Studies on the Flower Initiation in *Pharbitis* Seedlings I.

Ages of Shoot Apices and Initiation Rates of Leaf Primordia at the Shoot Apex

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Abstracts

Based on a microscopic observation of external-morphological changes of *Pharbitis* shoot apices, the shoot apex stages were determined, the ages of shoot apices were expressed numerically, and the initiation rates of leaf primordia were calculated graphically.

The initiation of leaf primordia at the shoot apex was accelerated by flower-inductive dark treatments. This acceleration was evident, even before the floral organ was recognized under dissecting microscope, within three to four days after an inductive dark period. It may be said that the first sign of flower initiation is an acceleration of the initiation rate of leaf primordia at the shoot apex.

Introduction

The Japanese morning glory, *Pharbitis nil*, strain VIOLET, is one of the most sensitive short day plants, and a single dark period of 16 hours is sufficient to induce a maximum flowering response^{1,2)}. The young seedlings are fully responsive, and experiments may be carried out three to four days after planting the seeds³⁾. Thus, this plant is very easy to work with, and many studies on the initiation of flower buds, especially on photoperiodic response, have been carried out with this plant.

However, certain problems remain to be investigated in more detail, e. g., (1) the metabolic changes involved in the establishment of flowering stimulus in the leaf, (2) the nature of the stimulus, (3) the transmission of the stimulus from leaf to shoot, and (4) the initiation and development of flower buds at the shoot apex. For a long time very little attention has been paid to the last problem. Anatomical studies on the flower initiation of *Pharbitis nil* have been made by Marushige⁴⁾.

A series of studies has been carried out by Wada (unpublished), to analyze the early stage of the initiation and development of flower primordia at the shoot apex of the *Pharbitis* seedling, using the following means: (1) external-morphological observations by a dissecting microscope, (2) X-ray and gamma-ray irradiations, (3) these irradiations localized to plant parts, and (4) the temperature treatment and the application of some chemical agents.

The experiments presented in this paper are concerned with the study on the ages of shoot apex and initiation rates of foliage and floral leaves at shoot apex.

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Material and Methods

Material: The material used was the Japanese morning glory, *Pharbitis nil* Chois., strain VIOLET. All experiments were performed with the seedlings, photo-periodic behaviours of which were reported by Kujirai and Imamura²⁹.

Germination of seeds: The seeds were treated with conc. H_2SO_4 for 30–40 minutes, washed in running water for 5 hours, sown on 2–4 sheets of wet filter paper in a Petri dish, and incubated in a laboratory or an incubator kept at 25–30°. Twenty-four hours after the H_2SO_4 -treatment, germinating seeds were sown in wooden boxes or in clay pots containing garden soil.

Short day treatment: On the fifth or the sixth day after the H_2SO_4 -treatment when the cotyledons fully expanded, the seedlings were transferred to a dark room kept at 25° for dark treatment. Each group of seedlings was subjected to a single dark period of various lengths. Some of the seedlings were kept under continuous illumination and served as controls. After the dark treatment, terminal and axillary buds of the seedlings were dissected daily under a dissecting microscope to examine the appearance of newly developing leaf- and flower-primordia.

Growth condition: All experiments were carried out in two air-conditioned rooms kept at 25–30°. One was a dark room and another was a room illuminated continuously with eight 100-watt incandescent lamps and six 40-watt fluorescent lamps. The light intensity at the level of the cotyledon surface was about 3000 lux.

Three substages in a single plastochron and numerical expression of shoot apex age: Abbe, Phinney and Baer³⁰ divided a single plastochron into three substages in the development of the corn shoot apex: early, mid and late substage. Although in *Pharbitis* the shoot apex differs morphologically from that of corn, the same classification of substages may be applied to *Pharbitis nil*, as shown diagrammatically in Fig. 1.

