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Floral Initiation of the Buds on Main Stem and the Axillary Shoots of *Pharbitis* Seedlings of Different Growth Stages with a Special Reference to Effects of Gibberellin A_3, Zeatin and Temperature

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Abstract

Flowering response of the buds on the main stem and that of the axillary shoots located below the first flower bud of the main stem of *Pharbitis* seedlings were examined in relation to their development.

The position of the node bearing the first flower buds on the main stem for 5-, 7- and 9-day-old plants was the second, third and fifth nodes, respectively, and the number of the flower buds decreased with the increase in plant ages.

A largest number of flower buds was produced on the axillary shoots from the cotyledonary, first and second nodes for 5-, 7- and 9-day-old plants, respectively. This suggests that the axillary shoots elongating when they are induced are massive sinks of the floral stimulus.

Gibberellin A_3 (GA_3) and zeatin applied to the plumule before the inductive dark period, and the exposure to low temperature (20°C) after the dark period, both the treatments resulted in an increase in the number of flower buds not only on the main stem but also on the axillary shoots, increasing the position of the nodes of flowering axillary shoots to the upper nodes on the main stem. The sensitivity of the axillary shoots to the floral stimulus may be stimulated by GA_3 and zeatin, and the floral-induced state at their apex may last longer at lower temperature.

Key words: Axillary shoots · Floral response · GA_3 · *Pharbitis* · Temperature · Zeatin.

I. Introduction

In many photoperiodically responsive plants, the floral stimulus generated in the induced leaf will bring about flower initiation at the shoot apex[11]. The floral stimulus from the induced cotyledons of *Pharbitis* seedlings causes flowering in the upper axillary buds on the main stem and finally in the apical bud as a terminal flower. The number of flower buds formed on the main stem is usually dependent on the flower inducing stimulation generated in the cotyledons[8], which decreases with their increasing age[7]. In others, flowering response of cotyledonary buds released from plumular dominance in 6-day-old to 9-day-old seedlings was much greater than that of the main stem[9], suggesting that number of flower buds on the main stem is determined by the sensitivity of plumular apex which decreases also with the increasing age of plants[9].

The position of node bearing the first flower bud on the main stem rises gradually with increasing in plant age when induced to flower[7]. The question of whether the axillary buds at the lower nodes than the node of the

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first flower bud on the main stem can respond to the floral stimulus.

Application of gibberellin A_3 (GA_3) to the plumule of *Pharbitis* seedlings before the inductive dark period stimulates the flowering response[3,4]. The application to the cotyledons with cytokinins such as kinetin and benzyladenine (BA) is also effective in stimulation of the flowering, but that to the plumules is not[4,10]. Exposure to a low temperature (20°C) after floral induction promotes also flowering[2,6].

In the present experiments, flowering response of the buds on the main stem and that of the axillary buds or shoots from the lower nodes than the node of the first flower bud to the floral stimulus from induced cotyledons of *Pharbitis* seedlings of different ages was studied. The effects on their flowering response of GA_3 and zeatin application to the plumules before the inductive dark period and those of exposure to various temperatures after the dark period were also examined.

II. Materials and Methods

Seedlings of *Pharbitis nil* Choisy, cv. Violet were grown under day-light of fluorescent lamps (80 μmol $m^{-2}s^{-1}$) at 28°C for 5, 7 and 9 days after planting the germinating seeds in vermiculite and perlite (1 : 1 v/v). The number of leaf primordia of all axillary buds including the cotyledonary one, and the lengths of the axillary buds and of the foliage leaves on the main stem were measured under a binocular microscope.

The flowering response of axillary shoots of different plant ages was examined by exposing the plants to a 16-h inductive dark period at 28°C. An aqueous solution of GA_3 or zeatin containing 0.01% Tween-20 was applied twice to the plumule (0.25 μg/plumule per each treatment) with a glasscapillary tube twenty-four hours and immediately before the dark treatment. For control plants distilled water with 0.01% Tween-20 was applied. After the inductive dark treatment, the plants were grown under continuous fluorescent light keeping at 20°, 24° and 28°C. The plants were watered daily and supplied with nutrient solution twice a week. About two weeks later, the main stem was cut off just below the node bearing the first flower bud and the lower axillary buds were allowed to grow. The number of nodes bearing flower buds on the main stem cut out was recorded. About one week after cutting, when the axillary shoots developed from the topmost axillary bud had elongated to an appropriate length, the shoots were cut out just below the node of them and the number of flower buds detected on thesre axillary shoots was counted. This procedure was repeated, and the number of flower buds on the axillary shoots one by one downwards to the cotyledonary node was recorded. The first flower bud of these axillary shoots was always formed on their first node. Control plants of different plant ages were treated with GA_3 and zeatin without exposure to the inductive dark period, and then grown at various temperatures. In this instance no flower buds were formed on the main stem as well as on the axillary shoots developed from the lower nodes. Two or three pots with 14 to 21 seedlings in total were used for each treatment. Flowering is expressed as the mean number of flower buds $\pm$ standard error.

