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ORIGINAL PAPER

Is the time of anthesis in rice (*Oryza sativa*) influenced by photoperiod?

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Abstract

Photoperiod sensitivity in rice cultivars is defined when the cultivar begins anthesis on a relatively invariant date, varying by < 7 days, regardless of the date of sowing or germination. While the date of flowering in photoperiod sensitive (PPS) rice cultivars is characteristically determined by the day length, especially during the short-day season (September–December), the response of the flower opening time (FOT) to photoperiod remains hitherto unexplored. This paper examines whether day length restrains year-to-year variation in FOT in PPS cultivars. We examined 105 PPS and 173 photoperiod insensitive (PPI) cultivars grown in different years and estimated their year-to-year FOT difference (or FOTD) and the year-to-year difference of sunrise to anthesis duration (or SADD). Wilcoxon signed rank test and bootstrap test were then performed to test whether these descriptors significantly differed between PPS and PPI groups of cultivars. The means of FOTD and SADD were detected to be significantly less in the PPS group than in the PPI group of cultivars, indicating significantly lesser variability of FOT in PPS than in PPI cultivars. This is the first report of a strong restraining influence of photoperiod on FOT variability in PPS cultivars.

Keywords Anthesis · Day length · Flower opening time · Photoperiod sensitivity · Rice

Introduction

Rice is a facultative short-day plant, which shows a wide range of variation in degree of sensitivity to photoperiod, influencing the flowering biology (Vergara and Chang 1985; Ogiso-Tanaka 2013). The date of anthesis or the first flowering date (FD) and 50% flowering date (usually 3–5 days after FD) of most *indica* landraces of rice (*Oryza sativa* L.) are known to be responsive to photoperiod, especially during the short-day seasons (Moldenhauer and Gibbons 2003; Yin and Kropff 1998; Padukkage et al. 2017). Decreasing day length is a critical environmental signal for transition from the vegetative to the reproductive phase in photoperiod sensitive (PPS) rice cultivars; beyond a critical day length (> 10 h), photoperiod sensitive (PPS) rice cultivars do not initiate their reproductive phase and cannot enter into the anthesis phase in long-day seasons (Vergara and Chang 1985; Moldenhauer and Gibbons 2003). Most of the *japonica* landraces and modern cultivars are photoperiod insensitive (PPI), capable of flowering both during short- and long-day seasons (Vergara and Chang 1985; Yin and Kropff 1998). Thus, photoperiodic sensitivity is a critical agronomic trait that can be modulated to improve the adaptability of rice varieties to different latitudes (Xu et al. 2014).

The knowledge of the degree of photoperiod sensitivity is also important for the purpose of varietal development, because several genes involved in the expression or suppression of photoperiod sensitivity have pleiotropic effects on yield characteristics of cultivars (Endo-Higashi and Izawa 2011; Xu et al. 2014). Moreover, cross-pollination between two different cultivars can take place only when their respective flowering durations (from the first flower opening date to the last flower opening date) overlap. However, synchronous flowering periods of different rice cultivars notwithstanding cross-pollination cannot be successful if the flower opening time (FOT) and the duration of the flowers remaining open (= flower exposure duration, FED) of the pollen and ovary parents of the rice cultivars also do not overlap. Clearly, if the florets of the ovary parent close even a second before the FOT of the pollen parent, the FED overlap between the two parents is zero, resulting in no cross-pollination (Deb and Bhattacharya 2021). Thus, overlap of both FD and FOT between the ovary and pollen parents is crucially important for success in cross-pollination and hybridization in rice.

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To ascertain the overlap of FED, it is crucial to examine the variability of FOT in different photoperiods.

While the days to heading and the FD are determined by photoperiod in PPS rice cultivars, FOT is known to be influenced by sunrise time, day temperature and CO_2 levels in air (Yin and Kropff 1998; Kobayasi et al. 2019; Deb et al. 2023). However, there has been no study on the association of FOT of PPS cultivars with photoperiod, perhaps on an implicit assumption that the influence of day length is confined to FD, but not to FOT. In fact, most of the studies that examined the influence of photoperiod sensitivity on "flowering time" actually measured the effects on either days to heading or days to flowering (e.g. Uwatoko et al. 2008; Hori et al. 2016; Padukkage et al. 2017; Molla 2022), but never on the FOT, the clock time of flower opening on the day of anthesis. We present here our 3-year long observations of the relationship between FOT and photoperiod in both PPS and PPI rice landraces conducted during short-day seasons.