In the early substage (s1), the apical dome or apical plateau expands to a maximum width and is about to initiate a new leaf primordium. In the mid substage (s2), the uppermost leaf primordium emerges as a small bulge on an open corner of the fully expanded apical plateau. In the late substage (s3), the apical plateau gradually narrows as the uppermost leaf primordium enlarges. The narrowed apical dome again enlarges and expands to the state of the early substage. When the uppermost leaf primordium is the eighth leaf (L8) and the shoot apex at that time is in the late substage (s3), the shoot apex stage will be expressed by L8-s3 in the present paper. The age of the shoot apex will be expressed numerically by the total number of substages of the shoot apex stages, for instance, $L8-s3 = (8-1) \times 3 +$

Diagram of shoot apex
Sub-stage	late	early	mid	late	early
Shoot apex stage	L7-s3	L8-s1	L8-s2	L8-s3	L9-s1

Fig. 1. Diagram of shoot apex showing three substages within a single plastochron

Fig. 2. Terminal shoot apices: Left, early substage; center, mid substage; right, the next early substage. 'H' and 'W' indicate height and width of leaf primordium, respectively.

$3=24$; or $L11-s2=(11-1)\times 3+2=32$. The age of the shoot apex is defined by a numerical expression of total shoot apex stages, which also indicates the age of the seedling at the time of dissection. In the present experiments, it was assumed that each floral leaf of two bracts, five sepals, five petals, five stamens, and three carpels is equivalent to one leaf primordium. Thus a flower having two bracts and 18 floral leaves possessed the total number of substages 60.

Initiation rates of leaf and flower primordia: In a study of corn embryogeny, Stein and Weber⁶⁾ used two practical methods for calculating leaf initiation rates. One was referred to as "the stage-per-day method" in which the average leaf stage per sampling date was determined, the other as "the mid-point method". This method has been used because it permits arithmetical calculation of the duration of each plastochron (leaf stage) by use of the formula, $t_x=1/2(X_2-X_1)+1/2(X_3-X_2)$, in which X is the plastochron number, and X_1 , X_2 , and X_3 represent the time values from planting to the mid-points of three successive stages respectively.

In the present experiments, a modified stage-per-day method was used because this was simple and convenient to express small changes of initiation rates. The shoot apex ages, i. e., the average total numbers of shoot apex stages on the ordinate, were plotted against the sampling dates on the abscissa, and the initiation rates of leaf- and flower-primordia were determined graphically.

Growth of leaf primordia: Heights and widths of leaf primordia were measured under a microscope at the positions as shown in Fig. 2. To determine fresh and dry weights, the plumules were cut off just above the cotyledonary nodes and weighed with a torsion balance. Dry weights were measured about three weeks after the plumules had been removed and the materials had attained constant weight in a desiccator after drying overnight in an electric oven at 90° .

Results and Discussion

1) *Shoot apex stages of terminal and axillary buds in the seedlings subjected to a single dark period of various durations:* On the sixth day after the H_2SO_4 -treatment, each group of seedlings was subjected to a single dark period of 8, 16, or 24 hours, followed by continuous illumination. Terminal and axillary buds in each seedling were dissected immediately, one, two, three, and four days after the end of the dark period. Control plants were kept under continuous illumination throughout the experimental duration and dissected in the same way as the experimental plants. This

Fig. 3

Fig. 4

Fig. 5

Fig. 3, 4 and 5. Advancement of shoot apex stage of *Pharbitis* seedlings subjected to a single dark period of various durations. All dark treatments were started at the same time. (0-hour, - -○- -; 8-hour, - -●- -; 16-hour, —●—; 24-hour dark period, —▲—)

experiment was undertaken three times, and the results are shown in Figs. 3, 4 and 5. In all three experiments, the advancement of shoot apex stages of the second and the third axillary buds were distinctly accelerated by the treatment of a 16- or 24-hour dark period. This effect of the dark treatment was not evident at first, but became clear with the lapse of time. The effect was quite clear three or four days after the end of the dark treatment. Eight hours of darkness did not produce such an effect, i. e., the plants either exposed to continuous light or subjected to an 8-hour dark period showed a similar change in the shoot apex stages throughout the experimental period. A single dark period of 16 or 24 hours is enough to initiate flower primordia, but that of 8 hours is not. This means that the shoot apex stages advance more rapidly in the seedlings induced to flower than in the non-induced seedlings, at least at the second and the third axillary buds.