III. Results

Fig. 1-a shows the growth of axillary shoots developed from the cotyledonary node and the first to fifth nodes, and the length of the foliage leaves on the main stem (3.0 mm $\pm$ 0.21 in plumule length) of 5-day-old seedlings. The axillary shoots from the cotyledonary node had three or four leaf primordia. In only a few plants, axillary buds were detected on the second node, but an axillary bud was not on the third and higher nodes.

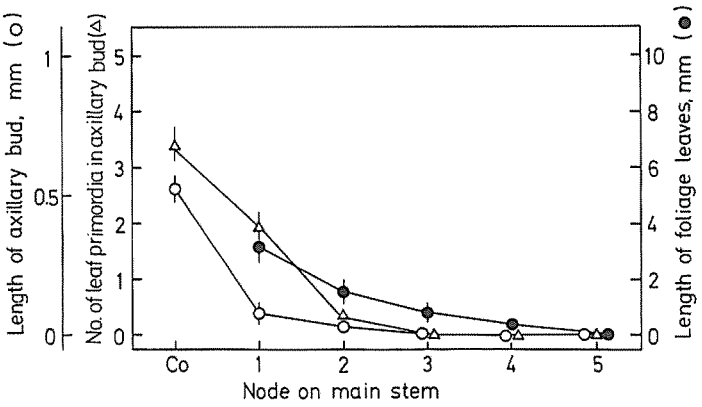

Fig. 1-a. Lengths of the axillary buds (○) and of the foliage leaves (●), and the number of leaf primordia in the axillary buds (△) at the cotyledonary (Co) and the first to fifth nodes on the main stem of 5-day-old *Pharbitis* seedlings of 3.0 mm ± 0.21 in plumule length.

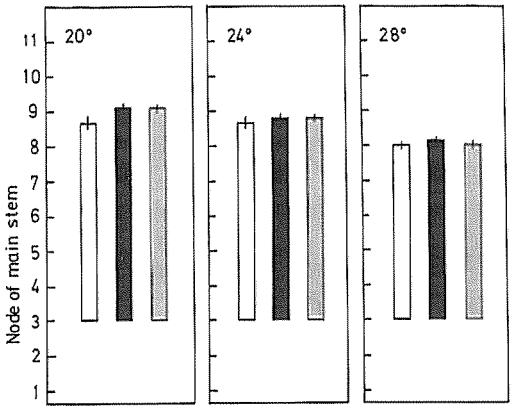

Fig. 1-b. Range of the nodes of the main stem bearing flower buds of 5-day-old *Pharbitis* seedlings when treated with distilled water (open), GA_3 (closed) and zeatin (dotted) before a 16-h inductive dark period, and grown at 20°, 24° and 28°C after the inductive dark period. Vertical bars on the histograms present standard errors.

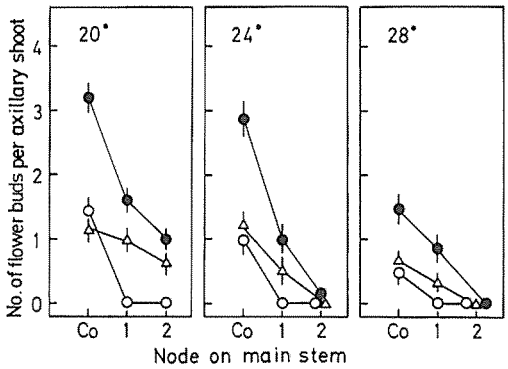

Fig. 1-c. Number of flower buds on the axillary shoots from the cotyledonary (Co), first and second nodes of 5-day-old *Pharbitis* seedlings when treated twice with GA_3 (●), zeatin (△) and distilled water (○) before a 16-h inductive dark period, and grown at 20°, 24° and 28°C after the inductive dark period.

