Bull. Fac. Bioresources, Mie Univ.
No. 12: 237-247
March 31. 1994

Development of Flower Buds in *Pharbitis nil* Choisy as Influenced by Various External Conditions and Growth Substances

Masahiro MAESAKA* and Yukiyoshi OGAWA

Faculty of Bioresources, Mie University

Abstract

The developmental stage of the flower buds of *Pharbitis nil*, cv. Violet was determined, and the effects of external conditions and exogenous gibberellin A_3 (GA_3) or abscisic acid (ABA) following a single flower-inducing dark period on their development were examined with axillary flower buds formed on the third node which were referred to as the first flower buds and with the terminal flower buds on the main stem.

1. The stage of development of the flower buds was represented by floral scores from 0 (vegetative bud) to 10 (petal unfurling).
2. Under continuous white-light of fluorescent lamps the first and terminal flower buds developed rapidly to the carpel initiation and carpel development, respectively. Thereafter, these flower buds hardly developed. The rate and pattern of development of these flower buds were not affected by the different lengths of the flower-inductive dark periods of 12-, 14- and 16-h, all of which caused the initiation of flower buds.
3. Exposure to short days caused both the first and terminal flower buds to develop further, where development of the formers preceded that of the latters. However, exposure to long days consisting of either continuous light or interruption of the dark period by light, inhibited or retarded the subsequent development of both the first and terminal flower buds, where development of the formers was retarded earlier than that of the latters.
4. The higher the light intensity, the more promoted the early development of both the first and terminal flower buds.
5. At 30°C the first flower buds ceased their early development. The terminal flower buds reverted earlier to vegetative buds. At 20°C both the flower buds continued to develop slowly later on.
6. Exogenous GA_3 promoted the early development of the terminal flower buds. However, ABA inhibited the early development of both the first and terminal flower buds.

These findings are discussed in relation to development of the lower axillary and terminal flower buds.

Key words: Development of flower buds · Long days · *Pharbitis* · Short days · Temperatures

I. Introduction

Most research on the processes of flowering has been concentrated on floral initiation. Arrival of the floral

Accepted September 28, 1993

* Present address: Experimental Farm, Faculty of Agriculture, Nagoya University (Aichigun, Aichi Prefecture).

stimulus at the shoot apical meristem from the leaves starts processes leading to the differentiation of floral organs including stamens and carpels. These processes of floral differentiation have been studied far less than the process of floral initiation itself although the formers are of considerable physiological interest and of great importance in floriculture as well as in the production of fruits and seeds of many plant species[18].

Pharbitis nil Choisy is a short-day plant which is induced to flower by a single long dark period, and it has been used extensively for studies of the physiology of flowering[5]. Some detailed anatomical observations have been reported on the photoperiodically controlled vegetative and reproductive shoot apical meristems of *Pharbitis* [1,7,8,9]. The structure and ontogeny of the foliage leaves, bracts, calyx and corolla of this plant have been investigated also in detail[11].

In the present experiments, the developmental stages of the flower buds of *Pharbitis nil* plants were represented by the floral scores from 0 to 10 with the morphological development of various floral organs. Effects of external conditions and GA_3 or ABA applied after exposure to an inductive dark period on the development of flower buds were examined with the lowest axillary and terminal flower buds formed on the main stem.

II. Materials and Methods

The plants were grown under the conditions described previously[13]. Seven seeds of *Pharbitis nil* Choisy, cv. Violet, were planted in a pot of 12 cm in diameter, which was filled with a mixture of equal parts of perlite and vermiculite. The plants were grown in continuous light of white light from fluorescent lamps at 70–80 μmol $m^{-2}s^{-1}$, photosynthetically active radiation (PAR). The seedlings, 4 days after planting, were exposed to an inductive dark period, 16 h long at 28°C unless stated otherwise. Flower buds were always formed at the axil of the third leaf which referred to as the first flower bud and at that of the higher leaves to the terminal as the terminal flower bud. The plants induced to flower were then exposed to the continuous fluorescent light at 28°C unless otherwise stated. In some cases the plants induced to flower were exposed either to short days or to long days utilizing sunlight or fluorescent light. To obtain long days, the dark period was interrupted by lighting for 1 h at midnight. These methods are described in more detail in an appropriate section of the results. In some cases, an aqueous solution of GA_3 containing 0.01% Tween-20 (0.05 and 0.5 μg GA_3 per plumule) or ABA, (+)-2 cis 4-trans ABA, (0.025 and 0.25 μg ABA per plumule) was applied twice with a glasscapillary tube onto the plumule of the plants just after and 24 h after the inductive dark period. As a control, distilled water with Tween-20 was applied. Plants were watered daily and with nutrient solution 3 days a week.

