

Movement of Floral Stimulus in the Cotyledon and Petiole of *Pharbitis nil* Seedlings

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Abstract

Flowering of *Pharbitis* seedlings was not prevented by the treatment that either the major veins or the lamina of the cotyledon was cut just before or after giving an inductive dark period. This suggests that the floral stimulus generated in the cotyledons is capable of moving through the mesophyll cells only, or through the vascular bundles only, and its route can change quickly if the occasion demands.

The flowering response of plants with a basal half cotyledon remained by 1.6 cm in length reached the maximum 4 h after the end of a 14-h inductive dark period, but that of the plants with the whole cotyledons removed did the maximum 6 h after the dark period. No difference in flowering was found in plants whose petiole of 1.2 cm length was cut at the top and at the base. This suggests that the floral stimulus moves at a rate of 0.8 cm/h or a little faster in the cotyledon, and that it moves quickly in the petiole. The movement of floral stimulus in the cotyledon did not occur at 10°C, but did readily at 15°C.

Plants with the petiole incised from both sides flowered as much as those with intact petiole, which suggests that the floral stimulus could move through a small number of cells remained intact at the incised portion of the petiole.

Key words: Cotyledon · Movement of floral stimulus · Petiole · *Pharbitis*

I. Introduction

It is well known that the leaves of many photoperiodic plants produce flowering hormones or floral stimuli in response to the length of day and night. These substances move to the shoot apex where they stimulate flowering.^{7,13)} Efforts to isolate these hormones were made by applying extracts from flowering to non-flowering plants, but they remained to be a limited success.^{3,6,11)} The chemical structure of these agents and their physiological function in plant tissue are entirely obscure.

Flowering of *Pharbitis* seedlings could be brought about by a single induced cotyledon,^{8,10,14)} and the floral stimulus could arrive at the plumule 16 to 20 h after the start of the dark period.^{5,9,15,16)}

Using *Pharbitis* seedlings with a single cotyledon, we studied their floral response as affected by cutting the veins or mesophyll tissues of the cotyledon and the different parts of the induced cotyledon at various times after floral induction. Effect of incising the petiole was also examined. Information on the pattern of the floral stimulus movement within the cotyledon and within the petiole might contribute to know about the nature of the floral stimulus itself.

II. Materials and Methods

Pharbitis nil seedlings, cv. Violet, were grown for 4 days after planting under the same conditions as reported previously⁸⁾. The one cotyledon with the longer petiole was used for the experiments, and the other one was detached. With a small razor blade, the cutting was made at different sites of the prominent veins of the cotyledon or the parts of lamina remaining the veins before or after various lengths of an inductive dark period. In some experiments the cotyledon was partly cut off to leave the one half or one fourth of the cotyledon of basal part, or the whole cotyledon was detached at various times after a 14 h inductive dark period. In other experiments the cotyledonary petiole was incised with a razor blade from both side to about half of its width just before or after the inductive dark period, but this treatment did not cause wilting of the cotyledon. After the dark period the plants were grown at 20°C for a week, and then kept at 28°C for two weeks until flowering was assessed by dissection. Two or three pots with 14 to 21 plants were used for each experimental lot. The data are shown as means of number of flowers per plant \pm the standard error of the mean.

III. Results

1. Effects of cutting cotyledon veins and mesophyll

Two principal lateral veins diverge from midrib at the lower and the upper point of the cotyledon, respectively, those being by about 5 mm apart. Immediately before an inductive dark period the veins were cut at various sites of the cotyledon, as shown in Fig. 1-a. The midrib was cut at the side of diverging point of upper-lateral veins or the other side of diverging point of lower-lateral veins. In addition to cutting the midrib at each point, the lower lateral veins including the basal margin of cotyledon were cut. After cutting the midrib and the veins at the lower point, the cotyledon surface was lined with cellophane tape between both ends of the

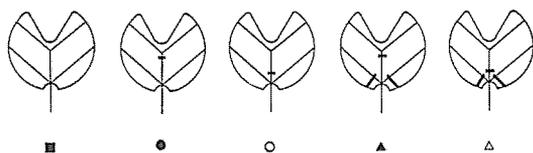


Fig. 1-a. Schematic presentation on the arrangement of prominent veins and the position of cutting (bold line) in *Pharbitis* cotyledon. Intact cotyledon (■), midrib cut just below the upper divergence of lateral veins (●), midrib cut just above the lower divergence (○), midrib cut in the upper portion and lower-lateral veins cut (▲), midrib cut in the lower portion and lower-lateral veins cut (△).

