
Biological Resource

Wisconsin Fast Plants, Ideal Plant Materials for Student Laboratories on Flower Initiation at the Secondary Level**Hideto Tojo¹⁾, Nobuyasu Katayama²⁾***¹⁾ *Shiraume High School, Japan*²⁾ *Tokyo Institute of Biology Education, Japan*

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For developing a student laboratory exercise on flower initiation (floral development) in senior high school biology, we selected the Wisconsin Fast Plants (WFPs) which are the new varieties of rape (*Brassica rapa*, syn. *campestris*) as our experimental material. We examined the effects of temperature, day length, light intensity and the plant hormone gibberellic acid (GA₃) on the flower initiation of WFPs. Under continuous light, the days required for detecting the first flower bud were dependent on the growth temperature being between 15°C and 35°C; the higher the temperature was, the faster the flower bud formation was. In the temperature range examined, the first flower bud could be detected within at least nine days (at 15°C) after sowing the seeds. Flower initiation was dependent on the day length at 15°C, but not at 25°C. At 15°C, flower buds formed nine days after sowing the seeds under continuous light, while no flower bud was detected nearly two weeks after the seeds were sown under a short-day condition (L:D = 8:16). The intensity of light in a long-day treatment (L:D = 16:8), from about 10 μmol·m⁻²·s⁻¹ (ca 1,500 lux) to 100 μmol·m⁻²·s⁻¹ (ca 13,000 lux) at 15°C, did not affect flower bud formation. The flower initiation in the plants treated with 0.01 μg·mL⁻¹ GA₃ was faster than in the untreated plants. The results obtained in the present study reveal that by using the WFPs students can get clear results on the effects of photoperiod, temperature, and the plant hormone gibberellin on the flower initiation of long-day plants within a shorter period than by using any other plant. Biology teachers possibly can organize a student laboratory on the photoperiodic response of plants referring to the present report.

Keywords: *Brassica rapa* (syn. *campestris*), flower initiation, laboratory exercise, photoperiodism, secondary biology, Wisconsin Fast Plants

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INTRODUCTION

Flower initiation (floral development) is the point at which vegetative growth turns to the reproductive stage. Therefore, it is one of the important biological phenomena to be learned at the secondary level (Lockard and Shortess, 1960). Students might be interested in studying this topic in the chapter “Plant Growth and Its Regu-

lation” in secondary biology because they can learn the relationships between plant morphogenesis and environmental factors, such as light, photoperiod and temperature.

In current biology textbooks for Japanese senior high school students, there are many plant names (e.g., *Ipomoea nil*, *Lemna* sp., *Xanthium occidentale* and *Kalanchoe* sp.) and terms (e.g.,

short-day and long-day treatments, short-day plants, long-day plants, indeterminate plants, and flowering hormone) in relation to flower initiation and photoperiodism. Some examples of experiments, such as leaf removal, grafting and stem girdling techniques, also appear in these textbooks. However, in practice, such experiments are rarely carried out as a student laboratory because the teaching of this topic through observation and experimentation is time-consuming. Therefore, learning of this topic has been mainly through lectures and deskwork. As a result, understanding of this topic has largely come by obtaining enumerated fragmentary knowledge of the phenomenon, and students mostly might be bored in studying this topic.

Among many plants, the Japanese morning glory (*Ipomoea nil*), a short-day plant, has been used as an experimental material for flower initiation and the flowering itself in research on plant physiology in Japan (Imamura, 1967; Takimoto and Kaihara, 1984). There have been a considerable number of trials to introduce the results of this serial research work to high school biology classes so far (e.g., Madrazo, Jr. and Hounshell, 1978). However, only a small number of teachers may carry out this experiment for student laboratories, because it takes at least a few weeks to get the experimental results.

Some experiments using other short-day plants, such as duckweed, *Lemna* sp., (Rhodes, 1968; Fujioka, 1986) and *Bryophyllum* sp. (Hibbs and Yokum, 1976; Hinata, 1988, 1989) were developed for high school student laboratories. Regarding duckweed, it seems difficult for students to detect its flower buds, because they are too small. The latter species are familiar as flower-pot plants and are very useful as teaching materials for vegetative reproduction. Unfortunately, these plants also require several weeks for

detecting the flower buds after a short-day treatment. Therefore, there has been no report on such trials in student laboratories.