The first flower buds at the third node and the flower buds at the terminal on the main stem were dissected with a needle under a binocular microscope (2×10) on various days from 2 to 20 or 30 days after the flower induction. Figure 1 shows the scheme for scoring their developmental stage. **Stage 0**; vegetative, small bald apex in the third axillary bud (A), and small apex with leaf primordia in terminal bud (T). **Stage 1**; relatively flat apex with equal length of the two bracts in the first axillary flower bud (F), and with unequal length of the two leafy bracts in the terminal flower bud (T). **Stage 2**; apex swollen and covered with bracts. **Stage 3**; five sepal primordia at the basal margin of the apex. **Stage 4**; five stamen primordia as protuberances at the margin of the apex. Initiation and development of the stamen primordia occurs somewhat earlier than those of the petal primordia. **Stage 5**; five petal primordia in position alternating with the sepals between the sepals and the stamens. **Stage 6**; carpel primordium at the apex center as a doughnut shaped protuberance consisting of three

parts. **Stage 7**; coniform carpel primordium of three parts whose top portions have been fused. Anther primordia divides into two or four parts. **Stage 8**; crest- (plume) like stigma at the top of the carpel. **Stage 9**; ball-like stigma at the top of the elongating style. **Stage 10**; petal unfurling.

Both the first and terminal flower buds of 6 plants were dissected at each time, and one bud with the largest variation was omitted from the account and developmental scores of 5 buds were averaged. The mean score of developmental stages ± the standard error of the mean is presented in the figures.

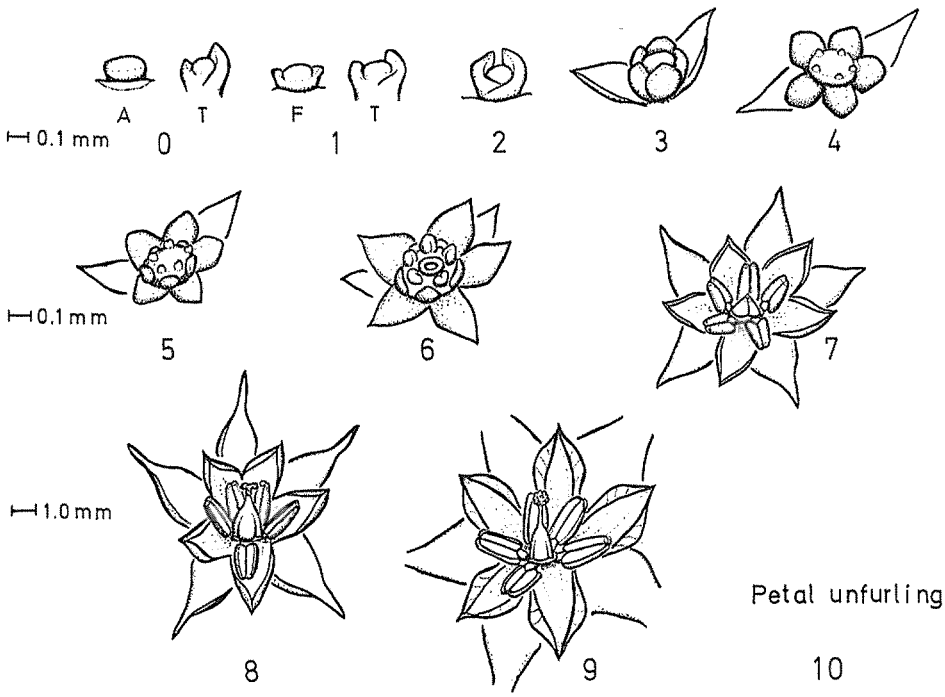

Fig. 1. Illustration of development of flower buds of *Pharbitis nil* and the scheme for scoring their developmental stage. The third axillary bud (A), the first axillary flower bud (F) and the terminal bud (T).

III. Results

1. *Exposure to dark period for flower induction*

Plants were exposed to a 12-, 14- or 16-h dark period, then returned to the continuous fluorescent light. These dark periods resulted in formation of 2.5±0.32, 5.3±0.22 or 6.2±0.25 flower buds per plant. A terminal flower bud was formed on only several plants exposed to a 14-h dark period and on all of the plants exposed to a 16-h dark period. No terminal flower bud was formed on the plants exposed to a 12-h dark period. The first axillary flower buds (Fig. 2-a) and the terminal flower buds (Fig. 2-b) were dissected on various days from the end of the inductive dark period. Rapid development of the first flower buds occurred shortly after flower induction and an initial carpel was detected (floral score; 6) at the 7th day after flower induction regardless of the length of the inductive dark period. The terminal flower buds in both the plants exposed to a 14- and 16-h