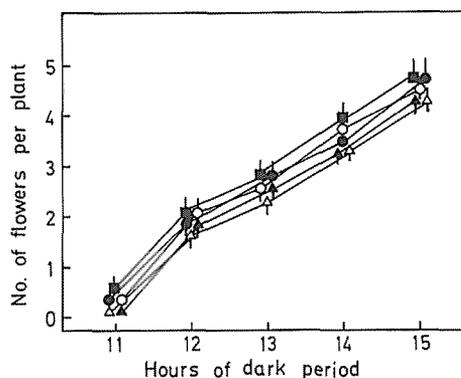


Fig. 1-b. Flowering response of *Pharbitis* with the cotyledon cut its veins to various durations of the dark period. Intact cotyledon (■), midrib cut just below the upper divergence (●), midrib cut just above the lower divergence (○), midrib cut in the upper portion and lower-lateral veins cut (▲), midrib cut in the lower portion and lower-lateral veins cut (△).

crosswise cuts to prevent the cotyledon lamina from hanging down. As shown in Fig. 1-b, no distinct difference of flower response was found between the plants with cotyledon disconnected veins and those with cotyledon of intact veins, although slightly less flowering was found in the plants whose midrib and lateral veins were cut at the lower point.

In a further experiment the cotyledon lamina was cut along an imaginary line to one half or one fourth in cotyledon area, taking care not to cut the midrib and the lateral veins. The control cotyledon was cut off along the same line (Fig. 1-c). As seen in Fig. 1-d, cutting the lamina did not affect flowering. Flowering response of the plants, whose cotyledon veins or lamina were cut immediately after 12 to 16 h inductive dark period, was also nearly the same level as that of control plants (data not shown).

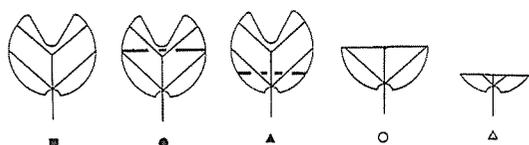


Fig. 1-c. Schematic presentation on sites where the lamina was cut at the upper (●) or lower line (▲) (bold line) with remaining the veins of *Pharbitis* cotyledon. Intact cotyledon (■) was cut off along the upper (○) or lower line (△).

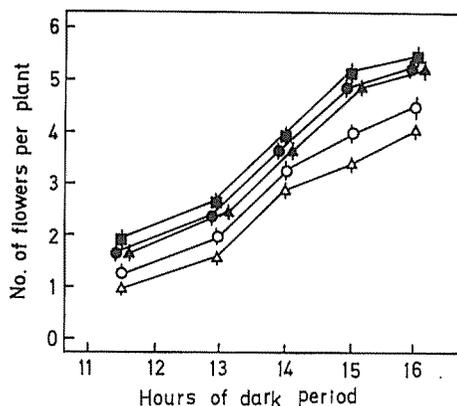


Fig. 1-d. Flowering response of *Pharbitis* cotyledon whose lamina was cut at the upper (●) or lower line (▲) with remaining the veins to various durations of the dark period. Intact cotyledon (■) was cut off along the upper (○) or lower line (△).

2. Effect of partial removal of the induced cotyledon

Various portions of the induced cotyledon ($8.9 \pm 0.25 \text{ cm}^2$) were cut off at various times after a 14 h dark period. Areas of cotyledon remained were $4.9 \pm 0.15 \text{ cm}^2$ (one half) and $2.4 \pm 0.1 \text{ cm}^2$ (one fourth), each being 1.6 and 0.8 cm in length. Whole cotyledons of the other plants were detached by cutting either at the top or the base of the petiole whose length was $1.2 \pm 0.05 \text{ cm}$ (Fig. 2-a). As seen in Fig. 2-b, removal of one half or three quarters of an intact cotyledon area at the end of the dark period resulted in less flower production comparing with plants with an intact cotyledon. The partial removal of the cotyledon also resulted in less flower production comparing with plants whose cotyledons were removed to the same extent just before the dark period. Defoliation, distal or proximal cutting of the petiole, at the end of the dark period resulted in no flowering, which indicates that the floral stimulus had not left the cotyledon by this time. Plants remaining one half or one fourth of a cotyledon had more flowers if cutting time was delayed. Flowering response reached a maximum when the time of cutting was delayed to 18 h from the onset of darkness, and then it leveled off. The number of flowers increased rapidly with delaying of time of defoliation, reaching a maximum at 20 h, and the flowering level was slightly lower comparing with that of plants with cotyledon intact. Less number of flowers produced by the

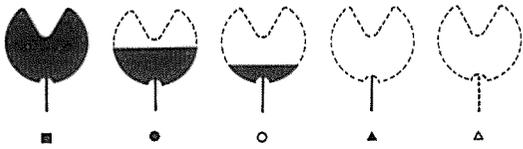


Fig. 2-a. Schematic presentation on sites where a cotyledon of *Pharbitis* was cut off. Broken lines indicate the areas cut off. Intact cotyledon (■) was cut to one-half cotyledon (●), to one-fourth cotyledon (○), at the top of petiole (▲) or at the base (△).

