Accurate Evaluation of Photoperiodic Sensitivity and Genetic Diversity in Common Buckwheat under a Controlled Environment

Takashi Hara and Ryo Ohsawa

(Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan)

Abstract: Photoperiodic sensitivity is one of the most important factors determining whether a crop can adapt to and be cultivated under a broad range of conditions. In common buckwheat (*Fagopyrum esculentum* Moench), flowering time (flowering of the first flower) is a complex trait influenced by photoperiod, light quality, and temperature, which change daily under natural conditions, and their interaction. Common buckwheat shows a large genetic variation because of the outcrossing reproductive strategy of this species. Thus, flowering time variation within a population reflects both environmental and genotypic variations, and accurate evaluation of photoperiodic sensitivity in common buckwheat requires cultivation under controlled environmental conditions. Here, we investigated photoperiodic sensitivity and its genetic diversity in two buckwheat cultivars, the autumn ecotype Miyazakizairai and the summer ecotype Botansoba, by controlling photoperiod during cultivation under the same temperature regime. Our results showed that (1) the summer ecotype consisted of early-flowering genotypes, including genotypes not found in the autumn ecotype; (2) the autumn ecotype consisted of various genotypes, including early-flowering genotypes and a large number of late-flowering genotypes not found in the summer ecotype; (3) the autumn ecotype showed larger genetic diversity than the summer ecotype in long-day treatments; and (4) genetic diversity first became evident in the 14.5-hr photoperiod in the autumn ecotype, and in the 15.0-hr photoperiod in the summer ecotype. These results support the hypothesis based on previous studies that common buckwheat summer ecotypes were derived from autumn ecotypes by adaptation to climate in northern Japan.

Key words: Allogamous plant, Common buckwheat, Controlled environment, Flowering time, Genetic diversity, Photoperiodic sensitivity.

Common buckwheat (*Fagopyrum esculentum* Moench, $2n = 16$) is cultivated throughout Japan, and its cultivars are classified into three ecotypes (autumn, intermediate, and summer ecotypes) according to their flowering time and yield under natural conditions. Common buckwheat is commonly considered a short-day plant. Namai (1990) reported the characters of each ecotype in Japan as follows: autumn ecotypes are grown in low-latitude regions, where the photoperiod is 11.0 to 13.5 hr during the cropping season from late August to early November, and their flowering is generally photoperiod-sensitive; summer ecotypes are grown in high-latitude regions, where the photoperiod is 13.5 to 15.5 hr during the cropping season from early June to late August, and they are generally photoperiod-insensitive; intermediate ecotypes show moderate photoperiodic sensitivity.

Many plants show very strict photoperiodic sensitivity. For example, Japanese basil (*Perilla frutescens*) and rice (*Oryza sativa*) are short-day plants that respond to a 15-min (Takimoto and Ikeda, 1961) and 30-min difference (Itoh et al., 2010) in photoperiod, respectively. Michiyama et al. (2005) suggested that common buckwheat responds to a 30-min difference in photoperiod. Although the relationship between light quality and flowering time is not clear in common buckwheat, flowering time is influenced by light quality (Reed et al., 1993; Delvin et al., 1998; Mockeler et al., 1999). Furthermore, the flowering time of common buckwheat is influenced by temperature (Xu, 1938; Hao et al., 1994). These findings suggest that flowering time in common buckwheat under natural conditions depends on both light (photoperiod and light quality) and temperature, which change daily during the cropping season. As a result, photoperiodic sensitivity cannot be accurately evaluated in common buckwheat merely by examining the flowering time of the plants sown at various times.
In previous studies on the photoperiodic sensitivity of common buckwheat (Onda and Takeuchi, 1942; Michiyama and Hayashi, 1998) the relationship between average photoperiod during a certain fixed period and the flowering time has been investigated. Therefore, to clarify the relationship between the flowering time and photoperiod and light quality, observations must be made under controlled temperature conditions and at a fixed photoperiod. Furthermore, analysis of photoperiodic sensitivity in common buckwheat is difficult because the genetic composition of buckwheat ecotypes is complex as a result of its outcrossing reproductive strategy. In general, a common buckwheat ecotype consists of various genotypes, and it can thus be expected to include various alleles related to photoperiodic sensitivity. Thus, the photoperiodic sensitivity of each plant might be different even in the same ecotype under the same environmental conditions. Therefore, to accurately evaluate photoperiodic sensitivity of common buckwheat ecotypes, it is necessary to observe many individuals under the same environmental conditions.