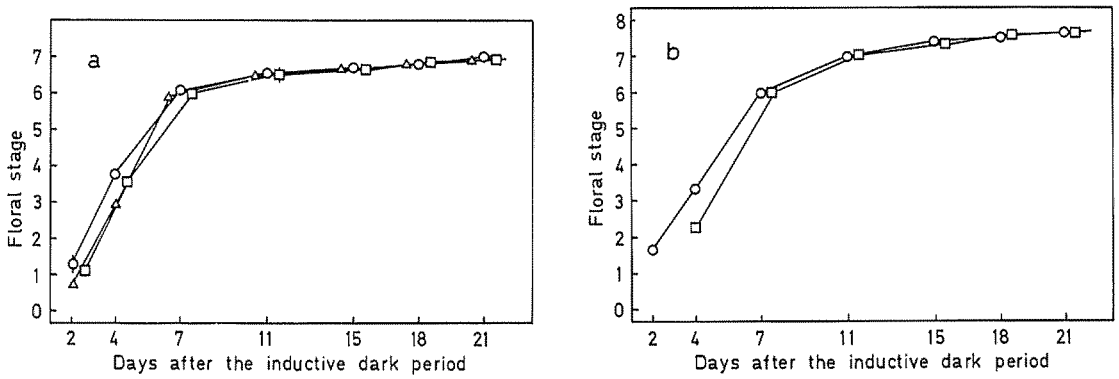

Fig. 2. Development of the first flower bud (a) and the terminal flower bud (b) of *Pharbitis* in continuous light after a flower-inductive 12-(△), 14-(□) or 16-h (○) dark period. Fig. 2-b (□) shows the mean of 2 terminal flower buds dissected each time for plants exposed to a 14-h dark period.

dark periods also developed rapidly shortly after the flower induction to carpel development (score; 7) at the 11th day. After carpel formation the flower buds developed very slowly, and some flower buds showed premature senescence and abscised by the 40th day after flower induction, though a few terminal flower buds developed to a score of 9 or 10.

2. *Effect of short days after the inductive dark period*

After the plants were exposed to an inductive 16-h dark period, one group of plants was grown in sunlight during the day (800 to 1,000 μmol $m^{-2}s^{-1}$ PAR, at midday) at temperatures ranging from 23° to 30°C and at 15° to 25°C at night in September, during which sunrise time was at 5:30–5:40 JST and sunset was at 18:30–18:40 (referred to as natural short days). The other group of plants was grown in the same sunlight during the day, but night darkness was interrupted by exposure to fluorescent light of 40 μmol $m^{-2}s^{-1}$ PAR for 1 h at midnight (referred to as long days). Fig. 3-a shows the development of both the first and terminal flower buds. The first flower buds began to develop by the 2nd day after floral induction and the terminal flower buds began to develop after the 4th day regardless of the daylength. In natural short days both the first and terminal flower buds developed continuously to petal unfurling at the 32th to 36th day as the development of the former buds preceded that of the latters.

In long days plant height increased much more than that in short days (data not shown). The first flower buds had developed to the carpel development at the 10th day and thereafter developed very slowly. The development of terminal flower buds preceded that of the first flower buds after the 22th day, thereafter the formers slowly devetoped to developed to the stigma development.

In another experiment, one group of plants induced to flower was exposed to a 16-h dark period with an 8-h light period of fluorescent light at 40 μmol $m^{-2}s^{-1}$ PAR for 6 days (referred to as experimental short days). The other group of plants was exposed for 6 days to long days consisting of 8-h of 40 μmol $m^{-2}s^{-1}$ fluorescent light and a 16-h dark period interrupted by 1-h of 40 μmol $m^{-2}s^{-1}$ light 8-h after the beginning of the dark period. Then, both groups of plants were transferred to continuous fluorescent light. As shown in Fig. 3-b, in short days both the first and terminal flower buds rapidly developed to the carpel development at the 9th day aftter the first inductive dark period. Then both the flower buds developed very slowly, some of them achieving the petal

unfurling at the 40th day. In long days both the first and terminal flower buds also developed slowly to the carpel initiation and the terminal flower buds developed more than the first flower buds after the 12th day.

The plants induced to flower were exposed either to the experimental short days or to long days consisting of 16-h dark period interrupted with light at midnight (Fig. 3-c). In short days, the first flower buds reached the petal unfurling and the terminal flower buds reached the stigma development at the 30th day.

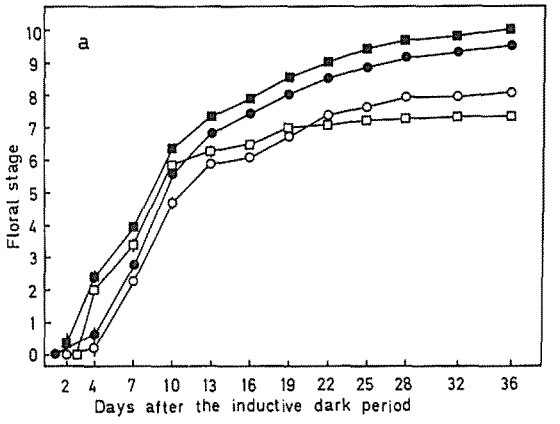

Fig. 3-a. Development of the first flower bud (□, ■) and the terminal flower bud (○, ●) of *Pharbitis* under natural short days (■, ●) or long days (□, ○) after exposure to an inductive 16-h dark period.