Regarding long-day plants, Hollis and Miller (1968) introduced *Silene pendula* into the laboratory exercise for university students. However, as it takes more than one month until flowering, the plant does not seem to be suitable for secondary school biology laboratories. Gotoh (1998) proposed the use of *Arabidopsis thaliana*, an ideal plant for research, for the secondary school student laboratory on flower initiation. This plant is suitable for this kind of experiment because of its shorter life cycle. Flowering in this plant is well-known to be induced by long-day photoperiods (Hayama and Coupland, 2003) and by plant hormones such as gibberellin (Boss *et al.*, 2004). But, there is a difficulty in detecting a flower bud at its early stages since the color of its petals is white, and it seems very hard for students to distinguish the juvenile flower bud from leaf primordia.

Friend and Helson (1966) indicated that the floral induction in rape (*Brassica rapa*, syn. *campestris*) occurs by one long-day treatment. Wisconsin Fast Plants (WFPs, see details in Appendices) which are the new varieties of rape have a short life cycle, as does *A. thaliana*. As the color of their petals is yellow, detecting their flower buds may be easier than with *A. thaliana*. However, the use of WFPs in students' laboratories on flower initiation has not been reported, yet. Therefore, in the present study, we examine the experimental conditions on the flower initiation of the WFPs for the sake of secondary biology teachers to introduce this plant material into their teaching of photoperiodism.

MATERIALS AND METHODS

Seeds

The seeds of the standard type of WFPs were purchased from Carolina Biological Supply Company, USA, through Nakamura Rika Kogyo Co. Ltd., Tokyo, Japan, or from *InTheWoods*: Kobayashi Hard Ware Co. Ltd., Aomori, Japan.

Plant Growth

Fifty seeds were sown on a wet vermiculite bed in a tray (planter). Although the plants are generally grown with the addition of some fertilizer to assist good growth, only water was given to the plants in the present study to restrict their height. The trays were placed in a temperature-controlled growth cabinet. The light was shone from above by four fluorescent light tubes (Toshiba FL15N, 100V, 15W). The light intensity was determined by a photometer, Memory Sensor MES-101 which can be equipped with the Quantum Sensor IKS-27/101 or the Illuminance Sensor IKS-17/101 (Koito Industries, Ltd., Japan). Each experiment was repeated four times.

Gibberellin Treatment

We used gibberellic acid (GA₃, Tokyo Chemical Industry Co. Ltd., Japan) for gibberellin treatment. GA₃ was given to the seedlings throughout. Twenty seeds were placed on 0.8% water-agar plates containing 0, 0.01, 1, 10 or 100 µg·mL⁻¹ GA₃, each in a 9 cm Petri dish or a Plant Box (BC-PB851, Bio Medical Science Co. Ltd., Tokyo). When a Petri dish was used, a plastic cover was placed on each Petri dish to prevent water loss from the agar plate. Then the Petri dishes and Plant Boxes were placed in the growth cabinet mentioned above. The experiment was repeated four times.

In our experiments, we did not conduct sterilization treatment because we did not notice any microbial infections on the seeds or seedlings.

Detection of Flower Buds

The presence of the first flower bud, whose color was pale yellow, of each plant was exam-

ined with the naked eye by opening juvenile leaves at the shoot apex of each plant with a pair of tweezers. The number of days required for detecting the first flower bud after sowing the seeds was recorded. Data were analyzed statistically: the mean value and significant difference (SD) of the number of days required for detecting the first flower bud were calculated for four replications.

RESULTS AND DISCUSSION

Effect of Temperature on Flower Initiation under Continuous Light

The plants were grown at different temperatures under continuous light illumination to see if the growth temperature would affect flower initiation. As shown in Figure 1, the days required for detecting the first flower bud after sowing the seeds were dependent on the growth temperature being kept between 15°C and 35°C; the higher the temperature was, the faster the flower bud formation was. In the temperature range examined, the first flower bud could be detected less than nine days after sowing the seeds.