Under long-day conditions (photoperiod > 14.5 hr), common buckwheat ecotypes include both flowering and non-flowering individuals (Nagatomo, 1961). Michiyama et al. (2005) reported that days-to-flowering (DTF) in the autumn cultivar Miyazakizairai sharply increases in response to the change in photoperiod from 14.0 to 14.5 hr. Therefore, in this study we considered that the critical photoperiod of common buckwheat is 14.0 to 14.5 hr and regarded 14.5 and longer photoperiod as long days. We then investigated the accuracy of evaluations of photoperiodic sensitivity under several long-day conditions.

In this study, we investigated the photoperiodic sensitivity of common buckwheat by examining the flowering time of in autumn and summer ecotypes grown under controlled environmental conditions (controlling photoperiod and light quality at the same temperature). Our study revealed important knowledge about the genetic diversity of photoperiodic sensitivity of common buckwheat, which enables this species to adapt to cultivation over a wide latitude range. We also examined the process by which summer ecotypes differentiated from autumn ecotypes.

**Materials and Methods**

We conducted experiments at the University of Tsukuba using the local common buckwheat cultivar in the Kyushu region, Miyazakizairai (called Autumn hereafter), an autumn ecotype that is not considered to be subject to selection by photoperiodic sensitivity, and a cultivar selected from local cultivars in the Hokkaido region, Botansoba (Summer hereafter), which is a summer ecotype considered to be selected by photoperiod (Fig. 1). We divided each ecotype into four groups and subjected them to different photoperiodic treatments: a short-day treatment (photoperiod fixed at 12.0 hr) and three long-day treatments (14.5, 15.0, and 15.5 hr). To eliminate the effects of differences in natural light and temperature, the plants were cultivated in a growth cabinet (210 × 210 × 240 cm: L × W × H, NK system Co., Ltd. Osaka, Japan) in a dark room (Fig. 2A), and the temperature regime was set at 12/12 hr 25ºC/20ºC. The number sown differed with the treatment because of restrictions on the cultivation area: 60 seeds each of Autumn and Summer were sowed.
and cultivated in the short-day treatment. In two of the long-day treatments (14.5 and 15.0 hr), 144 seeds each of Autumn and Summer were sown and cultivated, and in the 15.5-hr long-day treatment, 96 seeds each of Autumn and Summer were sowed and cultivated. All seeds were sowed in plastic boxes (59 × 16 × 19 cm; L × W × H), 12 seeds per box (Fig. 2B). Each box was filled with culture soil (Tsuchitaro, 120 mg L\(^{-1}\) N, 1000 mg L\(^{-1}\) P, 50 mg L\(^{-1}\) K, Sumitomo Forestry Co., Ltd., Tokyo, Japan). Two metal halide lamps (MLBOC400C-U, total flux: 32000 lm, luminance efficiency: 80 lm W\(^{-1}\), color temperature: 6000 K, Mitsubishi Electric Corp., Tokyo, Japan) were used in each
growth cabinet (Fig. 2A). The planter boxes were placed randomly in the growth cabinet. Photoperiodic treatments were begun just after sowing. The plants were watered as needed to ensure that the soil remained sufficient soil moisture.

Hagiwara et al. (1998) reported that flower bud differentiation in common buckwheat is delayed under a long-day (14.5 hr) condition compared with a short-day (8.0 hr) condition, and that this difference becomes more pronounced in the later growth stage. Thus, we investigated the photoperiodic sensitivity as follows: For each individual plant, we recorded the dates of cotyledon unfolding and the first flower in each flower cluster on the main stem. We defined the number of days from the expansion of the cotyledons to the flowering of the first flower as DTF and used DTF to indicate the photoperiodic sensitivity of each individual. We observed the plants daily until 100 d after sowing, when plant height reached the height of the metal halide lamp (170 cm). We then classified those individuals that had not flowered within 100 d after sowing as non-flowering. To evaluate differences in photoperiodic sensitivity among the treatments and between the ecotypes, we compared the average DTF in each treatment by analysis of variance (ANOVA) and Tukey-Kramer tests, using JMP 6.0 software (SAS Institute Inc., Cary, NC, USA). We assessed differences in the frequency of non-flowering individuals between the 15.0-hr and 15.5-hr treatments using a $\chi^2$ test. The average DTF differed greatly depending on the treatment and ecotype. Therefore, we calculated the coefficient of variation to characterize the genetic diversity of photoperiodic sensitivity among the treatments and between the ecotypes.

