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Effects of Cotyledon Area and Gibberellin A_3 on Photoperiodic Flower Induction in *Pharbitis nil* Seedlings

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Abstract

The relationship between the cotyledon area reduced before or after an inductive dark period and its photoperiodic response was examined with *Pharbitis nil* seedlings.

Plants with 0.25- to 1-cm^2 cotyledon area flowered, showing almost the same critical length of dark period, 11 or 12 h, as is the case with a single intact cotyledon (about 8.5 cm^2), but the floral response of plants with reduced cotyledon area did not increase with dark periods longer than 14 h, but it was far greater per unit area than that with the intact cotyledons. Application of gibberellin A_3 (GA_3) to the plumule enhanced the floral response but the promotion of GA_3 in plants with cotyledon area smaller than 1 cm^2 leveled off with the longer dark periods.

Plants with 0.12-cm^2 or 0.06-cm^2 cotyledons, if their plumules were given GA_3, flowered in response to one or two cycles of 15-h dark period. When plants with larger cotyledons than 0.12 cm^2 were exposed to several number of inductive dark period, number of flowers increased.

The floral response of plants whose cotyledons were reduced to 1-cm^2 area before 12- to 14-h dark periods took place by about 2 h earlier than that of plants whose cotyledons were reduced to 1-cm^2 after the dark period.

These data reveal some aspects of the generation of the floral stimulus in the cotyledon, especially in relation to its area.

Key words: Cotyledon area · GA_3 · Photoperiodic flowering · *Pharbitis*

I. Introduction

Flowering of photoperiodically sensitive plants depends on the day or night length to which leaves are exposed, whether flowering site is subjected to inductive conditions or not. This was first shown by Knott with spinach[8] and then by Chailakhyan with *Chrysanthemum morifolium*[2]. Since then, similar phenomena were found in many other short-day plants such as *Xanthium*[3], dill[4] and *Glycine*[1]. In *Xanthium*, a young developing leaf of 1 to 2 cm^2 in area could not induce to flower but a maximum response was obtained with the half expanded leaf[6]. However, only 2 cm^2 of a mature leaf was effective to cause flowering[3]. In *Pharbitis*[5] and *Perilla*[15,17] 1 cm^2 area cut out from a single foliage leaf could induce to flower when subjected to several dark periods. So far as we know, however, no experimental evidence has been shown as to the flowering response with smaller leaf area than 1 cm^2 in those short-day plants.

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On the other hand, *Pharbitis* seedlings with fully expanded cotyledons responded strongly to a single dark period[13], resulting in flowering. The application of gibberellin A_3 (GA_3) to the shoot apex before giving the inductive dark period enhanced flowering[11]. The high levels of endogenous bioassayable gibberellins (GAs) in expanding cotyledons of *Pharbitis* seedlings[10] suggest their involvement in photoperiodic flowering of this plants.

In the present experiments, we studied the flowering response of *Pharbitis nil* seedlings with a cotyledon reduced to smaller area than 1 cm^2 to different inductive dark periods and to the exogenous GA_3 applied to the plumule.

II. Materials and Methods

Seeds of *Pharbitis nil* Choisy, cv. Violet, were treated with concentrated H_2SO_4 for 40 min and then washed thoroughly in running water for 20 h. They were then allowed to sprout for 20 h on moist sand in a petri dish at 25°C. Germinating seeds were planted in a clay pot filled with mixture of perlite and vermiculite (1 : 1 v/v). The seedlings were grown under continuous light of day-light fluorescent lamps (80 μmol m^{-2} sec^{-1} PAR) at 28 ±1°C. Four days later, only a single cotyledon (about 8.5 cm^2) with the longer petiole was retained, the other one being removed. It was cut with a leaf punch so as to reduce to various areas ranging from 0.06 to 1 cm^2 in the form of either square or rectangle at the basal portion of the cotyledon. They were exposed to an inductive dark period of 11 to 16 h at 28°C in most experiments. No detectable increase in the area of the cotyledon was found during the dark period. After the dark period the seedlings were grown under continuous fluorescent light at 20°C for a week to allow maximum floral evocation at the apex,[7,12] and then transferred to 28°C until dissection for counting the number of flowers. The plants were applied with tap water daily and with nutrient solution three times a week. An aqueous solution of GA_3 with 0.05% (v/v) Tween-80 was applied to the plumule (0.25 μg GA_3/plumule) through a glasscapillary tube immediately before the inductive dark period. Control plants were applied distilled water containing Tween-80. Each group was consisted of two or three pots with 14 to 21 plants in each, and flowering response is shown as the mean number of flowers per plant ± the standard error.