Materials and method

Study site and materials

A total of 1440 rice landraces were cultivated every year on Basudha conservation farm (http://cintdis.org/basudha), located in Bissam Cuttack block, Rayagada district of southern Odisha (19° 42′ 32.0″ N, 83° 28′ 8.4″ E). These landraces, originally collected from different districts of Bangladesh, India, Myanmar, Nepal, Pakistan and Sri Lanka, in addition to 18 landraces from Southeast Asia and 2 from East Asia.

Cultivation schedule

The experimental cultivation was begun during the *aman* season (sown in June, harvested in winter) of 2020 with 969 landraces, followed by 117 landraces during *aman* of 2021, and 389 landraces during *aman* of 2022 (See Tables 1 and 2). The schedule of sowing of 1114 landraces from *aman* 2020 to *aman* 2022 is shown in Table 2.

Table 2 The Schedule of Sowing of 1114 Landraces from A*man* 2020 to *Aman* 2022. Numbers above the diagonal are the number of varieties repeat-sown in different seasons. Numbers in the diagonal (in boldface) are the number of landraces grown in disparate seasons. Adapted from Deb et al. (2023)

Cultivation Season	Aman 2020	Aman 2021	Aman 2022
Aman 2020	**969**	108	258
Aman 2021		**117**	5
Aman 2022			**391**

Procedure of recording FD and FOT

In this study, we examined only those landraces that were repeat-sown during the short-day (*aman*) season in different years, but not those flowering during long day season, because the cultivars flowering during both short-day and long- day season are not PPS, by definition. A total of 371 *aman* landraces were repeat-sown during the years 2020–2022. Among these, 108 landraces were sown during *aman* 2020 and Aman 2021, 5 were repeat-sown during *aman* of 2021 and 2022, and 258 landraces were repeat-sown during *aman* of 2020 and 2022 (Table 2). For each of these landraces,

(a) We recorded the FD and the first flower opening time (FOT), one day after heading. The 50% flowering stage of the landraces arrived 3–5 days after FD.
(b) We recorded the exact time of opening of an apical floret in one of the first 50% exserted panicles in each cultivar population (consisting of 64 plants), and tagged the stalk of the panicle with a coloured thread, without touching any floret. Because our objective was to record the earliest flower opening time, we considered only panicles that had attained at least 50% heading. Among the selected panicles, we recorded the FOT of the floret that opened the earliest, in each varietal plot. We often missed the FOT of several cultivars that had flowered on the same day simultaneously in different plots at the same time. We were able to record the FOT of 1114 rice landraces in total (Deb 2022).
(c) For estimating the length of sunrise to anthesis duration (SAD), the exact sunrise time at Bissamcuttack Block was obtained from https://www.timeanddate.

Table 1 Life History Stages of the Aman Landraces. Adapted from Deb et al (2023)

Season	No. of landraces examined	Sowing dates	Transplanting dates	Flowering dates	Harvesting dates
Aman 2020	969	15 Jun–30 Jun	6 Jul–16 Jul	8 Aug–22 Jan	2 Sep–5 Feb
Aman 2021	117	28 Jun–4 Jul	15 Jul–19 Jul	1 Sep–22 Oct	17 Sep–2 Jan
Aman 2022	391	21 Jun–31 Jun	10 Jul–18 Jul	31 Aug–27 Nov	13 Sep–8 Jan

com/sun/@10775335 and https://isubqo.com/prayer-time/india/odisha/bishama-katek/

Identifying PPS cultivars

Since our objective was to determine the strongly PPS cultivars and the influence of photoperiod on their FD as well as FOT, we made a binary classification of PPS and PPI, so as to avoid possible confusion between “weakly PPS” and “moderately PPI” entities. To identify the (strongly) photoperiod sensitive (PPS) landraces, we considered two criteria. Firstly, we followed the traditional farmer’s method of directly measuring the proximity of the date of anthesis during the short day (*aman*) season in different years: If the year-to-year variation of a cultivar’s FD does not vary beyond 6 d, despite different dates of its sowing in different years, the cultivar was decided to be PPS. All other cultivars (including “weakly PPS”) are considered to be PPI. Second, any landrace that flower both during short-day and long-day seasons is PPI, whereas the landraces that do not flower during the long-day season are PPS, adapted to short-day photoperiods (Vergara and Chang 1985; Molla 2022). The classification was confirmed by Deb’s (2023b) index of photoperiod sensitivity.

