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Flowering Response of Cotyledonary Buds in *Pharbitis nil* Seedlings

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Abstract

Cotyledonary axillary buds (cotyledonary buds/shoots) of *Pharbitis* became visible in 3-day-old seedlings, and grew rapidly until the seedlings were 6 days old. Then, their growth slowed.

Flowering of the cotyledonary buds released from plumular dominance by removing the plumule just before an inductive dark period was compared with that of the plumule intact (epicotyl shoot).

Flowering of the cotyledonary buds was mainly determined by stimulus from the induced cotyledon on the same side. The flowering of each of cotyledonary buds at the base of a pair of induced cotyledons was almost the same. Flowering of cotyledonary buds increased with age of the plant to a maximum at 6 or 7 days of age and then decreased gradually. The flowering of each cotyledonary bud with one cotyledon at this later stage was much greater than that of the plumule with a pair of cotyledons.

When the plumules were removed at different times during a 16-h dark period, flowering of the cotyledonary buds decreased at 8 to 20 h after the start of the dark period.

The data are discussed in relation to action of the floral stimulus from the induced cotyledon at the shoot apex of *Pharbitis* seedlings and the sensitivity of apex to the floral stimulus.

Key words: Cotyledonary bud · Floral stimulus · *Pharbitis* · Plumule · Sensitivity of apex

I. Introduction

The leaves of short-day plants respond to the duration of dark period and generate a floral stimulus. This stimulus then moves to the shoot apex where it causes flowers to form. The activity of this stimulus at the apex is influenced by the physiological state of the apex[3] and environmental conditions, particularly temperature[2,4]. The plumular apex of 3-day-old *Pharbitis* seedlings can flower in response to the floral stimulus from just-expanded and induced cotyledons. The magnitude of this floral response decreases as the cotyledons enlarge with increasing age[5]. Development of the cotyledonary buds of *Pharbitis* seedlings is usually retarded as the plumule developes, but the buds grow out immediately after the plumule is removed. Kujirai and Imamura[1] reported that the cotyledonary buds of *Pharbitis* seedlings flower if they are freed from plumular dominance before the inductive dark period.

In the present experiments, we studied the effect of developmental age of the cotyledonary buds of *Pharbitis* seedlings on their floral response after released from the inhibition of correlation growth, and floral response of the cotyledonary buds was compared with that of the plumule.

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II. Materials and Methods

Growing conditions were the same as described previously[6]. Seedlings of *Pharbitis nil* Choisy, cv. Violet were grown under continuous-white light of fluorescent lamps (80 μmol m^{-2} s^{-1}) at 28°C for various durations after planting (2 to 9 days) unless stated otherwise. Length and leaf number of plumule and cotyledonary buds were measured by observation of the shoot apices and the cotyledonary axils under a binocular microscope. There was usually cotyledonary bud at the base of a pair of cotyledons, two buds being in each plant. But the bud was rarely absent at the base of any one cotyledon. To make the cotyledonary buds flower, the plumule was removed with a small forceps just before or after an inductive dark period, usually 16 h long. After the dark period the plants were grown in the continuous light at 20°C for a week and then at 28°C until they were dissected. Each group was consisted of two or three pots with 14 to 21 seedlings for each. Flowering is shown as the mean number of flowers ± the standard error per one cotyledonary bud (cotyledonary shoot) or one plumule (epicotyl shoot).

III. Results

1. *Development of plumule and cotyledonary buds*

Developmental patterns of the plumule and cotyledonary buds are shown in Fig. 1. The length of the plumule increased gradually for 4 days, and then increased rapidly. One new leaf blade emerged each day. The primordia of the cotyledonary buds were detectable by microscopy on the third day and their length and leaf number increased until the plants were 6 days old, but their growth slowed soon.