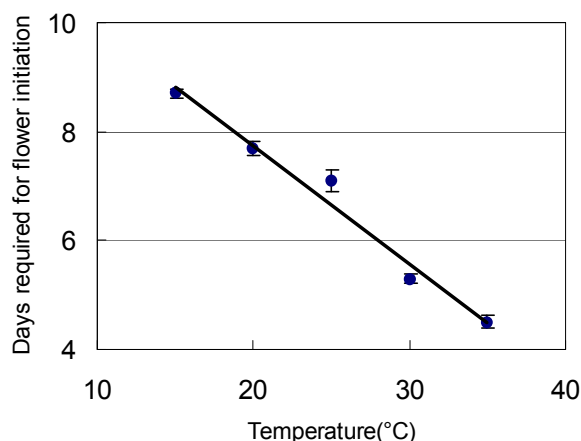


Figure 1: The effect of temperature on flower initiation in the standard type of WFPs

The vertical axis indicates the number of days required for detecting the first flower bud after placing the seeds under continuous light illumination (n = 50, four replications).

Flower Initiation under Different Photoperiods

According to King and Kondra (1986), *B. campestris* (= *B. rapa*), as well as *B. napus*, shows photoperiodic responses. However, Weis (2015) reported that the standard purple-stemmed line of WFPs was insensitive to day length. As he did not mention the temperature examined, we examined the effects of day length on flower initiation at different temperatures of 15°C, 20°C and 25°C. As shown in Figure 2, at 15°C, the number of days required for detecting the first flower bud after sowing the seeds was dependent on the day length. Under continuous light at 15°C, the first flower bud was detected nine days after sowing the seeds (Fig. 1). However, under a short-day condition (8-hour light and 16-hour dark; L:D = 8:16), it took nearly two weeks to detect the first flower bud. On the other hand, at 20°C (data not shown) and 25°C, the number of days required for detecting the first flower bud after sowing the seeds was constant (about one week) (Fig. 2). From the results, the standard type of WFPs is considered to be a tempera-

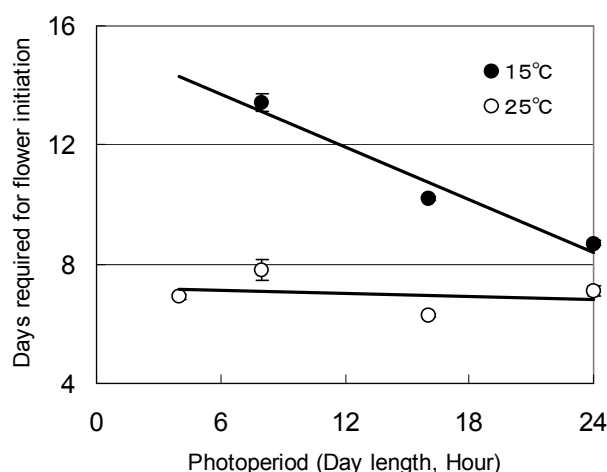


Figure 2: The effect of photoperiod (day length) on flower initiation in the standard type of WFPs at 15°C and 25°C

The vertical axis indicates the number of days required for detecting the first flower bud after sowing the seeds ($n = 50$, four replications).

ture-dependent “quantitative long-day plant (see details in Appendices)” for which flowering is accelerated or delayed by the length of the photoperiod at lower temperatures. The critical temperature for its photoperiodic response is considered to be between 15°C and 20°C. Therefore, it is recommended that the temperature must be kept below 15°C for this experiment.

Flower Initiation under Different Light Intensities

To see if the light intensity would affect flower initiation, plants were grown at 15°C under different light intensities in a long-day condition (L:D = 16:8). The light intensity which plants received was changed by placing each planter a different distance from the light source. Under light intensity ranging from $13 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (ca 1,500 lux) to $103 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (ca 13,000 lux), there was no significant difference in the length of the period (ca 10 days) until the first flower bud was detected. From the results, the experiment possibly can be carried out without considering the strength of the light. As the lowest light intensity examined in the present study is easily obtainable by using an ordinary fluorescent desktop light stand, no special light source is required for conducting the experiments.

