FLORAL INITIATION OF *PHARBITIS NIL* AT LOW TEMPERATURES

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I. INTRODUCTION

According to the studies in the field of photoperiodism, plants can be classified, with some exceptions, into three groups, short-day plants, long-day plants and day-neutral plants (8, 9, 33, 39, 43, 47). Environmental factors, especially the temperature greatly influences the photoperiodic behavior (19, 67). With many short-day and long-day plants, it has been shown that the critical photoperiod for flowering and also the time of initiation of floral primordia are influenced by temperatures (1, 35, 40, 41, 43, 47, 49, 53, 54, 55, 66, 68, 69, 70, 80, 84). *Hyoscyamus niger* grown at 28.5° required at least 11 1/2 hours of light per day to flower, whereas the critical daylength is reduced to 8 1/2 hours when grown at 15° (35). At relatively high temperature of 32° *Rudbeckia bicolor*, a sensitive long-day plant, will flower under photoperiods too short to permit flowering under cool conditions (46). Some workers also reported that short-day plants initiated floral primordia under long day by submitting the plants to cold treatments (5, 50, 63, 64, 65, 73, 75). For example, Roberts and Struckmeyer found that *Nicotiana* and *Datura* were short-day plants only at 24° or higher temperatures, but tended toward dayneutral at about 13° (63, 64, 65). Strawberry, *Frageria*, *Xanthium* and *Kalanchoe* show a similar response (5, 14, 17, 19, 50, 52, 67).

Japanese morning glory, *Pharbitis nil Chois*, is known as one of the most sensitive short-day plants (20, 21, 22, 32, 48, 73, 74). The material used in the present research was strain "Violet" which initiates floral primordia by the application of a single dark period of adequate length. This plant remains under continuous illumination strictly vegetative for months, and attains a large size without differentiating any floral primordia. However, it was found recently that the plant flowers even in continuous light by low temperature treatments. In view of the importance of such a claim, experiments were designed to investigate floral initiation of *Pharbitis nil* at relatively low temperature.

II. MATERIAL AND METHODS

In all the experiments presented here, plants were cultured in test tubes aseptically. This procedure was inevitable. As genus is presumably of tropical or subtropical origin, the plant requires relatively high temperature for growth. At low temperature it grows slowly and is often invaded by pathogenous

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fungi; therefore the experiment at low temperature must be undertaken in aseptic condition.

Unless otherwise mentioned, the basic culture medium was a modified White's solution. The medium consisted of 200 mg $Ca(NO_3)_2$, 360 mg $MgSO_4$, 200 mg Na_2SO_4, 80 mg KNO_3, 65 mg KCl, 16.5 mg Na_2HPO_4, 4.5 mg $MnSO_4$, 1.5 mg $ZnSO_4$, 0.75 mg KI, 4 mg Fe-citrate, 8 mg agar and 1000 ml dist. water. The medium was placed in test tubes and autoclaved under 1.5 kg/cm^2 overpressure for 15 minutes.

The "Violet" strain of *Pharbitis nil* was used as the main material. To obtain uniform germination, well-matured seeds of medium size were treated with conc. H_2SO_4 for 40 minutes and then washed in running water for 1 hour. They were sterilized by immersing in 10% calcium hypochlorite for 30 minutes, washed with sterilized water several times, and soaked in sterilized water for 24 hours at 25°. Then the seeds were again treated in 10% calcium hypochlorite solution for 10 minutes, washed with sterilized water repeatedly, and the seed coats were removed aseptically by hand. These seeds were then sown in cotton-stoppered 18 × 150 mm test tubes containing 10 ml of culture medium. The tubes with one seed for each were immediately subjected to continuous illumination from daylight fluorescent lamps at various experimental temperatures. The intensity of illumination at the plant level was about 1500–2000 lux. After the temperature treatment, the plants were transferred to 25° under continuous illumination. They were dissected to observe floral primordia with the aid of a binocular microscope 40–50 days after germination. The methods mentioned here were more or less modified as described in each experiment.

III. EFFECT OF TEMPERATURE

Pharbitis plants were cultured aseptically on the basic medium either containing 5% sucrose or without sucrose. The cultures were subjected to various constant temperatures or alternating temperatures under continuous illumination, and the flowering responses were examined.

1. *Constant Temperature Treatments*

The tubes containing germinating seeds were continuously illuminated at constant temperatures of 10°, 12°, 15°, 18° and 25° for various durations (Tables 1-a and 1-b). After the treatment, the seedlings were transferred to 25° and were dissected 40 days after germination.