These phenomena were not observed at the first axillary buds in all experiments. In the terminal buds a similar acceleration was observed but was not so clearly evident as in the second and the third axillary buds. Therefore, the experiments were repeated six more times, and the shoot apex stages of the terminal buds of the seedlings subjected to a 16-hour dark period were compared with those of the seedlings kept under continuous illumination. Results shown in Table 1 indicate that the shoot apex stages of the terminal buds are significantly accelerated by the dark treatment of 16 hours. In Table 2 are shown the initiation rates of the leaf primordia at the terminal buds calculated from the data shown in Table 1. It is clear that the rates were raised by a 16-hour dark period in the terminal bud as well.

Table 1. Comparison of shoot apex stages between terminal buds kept under continuous illumination (CL) and those subjected to a single dark period of 16 hours (16^hd).

No. of experiment	Time of dissection (Days after a 16 ^h dark period)									
	0		1		2		3		4	
	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d
1	14	14	15	15	17	17	18	20	—	—
2	14	14	14	15	—	—	—	—	—	—
3	15	15	17	17	19	20	21	21	23	24
4	14	15	17	16	19	21	21	23	—	—
5	15	15	17	18	20	22	21	25	23	32
6	14	14	—	—	20	23	—	—	26	32

Table 2. Initiation rate in terminal buds (advanced shoot apex age per day) during 4 days after a 16^h dark period (calculated from the data shown in Table 1).

No. of experiment	Continuous illumination	A 16 ^h dark period
1	1.3	2.0
3	2.0	2.5
4	2.3	2.7
5	2.0	4.3
6	3.0	4.5

The results presented here are interesting in connection with the well-known facts that the second or the third axillary bud responds most sensitively to a single dark period, but the terminal bud is less sensitive, and the first axillary bud is almost insensitive in seedlings of the same age as the ones used in the present experiments.

2) *Initiation rates of leaf and flower primordia at shoot apex*: Six days after the H_2SO_4 -treatment, seedlings were subjected to a single dark period of 16 hours and returned to continuous illumination. During 16 days after the dark treatment, 20–30 plants were harvested every day and dissected to determine the shoot apex ages. Control plants were kept under continuous illumination throughout the experimental

Fig. 6. Shoot apex ages and the initiation rates of primordia at shoot apices of terminal and axillary buds in the seedlings subjected to a single 16-hour dark period (16^{hd} , broken lines), and those in the seedlings grown under continuous light (CL, solid lines).

period and dissected in the same way as the experimental plants. Results shown in Fig. 6 indicate that the shoot apex age of the flower-induced apex (plants subjected to a 16-hour dark period) proceeds faster than that of the vegetative apex (control). This difference in the progress of the shoot apex stages was observed three to four days after the end of the dark treatment and became quite clear thereafter.

The flower-induced apex: The times of initiation of bracts, sepal and anther primordia are shown by B, S and A, respectively, in Fig. 6. After the initiation of bracts, the shoot apex stages advanced rapidly, because the bracts appeared in closer succession than the vegetative leaves. Thereafter five sepals appear in very close succession, and the shoot apex stages at this time advanced very rapidly. Furthermore, five anthers and then five petals appear almost at the same time, and the rates of advancement of the shoot apex ages reached their maximum at this time. In Fig. 6, the shoot apex ages of the second, the third, the fourth, and the fifth axillary buds advanced at the same rate, i. e., curves for these buds in Fig. 6 run parallel at about 24- to 31-hour intervals. The curve for the first axillary bud is not parallel to the others, i. e., the development of the first axillary bud was slower than that of the others, and it stopped about two weeks after the end of the dark treatment. If the first axillary bud was forced to develop by removal of the main axis, it gave rise to a vegetative shoot with various malformed leaves as shown in Fig. 7. The shoot apex stage of the first axillary bud in the flower-induced plants advanced more rapidly than that in the vegetative plants from the fourth to the twelfth day after the inductive dark period. This indicates that this bud had also been affected by flowering stimulus and had begun to develop floral organs. Nevertheless, it gave rise to a vegetative shoot with malformed leaves after the removal of the main axis. This means that the bud which was once flower-induced reversed to a vegetative shoot under the conditions mentioned above. With respect to *the vegetative bud*, the curves on the ages of vegetative plants (Fig. 6), which were grown under continuous illumination, were almost linear during the first 22 days. The initiation rate of the terminal bud was higher than that of the axillary bud because of apical dominance. The development of successive axillary buds proceeded