With the background understanding of possible influences of day temperature and sunlight exposure (Yin and Kropff 1998; Kobayasi et al. 2010; Deb et al. 2023), we excluded the FOT and SAD data gathered on cloudy days and analysed the FOT and SAD of all landraces only on sunny days in each year from our data repertoire (Deb 2022). This served to eliminate the deviations of FOT of the landraces due to incomplete solar exposure and temperature drop at FOT on cloudy days. The table of FOT difference between years (FOTD) and SAD difference between years (SADD) of 278 landraces flowering on sunny days in different years, extracted from Deb (2022), is freely available from Deb (2023a).

Statistical analyses

To test our null hypothesis that the the degree of year-to-year difference in FOT (and SAD) is no different between PPS and PPI cultivars, FOTD was measured as $|FOT(i) - FOT(j)|$ between years i and j. We also measured $SADD = |SAD(i) - SAD(j)|$ between years i and j and compared both FOTD and SADD between the PPS and PPI cultivar groups.

All statistical analyses were made on a desktop using Open Office Calc and by using *R* version 4.1.3. We chose $p < 0.01$ for the level of significance as well as confidence intervals. The assumption of normality of distribution of FOTD and SADD of the PPS and PPI cultivars was tested by using Shapiro–Francia *W*’ test, supported by Anderson–Darling *W* test, with a two-sided type-I error of 1%. These tests are more sensitive and powerful than other tests of normality (Georgiev 2023; Mbah and Paothong 2014).

Following Gibbons and Chakraborti (2021), we performed Wilcoxon signed rank test to measure the significance of the difference between mean values of FOTD in PPS and PPI cultivars and also between the means of SATD in PPS and PPI cultivars, regardless of their distributions. To confirm the test result, we further performed the bootstrap mean test. The bootstrap method has a close synergy with simple random sampling with replacement process (Efron and Tibshirani 1994). So, we designed 1000 random samples from the PPI and PPS populations and tested the equality of the mean. Subsequently, we perform a permutation test, which is the exact statistical hypothesis test involving two or more samples. The null hypothesis is constructed to test whether all samples of the random variables PPS and PPI came from the same distribution.

Results

The total number of *aman* landraces sown repeatedly (at least twice) in the 3 years totalled 361, from which we selected 278 that flowered on sunny days. Based on the close proximity (< 7 days) of the FD between different years (see “Methods” Section), 105 landraces were identified to be strongly PPS, and 173 were photoperiod insensitive (PPI) cultivars with $|FDi - FDj| > 6$ days, where i and j are different years. The frequency distribution of FOTD of PPI landraces appears slightly different from that of the PPS landraces (Fig. 1A). A two-sample median test confirmed that the medians of FOTD and SADD for PPI are greater than the medians of FOTD and SADD of the PPS cultivars. As SAD strongly corresponds to FOT (Deb et al 2023), the year-to-year difference between FOTD closely corresponds to SADD (Table 3). The pattern of distribution of SADD (Fig. 1B) matches that of the FOTD. The mean and median values of both FOTD and SADD vary between the PPS and PPI groups (Table 3).

The Shapiro–Francia *W*′ and Anderson–Darling *W* tests (Table 4) suggest rejection of the assumption of normality of distribution of FOTD and SADD in both PPI and PPS groups. While the distributions of both FOTD and SADD were not normal, the difference in the distributions of FOTD as well as SADD between the PPS and PPI groups is significant. A two-sample permutation test (with 1000 random iterations) showed a highly significant difference in the distributions of FOTD ($p = 0.002$) and SADD ($p = 0.0001$) between PPS and PPI samples (Table 5).

