には、本研究を進めるうえで多くの支えをいただきました。社会人博士として十分な研究時間を確保することが難しい中、皆様の温かいご協力のおかげで研究を続けることができました。特に、同じ研究テーマを持ち、ともに議論し助け合った Md. Imadadul Hoque さんには、多大なるご助力をいただきましたこと、心より感謝申し上げます。

また、ホクレン農業協同組合連合会の皆様には、社会人博士として研究を進めるうえで、深いご理解とご支援をいただきました。業務との両立に際し、温かく励ましのお言葉を頂戴し、貴重なアドバイスをいただいたことに、改めて深く感謝申し上げます。

最後に、家族にはこれまでの学業を支えてもらい、どんな時も変わらぬ応援を送ってくれました。その支えがあったからこそ、今日まで研究を続けることができました。心より感謝申し上げます。