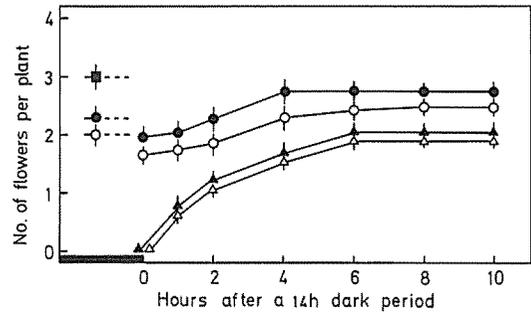
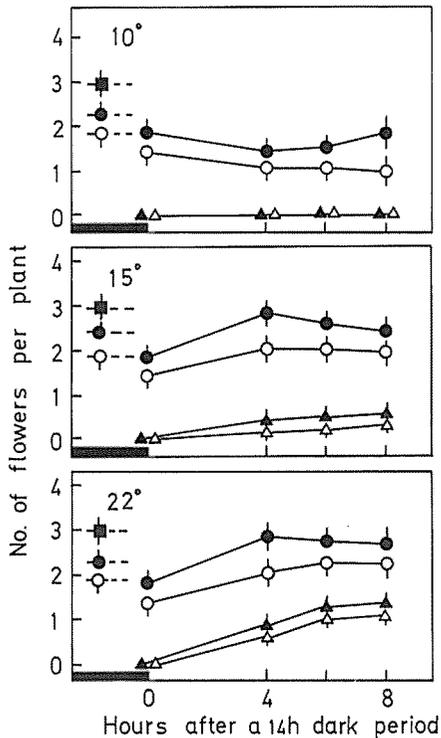


Fig. 2-b. Flowering response of *Pharbitis* with the cotyledon cut off at various times after 14-h inductive dark period (darkened bar). Intact cotyledon (---■---), one-half cotyledon cut before (---●---) or after the dark period (—●—), one-fourth cotyledon cut before (---○---) or after the dark period (—○—), whole cotyledon removed by cutting at the top of petiole (—▲—) or at the base (—△—).

Fig. 2-c. Flowering response of *Pharbitis* with the cotyledon cut off after various times at different temperatures (10°, 15° or 22°C) after 14-h inductive dark period (darkened bar). Intact cotyledon (---■---), one-half cotyledon cut before (---●---) or after dark period (—●—), one-fourth cotyledon cut before (---○---) or after the dark period (—○—), whole cotyledon removed by cutting at the top of petiole (—▲—) or at the base (—△—).

removal of the cotyledon might be ascribable to a shortage of the floral stimulus to form the terminal flower, or to a lack of any other necessary factors for flower production as suggested by King et al.⁴⁾ and Ogawa⁸⁾. Flowering of plants whose cotyledon was removed at the top and at the base of the petiole was quite the same.

Floral response as affected by cutting off a part of cotyledon at various times after 14 h dark period at different temperatures (10°, 15° or 22°C) was examined (Fig. 2-c). At 10°C, no increase in flowering response was observed in plants with cotyledon cut partially, and no flowering did occur in plants with cotyledon detached even if the time of cutting or detaching was delayed. At 15°C, plants whose cotyledon was partially cut flowered to the same degree as those at 22°C, but with complete cotyledon removal there were far fewer flowers.

3. Effect of incision of cotyledonary petiole

In one group of plants, the cotyledonary petiole was incised to about half of its width but the cotyledon did not wilt. A droplet of sap exuded from the incision. In some experiments, a 2 mm² mica sheet was inserted into the incision. Observation through a microscope revealed that a large part of the vascular bundles had been cut by the incision. In another group, the petiole was folded down with a nipper, keeping in place until dissection for flowering. These plants were exposed to various durations of the inductive dark period. These treatments had no effect on flowering (Fig. 3). Similar manipulation just after the dark period also did not affect the flowering (data not shown).

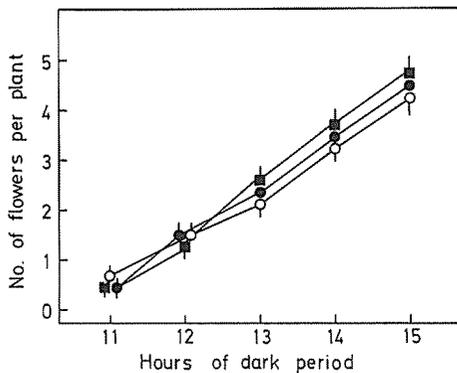


Fig. 3. Flowering response of *Pharbitis* with cotyledonary petiole incised from both sides (○) or folded down with a nipper (●), before the dark period of various durations. Control plants had intact cotyledonary petioles (■).