A strong effect of day length on the FOT and SAD in the PPS group is expected to be reflected in a consideraly

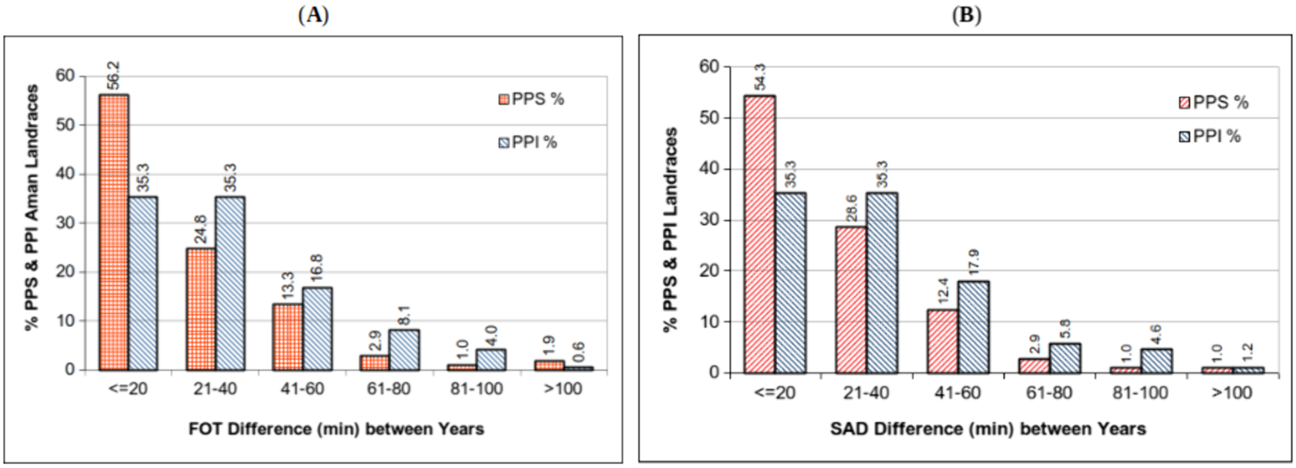

Fig. 1 **A** The range of year-to-year difference of FOT (FOTD, in minutes) and **B** year-to-year difference of SAD (SADD, in minutes) of photoperiod sensitive (PPS) and photoperiod insensitive (PPI) landraces

Table 3 The Range, Mean, Median and Variance of Year-to-Year Difference in FOT and SAD between PPS and PPI Cultivars

	FOTD (min)		SADD (min)	
	PPS ($n = 105$)	PPI ($n = 731$)	PPS ($n = 105$)	PPI ($n = 173$)
Min	0	0	0	0
Max	101	115	101	111
Mean	23.8	31.9	23.3	33.0
Median	18	28	18	30
Variance	443.9	536.0	371.5	558.9

narrow range of FOT compared to a wider range of FOT in the PPI group of cultivars. Thus, the means of both FOTD and SADD are likely to be significantly smaller in PPS than in PPI cultivars. Wilcoxon's rank sum test (Table 6) established that the mean of FOTD (=23.8 minutes) is significantly ($p < 0.001$) less in PPS landraces than the mean (=31.9 min.) in PPI landraces. Equivalently, the mean of SADD (=23.3 min.) in the PPS group is also significantly ($p < 0.001$) less than the mean (=33 min.) in the PPI group (Table 6). This strong difference was further corroborated by a bootstrap test using 1000 randomised iterations (with replacement) from the 109 FOTD as well as SADD values

Table 4 Shapiro–Francia and Anderson–Darling tests of normality of distribution

Group	Descriptor	N	Shapiro–Francia W'	p	Anderson–darling W	p
PPI	FOTD	173	0.0021	0	2.9372	0
PPS	FOTD	105	0.0028	0	3.3009	0
PPI	SADD	173	0.0067	0	3.4019	0
PPS	SADD	105	0.0080	0	2.6453	0

Table 5 Two-sample permutation test for PPS and PPI landraces

Descriptor	Hypothesis	p	Inference
FOTD	H_0: PPS = PPI against H_1: not H_0	0.002	Alternative hypothesis (H_1) is accepted
SADD	H_0: PPS = PPI against H_1: not H_0	0.000	Alternative hypothesis (H_1) is accepted

Table 6 Wilcoxon's rank sum test and bootstrap test for difference between means (μ) of FOTD and SADD between PPS and PPI groups of cultivars

Variable	hypothesis	Wilcoxon's rank sum test	Bootstrap test
FOTD	H_0: $\mu(\text{PPS}) = \mu(\text{PPI})$ H_1: $\mu(\text{PPS}) < \mu(\text{PPI})$	$p < 0.0006$	$p < 0.005$
SADD	H_0: $\mu(\text{PPS}) = \mu(\text{PPI})$ H_1: $\mu(\text{PPS}) < \mu(\text{PPI})$	$p < 0.0005$	$p < 0.005$

from PPS cultivars, and the 173 FOTD as well as SADD values from PPI cultivars (Table 6).