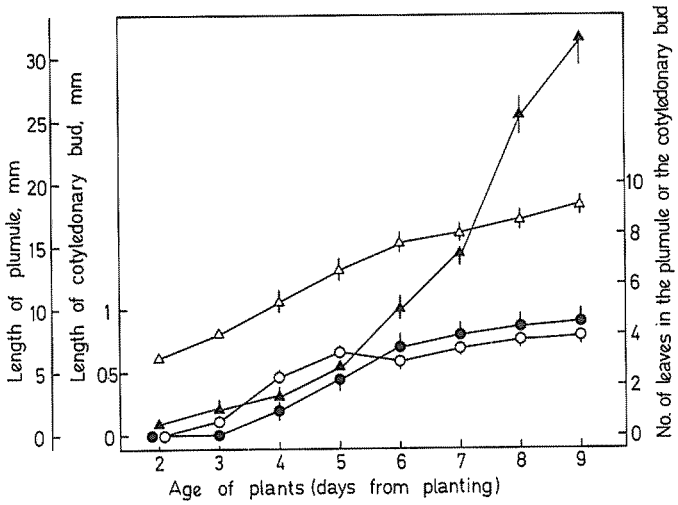


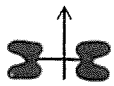
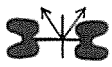
Fig. 1. Length (▲, ●) and number of leaves (△, ○) of the plumule (▲, △) and cotyledonary bud (●, ○) in *Pharbitis* seedlings as a function of plant age.

2. *Flowering of cotyledonary buds*

The two cotyledons of an individual seedling have different petiole lengths. The upper and wrapping cotyledon in the seed has the shorter petiole. However, there was no significant difference in area of the expanded heterocotyledons. In 7-day-old seedlings exposed to a 16-h inductive dark period, flowering of each of the buds at the base of a pair of cotyledons and flowering of the bud at any one cotyledon with the other one

removed were examined. In this experiment the flowering of the plumule with a pair of cotyledons was compared also to that with only a single cotyledon. These results are shown in Table 1. Flowering of each of the cotyledonary buds was similar for plants with two buds and a pair of cotyledons (B, *l* vs. *s*). There was also no difference between the flowering of the cotyledonary buds at a pair of cotyledons and that at any single cotyledon with the other one removed (B vs. D, *l* and F, *s*). Flowering was much less in the cotyledonary buds where the adjacent cotyledon had been removed (D, *s* and F, *l*). Flowering of the plumule with a pair of cotyledons (A) was similar to flowering in degree with only a single cotyledon (C, E), as described previously[6], although this flowering was less than flowering of the cotyledonary buds (shoots) with only a single cotyledon. The cotyledonary shoots with a single or a pair of cotyledons were much shorter than the epicotyl shoot with a single cotyledon, which was in turn shorter than the epicotyl shoot with a pair of cotyledons. However, the flowering of these shoots was not significantly correlated with their shoot length. It is obviously not critical for flowering whether the cotyledonary bud is at the base of a cotyledon with longer petiole or at that with shorter one. In subsequent experiments the plumule was removed just before an inductive dark period so that cotyledonary buds at a pair of cotyledons would flower in response to stimulus from the respective induced cotyledon.

Table 1. Flowering response of plumule (A, C, E) and cotyledonary buds (B, D, F) in *Pharbitis* seedlings at 7 days old exposed to a 16 h dark period. *l*: longer petiole. *s*: shorter petiole

Shoots / Flowers	A	B		C	D		E	F	
									
		l	*s*	*l*	*l*	*s*	*s*	*l*	*s*
No. of flowers[x]	3.8^{a} ±0.2	4.7^{b} ±0.3	4.4^{b} ±0.3	3.7^{a} ±0.2	4.6^{b} ±0.3	2.6^{c} ±0.3	3.6^{a} ±0.2	2.6^{c} ±0.2	4.3^{b} ±0.3
Shoot length cm	46.0 ±3	5.2 ±2	4.2 ±0.5	37.6 ±3	8.2 ±3	5.4 ±3	28.3 ±3	5.5 ±0.5	5.0 ±0.5

x: Means followed by same letter not significantly different at P=0.05 based on one-way analysis of variance.

3. *Ages of the plants*

The plumule of 2- to 9-day-old seedlings was removed just before or after a 16-h inductive dark period and the floral response of cotyledonary buds was compared with that of the plumule. The results are shown in Fig. 2. Cotyledonary buds released before the dark period did not flower until they were 3 days old. Thereafter their flowering increased with the age of plants to a maximum at 6 or 7 days, and then decreased gradually for 2 more days. At all ages the first flower was always formed on the first node of the cotyledonary shoots. However, the plumule of the plants at 2 to 4 days old flowered strongly. Thereafter the flowering decresed, first slowly and then rapidly. Node of the first flower on the epicotyl shoot was progressively higher, up to the seventh node in the older plants. Thus, flowering of the cotyledonary buds at the later stage than 5 days old was much greater than that of the plumule. When the plumule was removed after the dark period flowering of the cotyledonary buds at all ages was much less.