Table 1. Flowering of Autumn and Summer ecotypes of common buckwheat in each photoperiod.

| Ecotype | Photoperiod treatment (hr) | Days-to-flowering (d) | No. of individuals |
|---------|---------------------------|----------------------|-------------------|
|         |                           | Average | Shortest | Longest | Flowering (%) | Non-flowering (%) | Total 2 |
| Autumn  | 12.0                      | 19.3     | 17       | 22      | 51             | 0 (0.0)           | 51     |
|         | 14.5                      | 34.7     | 22       | 50      | 134            | 0 (0.0)           | 134    |
|         | 15.0                      | 54.3     | 26       | 90      | 116            | 6 (5.2)           | 122    |
|         | 15.5                      | 55.4     | 34       | 92      | 52             | 32 (38.0)         | 84     |
| Summer  | 12.0                      | 18.4     | 17       | 21      | 59             | 0 (0.0)           | 59     |
|         | 14.5                      | 22.9     | 19       | 28      | 131            | 0 (0.0)           | 131    |
|         | 15.0                      | 30.7     | 21       | 52      | 101            | 2 (1.9)           | 103    |
|         | 15.5                      | 33.5     | 21       | 57      | 71             | 6 (7.8)           | 77     |

1) Values with the same superscript do not differ significantly at the level of $P = 0.05$ level (Tukey-Kramer test) between photoperiod and ecotype.

2) The total number of individuals in each treatment differs from the number of seeds sown because some seeds did not germinate and some individuals withered.

![Fig. 4. Coefficients of variation for each ecotype in each photoperiod. Because many individuals were non-flowering in the Autumn ecotype in 15.5-hr photoperiod (32 of 84, Table 1), the variation was not calculated.](image-url)

**Results**

We examined the differences in the frequency distributions of DTF (Fig. 5), the average DTF (Table 1), and the coefficients of variation (Fig. 4) between the ecotypes and among the treatments.

In Autumn, the average DTF was 19.3 d under the short-day treatment (12.0 hr, Table 1). In the three long-day treatments (14.5, 15.0, and 15.5 hr), the average DTF was 34.7, 54.3 and 55.4 d, respectively (Table 1). There were significant differences in the average DTF with the treatments, except between the 15.0-hr and 15.5-hr photoperiods (Tukey-Kramer test, Table 1) and the average DTF increased (i.e., flowering was delayed) as the photoperiod was prolonged.

In Autumn, the shortest and longest DTF were 17 and
22 d, respectively, under the short-day treatment (12.0 hr, Fig. 3 and Table 1), and the DTF increased under a photoperiod prolonged up to 15.0 hr but not with further prolongation (14.5 hr, 22 and 50 d; 15.0 hr, 26 and 90 d; 15.5 hr, 34 and 92 d; Fig. 3 and Table 1). Six individuals (5.2\%) in the 15.0-hr treatment and 32 individuals (38.0\%) in the 15.5-hr treatment were non-flowering (Table 1). The difference was significant ($\chi^2$ test). The coefficient of variation under the short-day treatment was 6.7\% (Fig. 4), and that in 14.5 and 15.0 hr was 20.4\% and 29.3\%, respectively. Thus, genetic diversity was manifest in the 14.5-hr and longer photoperiod. These results show that in Autumn, the longer the photoperiod, the greater the DTF (i.e., flowering was delayed); the underlying genetic diversity in DTF within the ecotype was more obvious, and non-flowering individuals became more frequent under a longer photoperiod.

In Summer, the average DTF was 18.4 d under the short-day treatment (12.0 hr, Table 1), and it also increased with prolonging photoperiod up to 15.0 hr, but not with further prolongation from 15.0 to 15.5 hr (Table 1).