Fig. 7. Various malformed leaves developed on the first axillary shoot, which grew after the removal of main axis (for details see text).

Fig. 8. Fresh and dry weights of plumules (shoots) in the seedlings subjected to a single dark period of various durations.

Fig. 9. Advancement of shoot apex ages of terminal and axillary buds in the seedlings subjected to a single dark period of various durations.

almost parallel at intervals of 30-42 hours (Fig. 6).

Initiation rates of vegetative and reproductive apices which were calculated from Fig. 6 are shown in Table 3. The initiation rate of the vegetative apex (advance-ment of shoot apex stages per day) was 1.3-2.3 throughout the experimental duration, while that of the reproductive apex reached 9.6-10.1 when the anthers began to initiate. During the first 3-4 days after a flower inductive dark period, morphological differences between vegetative and reproductive shoot apices were not observed.

Table 3. Advancement of shoot apex stages per day, initiation rates, at different growth phases (calculated from Fig. 7).

Light condition	Continuous illumination	A single 16-hour dark period		
	Vegetative	Vegetative to Bract	Bract to Sepal	Sepal to Anther
Axillary bud				
1st	1.3	2.8	3.9	—
2nd	1.3	3.1	4.8	10.1
3rd	1.3	2.2	3.6	9.6
4th	1.4	2.2	3.6	9.8
5th	1.5	2.5	3.6	9.7
Terminal bud	2.3	2.3	3.8	10.1

Table 4. Growth of leaf primordia in the seedlings subjected to a single 16-hour dark period (16^hd) and those kept under continuous illumination (CL).

a. Basal width of leaf primordium.

Sampling date	18/V		19/V		20/V		21/V		22/V		23/V	
Light condition	CL	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	
Leaf number												
3	1	1.2	1.2	1.7	1.5	1.8	1.8	2.1	1.9	2.4	2.4	
4	1	1.2	1.4	1.6	1.5	1.9	1.8	1.8	2.0	2.3	2.1	
5	1	1.2	1.3	1.8	2.1	2.4	2.4	2.9	2.7	3.3	2.3	
Mean.....	1	1.2	1.3	1.7	1.7	2.0	2.0	2.4	2.2	2.7	2.3	

b. Height of leaf primordium.

Sampling date	18/V			19/V		20/V		21/V		22/V		23/V	
Light condition	CL	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d	CL	16 ^h d
Leaf number													
3	1	1.3	1.5	2.0	2.0	2.5	2.7	3.8	3.5	—	—		
4	1	1.3	1.3	2.0	1.8	2.6	3.1	4.0	3.8	—	—		
5	1	2.0	2.0	3.5	4.3	6.5	6.3	9.8	10.3	—	—		
Mean.....	1	1.5	1.6	2.5	2.7	3.9	4.0	5.9	5.9	—	—		

Numbers in the table indicate the ratio of length measured every day after a 16-hour dark period to that measured on the 18th day, May.

But, the initiation rate of leaf primordia during this phase was accelerated in the seedlings subjected to the dark treatment. It may be said that the first sign of flower initiation is an acceleration of the initiation rate of leaf primordia at the shoot apex. A similar phenomenon was observed by Schwabe⁷⁾ in the *Chrysanthemum* and also by Thomas⁸⁾ in *Chenopodium amaranticolor*. Wetmore *et al.*⁹⁾, Jacobs and Raghavan¹⁰⁾, and Sunderland¹¹⁾ reported that the first sign of flower initiation is an increased rate of cell division at the shoot apex. In the present experiments with *Pharbitis nil*, the rate of cell division was not measured, but the accelerated initiation rate of primordia must be accompanied by an increase in the rate of cell division.