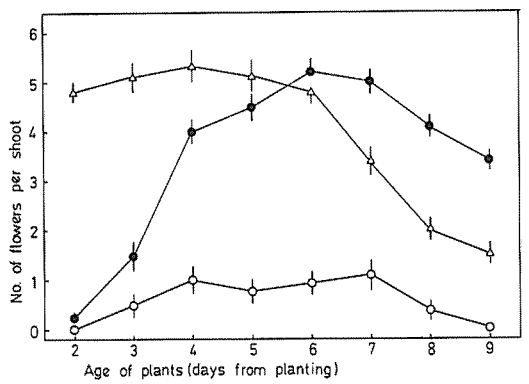


Fig. 2. Flowering response of the plumule (△) and cotyledonary bud (●, ○) of *Pharbitis* seedlings of various ages to a 16 h dark period, before (●) or after (○) which the plumule was removed to examine the cotyledonary bud response.

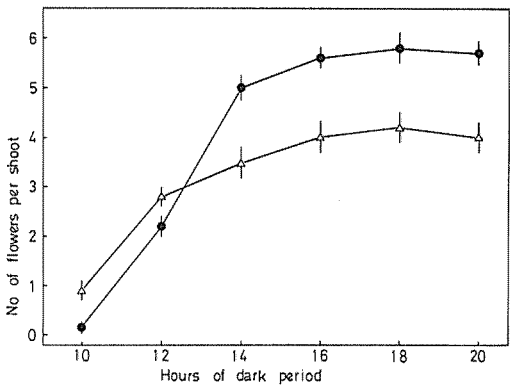


Fig. 3. Flowering response of the plumule (△) and cotyledonary bud (●) removed the plumule in 7 day-old *Pharbitis* seedlings to various durations of an inductive dark period.

4. *Durations of the dark period*

Effect of various durations of the dark period on flowering of the cotyledonary buds released before the dark period in 7-day-old plants was compared with that on the flowering of the plumule. As shown in Fig. 3, flowering of the cotyledonary buds after 10-h and 12-h dark periods was somewhat less than that of the plumule but the reverse was true if the dark period was at least 14 h long.

5. *Times of removal of the plumule*

Effect of removal of the plumule at various times during a 16 h inductive dark period on flowering of the cotyledonary buds was examined with 7-day-old seedlings. The plumules were removed under a safe green light during the dark period. In other plants whose plumules were removed just before the dark period, the induced cotyledons were removed later, both during the dark period and afterward, in order to find out when the floral stimulus moved out of the induced cotyledons. The results are shown in Fig 4. Flowering of the

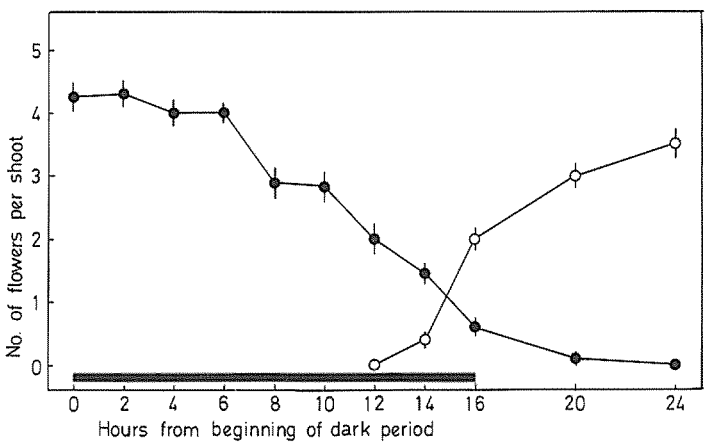


Fig. 4. Flowering response of the cotyledonary bud in 7-day-old *Pharbitis* seedlings exposed to a 16-h dark period, (darkened bar) as function of different times of removal of the plumule (●) and of removal of the induced cotyledons (○) whose plumule was removed just before the dark period.

cotyledonary buds decreased when the plumule removal was carried out at 8 h or later in darkness. Thereafter the flowering decrease continued to 20 h and no flowering was at 24 h. Removal of induced cotyledons was followed by flowering only if it was done after at least 14 h of dark period. The decreasing pattern in the flowering of the cotyledonary buds with time of removal of the plumule was not influenced by prior application of an aqueous solution of gibberellin A_3 or zeatin to the buds even though these substances enhanced flowering level (data not shown).