Effect of Gibberellic Acid on Flower Initiation

Whittwer and Bukovac (1957) firstly reported that a group of plant growth regulators, gibberellins, affected the temperature and photoperiodic requirements for flowering in some plants including *Brassica*. Thereafter, gibberellins have been shown to promote flower initiation in many plants (Lang, 1957; Boss *et al.*, 2004). Therefore, we examined whether GA₃ stimulates flower initiation in the standard type of WFPs. The flower bud formation in the plants treated with $0.01 \mu\text{g}\cdot\text{mL}^{-1}$, $1 \mu\text{g}\cdot\text{mL}^{-1}$ GA₃ (Table 1) or

higher concentrations of GA₃ (data not shown) was faster than in the GA₃-untreated plants. The application of 100 µg·mL⁻¹ GA₃ induced deformity of apical leaves, and so, such a high concentration of GA₃ may cause some negative effects on plant development. As treatment with a GA₃ concentration higher than 0.01 µg·mL⁻¹ resulted in the same stimulation effect on flower initiation, further examinations of GA₃ concentrations lower than 0.01 µg·mL⁻¹ are required.

Advantages of WFPs as an Experimental Material for Flower Initiation

As well as *A. thaliana*, the WFPs are small in plant size and, therefore, many plants can be cultivated in a narrow space. The seeds of WFPs are non-dormant, so the seeds collected from mother plants can be used immediately. Flower initiation in WFPs does not require any vernalization treatment (pre-chilled treatment). Flower initiation starts just after the complete expansion of the cotyledons; the color of flower bud is pale yellow so that one can identify it with the naked eye more easily than in *A. thaliana*, whose flower bud is white. Flower initiation can be detected 1-2 weeks after sowing the seeds, as shown above, and the flower opening occurs 2-3 weeks after the seeds have been sown (See Appendices).

So far, plant materials for teaching flower initiation have mainly been short-day plants, such as the morning glory (*Ipomoea nil*) and *Xanthium occidentale*. By using WFPs, teachers can show flower initiation in a long-day plant to their students easily. In addition, the temperature dependence of photoperiodic responses and the involvement of plant growth regulators in photoperiodic responses can be taught (Our results may be helpful for the teachers). Compared with the other plants commonly used as experimental materials for flower initiation, the flower bud forma-

Table 1: The effect of gibberellic acid (GA₃) on flower initiation in the standard type of WFPs

GA ₃ concentration (µg·mL ⁻¹)	Days* (± SD)
0	11.8 ± 0.05
0.01	8.5 ± 0.09
1	8.5 ± 0.19

15°C, 13000 lux (103µmol·m⁻²·s⁻¹), L:D = 8:16

* Days after sowing the seeds when the first flower bud was detected (n = 20, four replications).

tion in WFPs occurs faster. Thus, inquisitive students can carry out experiments on flower initiation repeatedly.

In addition, both *Ipomoea* and *Xanthium* are “strict” short-day plants, which have an apparent critical night (dark) length for flower initiation. On the other hand, WFPs do not seem to have such a critical night length for flowering. The combination of these plants in teaching flower initiation will lead students to more effective learning of its mechanisms and its relationship to environmental factors.

FURTHER EXPERIMENTS RECOMMENDED

Further research should be carried out to examine the experimental conditions in preparation for more student laboratory experiments to provide students with stimulating activities. These experiments are as follows:

- (1) On the effect of light-break or dark-break on flower initiation;
- (2) On the determination as to whether the WFPs have the critical night length;
- (3) On the effects of light quality (blue, red or far-red light) on flower initiation (Friend 1968b);
- (4) On the determination of photosensitive plant parts by the excision of leaves, grafting, etc.;
- (5) On the effects of chemical substances, such as

sugars (Friend 1984) and plant growth regulators other than gibberellins, on flower initiation.

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WEBSITES

- Wisconsin Fast Plants (WFPs): http://www.fastplants.org/home_flash.html <accessed March 27, 2018>
- Science & Plants for Schools (SAPS): <http://www.saps.org.uk/secondary/teaching-resources>
<http://www.saps.org.uk/secondary/teaching-resources/282-fast-plants-for-finer-science-an-article-from-the-journal-of-biological-education> <accessed March 27, 2018>

APPENDICES

What are WFPs?