IV. Discussion

Chailakhyan¹⁾ concluded that the floral stimulus moves through mesophyll tissues of the leaf blade in *Perilla*, because normal flowering occurred even after the basal veins were cut. Similarly, for *Pharbitis* cotyledons, cutting either the veins or lamina mesophyll allowed to flower as much as in plants with an intact cotyledon (Figs. 1-b and 1-d). If the cuts were made just after the dark period at which time the floral stimulus was still in the cotyledon, flowering was normal. This indicates that floral stimuli in the cotyledons can move either through mesophyll cells only or through vascular bundles only, and that their pathway can change quickly as the occasion demands.

In the plants whose cotyledons were cut off to a half of the cotyledon, the time required for maximal flowering was 18 h, while in those defoliated completely it was 20 h (Fig. 2-b). Floral stimulus from the apical half of the cotyledon had moved a distance of 1.6 cm to the basal half within 18 h (within 4 h after a 14 h dark period). Then, the stimulus moved out of the basal half of the cotyledon within the next 2 h. Thus, the speed at which the floral stimulus moved in the cotyledon could be estimated as about 0.8 cm/h, or more. The speed of the stimulus movement in the *Pharbitis* cotyledon is similar to that in the *Lolium* leaf blade (1–2 cm/h), as estimated by Evans and Wardlaw²⁾. Because the flowering response of the *Pharbitis* cotyledon cut at the top of petiole did not differ from those cut at the base (Fig. 2-b), the speed of the stimulus movement in the petiole must be as quick as that in the stem, which was estimated by Takeba and Takimoto¹²⁾, and by King et al.⁴⁾ to be from 35 to 60 cm/h. This would explain the reason why enough of the floral stimulus generated in an intact cotyledon could arrive at the plumule within 4 or 6 h after the end of a 14 h inductive dark period.

Flowering of plants with cotyledon cut to one half or one fourth at various times after 14 h dark period was examined using plants with the cotyledon whose midrib and lateral veins were cut at the lower point, and compared with that of plants with cotyledon intact. No difference in pattern of flowering was found between these two kinds of plants (data not shown). This suggests that cutting the veins does not influence on the speed of the movement of floral stimulus in the cotyledon.

Zeevaart¹⁷⁾ reported that export of the floral stimulus out of the cotyledon was retarded when the plants were placed at lower temperature after floral induction. At 10°C, no increase in flowering in plants with partially cut cotyledon and no flowering in defoliated plants occurred even when the cutting time was delayed (Fig. 2-c, 10°). This suggests that the floral stimulus is stationary within the cotyledon at the low temperature. However, at 15°C flowering of the plants with partially cut cotyledon occurred nearly to the same degree as that of plants placed at 22°C but it did very slightly after defoliation (Fig. 2-c, 15° vs. 22°). This suggests that the movement of the floral stimulus from the cotyledon into the petiole is more sensitive to temperature than that within the cotyledon.

Microscopic observation on the incised petiole revealed that a considerable portion of the cortex and vascular bundles including phloem had been disconnected. However, the flowering was not affected by the incision at all (Fig. 3). This indicates that the floral stimulus passed well through cells remaining around the incised part of the petiole, and the stimulus could move smoothly through the folded portion of petiole (Fig. 3). Further studies should concentrate on the mechanisms of cell-to-cell movement of floral stimulus in the cotyledon and rapid transport in the petiole.

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アサガオの花成刺激の子葉ならびに葉柄内の移動

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アサガオ、品種 Violet の幼植物の子葉を1枚にした後、花成誘導暗期の直前に、葉脈を切断するか、あるいは葉脈以外の葉身を切断して、花成に対する影響を調べた。いずれの切断も花成への影響は殆どなかった。また、暗期の直後に行なったいずれの切断も、花成に影響しなかった。これらの結果は、子葉内に生じた花成刺激は、それぞれの切断によって葉肉組織を、あるいは維管束組織を移動することを示している。また、これらの移動組織間では、短時間に変更が可能であることを示唆している。

14時間の誘導暗期後、子葉の基部1.6 cmの長さを残して切断した場合の花成は、暗期後4時間で最高になったが、全子葉を切断した場合の花成は、6時間で最高になった。また、1.2 cm長さの子葉柄の先端と基部での子葉切断は花成反応に殆ど差がなかった。この実験結果は、花成刺激は、子葉内を0.8 cm/時間、あるいはそれより少し速い速度で移動し、葉柄内は非常に速い速度で移動することを示唆している。10°Cでは、花成刺激は、子葉内を殆ど移動しなかった。15°Cでは、子葉内を正常に移動するが、葉柄内への移入が抑制された。

葉柄を両側から切り込みをつけても、花成に殆ど影響しなかった。これは花成刺激が、切り込み面に僅かに残っている無傷の組織を容易に移動することを示唆している。