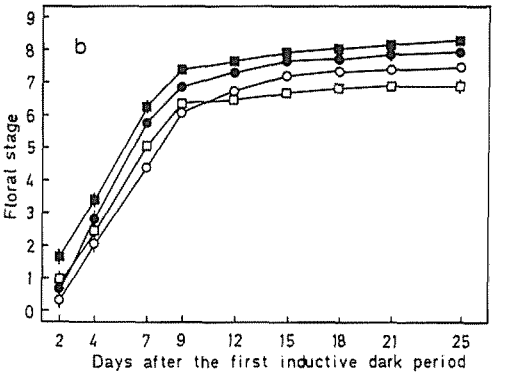

Fig. 3-b. Development of both the first flower bud (□, ■) and the terminal flower bud (○, ●) of flower-induced plants exposed to a 16-h dark period with 8-h of fluorescent light for 6 days (■, ●) or to long days (□, ○), and then exposed to continuous light.

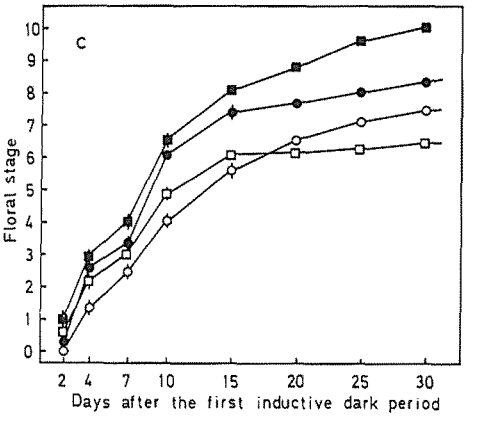

Fig. 3-c. Development of both the first flower bud (□, ■) and the terminal flower bud (○, ●) of flower-induced plants exposed to a 16-h dark period with 8-h fluorescent light for 30 days (■, ●) or to long days (□, ○).

3. *Effect of light intensity*

Plants induced to flower were grown in continuous fluorescent light of 15, 40 and 80 μmol $m^{-2}s^{-1}$ PAR. As shown in Fig. 4-a, on the 9th day the first flower bud in 40 and 80 μmol $m^{-2}s^{-1}$ light developed to the petal primordium formation and carpel initiation, respectively. Then, these flower buds developed very slowly or not at all. The first flower buds in 15 μmol $m^{-2}s^{-1}$ light began to develop latest and developed slowly to the petal-primordium formation at the 15th day, and then continued to develop, but very slowly. As shown in Fig.

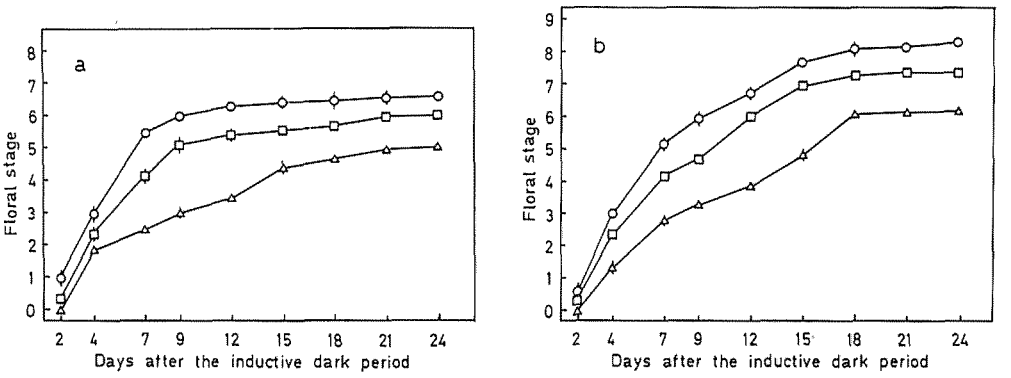

Fig. 4. Development of the first flower bud (a) and the terminal flower bud (b) of *Pharbitis* in continuous light of 15 (△), 40 (□) or 80 μmol $m^{-2}s^{-1}$ (○) after exposure to an inductive 16-h dark period.

4-b, the terminal flower buds in 15, 40 and 80 μmol $m^{-2}s^{-1}$ light also developed to the carpel initiation, carpel development and stigma development at the 18th day, respectively. Then, these flower buds ceased to develop.