In Summer individuals the DTF in a 12-hr photoperiod was between 17 and 21 d, but the frequency distribution range of DTF increased with prolonging photoperiod (14.5 hr, 19 d and 28 d; 15.0 hr, 21 d and 52 d; 15.5 hr, 21 d and 57 d; Fig. 3 and Table 1). Two individuals (1.9\%) in the 15.0-hr treatment and six individuals (7.8\%) in the 15.5-hr treatment were non-flowering (Table 1), but this difference was not significant ($\chi^2$ test). The coefficient of variation under the short-day treatment was 5.2\% (Fig. 4), and the coefficients of variation under the long-day treatments were 9.0\% (14.5 hr), 18.0\% (15.0 hr), and 26.2\% (15.5 hr). Thus, genetic diversity was manifested in the 15.0-hr photoperiod, and was more obvious in a longer photoperiod. Thus, in Summer, DTF increased the longer the photoperiod, and the underlying genetic diversity in DTF was observed within the ecotype.

The average DTF in Autumn and Summer was 19.3 and 18.4 d (12.0 hr), 34.7 and 22.9 d (14.5 hr), 54.3 and 30.7 d (15.0 hr), and 55.4 and 33.5 d (15.5 hr), respectively (Table 1). In the long-day treatments (14.5 hr and longer photoperiod), the average DTF in Autumn was significantly different from that in Summer. Also, the difference in DTF between treatments was larger in Autumn than in Summer (Table 1). In the long-day treatments, especially in 15.0 and 15.5 hr photoperiods, Summer included early-flowering genotypes, in which DTF was 20 – 24 d at 15.0 hr and at 20 – 29 d at 15.5 hr, which were not found in Autumn (Fig. 3). On the other hand, Autumn consisted of more varied genotypes: from early- to late-flowering genotypes including individuals that flowered in 55 – 94 d at 15.0 hr and 60 – 94 d at 15.5 hr, which were not found in Summer. Moreover, the frequency of non-flowering individuals (5.2\% in 15.0 hr and 32.0\% at 15.5 hr) was higher in Autumn than in Summer (1.9\% at 15.0 hr and 7.8\% in 15.5 hr; Table 1 and Fig. 3). In long-day treatments, Autumn showed larger genetic diversity (20.4\% at 14.5 hr and 29.3\% at 15.0 hr) than Summer (9.0\% at 14.5 hr and 18.0\% at 15.0 hr; Fig. 4). In addition, the genetic diversity first became evident at 14.5 hr in Autumn, and at 15.0 hr in Summer (Fig. 4).

**Discussion**

We were able to accurately evaluate photoperiodic sensitivity and genetic diversity in two common buckwheat ecotypes by cultivating them under controlled environmental conditions (controlling photoperiod and light quality under the same temperature regime). From our results we gained important knowledge about the genetic diversity of photoperiodic sensitivity in common buckwheat that enables this species to adapt to cultivation over a wide latitude range. Moreover, the results suggested the process by which summer ecotypes differentiated from autumn ecotypes.

Previous studies (Onda and Takeuchi, 1942; Michiyama and Hayashi, 1998), conducted under natural conditions, showed that the flowering time of common buckwheat is delayed by the lengthening of the photoperiod. Onda and Takeuchi (1942) and Michiyama and Hayashi (1998) also showed that the flowering time is delayed by a long photoperiod more in autumn than in summer ecotypes. The results of our experiment, conducted under controlled environmental conditions, strongly support the results of these previous studies: Autumn and Summer showed a delay in flowering (an increase in the average DTF) under longer photoperiods, and the difference in the average DTF between the photoperiodic treatments was larger in Autumn than Summer.

Summer and intermediate ecotypes were probably derived from autumn ecotypes by natural selection (Matano and Ujihara, 1979). Minami and Namai (1986a, 1986b) suggested that the summer and intermediate ecotypes were selected by adaptation to the climatic conditions of northern Japan. Ohsawa (1997) also suggested that photoperiodic sensitivity is a key factor for this adaptation. In rice, the difference in the genetic variation was associated with the degree of selection pressure; stronger selection pressure leading to decreased variance (Kikuchi, 1979). These considerations led us to hypothesize that autumn ecotypes have greater genetic diversity with regard to photoperiodic sensitivity than summer ecotypes. Our results show that Autumn consisted of more varied genotypes than Summer: Autumn included early-flowering genotypes and late-flowering genotypes not found in Summer and a higher frequency of non-flowering individuals. These results clearly show that Autumn consisted of individuals with more varied photoperiodic sensitivity (i.e., various short-day requirement) than
A: Genotype with early flowering in any photoperiod.
B: Genotype with late flowering when the photoperiod is more than 15.0 hr.
C: Genotype with late flowering when the photoperiod is longer than 12.0 hr.
D: Genotype with late flowering when the photoperiod is longer than 14.5 hr.