III. Results

1. *Cotyledon-area manipulation before the dark period*

In a preliminary experiment, the flowering response of plants with either a single or a pair of cotyledons each of which was reduced to 1 cm^2 was compared with that of plants with a single or a pair of intact cotyledons. As seen in Fig. 1, the number of intact cotyledons had little effect on the number of flowers for any given length of dark period. A single intact cotyledon was fully effective to induce flowering of axillary buds from the third node to the terminal bud of the main shoot after exposure to 14 to 20 h dark periods. Similar trend in flowering response of a pair of cotyledons was found. The reduction of cotyledon area to 1 or 2 cm^2 caused to a considerable decrease in flowering. Dark periods longer than 14 h did not increase the number of flowers per plant.

In a further experiment, only a single cotyledon with a longer petiole was used and the other one was removed. The remaining single cotyledon was reduced to various small areas and the plumule was treated with

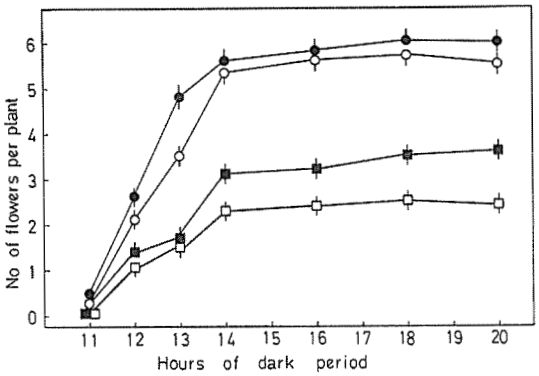

Fig. 1. Flowering of *Pharbitis* with a single intact cotyledon (8.5 cm^2) (○) or a pair of the cotyledons (●), and that with a single (□) or a pair (■) of cotyledons reduced to 1 cm^2 each, when exposed to different lengths of an inductive dark period.

either GA_3 or distilled water. Then the seedlings were exposed to various durations of dark period. As shown in Fig. 2, the plants with the small cotyledon area flowered after 11 or 12 h of dark period, being a critical length of the dark period. However, with the dark period longer than 13 h the plants with smaller cotyledon areas produced a less number of flowers, and further lengthening the dark periods could not increase the number of flowers. GA_3 application to plumules of the plants with 1 cm^2 cotyledons resulted in as much flowering as the control plants with 8.5 cm^2 intact cotyledons and in both plants the number of flowers was dependent on the length of the dark period. However, the promotion of flowering by GA_3 in plants with cotyledon areas reduced to less than 0.5 cm^2 leveled off with lengthening the dark periods.

A single cotyledon was reduced to an area as small as 0.06 cm^2, and the plumule was treated with either GA_3 or distilled water. These plants were then exposed to various cycles of 15-h dark period and 9-h light period. The results are shown in Fig. 3. In general, the plants exposed to more inductive cycles produced more number of flowers, and GA_3 application promoted flower production. When plants with 0.12-cm^2 cotyledon area were treated with GA_3, they flowerd in response to only one dark period and those with 0.06-cm^2 flowered after two inductive cycles, if treated with GA_3. However, without GA_3 application the latters did not

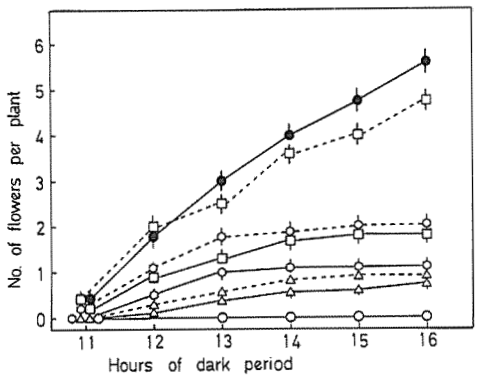

Fig. 2. Flowering of *Pharbitis* with a single intact cotyledon (●) or with reduced cotyledon area of 1- (□), 0.5- (○), 0.25- (△), or 0- (○) cm^2, when exposed to different lengths of an inductive dark period. Plumule was either treated with (·····) or without GA_3 (——).