Fig. 1-b shows the range of the nodes bearing flower buds on the main stem of 5-day-old plants when exposed to the inductive dark period preceded by water, GA_3 and zeatin treatments and followed by exposure to different temperatures. In all treatments flower buds were formed at the third and higher nodes to the terminal on the main stem. Fig. 1-c shows the number of flower buds on the axillary shoots from the cotyledonary, first and second nodes. The control plants without application of GA_3 and zeatin produced flower buds only on the cotyledonary shoots. The axillary shoots from the first and second nodes did not produce flower buds at any temperature examined. However, application of GA_3 and zeatin stimulated flowering and produced flower buds even on the axillary shoots from the first and second nodes. GA_3 was more effective than zeatin. The lower the temperature, the greater was the number of flower buds produced.

Fig. 2-a shows the growth of axillary shoots developed from the cotyledonary to sixth nodes and the length

of the foliage leaves on the main stem (7.7 mm±0.57 in plumule length) of 7-day-old seedlings. The axillary shoots of the first node were elongating with three or four leaf primordia and the axillary shoots of the third node had with only one leaf primordium. No axillary buds were detectable on the fourth node and above. Fig. 2-b shows the range of the nodes bearing flower buds on the main stem of 7-day-old plants exposed to the inductive dark period. The flower buds were formed at the fourth and higher nodes. The treatment with GA_3 and zeatin and that with lowering temperatures increased the number of flower buds. Fig. 2-c shows the number of flower buds produced on the axillary shoots from the cotyledonary to third nodes. Control plants grown at 28°C produced flower buds on only the axillary shoots from the first node. The application of GA_3 and zeatin increased the number of flower buds on the axillary shoots and was besides effective in producing flower buds on the shoots from the second node.

Fig. 3-a shows the growth of axillary shoots from the cotyledonary to sixth nodes and the length of the foliage leaves on the main stem (12.5 mm±1.5 in plumule length) of 9-day-old plants. The axillary shoots with

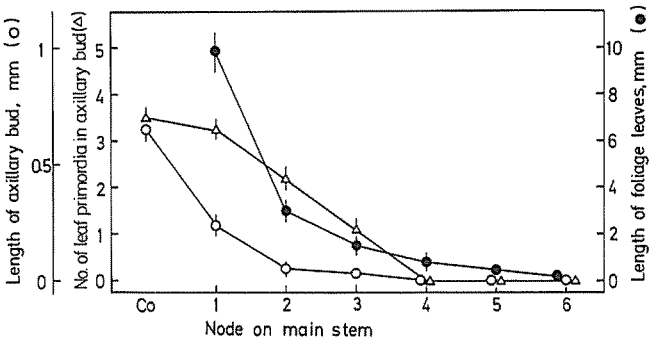

Fig. 2-a. Lengths of the axillary buds (○) and of the foliage leaves (●), and the number of leaf primordia in the axillary buds (△) at the cotyledonary (Co) and the first to sixth nodes on the main stem of 7-day-old *Pharbitis* seedlings of 7.7 mm±0.57 in plumule length.

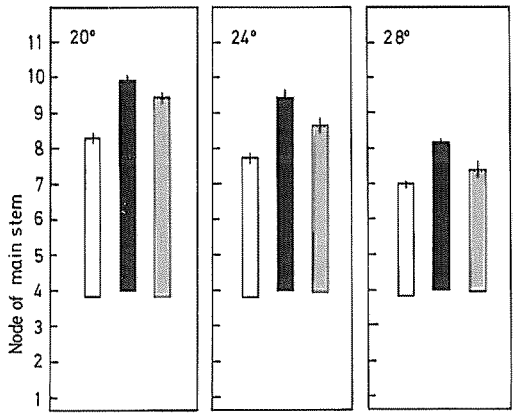

Fig. 2-b. Range of the nodes of the main stem bearing flower buds of 7-day-old *Pharbitis* seedlings when treated with distilled water (open), GA_3 (closed) and zeatin (dotted) before a 16-h inductive dark period, and grown at 20°, 24° and 28°C after the inductive dark period. Vertical bars on the histograms present standard errors.

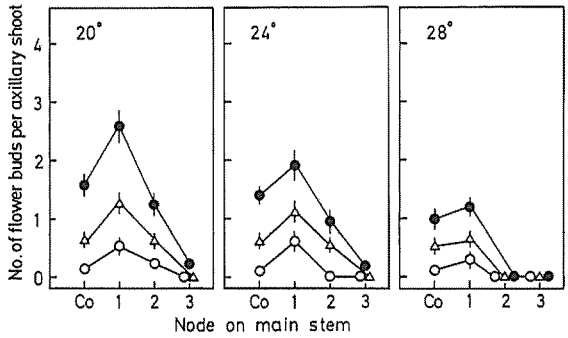

Fig. 2-c. Number of flower buds on the axillary shoots from the cotyledonary (Co), first, second and third nodes of 7-day-old *Pharbitis* seedlings when treated twice with GA_3 (●), zeatin (△) and distilled water (○) before a 16-h inductive dark period, and grown at 20°, 24° and 28°C after the inductive dark period.