4. *Effect of temperature*

Plants induced to flower were grown in continuous fluorescent light of 40 μmol $m^{-2}s^{-1}$ PAR kept at 15°, 20°, 25° or 30°C. The higher the temperature, the greater the plant height increase (data not shown). As shown in Figs. 5-a and b, the optimum temperature for development of both the first and terminal flower buds was between 20° and 25°C. The early development of both the flower buds was retarded slightly at 20°C and severely at 15°C. However, at 20°C both the flower buds continued to develop to a stage beyond that of the buds at 25°C after the carpel development. At 30°C early development of the first flower buds was slightly promoted by the 9th day, but then the flower buds at the carpel initiation ceased to develop further. The high temperature had a storonger inhibitory effect on the terminal flower bud development so that the flower buds was reverted to a vegetative bud at the 12th day.

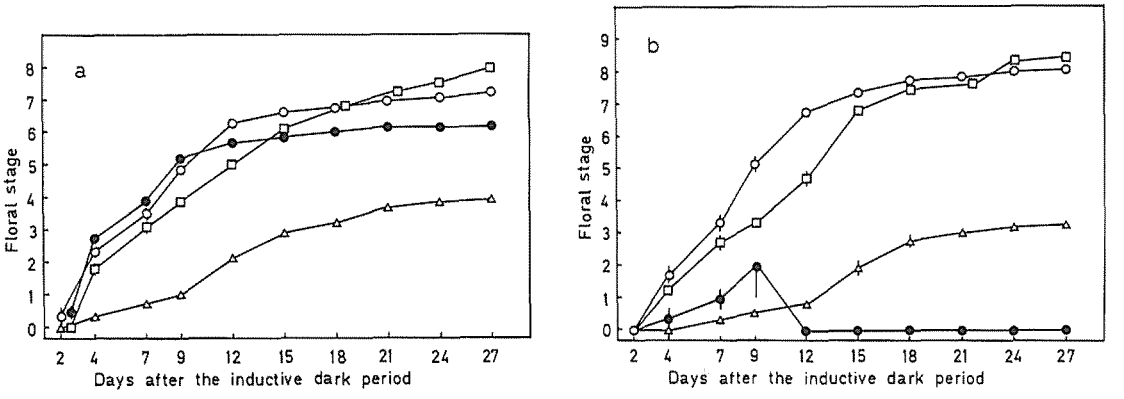

Fig. 5. Development of the first flower bud (a) and the terminal flower bud (b) of *Pharbitis* in continuous light at 15° (△), 20° (□), 25° (○) or 30°C (●) after exposure to an inductive 16-h dark period.

5. *Effects of exogenous GA_3 and ABA*

Figs. 6-a and b show the effect of GA_3 on the development of both the first and terminal flower buds in continuous fluorescent light at 28°C. The treatment with GA_3 had no effect on the development of the first flower buds, but it significantly promoted the early development of the terminal flower buds. The effects of

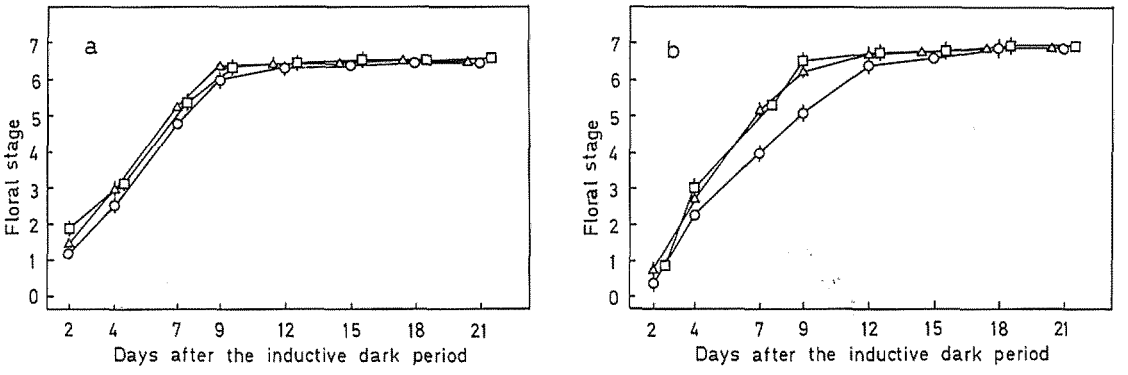

Fig. 6. Development of the first flower bud (a) and the terminal flower bud (b) of *Pharbitis* treated twice with GA_3 at 0.05 μg/plumule (△), 0.5 μg/plumule (□), or distilled water (○) in continuous light after exposure to an inductive 16-h dark period.