None of the plants cultured on sucrose-deficient medium initiated floral primordia. Some of the plants cultured on sucrose medium and subjected to either 10°, 12° or 15°, initiated floral primordia (Fig. 1). The flowering percentage became greater as the duration of the low temperature treatments increased. All of the plants which were cultured on sucrose medium and subjected to 15° for 30 days, initiated flower buds. The plants cultured at 10° and 12°, however, grew poorly and only a few of them initiated floral primordia even by

TABLE 1-a.

Effect of constant temperatures on floral response of *Pharbitis nil* cultured under continuous illumination.

Temperature	Sucrose conc. (%)	Duration of treatment	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
10°	0	10	12	0	0	0
		20	10	0	0	0
		30	9	0	0	0
	5	10	10	0	0	0
		20	10	0	0	0
		30	10	20.0	0.2	20.0
15°	0	10	15	0	0	0
		20	11	0	0	0
		30	15	0	0	0
	5	10	21	4.8	0.5	4.8
		20	21	71.3	2.1	71.3
		30	20	100	3.5	100
25°	0	30	23	0	0	0
	5	30	23	0	0	0

TABLE 1-b.

Temperature	Sucrose conc. (%)	Duration of treatment in days	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
12°	0	10	22	0	0	0
		20	19	0	0	0
		30	15	0	0	0
	5	10	23	0	0	0
		20	20	0	0	0
		30	16	12.5	0.1	12.5
15°	0	10	19	0	0	0
		20	13	0	0	0
		30	15	0	0	0
	5	10	24	0	0	0
		20	23	78.3	3.8	78.3
		30	20	100	4.0	100

Table 1-b (continued)

18°	0	10	22	0	0	0
		20	23	0	0	0
		30	22	0	0	0
	5	10	24	0	0	0
		20	23	0	0	0
		30	20	0	0	0
25°	0	30	23	0	0	0
	5	30	23	0	0	0

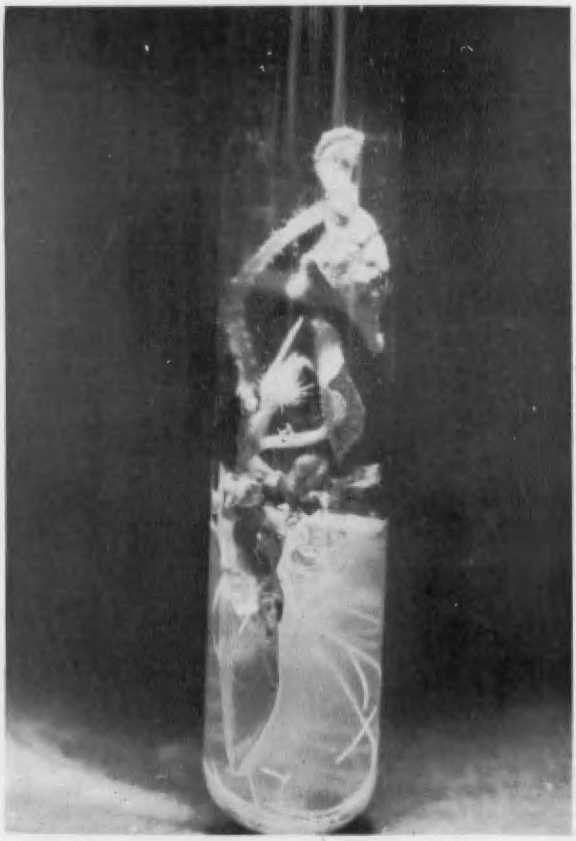


Fig. 1. Floral initiation of *Pharbitis nil* subjected to continuous illumination at 15° for 30 days. Photographed 20 days after the treatment.

the treatment of 30 days. The plants cultured at 18° and 25° grew well, but remained strictly vegetative.

Under field condition *Pharbitis* develops axillary flower buds, and terminal flower buds are formed only when the plants received a strong flowering stimulus. In the present experiment, however, all the plants that showed any floral initiation developed terminal flower buds.

2. *Alternating Temperature Treatments*

(1) Plants were cultured on sucrose-enriched or sucrose-deficient media and exposed to alternating temperatures (16 hours at 10° and 8 hours at 25°) for several durations. The results presented in Table 2 are similar to those obtained in the previous experiment in which the plants were exposed to the constant temperature of 15°. The percentage of flowering plants became greater as the number of exposures to alternating temperatures increased. A 30-day exposure

TABLE 2.