three or four leaf primordia were elongating on the first and second nodes. In some plants, axillary shoots with a leaf primordium were on the fifth node. No axillary buds were detectable on the sixth node and above. Fig. 3-b shows the range of the nodes bearing flower buds on the main stem of 9-day-old plants exposed to the inductive dark period. The flower buds were formed on the sixth node and above. Number of flower buds formed on the main stem of those plants was less than that of the younger plants. Fig. 3-c shows the number of flower buds produced on the axillary shoots from the cotyledonary to fitth nodes. The number of flower buds was greatest on the axillary shoots from the first and second nodes, especially GA_3 treatment was remarkably effective in producing the flower buds on these axillary shoots. The lower the temperature, the greater was the promotion of flower production by GA_3 and zeatin.

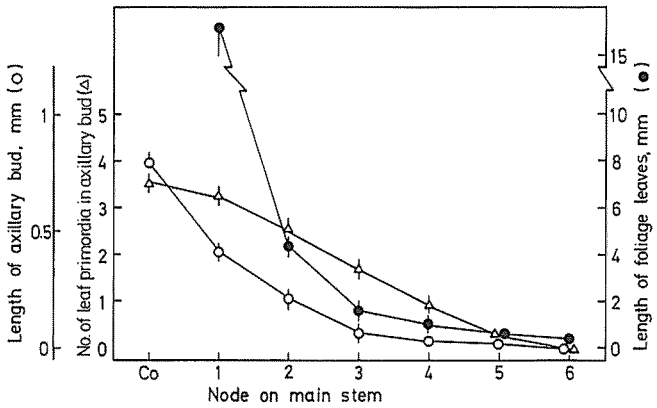

Fig. 3-a. Lengths of the axillary buds (○) and of the foliage leaves (●), and the number of leaf primordia in the axillary buds (△) at the cotyledonary (Co) and the first to sixth nodes on the main stem of 9-day-old *Pharbitis* seedlings of 12.5 mm±1.5 in plumule length.

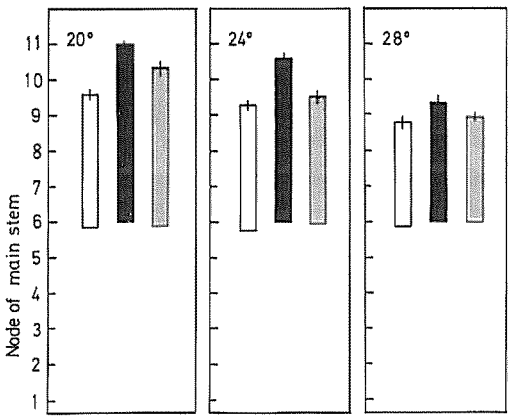

Fig. 3-b. Range of the nodes of the main stem bearing flower buds of 9-day-old *Pharbitis* seedlings when treated with distilled water (open), GA_3 (closed) and zeatin (dotted) before a 16-h inductive dark period, and grown at 20°, 24° and 28°C after the inductive dark period. Vertical bars on the histograms present standard errors.

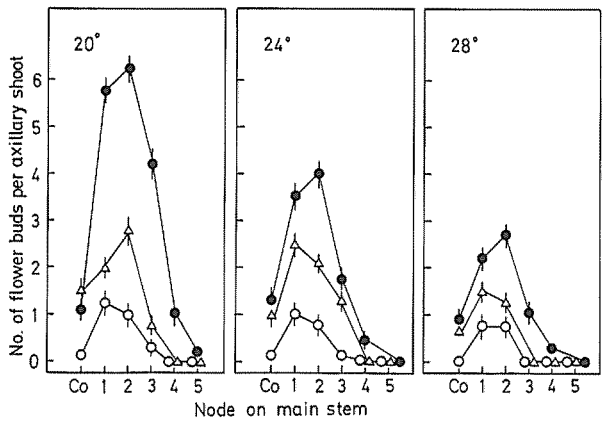

Fig. 3-c. Number of flower buds on the axillary shoots from the cotyledonary (Co) to fifth nodes of 9-day-old *Pharbitis* seedlings when treated twice with GA_3 (●), zeatin (△) and distilled water (○) before a 16-h inductive dark period, and grown at 20°, 24° and 28°C after the inductive dark period.