ABA on both the flower buds in continuous light and those in experimental short days consisting of 16-h darkness with 9-h light are shown in Figs. 7-a and b, and 8-a and b, respectively. ABA substantially inhibited the early development of the first and terminal flower buds in the long days as the well as short days. ABA had a marked inhibitory action on the terminal flower buds in the long days, such that a few of the plants treated with ABA had no flower bud. However, the promotive effect of GA_3 and the inhibitory effect of ABA were evident only for the early stages and were not for the later stages. These substances would be metabolized gradually to inactive

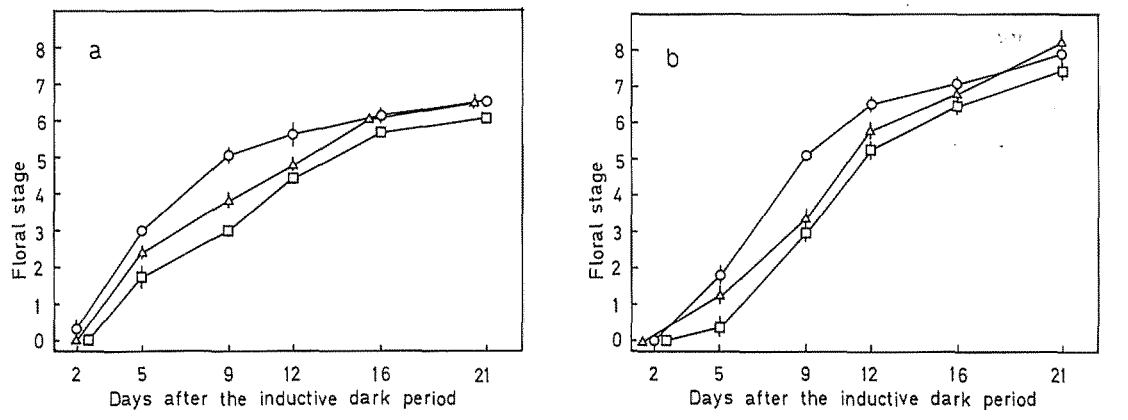

Fig. 7. Development of the first flower bud (a) and the terminal flower bud (b) of *Pharbitis* treated twice with ABA at 0.025 μg/plumule (△), 0.25 μg/plumule (□), or distilled water (○) in continuous light after exposure to an inductive 16-h dark period. Fig. 7-b. (△, □) shows the mean of 3 or 4 terminal flower buds treated with ABA and dissected on the 9th day and later.

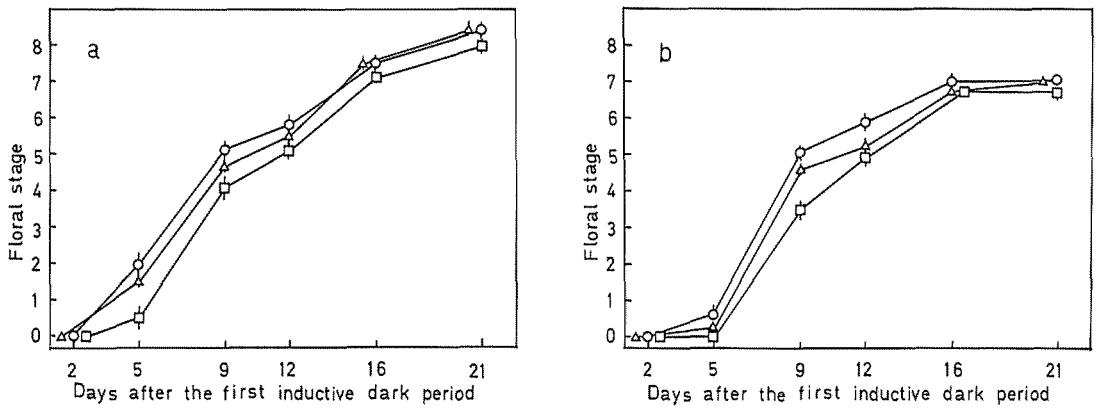

Fig. 8. Development of the first flower bud (a) and the terminal flower bud (b) of *Pharbitis* treated twice with ABA at 0.025 μg/plumule (△), 0.25 μg/plumule (□), or distilled water (○) in continuous short days consisting of 16-h dark period and 8-h fluorescent light period after exposure to an inductive 16-h dark period.

substances within the plant tissue. Indoleacetic acid (0.05 or 0.5 μg IAA per plumule) and zeatin (0.05 or 0.5 μg per plumule) had no effects on the development of both the first and terminal flower buds (data not shown).