Floral initiation of *Pharbitis nil* cultured under continuous illumination at alternating temperature (10° for 16 hours and 25° for 8 hours per day) for various durations.

Sucrose conc. (%)	Duration of treatment in days	No. of plants	% of plant with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
0	0	15	0	0	0
	10	16	0	0	0
	20	16	0	0	0
	30	15	0	0	0
5	0	15	0	0	0
	3	13	0	0	0
	5	15	0	0	0
	10	15	0	0	0
	15	15	33.3	0.9	33.3
	20	12	83.3	3.4	83.3
	30	15	100	4.0	100

TABLE 3.

Flowering response of *Pharbitis nil* subjected to continuous illumination at various alternating temperatures (10°, 12°, 15°, 18° for 16 hours and 25° for 8 hours) for 30 days.

Sucrose conc. (%)	Alternating temperature		No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
	16 hrs.	8 hrs.				
0	10°	25°	12	0	0	0
	12°	25°	10	0	0	0
	15°	25°	20	0	0	0
	18°	25°	24	0	0	0
5	10°	25°	15	100	3.8	100
	12°	25°	19	100	4.0	100
	15°	25°	23	13.0	0.3	4.3
	18°	25°	23	0	0	0

to alternating temperatures was necessary for 100% flowering on sucrose medium. In this experiment also, all the plants cultured on sucrose-deficient media remained vegetative.

(2) Plants were exposed to various alternating temperatures (i.e. 10°, 12°, 15° or 18° for 16 hours and 25° for 8 hours) for 30 days, and flowering responses were examined (Table 3).

All the plants subjected to 10° for 16 hours and 25° for 8 hours* and those subjected to 12° for 16 hours and 25° for 8 hours* initiated floral primordia. Only 13% of those subjected to 15° (16 hrs.)–25° (8 hrs.) initiated flowers.

(3) To obtain more detailed information of the effect of alternating temperatures, the following experiment was carried out. The plants of one group were exposed daily to 1° for 4, 8, 12, 16, 20 or 24 hours, being kept at 25° for the remaining hours. The other 4 groups were treated in the same way but exposed to 5°, 10°, 15° and 20°, respectively, instead of 1°. The treatments were repeated for 30 days, after which the plants were grown at 25° for 20 days and then dissected to examine floral initiation (Table 4).

TABLE 4.

Flowering responses of *Pharbitis* plants subjected to continuous illumination at various alternating temperatures for 30 days. Plants were exposed to 1°, 5°, 10°, 15° or 20° for various hours, the remaining hours of each day being kept at 25°. The results are shown by percentage of flowering plants. Each lot contained about 20 plants.

a) Sucrose medium

Temperature	Hours of low temperature per day					
	4	8	12	16	20	24
1°	0	0	36.4	47.1	0	0
5°	0	0	42.8	69.6	0	0
10°	0	0	23.8	90.5	100	7.7
15°	0	0	0	0	95.4	100
20°	0	0	0	0	0	0

b) Sucrose-deficient medium

1°	0	0	0	0	0	0
5°	0	0	0	33.3	0	0
10°	0	0	0	0	11.1	0
15°	0	0	0	0	0	0
20°	0	0	0	0	0	0

* These treatments will be designated hereafter by the symbols 10°(16 hrs.)–25°(8 hrs.) and 12°(16 hrs.)–25°(8 hrs.) respectively.

On sucrose medium almost all the plants subjected to alternating temperatures of 10° (16 hrs.)–25° (8 hrs.), 10° (20 hrs.)–25° (4 hrs.) and 15° (20 hrs.)–25° (4 hrs.), and to a constant temperature of 15°, initiated floral primordia. Daily alternation of 10° and 25° was effective in causing floral initiation when the plant were exposed to 10° for more than 12 hours. However, at a constant temperature of 10°, only 7.7% of the plants initiated floral primordia. Alternation of 1° and 25° or 5° and 25° was also effective to some extent when the plants were subjected to those low temperatures for 12–16 hours. Daily alternation of 20° and 25° was not effective on floral initiation.

On sucrose-deficient medium a few plants initiated floral primordia when they received the treatment of 5° (16 hrs.)–25° (8 hrs.) or 10° (20 hrs.)–25° (4 hrs.). The experiments were repeated several times and always gave similar results.