IV. Discussion

In *Pharbitis* seedlings exposed to an inductive dark period, the position of the node bearing the first flower bud on the main stem rose to the higher nodes with increasing the ages of plants when exposed to the inductive dark period (Figs. 1, 2, 3; b). Whether an axillary bud develops into a flower bud itself or axillaty shoot may depend on the developmental stage of the axillary bud meristem when it perceives the floral stimulus. Axillary meristem on the node of foliage leaves smaller than about 1.0 mm long, being not yet visible bud primordium, may develop into flower buds. Larger buds with a visible leaf primordium could not develop into flower bud, but could develop into axillary shoots with or without flower buds (Figs. 1, 2, 3; a vs. b and c). The axillary meristem just below the node of the first flower bud in the control plants without GA_3 and zeatin treatments did not produce flower buds even though the lower axillary meristem were able to produce easily the flower buds (Figs. 1, 2, 3; c). This suggests that the axillary meristem at the early stage specified could not respond to the floral stimulus. Fontaine[1] has reported on the changes of meristem sensitivity to the floral stimulus in *Anagallis* as a function of the plastochrone stage at the apex.

The nodal position of the axillary shoots bearing flower buds was restricted to the cotyledonary node for 5-day-old plants, but it shifted upwards to the first node for 7-day-old plants and the second or third node for 9-day-old plants (Figs. 1, 2, 3; c). This suggests that the elongating axillary shoots with a few leaf primordia in the plumule could be large sinks for the stimulus or very sensitive to the stimulus (Figs. 1 vs. 2 vs. 3; a and c). On the other hand, the increase in number of developing axillary shoots will bring about an increase in the sink size for the transport of the floral stimulus from the cotyledons, thus may result in a competitive decrease in the import of the floral stimulus into the main stem, as indicated by the decrease of the number of flower buds on the main stem with the increase of plant age (Figs. 1, 2, 3; b and Ref. 7).

Administration of GA_3 and zeatin to the plumule promoted further flowering not only on the main stem (Figs. 2 and 3; b), but also on the axillary shoots (Figs. 1, 2 and 3; c). This effect of these growth substances was intensified when the plants were grown at the lower temperature after the inductive dark period (Figs. 1, 2 and 3; b and c). The promotive effect of these growth substances was more evident in relatively older plants such as 7- and 9-day-old plants when they were induced (Figs. 1 vs. 2 vs. 3; b and c). In the previous study cytokinins such as kinetin and BA applied to the cotyledons promoted flowering but those applied to the plumule did not[4,10]. The difference in the flower-promoting effect of zeatin applied to the plumule seems to result from the difference of the age of the plumule between the previous and present experiments, though further study is necessary before any conclusion is derived. GA_3 is known to stabilize the floral stimulus at the apex[5,6]. Thus, GA_3 and probably zeatin may function to increase the sensitivity of the axillary buds or shoots to the floral stimulus. As a result, the range of nodes bearing sensitive apices of axillary buds (shoots) in the plumule treated with GA_3 or zeatin could be extended to upper nodes. The flower-induced state of the apices may last longer at lower temperatures and it may be further prolonged if the apices had been treated with GA_3 or zeatin.

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アサガオの主茎及び腋生枝条の光周期的花成反応，特に植物齢，ジベレリン A_3，ゼアチン及び温度の影響について

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アサガオ，品種 Violet の各種草齢の幼植物の主茎の上位節及び初花節位より下位の腋生枝条の，光周性花性刺激による花成反応を調査した。

播種後5日，7日そして9日の草齢の主茎の花芽は，それぞれ第3節位，第4節位そして第6節位から上位に形成された。その花芽数は草齢が進むにつれて減少した。他方，5日，7日そして9日草齢の最も多数の花芽を形成する腋生枝条の主茎上の節位は，それぞれ子葉節，第1節そして第2節であった。この節位は，花成誘導の際，幼芽中で数個の葉原基をもった伸長中の腋生枝条の節位と一致した。この結果は，伸長中の腋生枝条が，花成刺激の強い受容組織であり，また，このような枝条は，花成刺激に強く反応することを示唆している。

ジベレリン A_3 (GA_3)，あるいはゼアチンを花成誘導前に幼芽に与えるか (0.5 μg/幼芽)，あるいは花成誘導後，低い温度下に (20°C) 置くと，主茎の花芽数のみならず下位節の腋生枝条の花芽数が増加した。さらに，花成腋生枝条の主軸上の節位の範囲は上位節へ広がった。これらの結果は，主茎上の芽及び腋生枝条の花成刺激に対する反応が GA_3 やゼアチンによって促進され，これら茎（枝条）頂の花成誘導状態は低い温度で長く続くことを示唆している。