**Fig. 5.** Pattern diagram of selection due to differences in the short-day requirement (A) and phenotypic diversity (B).

- A: In the short-day treatment (12.0 hr), all short-day requirement genotypes in the autumn ecotype flower at about the same time, so the phenotypic variation is low. As the photoperiod becomes longer, as it does at more northern latitudes in Japan, the autumn ecotype would be subjected to selection. After natural and artificial selection, first, the genotype for late flowering when the photoperiod is more than 12.0 hr is eliminated from the population (a), then, the genotype for late flowering when the photoperiod is more than 14.5 hr is eliminated (b), and finally, the genotype for late flowering when the photoperiod is more than 15.0 hr is eliminated (c).

- B: When selected genotype group (a), which was selected by the 14.5-hr treatment (Fig. 5A), is cultivated under each photoperiod, the level of phenotypic diversity is revealed under photoperiods of more than 14.5 hr and is comparable under photoperiods of less than 14.5 hr; when selected genotype group (b), which was selected by the 15.0-hr treatment (Fig. 5A), is cultivated under each photoperiod, the phenotypic diversity becomes higher at photoperiods of longer than 15.0 hr and is comparable at photoperiods of 15.0 hr or less; when selected genotype group (c), which was selected by the 15.5-hr treatment (Fig. 5A), is cultivated under each photoperiod, the phenotypic diversity is comparable at all photoperiods.
Summer. Moreover, in each long-day treatment, genetic diversity in the short-day requirement (indicated by the coefficient of variation of DTF) was higher in Autumn than in Summer. These results suggest that Summer has been subjected to greater selection pressure in terms of the photoperiod during the cultivation period than Autumn.

We showed the presumed pattern diagram of the selection based on from the difference in the short-day requirement (Fig. 5A). In the short-day treatment (12.0 hr), all short-day requiring genotypes in the autumn ecotype flower at almost the same time, resulting in a small phenotypic variation. In the long-day treatments (14.5, 15.0, and 15.5 hr), the differences in the flowering time among the genotypes becomes clear. Michiyama and Hayashi (1998) considered that seed set percentage is reduced under long-day condition because of the frequent occurrence of malformed pistil in the late flowering genotype and also other physiological factors. As a result, the late flowering genotype would be eliminated from the population by selection. Thus, the genotypes of autumn ecotype would have been subjected to strong selection as the photoperiod became longer, i.e., as they were cultivated further and further north. Under the 14.5-hr photoperiod, the genotype with late flower when the photoperiod is longer than 12.0 hr is flowered later than the other genotypes. After natural and artificial selection, these genotypes would be eliminated from the population (Fig. 5A, (a)). Similarly, under the 15.0-hr photoperiod, the genotype with late flowering when the photoperiod is longer than 14.5 hr would be eliminated from the population after natural and artificial selection (Fig. 5A, (b)), and under the 15.5-hr photoperiod, the genotype with late flowering when the photoperiod is longer than 15.0 hr would be eliminated after natural and artificial selection (Fig. 5A, (c)). Thus, the level of genetic diversity in a population with regard to the short-day requirement depends on the latitude at which it is cultivated. Hara et al. (2011) showed that two gene regions are associated with photoperiodic sensitivity in common buckwheat. In future studies on photoperiodic sensitivity of common buckwheat, the above-mentioned selection process should be clarified and confirmed by evaluating the genetic diversity of common buckwheat in different latitudes by using these genes associated with the photoperiodic sensitivity.

We designed a frame format of phenotypic diversity for each genotype group (a), (b) and (c) shown in Fig. 5A in each photoperiod (Fig. 5B). In group (a), which was selected by the 14.5-hr photoperiod, the level of phenotypic diversity is increased under a photoperiod of longer than 14.5 hr and is comparable to that under a photoperiod of less than 14.5 hr (the genetic diversities of Summer, shown in Fig. 4, is classified here). Similarly, in group (b), which was selected by the 15.0-hr photoperiod, the phenotypic diversity at photoperiods longer than 15.0 hr is larger than that under a photoperiod of 15.0 hr or shorter photoperiod. In group (c), which was selected by the 15.5-hr photoperiod, the phenotypic diversity is comparable under all photoperiods.