3) *Growth of plumule*: Fresh and dry weights of plumules in the seedlings subjected to a single dark period of various durations were measured, and the average values are shown in Fig. 8. Both fresh and dry weights decreased with lengthening duration of the dark period. This relation was especially noticeable in the fresh weight, and became the more evident the longer the observation time was delayed after the dark period.

On the other hand, as shown in Fig. 9, the shoot apex ages at the terminal buds were scarcely influenced by the flower inductive dark treatment during the first three days, but those of the second axillary buds were promoted by the inductive dark treatment. Thus, the advancement of shoot apex ages was not accompanied by an increase in fresh or dry weights of the shoots. The height and width of the leaf primordium were also measured. As shown in Table 4, the size of the leaf primordium was not influenced by the flower inductive dark treatment, at least during the first five days.

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摘 要

和田清美：アサガオの花芽分化についての研究——とくに茎頂の令 (age),
および葉・花葉原基の茎頂での分化速度の比較。

アサガオ (紫) の芽ばえを用いておこなった花芽分化についての実験の一部として、頂芽と腋芽の茎頂の令をかんだ数値で表現する試みをおこなった。芽ばえの発育に伴って茎頂は葉原基を順次分化するが、それらの初期発生はそれぞれ、初・中・後の3段階 (early, mid, and late substage) を経ておこなわれ

ることを解剖顕微鏡下で観察した。これにもとづき、観察時の茎頂が第何葉目の葉原基をどのような段階でもつかを知ることによって、茎頂がそれまで経験した葉原基分化の段階数を総計し、その数値で茎頂の令をあらわした。例えば、観察時に茎頂がすでに8枚の葉を分化しており、第9葉原基をその分化段階の中期の状態でもつとき ($L9-s2$) と記号であらわす。これは、 $8 \times 3(\text{substages}) + 2(\text{substages}) = 26(\text{substages})$, すなわち ($L9-s2=26$) で茎頂の令をあらわす。又、花葉についてはそれぞれを一枚の葉と同じとしてあつかった。さらに各原基の分化速度は、日数あたりの茎頂の令の進行数であらわした。

芽ばえが連続光下におかれたとき、花芽分化は全く見られず、頂芽・腋芽ともに葉芽として葉原基を分化しつづける。腋芽の場合、その葉原基分化速度は腋芽間でほぼ等しく、茎頂令を縦軸に、観察日数を横軸にしてグラフを描くと、同じ勾配をもつ直線的なグラフがえられる。これらのグラフは互いに平行でその間隔は30~42時間である。頂芽についてのグラフも直線的であるが、頂芽優勢を示して腋芽のものにくらべて勾配が急となっている。

一回の16~24時間の暗期によって開花誘導をうけた芽ばえの頂芽・腋芽の茎頂では、葉原基分化にひきつづいて花葉原基の分化が始まる。茎頂でのそれらの分化速度は、暗期後1~2日は連続光下のものと差が見られないが、2, 3, 4日と日数のますにつれて差が生じとくに3~4日後からは全く大きな差となる。暗期後からの3~4日間を花芽分化の初期相とよぶことにする。さきの場合と同様にしてグラフを描くと、頂芽と各腋芽（第1葉腋芽を除く）で同じ勾配をもつ同じ型のグラフがえられることより、花芽分化にさいして茎頂でおこる変化は頂芽・腋芽で同一の過程を経て進行すると思われる。又、各腋芽についてのグラフは互いに平行し、その間隔は24~32時間で葉芽分化の場合にくらべ8~10時間だけその間隔がせばまっている。これらより開花誘導後、花芽分化初期相において、はやくも茎頂で原基形成能が高まることが示された。この増大と幼芽重量の増大および葉原基の伸長との関係についてもしらべた。（静岡大学理学部生物学教室）