IV. Discussion

In the short-day plants, *Xanthium pennsylvanicum, Chrysanthemum morifolium* and *Oryza sativa,* the main or terminal shoot apex is induced to from a flower or an inflorescence. Development of these flower buds is a function of the length of one inductive dark period or the number of cycles of the inductive dark period. Therefore, in *Xanthium*[16], *Chrysanthemum*[15] and *Oryza*[4,17] floral developmental stages at an arbitrary time after the flower induction have been used as a criterion of their floral response to the inductive dark period. On the other hand, in *Pharbitis* plants flower buds are formed usually at the axillary bud sites. The terminal flower buds are formed only in the plants exposed to longer inductive dark period. When the *Pharbitis* plants are kept in continuous light after an inductive 12-, 14- or 16-h dark period, the first or terminal flower buds formed on the main stem developed rapidly to the carpel stages, and then both the flower buds ceased to develop or developed very slowly (Figs. 2, 3, 4, 5, 6) so that some flower buds showed gradual senescence and abscission. The rate and pattern of development of both the flower buds were not dependent on the length of the first floral-inductive dark period (Figs. 2-a and b). Therefore, the stage of floral development in *Pharbitis* can not be used as the criterion of its floral response to the inductive dark period.

Exposure to short days (natural and experimental) after flower induction promoted later development of the first flower buds as well as the terminal ones (Figs. 2 vs. 3). The flower bud development was also advanced with an increase in the number of short-day cycles after flower induction (Figs. 3: b vs. c). These line of evidence are in accordance with Eguchi's early report (1937)[3] that development of flower buds of *Pharbitis* plants is promoted by subsequent exposure to short days, hence this plant is referred to as an S-S plant. Development of inflorescence of other short-day plants such as soybean[2], *Xanthium*[10] *Chrysanthemum*[15] and *Oryza*[4] is also substantially promoted by exposure to short days after flower induction, thus these plants also

could be referred to as S-S plants. This evidence suggests that in these plants the floral stimulus generated in their leaves under short days promotes any developmental processes of the flower buds. In *Pharbitis* plants development of the first flower buds always preceded that of the terminal flower buds when the plants were exposed to continuous short days (Figs. 3-a and c). This shows that the axillary flower buds present at lower nodes on the main stem could develop faster than the flower buds at higher nodes, if supplied continuously with flotral stimulus from the leaves.

In long days consisting of continuous light or the dark period interrupted by light, both the first and terminal flower buds developed very slowly after they developed to the carpel stages. Exposure to long days either after flower induction or during floral development retarded subsequent development of inflorescence of *Xanthium*[10], *Chrysanthemum*[15] and *Oryza*[4]. Ogawa and King[14] showed that *Pharbitis* cotyledons interrupted the dark period by short-term red light generate a photoperiodically sensitive inhibitor. In contrast with short days, exposure to long days usually stopped earlier the development for the first flower buds than for the terminal ones (Figs. 2, 3 and 4: a vs. b). In long days development of the axillary flower buds present at lower nodes might be affected more by a correlative inhibition of the elongating stem. However, the inhibitory effect of long days at 30°C was much more severe on the terminal flower buds than the axillary buds (Fig. 5: a vs. b). At 20°C even in long days both the first and terminal flower buds developed continuously and some of the terminal flower buds reached the petal unfurling stage (Figs. 5-a and b). This evidence indicates that the action of inhibitor (s) arising from leaves in long days has been depressed at 20°C. This is also in accordance with the previous reports by King and Evans[6], and Ogawa[13] that floral evocation occurs optimally at 20°C after the flower induction, and the higher the temperature, then the less the number of flower buds evoked.

The higher the light intensity, the greater the early floral develpment (Figs. 4-a and b), which means that at the early stages floral development partially depends on supply of the photosynthates.

Exogenous GA_3 had a promotive effect on the early development of the terminal flower buds (Fig. 6-b), whereas ABA had an inhibitory effect on the development of the first and terminal flower buds (Figs. 7 and 8: a and b). These lines of evidence may suggest that the promotion of flower bud development by short days is involved with a role of endogenous gibberellins from the leaves and that the inhibition by the long days or the high temperatures such as 30°C is done with a role of endogenous ABA. The lack of effect of exogenous IAA and zeatin on the flower bud development might be an indication that the flower bud once initiated could itself produce these growth substances, or that it could obtain a sufficient supply of them from elsewhere in the plant. The physiology of the development of flower buds should be further studied with isolated flower buds and flower parts grown *in vitro*.

References

1) Bhar, D. S. and N. W. Radforth. Vegetative and reproductive development of shoot apices of *Pharbitis nil* as influenced by photoperiodism. Can. J. Bot. 47: 1403–1406 (1969).
2) Borthwick, H. A. and M. W. Parker. Influence of photoperiods upon the differentiation of meristems and the blossoming of Bioloxi soybeans. Bot. Gaz. 90: 825–839 (1938).
3) Eguchi, T. Effects of daylength upon the time of differentiation of flower bud and subsequent development to flowering. Proc. Imp. Acad. Japan. 13: 332–333 (1937).
4) Ikeda, K. Studies on initiation and subsequent development of floral bud in rice plants. 1. Floral development as influenced by the photoperiodic conditions. Bull. Fac. Agr. Mie Univ. 40: 1–9 (1970).