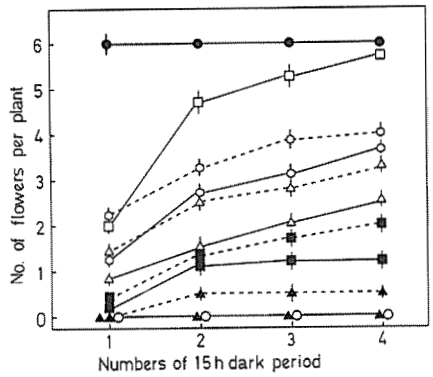

Fig. 3. Flowering of *Pharbitis* with a single intact cotyledon (●) or with reduced cotyledon area of 1- (□), 0.5- (○), 0.25- (△), 0.12- (■), 0.06- (▲) or 0- (○) cm^2, when exposed to different numbers of 15-h inductive dark periods. Plumule was either treated with (·····) or without GA_3 (——).

flower at all even if four inductive cycles were given.

2. *Cotyledon-area manipulation after the dark period*

In one group of seedlings their cotyledons were reduced to 1 cm^2 immediately before dark periods of 11 to 16 h. In another group the cotyledons were either reduced to 1 cm^2 or removed immediately after the dark periods or 4 h after 16-h dark period. The plumules of one group of plants were treated with GA_3 before the dark periods. The flowering responses of these plants are shown in Fig. 4. Whether or not GA_3 was applied, over 12 to 14 h dark periods the flowering response of plants with 1 cm^2 cotyledon reduced before the dark period occurred with about 2-h shorter dark periods than those reduced after the dark periods. In addition, the plants without GA_3 flowered when their intact cotyledon was removed after 14 h dark period, while those treated with GA_3 flowered after 13 h dark period.

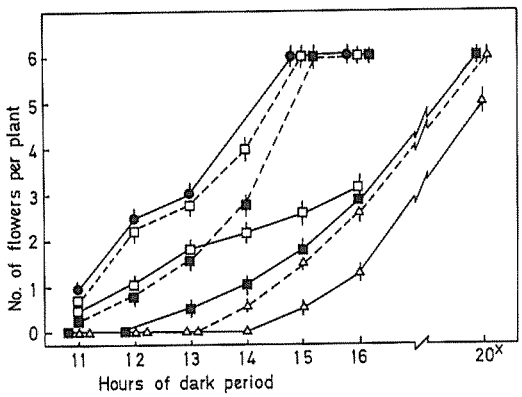

Fig. 4. Flowering of *Pharbitis* with intact (●) or with reduced cotyledon area of 1-cm^2 (□), when exposed to different lengths of an inductive dark period (11 to 16 h). At the end of the dark periods or 4 h after a 16-h dark period (20×), the intact cotyledon was reduced to 1 cm^2 (■) or removed (△). Plumule was either treated with (·····) or without GA_3 (——).

IV. Discussion

Over a wide range of dark periods, the number of intact cotyledon had a little or no relation to the production of flowers in *Pharbitis* seedlings (Fig. 1). Similar results have been shown also in other reports.[14,16] These show that floral stimulus from a single intact cotyledon exposed to 14- to 16-h dark period is sufficient to cause flowering in all axillary buds and the terminal bud on the main shoot of *Pharbitis* seedlings. Kujirai and Imamura[9] reported that flowering in *Pharbitis* seedlings with a single intact cotyledon was much less than in those with a pair of the cotyledons. However, we cannot compare the present results with their data, because under their experimental conditions plant growth was very slow; the node bearing the first flower bud on the main shoot did not so greatly advance with age as it did in the present experimental condition.