3. *Discussion*

Pharbitis nil, strain "Violet", when subjected to a single dark period of an adequate length, initiates floral primordia (20, 21, 22, 74). However, under long photoperiods or continuous illumination at 25° the plants remain vegetative. Roberts and Struckmeyer have reported that, in many short-day plants, flowering was observed under long photoperiods at certain temperatures (63, 64, 65). Hartmann (14), Ogawa (51), de Zeeuw (5) and Takimoto (73) have also reported similar results.

As shown in the present experiments, *Pharbitis* plants also initiate floral primordia under continuous illumination at relatively low temperature when cultured on a sucrose-containing medium. Constant temperature treatment of 15° seems to be the most favorable for floral initiation. Flowering occurs easily when the plants are subjected to this temperature for 30 days. Temperature-alternation is also effective, and in this case also, 30 days of treatment are required to cause maximum flowering response.

As *Pharbitis* is presumably of subtropical origin (45), it grows poorly and flowers sparingly at 10° or 12°. Takimoto has reported that many plants died when cultured on sucrose medium at 10° for three months, but that all of the surviving plants initiated floral primordia under continuous illumination (73). One may assume that the reduced flowering response of the plants in the present experiment maintained at 10° for 30 days is due to their poor growth and that longer durations of treatment are therefore necessary for floral initiation.

The above experiment indicates that sucrose is favorable for floral initiation. When exposed to alternating temperatures of 5° (16 hrs.)–25° (8 hrs.) or 10° (20 hrs.)–25° (4 hrs.) on sucrose-deficient medium, only a few of the plants initiated flower buds. Plants subjected to temperatures higher than 18° do not flower under continuous illumination, irrespective of whether the temperature is constant or not, and of whether the medium contains sugars or not.

Pharbitis nil is a typical short-day plant at ordinary temperature. But it

initiates floral primordia under continuous illumination at relatively low temperature. Two explanations are possible: 1) At low temperature the plants may be insensitive to the inhibitory effect of long-day conditions, which at ordinary temperatures may suppress the flowering response, or 2) the low temperature treatment itself has a positive promoting effect on floral initiation. Whichever of these alternatives may be correct, it is obvious that this phenomenon results from the conditions caused by low temperature.

A 30-day exposure to low temperature was necessary for 100% flowering. At the end of the 30-day treatment, small flower buds were observed with naked eye (cf. Chapter X). That is, floral primordia may be formed during the low temperature treatment.

With regard to the effect of temperature on flower formation, two different effects ought to be distinguished: 1) a direct or non-inductive effect, and 2) an indirect or inductive effect, the latter being called vernalization (67, 90). The term vernalization in its narrow sense is restricted to causative, inductive promotions of flowering by low temperature and its action is not visible at first and appears as an after-effect. It seems the effect of low temperature on flowering in *Pharbitis nil* is direct, i.e., non-inductive.

IV. EFFECT OF CULTURE MEDIUM

In the present experiments, plants were cultured on various kinds of medium, and their flowering responses at low temperatures were examined. All plants were grown under continuous illumination from daylight fluorescent lamps, the luminosity of which was 1500 to 2000 lux at the plant level. They were kept at 15° for 30 days, and then at 25° for additional 15 days, at the end of which time they were dissected for the observation of floral initiation. Control plants were exposed to continuous illumination at 25° throughout the experimental duration and dissected 45 days after germination. As the controls did not initiate floral primordia in any experiments, they will be left out in the following tables.

1. *Effect of Agar Concentration*

The effects of varying concentrations of agar added to the basal medium containing either 5% or no sucrose on the flowering and growth of the plants are shown in Table 5. Better growth was obtained with 0.75 or 1% agar than with other agar concentrations. The culture medium with 0.3% agar is fluid and those with 1.5% and 2% are very hard in consistency. On such media the plants grew poorly. Dry weight of plants grown on media containing 5% sucrose was significantly heavier than that of plants grown on media without sucrose, but no significant differences in fresh weight and shoot length were found between the plants grown on media with and without sucrose. None of the plants cultured on sucrose-free media initiated floral primordia. The maximum promotion of flowering occurred on sucrose media containing 0.75 or 1%

TABLE 5.

Effect of agar concentrations on floral initiation and growth of *Pharbitis nil* subjected to continuous illumination at 15° for 30 days.