WFPs are also known as “Rapid-cycling brassicas (Hafner, 1990)” because of their short life cycle.

They are new varieties of rape (*Brassica rapa*, syn. *campestris*) developed by Professor Williams of the University of Wisconsin, Madison (Williams, 1985; Williams and Hill, 1986). These varieties were at first developed as experimental materials for studying genetics. So far, many strains, such as the dwarf type and the high-anthocyanins-producing type, have been obtained (Rood *et al.*, 1989).

WFPs have been used widely for plant biological research as one of the ideal model organisms (Musgrave, 2000). Furthermore, WFPs have been used as experimental materials from the primary level to the tertiary level in teaching not only genetics, but also morphology, physiology and reproduction (Tomkins and Williams, 1990; Price, 1991; Price and Harding, 1993; Himelblau *et al.*, 2004; WFPs' website; SAPS's website). However, there has been no report on the use of WFPs in students' laboratories on photoperiodism.

As well as *A. thaliana*, *B. rapa* is well known as a long-day plant (Friend and Helson, 1966; Friend, 1968a, 1968b, 1984; King and Kondra, 1986). Its floral development is induced by one long-day treatment (Friend and Helson, 1966). Its growth stages were defined well by Harper and Berkenkamp (1975), and its floral development process was studied well by Orr (1978, 1981). Therefore, we have enough information about the flower initiation of *B. rapa*, and thus, WFPs might be an ideal long-day plant material to study flower initiation. The following characteristics (Williams and Hill, 1986) make them a suitable experimental material for studying flower initiation: (1) Under optimal conditions, their life cycle (from seed germination to the seed maturation of the next generation) completes itself within about five weeks; (2) flower-opening is observed 13 days after sowing the seeds when the plants are grown under continuous illumination of a sufficient light intensity at a temperature of 22°C; (3) Even in a mature plant, its height is shorter than 20 cm, so one can cultivate many plants in a small space or in a growth cabinet.

Quantitative long-day plants and qualitative long-day plants

Long-day plants are those whose flowering is promoted by a long-day condition. Among them, the plants which can flower even under inappropriate photoperiods, though their flowering is delayed, are called "quantitative long-day plants" or "facultative long-day plants." The plants whose flowering is absolutely dependent on a long-day condition are called "qualitative long-day plants" or "obligate long-day plants." See details in the following websites:

Bareja, B. G. (2011) What is photoperiodism, crop types and significance

<https://www.cropsreview.com/photoperiodism.html> <accessed March 27, 2018>

Cox, D. (2009) Photoperiod and bedding plants

<https://ag.umass.edu/greenhouse-floriculture/fact-sheets/photoperiod-bedding-plants> <accessed March 27, 2018>

Wada, K. (2003) Physiology of flowering in *Pharbitis nil*

<https://www.sc.niigata-u.ac.jp/biologyindex/wada/english/index2.html> <accessed March 27, 2018>

At present, in biology textbooks for Japanese senior high school students, only qualitative long-day plants as well as qualitative short-day plants are described, and there is no reference to quantitative long-day plants and quantitative short-day plants. We consider that the difference between "qualitative" and "quantitative" is not all that essential for secondary students to understand photoperiodism.

Light intensity conversion

Few secondary schools have light meters (quantum-meters) which can measure photon flux density (PFD) because the equipment is expensive. On the other hand, schools can have illuminometers which are generally cheaper than any quantum-meter and can be purchased easily anywhere, *e.g.*, at camera shops or local science equipment suppliers. Therefore, in the present paper, we show light intensities in both PFD and illuminance for the convenience of teachers to convert the illuminance to PFD. However, the factor for conversion is not constant; it is dependent on the light source. For example, in the case of the fluorescent lamp we used in the present study, 1 klux equals $9 \mu\text{mol m}^{-2} \text{s}^{-1}$, while with light from a projector lamp (Phillips FP-10S, 100V, 300W), 1 klux equals $18.5 \mu\text{mol m}^{-2} \text{s}^{-1}$.