5) IMAMURA, S. Ed. Physiology of Flowering of *Pharbitis nil*. Japan. Soc. Plant Cell Physiol., Tokyo (1967).
6) KING, R. W. and L. T. EVANS. Timing of evocation and development of flowers in *Pharbitis nil*. Aust. J. Biol. Sci. 22: 559–572. (1969).
7) MARUSHIGE, Y. Ontogeny of the vegetative and reproductive apices in *Pharbitis nil* Chois. 1. Development of the vegetative apex. Bot. Mag. Tokyo, 78: 353–359 (1965).
8) MARUSHIGE, Y. Ontogeny of the vegetative and reproductive apices in *Pharbitis nil* Chois. II. Development of the terminal flower bud. Bot Mag. Tokyo, 78: 397–406 (1965).
9) MARUSHIGE, Y. Ontogeny of the vegetative and reproductive apices in *Pharbitis nil* Chois. III. Development of axillary buds. Bot Mag. Tokyo, 78: 407–411 (1965).
10) NAYLOR, F. L. Effect of length of inductive period on floral development of Xanthium pennsylvanicum. Bot. Gaz. 103: 146–154 (1941).
11) NISHINO, E. Developmental anatomy of foliage leaves, bracts, calyx and corolla in *Pharbitis nil*. Bot. Mag. Tokyo, 89: 191–209 (1976).
12) OGAWA, Y. Effect of temperature and gibberellin A_3 on flowering in *Pharbitis nil* Choisy. J. Japan. Soc. Hort. Sci. 59: 371–376 (1988).
13) OGAWA, Y. Effects of cotyledon area and gibberellin A_3 on photoperiodic flower induction in *Pharbitis nil* seedlings. Bull. Fac. Bioresources, Mie Univ. 10: 195–200 (1993).
14) OGAWA, Y. and R. W. KING. The inhibition of flowering by non-induced cotyledons of *Pharbitis nil*. Plant Cell Physiol. 31: 129–135 (1990).
15) OKADA, M. Studies on flower bud defferentiation and flowering in Chrysanthemums. Mem. Fac. Agr. Tokyo Univ. Educat. 9: 63–202 (1963). (In Japanese with English summary).
16) SALISBURY, F. B. The dual role of auxin in flowering. Plant Physiol. 30: 327–334 (1955).
17) SUGE, H. and A. OSADA. Physiology of flowering in rice plants. 1. Synthesis and translocation of floral stimulus. Proc. Crop. Sci. Soc. Japan. 36: 32–36 (1967).
18) VINCE-PRUE D. Photoperiodic effects on flower differentiation and growth. *In* Photoperiodism in Plants. pp. 293–305. McGraw-Hill Book Company (UK) London (1975).

アサガオの花芽の発達に及ぼす各種の外的条件と生長物質の影響

前坂　昌宏*・小川　幸持
三重大学生物資源学部

アサガオ，品種 Violet の幼植物における，1回の花成誘導暗期によって形成された主茎上の第3節位の腋生花芽（初生花）と頂生の花芽（頂花）の両者の発達に及ぼす花成誘導後の各種の外的要因，および外生ジベレリン A_3 (GA_3) とアブシジン酸 (ABA) の影響を調べた。

1. 花芽の発達を，0（栄養芽）および1（花芽の分化）から10（花弁の展開）までの段階で示した。
2. 昼色蛍光灯の連続照明下で，初生花は心皮の形成段階，頂花は心皮の発達段階までそれぞれ急速に発達した。しかしその後，両者の発達は遅くなるか，あるいは停止した。この花芽の発達の速度と形式は，花成誘導暗期の長さに（12，14，16　時間）影響されなかった。
3. 短日条件下では，初生花及び頂花は花弁の展開まで連続的に発達した。この際，初生花が，頂花より優先的に発達した。これに対して，連続照明及び暗期の光中断による長日条件下では，心皮の形成と発達段階まで急速に発達するが，それ以後の発達は遅くなった。この際，初生花の発達抑制が頂花より早く起った。
4. 照射光の光度が強いほど，初生花及び頂花の初期発達は促進された。
5. 20°C では，初生花及び頂花の発達は緩慢であるが，連続的に進行した。これに対して，30°C では初生花の発達は早期に停止し，頂花は栄養芽に戻った
6. GA_3 は，頂花の初期発達を促進した。ABA は，初生花と頂花の両者の発達を抑制した。

上記の実験結果から，アサガオにおける下位節の腋生花芽と頂生花芽の相互の発達形式について考察した。

*現在，名古屋大学農学部　附属農場（愛知県，愛知郡）