Discussion

Photoperiod sensitivity in cereals is an important trait in tropical rice landraces, most of which are adapted to short-day season of cultivation. Investigations in the complexity of photoperiod sensitivity in rice are confined to the detection of the anthesis initation dates (Vergara and Chang 1985; Deb 2023b), but the effect of day length on the flower opening time (FOT) has never been suspected nor indicated by hitherto published studies in rice biology. This study is the first evidence of photoperiod sensitivity of FOT and SAD of a large number of *indica* rice landraces and expands the connotation of photoperiod sensitivity in rice to imply relative invariance of the first flowering time, in addition to FD (Table 6).

As seasonal day length is known to turn on a group of genes which interact to initiate anthesis in PPS cultivars on specific days during the short-day season (Yano et al. 2000; Zhang et al. 2019; Zong et al. 2021), it seems likely that for the same cultivars, the FOT is also triggered by the same set of genes in response to day length. Several genes have been reported to be involved in the control of rice flowering time by modulating the day-length response (Hori et al. 2016; Molla 2022), which may be operative in PPS landraces. The variability of FOT in PPS cultivars is an outcome of the interactions of diverse alleles of the 'flowering time' genes (Hori et al. 2016). As the PPI group of cultivars is not responsive to day length for flowering date, the FOT and SAD in this group are unlikely to be influenced by day length. Our analysis indicates that in the PPS group of rice cultivars, both the flowering date and FOT are strongly influenced by photoperiod and that the variability of FOT in PPS cultivars is significantly narrower than that in PPI cultivars.

Conclusions for future biology

Recent discoveries of the genetic basis of photoperiod sensitivity in cereals do not provide empirical evidence of any gene(s) influencing FOT being triggered by photoperiod. This study establishes that in photoperiod-sensitive cultivars of rice, not only the flowering date (FD), but also the FOT is responsive to day length, and indicates that certain genes for anthesis initiation in these landraces may be involved in flowering time. Future research may explore the functions of the candidate genes (Zong et al. 2021; Molla 2022) by incorporating their different alleles into photoperiod-insensitive cultivars and monitoring the plasticity of FOT in the cultivars. Future examinations of different combinations of the effect of the photoperiod-triggered genes (i.e. effect on FD alone, on FOT alone, on both FD and FOT) are likely to open new windows to understanding the complex interactions between cereal floral biology and environmental factors.

Identification of the genetic basis of FOT and FED variability in PPI varieties is also important in improvement of selected agronomic characters in rices. In PPI cultivars, several alleles of a set of genes governing photoperiod insensitivity have significant pleiotropic effects on yield characters, such as panicle density and grain weight (Xu et al. 2014). These alleles can be modulated in specific crosses between PPI and PPS cultivars to improve yields in different rice varieties.

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Data availability The primary data of flowering date and time of all landraces are available from Harvard Dataverse (Deb 2022). The calculation of the year-to-year differences in FOT and SAD in PPS and PPI are freely available from Harvard Dataverse (Deb 2023a).

Declarations

Conflict of interest Authors declare no competing interests.

References

Deb D (2022) Rice flower opening time and flower opening duration data. Harvard Dataverse, V10. https://doi.org/10.7910/DVN/KYW1UC

Deb D (2023a) Flower opening time of photoperiod sensitive and photoperiod insensitive rices on sunny days. Harvard Dataverse, V1. https://doi.org/10.7910/DVN/QRCNMD

Deb D (2023b) A farmer's approach to detecting photoperiod sensitivity in rice (*Oryza sativa* ssp. indica) landraces. J Bot Res 5(3):60–66. https://doi.org/10.30564/jbr.v5i3.5737

Deb D, Bhattacharya D (2021) Unusually high frequency of cross pollination in rice landraces Shiuli and Kharah with co-incident flower opening times. Curr Sci 121(1):121–126. https://doi.org/10.18520/cs/v121/i1/121-126