Flowering of plants with a single or a pair of cotyledons whose area was reduced to 1-cm^2 each was much less than that with a single intact cotyledon, but it was still far greater if based on unit area (Fig. 2). Imamura and Takimoto[5] also found that a small area cut from foliage leaf of *Pharbitis* plants was more effective for flower induction than expected from the leaf area. Even plants with cotyledons as small as 0.25 cm^2 flowered under almost the same critical dark period as the plants with intact cotyledons of full size, although far less flowers were formed and their number did not increase with the increase in length of the dark periods (Figs. 1 and 2). This suggests that starting time of the floral stimulus generation in the cotyledons is not affected by their size, but

their potential magnitude is limited in a small area. The applications of sucrose (1 to 6%), various amino acids, vitamins, and minerals to both the cotyledons reduced to small area and the plumule did not affect flowering (data not shown), which suggests that the shortage of these nutrients do not limit the floral processes in the cotyledon or at the apex. Incidentally, ethrel (0.15 to 1.5 μg) applied to the basal portion of cotyledon either before or after the inductive dark period did not affect flowering (data not shown). This means that flowering of the plants with cotyledons cut to small area is not affected by endogenous ethylene even if it is released from the cut surface of the cotyledon.

Application of GA_3 to the plumule increased the number of flowers in the plants with cotyledon reduced in area. In fact, plants with cotyledons reduced to 1 cm^2 formed so many flowers as those with intact cotyledons, if GA_3 was applied to the plumule (Figs. 3 and 4). This could mean that the principal stimulus generated in a 1-cm^2 cotyledon is sufficient to cause the same level of flowering as a single intact cotyledon, if another factors such as GAs are supplied to the apex.

Plants with cotyledons as small as 0.12 and 0.06 cm^2 flowered after one or two inductive cycles, if GA_3 was applied to the plumule (Fig. 3). GA_3 itself acts not on the cotyledon but on the shoot apex, as shown by Ogawa[11]. Thus, the present data (Fig. 3) indicate that these cotyledons reduced to 0.12 to 0.06 cm^2 are able to generate floral stimulus in response to the dark period and to cause flower initiation at the apex in cooperation with GA_3 action. The data also show that the cotyledons reduced to very small area can generate the floral stimulus with the repetition of inductive cycles and the stimulus can act additively on the apex.

Cutting cotyledons to 1 cm^2 before the dark period resulted in greater flowering than that at the end of darkness of 12 to 14 h (Fig. 4). By the time of 13- or 14-h dark periods the floral stimulus had not moved out of the induced cotyledon, as is indicated by the data on the removal of the cotyledon at different times (Fig. 4). Thus, generation of the floral stimulus in the cotyledon reduced in area before the dark period is supposed to be promoted as compared with that in the intact cotyledon. This idea is also supported by the data that the generation of the floral stimulus per unit area in a narrowed cotyledon is greater than that of an intact cotyledon (Figs. 1 and 2).

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アサガオの光周性花成誘導に及ぼす子葉面積とジベレリン A_3 の影響

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アサガオ，品種，Violet の播種後4日目の幼植物の子葉を1枚（約8.5 cm^2）にした後，その基部を 0.06 cm^2 から 1 cm^2 の小面積の方形に切り，花成誘導暗期における花成反応を調べた。一部のグループは，暗期前幼芽にジベレリン A_3 の溶液を滴下して（GA_3, 0.25 μg/幼芽），その影響を調べた。

0.25 cm^2 から 1 cm^2 の子葉は1回の12時間暗期に感応し花成が生じた。子葉面積が小さくなるにつれて花成は減少した。また，小面積にした子葉の花成は14時間以上の暗期で増加しなかった。GA_3 処理によって花成は増加した。しかし，0.5 cm^2 より小さくした子葉での GA_3 による促進は，14時間以上の暗期で増加しなかった。これは小面積にした子葉での花成刺激の生成は，あるレベル以上は制限されることを示唆している。しかし，これら小面積にした子葉の単位面積当りの花成誘導効果は，完全な子葉の場合より大であった。

0.12 cm^2 と 0.06 cm^2 にした子葉は，幼芽に GA_3 を処理すると，1回および2回の15時間暗期にそれぞれ反応して花成が生じた。0.12 cm^2 以上の子葉では暗期回数の増加に伴い花成が増加した。1回の暗期に反応して花成が生じる最小の子葉面積は，0.06 cm^2 から0.12 cm^2 の間であると思われる。

12時間から14時間の暗期前に 1 cm^2 にした子葉の花成は，各暗期直後に 1 cm^2 にした場合より大であった。このことは，暗期前に小面積にすると，暗期花成反応が完全子葉の場合に比べて促進されることを示唆している。