IV. Discussion

The flowering response of the two buds of a pair of cotyledonary buds was nearly the same and was independent of inherent growth differences in cotyledonary petiole (B, *l* vs. *s* in Table 1). The flowering of cotyledonary bud at a single cotyledon with the opposite cotyledon removed was similar to that of the cotyledonary bud at a pair of cotyledons (B vs. D, *l* and F, *s* in Table 1), but the flowering of the cotyledonary bud on the side with a cotyledon removed was very weak (B vs. D, *s* and F, *l* in Table 1). This suggests that the flowering of the cotyledonary bud is caused mainly by a stimulus from the induced cotyledon on the same side, and also that the conductive function between the bud and the adjacent cotyledon is more efficient than that between the bud and the opposite cotyledon.

Cotyledonary buds of 3-day-old seedlings flowered, and the flowering increased to a maximum at 6 or 7 days of age. By contrast, the plumule with a pair of cotyledons at day 3 or day 4 flowered maximally (Fig. 2), which suggests that 6- or 7-day-old cotyledonary buds can respond as much as 3- or 4-day-old plumules. Thus, in the plants older than 5 days flowering of cotyledonary buds was stronger than that of the plumules even though the formers have a two apex-sink for stimulus import, while the latters have one apex-sink for stimulus from the same cotyledons (Table 1, Figs. 2 and 3). This suggests that the action of the floral stimulus at the apex is qualitative rather than quantitative, and also that establishment of the stimulus at the apex depends on the age of apex. Also, it is obvious that the decrease in flowering of epicotylar shoots with age[5,7] can result not only from ageing of the cotyledon in which the floral stimulus is generated but also from ageing of the shoot apex which recieves the stimulus from the cotyledon.

The results of the removal of induced cotyledons at different times during the dark period show that the floral stimulus is exported from the induced cotyledons to the cotyledonary buds no sooner than 14 h after the start of the dark period (Fig. 4), which is similar to the other reports regarding export of floral stimulus from the cotyledon to the plumule.[6,8,9] However, in these previous cases there may be some reduction in flowering due to physiological shock at the apex caused by surgical removal of the cotyledon, and there also may be a deficiency of some secondary factor for floral evocation at the apex as indicated by Ogawa[6]. The pattern of flowering of the cotyledonary bud when the plumule was removed at different times (Fig. 4) may provide a pattern of stimulus movement arriving at the buds from the induced cotyledon, that is not influenced by the shock at apex as caused by cotyledon removal. However, decreases in flowering when the plumule was removed at 8 to 16 h may indicate competition of the plumule for this floral stimulus, and that the effect of this competition is greater if the plumule is removed later. Another interpretation of the data is that an active bud is needed for the cotyledonary buds to respond to the floral stimulus and if this activation occurs too late then the stimulus loses strength.

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アサガオの子葉えき芽の花成反応

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アサガオ，品種 Violet の子葉えき芽は播種後３日で認められ，その後６日まで急速に生長した。その後，緩慢に生長した。

幼芽（上胚軸）を切除して，子葉えき芽に対する幼芽優勢を除いた後，花成誘導暗期を与えた。子葉からの花成刺激による子葉えき芽の花成反応を調べ，幼芽の花成反応と比較した。

２枚の子葉の各えき芽の花成は，おもに同じ側にある子葉の花成刺激によって生じた。５日以後の草令の子葉えき芽の花成は（えき芽/１枚子葉），両側の子葉による幼芽の花成（幼芽/２枚子葉）より大であった。この両者における花成反応の違いは，花成刺激に反応する芽頂の令の相違による。このことは，草令の進行に伴う幼芽の花成の減少が（小川，1992）[5]，子葉の生理的変化のみならず，幼芽自体の変化も関係していることを示唆している。

幼芽の切除が，16時間の誘導暗期開始後８時間から20時間へと遅れると，子葉えき芽の花成は著しく減少した。この花成の減少は，花成刺激に反応するえき芽の活性がまだ弱い状態にあること，さらに幼芽切除が遅れると子葉えき芽の花成刺激の受容に幼芽との競争があることによると思われる。