Deb D, Joshi NV, Bhattacharya D, Nauri M, Ganguly R (2023) Diversity of flower opening time and duration in rice (*Oryza sativa* ssp. indica) landraces of South and Southeast Asia in different cultivation seasons. J Bot Res 5(3):36–48. https://doi.org/10.30564/jbr.v5i3.5724

Efron B, Tibshirani RJ (1994) An introduction to the bootstrap. Chapman and Hall/CRC Press, New York

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(6):1083–1094

Georgiev GZ (2023) Normality calculator. https://www.gigacalculator.com/calculators/normality-test-calculator.php

Gibbons JD, Chakraborti S (2021) Nonparametric statistical inference, 6th edn. CRC Press, New York

Kiyosumi H, Matsubara K, Yano M (2016) Genetic control of flowering time in rice: integration of Mendelian genetics and genomics. Theor Appl Genet 129(12):2241–2252. https://doi.org/10.1007/s00122-016-2773-4

Kobayasi K, Matsui T, Yoshimoto M, Hasegawa T (2010) Effects of temperature, solar radiation, and vapor-pressure deficit on flower opening time in rice. Plant Product Sci 13:21–28

Kobayasi K, Sakai H, Tokida T, Nakamura H et al (2019) Effects of free-air CO_2 enrichment on flower opening time in rice. Plant Product Sci 22(3):367–373. https://doi.org/10.1080/1343943X.2019.1569472

Mbah AK, Paothong A (2014) Shapiro–Francia test compared to other normality test using expected *p*-value. J Stat Comput Simul 85(15):3002–3016. https://doi.org/10.1080/00949655.2014.947986

Moldenhauer KAK, Gibbons JH (2003) Rice morphology and development. In: Smith CW, Dilday RH (eds) Rice: Origin, history, technology and production. Wiley, Hoboken, pp 103–127

Molla KA (2022) Flowering time and photoperiod sensitivity in rice: key players and their interactions identified. Plant Cell 34(10):3489–3490. https://doi.org/10.1093/plcell/koac230

Ogiso-Tanaka E, Matsubara K, Yamamoto S, Nonoue Y, Wu J (2013) Natural variation of the *RICE FLOWERING LOCUS T 1* contributes to flowering time divergence in rice. PLoS ONE 8(10):e75959. https://doi.org/10.1371/journal.pone.0075959

Padukkage D, Senanayake G, Geekiyanage G (2017) Photoperiod sensitivity of very early maturing Sri Lankan rice for flowering time and plant architecture. Open Agric 2:580–588

Uwatoko N, Onishi A, Ikeda Y, Kontani M, Sasaki A, Matsubara K, Itoh Y, Sano Y (2008) Epistasis among the three major Xowering time genes in rice: coordinate changes of photoperiod sensitivity, basic vegetative growth and optimum photoperiod. Euphytica 163:167–175. https://doi.org/10.1007/s10681-007-9584-2

Vergara BS, Chang TT (1985) The Flowering response of the rice plant to photoperiod, 4th edn. International Rice Research Institute, Los Baños

Xu Q, Saito H, Hirose I, Katsura K, Yoshitake Y, Yokoo T, Tsukiyama T, Teraishi M, Tanisaka T, Okumoto Y (2014) The effects of the photoperiod-insensitive alleles, *se13*, *hd1* and *ghd7*, on yield components in rice. Mol Breed 33:813–819. https://doi.org/10.1007/s11032-013-9994-x

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

Yin X, Kropff MJ (1998) The effect of photoperiod on interval between panicle initiation and flowering in rice. Field Crops Res 57:301–307. https://doi.org/10.1016/S0378-4290(98)00074-4

Zhang B, Liu H, Qi F, Zhang Z, Li Q, Han Z, Xing Y (2019) Genetic interactions among *Ghd7*, *Ghd8*, *OsPRR37* and *Hd1* contribute to large variation in heading date in rice. Rice (N Y) 12:48. https://doi.org/10.1186/s12284-019-0314-x

Zong W, Ren D, Huang M, Sun K et al (2021) Strong photoperiod sensitivity is controlled by cooperation and competition among *Hd1*, *Ghd7* and *DTH8* in rice heading. New Phytol 229(3):1635–1649. https://doi.org/10.1111/nph.16946