These results support the previous hypotheses for adaptation and differentiation of common buckwheat: namely, summer ecotypes were derived from autumn ecotypes by natural selection (Matano and Ujihara, 1979); this selection occurred by adaptation of common buckwheat to the climatic conditions in northern Japan (Minami and Namai, 1986a, 1986b); and photoperiodic sensitivity is a key factor of adaptation and differentiation of common buckwheat (Ohsawa, 1997).

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References

Delvin, P.F., Patel, S.R. and Whitelam, G.C. 1998. Phytochrome E influences internode elongation and flowering time in Arabidopsis. Plant Cell. 10: 1479-1487.

Hagiwara, M., Inoue, N. and Matano, T. 1998. Variability in the length of flower bud differentiation period of common buckwheat, Fagopyrum, 15: 55-64.

Hao, X., Yang, W. and Bi, R. 1994. Quantitative relationships between the growth and development of buckwheat and temperature and light. Fagopyrum. 14: 49-54.

Hara, T., Iwata, H., Okuno, K., Matsui, K. and Ohsawa, R. 2011. QTL analysis of photoperiod sensitivity in common buckwheat by using markers for expressed sequence tags and photoperiod-sensitivity candidate genes. Breed. Sci. 61: 394-404.

Itoh, H., Nonoue, Y., Yano, M. and Izawa, T. 2010. A pair of floral regulators sets critical day length for Hd3a florigen expression in rice. Nature genetics. 42: 635-639.

Kikuchi, F. 1979. Adaptive changes in rice hybrid populations in sensitivity to different environments. Bull. Natl. Inst. Agric. Sci., Ser. D. 30: 69-179.

Matano, T. and Ujihara, A. 1979. Agroecological classification and geographical distribution of the common buckwheat, Fagopyrum esculentum M. in the East Asia. Japan Agricultural Research Quarterly. 13: 157-162.

Michiyama, H. and Hayashi, H. 1998. Differences of growth and development between summer and autumn-type cultivars in common buckwheat (Fagopyrum esculentum Moench). Jpn. J. Crop Sci. 67: 323-330*.

Michiyama, H., Tsuchimoto, K., Tani, K., Hirano, T., Hayashi, H. and Campbell, C. 2005. Influence of day length on stem growth, flowering, morphology of flower clusters, and seed-set in buckwheat (Fagopyrum esculentum Moench). Plant Prod. Sci. 8: 44-50.

Minami, H. and Namai, H. 1986a. Potential genetic variation of flowering time in late-summer type cultivar of buckwheat (Fagopyrum esculentum Moench) in Kyushu region [Japan]. Jpn. J. Breed. 36: 67-74**.
Minami, H. and Namai, H. 1986b. Populational change in flowering time caused by different harvesting date observed in the late-summer type cultivar Miyazakizairai of buckwheat (Fagopyrum esculentum). Jpn J Breed. 36: 155-162**.

Mockeler, T.C., Guo, H., Yang, H., Duong, H. and Lin, C. 1999. Antagonistic actions of Arabidopsis cryptochromes and phytochrome B in the regulation of floral induction. Development. 126: 2073-2082.

Nagatomo, T. 1961. Studies on physiology of reproduction and some cases of inheritance in buckwheat. Rep. Breed. Sci. Lab., Faculty Agric., Miyazaki Univ. 1: 1-213**.

Namai, H. 1990. Pollination biology and reproductive ecology for improving genetics and breeding of common buckwheat, Fagopyrum esculentum (1). Fagopyrum. 10: 23-46.

Ohsawa, R. 1997. Evaluation of a Japanese germplasm collection of common buckwheat using a multivariate approach. Proc. 8th SABRAO Congress. 107-108.

Onda, S. and Takeuchi, T. 1942. Ecotypes of Japanese buckwheat varieties. Nogyo oyobi Engei. 17: 971-974***.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M. and Chory J. 1993. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell 5: 147-157.

Takimoto, A. and Ikeda, K. 1961. Effect of twilight on photoperiodic induction in some short day plants. Plant Cell Physiol. 2: 213-239.

Xu, Q. 1938. Studies on the effects of seasonal change of day length and temperature on reproductive period in crop. 2. Flowering time and its uniformity in buckwheat. Nogyo oyobi Engei 13: 1601-1612***.

* In Japanese with English abstract.
** In Japanese with English summary.
*** In Japanese.