Sucrose conc. (%)	Agar conc. (%)	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm	Fresh weight in mg	Dry weight in mg
0	0.3	13	0	0	8.0	242.0	24.0
	0.75	13	0	0	23.2	434.0	25.0
	1.0	14	0	0	17.1	286.5	23.1
	1.5	3	0	0	16.4	260.0	23.3
	2.0	—*	—*	—*	—*	—*	—*
5	0.3	13	38.5	1.7	10.0	283.7	43.3
	0.75	15	100	5.0	22.6	430.0	63.8
	1.0	14	85.7	3.9	18.0	341.2	54.8
	1.5	13	46.1	0.7	15.6	271.2	40.3
	2.0	12	33.3	1.0	15.1	257.0	39.0

*All plants died.

agar, and only a few plants initiated floral primordia on media with 0.3% or 1.5% and 2% agar: i.e., on media with too soft or too hard consistency.

2. *Effect of pH*

The pH values were adjusted before autoclaving by the addition of 1N-HCl and 1N-Na_2CO_3, using glass electrodes for their determinations. As shown in Table 6, at pH 3.59 the growth of the plants was inhibited strikingly and almost all of them died probably because of the fluid and strong acidic conditions of the medium at this pH level. The plants grew fairly well and developed flowers on the media with pH values ranging from 4.32 to 6.86. Most of the plants in-

TABLE 6.

Effect of pH of the medium containing 5% sucrose. The plants were cultured under continuous illumination at 15° for 30 days. The pH values were adjusted with 1N-HCl and 1N-Na_2CO_3 before autoclaving.

pH values		No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm
Before autoclaving	After autoclaving				
3.01	3.59	3	0	0	8.6
4.21	4.32	16	100	4.2	28.8
5.22*	5.02	16	100	4.1	28.0
6.22	5.50	16	87.5	2.3	26.6
6.98	5.92	15	73.3	2.1	22.9
8.85	6.86	16	75.0	1.8	20.6

*Original pH value of White's solution without adjustment.

itiated floral primordia on sucrose media, the pH values of which ranged from 4.32 to 5.50. At pHs 5.92 and 6.86, the percentages of flowering plants were 73.3 and 75.0, respectively.

3. *Effect of Mineral Nutrition*

In order to examine the effect of nutritive components, the following series of culture media were used. A, 0.8% plain agar; MA, minerals+0.8% agar; SA, 5% sucrose+0.8% agar; MSA, minerals+5% sucrose+0.8% agar.

The results of the experiment are presented in Table 7. Shoot length and fresh weight showed relatively higher values on MA and MSA media, both of which contained minerals, but dry weight showed higher values on media containing sucrose, i.e., SA and MSA. On media containing sucrose, floral primordia were initiated irrespective of whether minerals were added or not. This indicated that mineral nutrients from the seed and agar were sufficient for the development of floral primordia under the present experimental condition.

TABLE 7.

Effect of minerals and sucrose in the media. The plants were cultured under continuous illumination at 15° for 30 days. A, 0.8% plain agar; MA, minerals+5% sucrose; SA, 5% sucrose+0.8% agar; MSA, minerals+5% sucrose+0.8% agar.

Components of culture media	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm	Fresh weight in mg	Dry weight in mg
A	22	0	0	15.1	300.0	22.2
MA	24	0	0	17.3	421.0	25.0
SA	18	100	4.0	15.9	295.4	50.0
MSA	21	100	3.7	22.7	461.3	60.5

4. *Effect of Carbon-nitrogen Ratios*

Agar media containing 0, 1, 2 or 5% sucrose and 0, 0.05, 0.1 or 0.5% KNO_3 (without the other minerals of White's solution) were used for examination of the effect of the carbon-nitrogen ratio on floral initiation. The results of the experiment are presented in Tables 8 and 9. Shoot length showed maximum values on media containing 0.05% KNO_3 irrespective of the concentrations of sucrose added, and relatively high values were obtained on 1% and 2% sucrose media containing 0.1% KNO_3. Although the media with 0.05–1% KNO_3 caused an increased shoot length, they were unfavorable for flowering. On media containing 1% KNO_3 the growth was quite retarded irrespective of sucrose concentrations, and floral initiation did not occur. Plants remained vegetative on sucrose-free media whether KNO_3 was added or not. It seems that floral initiation under continuous illumination at relatively low temperature may result from conditions causing poor vegetative growth, but the reproductive

TABLE 8.

Effect of sucrose-KNO_3 ratios on floral initiation. Plants were cultured under continuous illumination at 15° for 30 days. The flowering response in each medium is given as the percentage of plant with flower buds. The number of plants observed is given in parentheses.

Sucrose conc. (%)	KNO_3 concentration (%)				
	0	0.05	0.1	0.5	1
0	0 (12)	0 (16)	0 (14)	0 (11)	0 (2)
1	50.0 (16)	14.3 (21)	25.0 (20)	52.9 (17)	0 (4)
2	72.8 (22)	21.8 (23)	59.2 (22)	70.0 (20)	0 (3)
5	94.5 (18)	70.1 (20)	95.0 (20)	87.5 (16)	0 (4)

TABLE 9.

Shoot length (mm) on media with various sucrose-KNO_3 ratios. Plants were subjected to continuous illumination at 15° for 30 days.

Sucrose (%)	KNO_3 concentration (%)				
	0	0.05	0.1	0.5	1
0	21.8	58.5	33.1	27.0	7.3
1	33.5	75.1	53.3	26.9	6.2
2	35.9	74.3	47.2	33.1	7.0
5	25.6	52.1	35.1	23.8	8.2

TABLE 10.

Effect of $Ca(NO_3)_2$ concentration on floral initiation and growth in *Pharbitis nil*. Plants were grown under continuous illumination at 15° on media containing 2% sucrose for 30 days.

$Ca(NO_3)_2$ conc. (%)	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm
0	23	73.9	3.5	47.6
0.05	20	45.0	2.4	61.4
0.1	23	65.3	2.7	60.1
0.2	20	80.0	3.8	35.9

development does not take place when the growth is too much inhibited.

To investigate further the influence of the "C–N" ratio on flowering, $Ca(NO_3)_2$ in place of KNO_3 was added to agar media with 2% sucrose (Table 10). On the media containing 0.05 or 0.1% $Ca(NO_3)_2$, shoot length showed higher

values, but the flowering response was suppressed. These results are similar to those obtained in the above experiment with KNO_3.

5. *Effect of Sucrose Concentration*

The effect of sucrose concentration in the medium on floral initiation and growth is shown in Table 11. Shoot length showed higher values on media containing 1–4% sucrose. Fresh weight was nearly the same in all the lots, except for a somewhat lower value in the medium containing 10% sucrose. The dry weight of plants grown on sucrose medium was 2 to 3 times as great as that on sucrose-free medium, and increased with increasing sucrose concentrations. Floral initiation was not observed when the plants were grown on sucrose-free medium. On media with sucrose at concentrations from 4 to 10%, all the plants flowered with terminal flower buds. However, the number of flower buds per plant was the largest when grown on the medium containing 4% sucrose.

TABLE 11.

Effect of sucrose concentration on floral initiation and growth. Plants were subjected to continuous illumination at 15° for 30 days.

Sucrose conc. (%)	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm	Fresh weight in mg	Dry weight in mg
0	14	0	0	17.5	421.0	52.0
1	16	50.0	1.2	21.8	443.0	41.0
2	19	79.0	2.6	22.1	492.2	47.0
4	19	100	4.1	24.5	460.7	52.3
6	15	100	3.5	18.4	448.5	64.5
8	16	100	3.0	15.6	446.7	78.6
10	17	100	2.9	15.1	325.5	70.5

6. *Effect of Various Sugars*

Table 12 shows the effect of different sugars (0.15 M) on floral initiation and growth. The plants grown on media containing xylose, galactose, mannose, mannitol or soluble starch showed lower values in shoot length and fresh weight, as compared with those grown on media without sugars. Dry weight increased on all the media containing sugars. Fructose, mannose, sucrose, maltose, lactose and raffinose were found to be very effective in causing floral initiation. Only 66% of plants cultured on glucose medium initiated floral primordia. Xylose and mannitol were less effective and galactose and soluble starch had no effect.

In another experiment, five effective sugars, glucose, fructose, mannose, maltose and sucrose, were sterilized by filtrating through a Seitz filter and added aseptically to the autoclaved basal medium. The sugar concentration was 0.15M (Table 13). The above sugars sterilized by Seitz filtration were as effective as the autoclaved sugars in causing floral initiation. In this experiment, however, filtrated glucose gave somewhat higher percentage of flowering plants than the

TABLE 12.

Effect of various sugars on floral initiation and growth. Plants were subjected to continuous illumination at 15° for 30 days.

Sugars (0.15 M)	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm	Fresh weight in mg	Dry weight in mg
Sugar-free	14	0	0	22.7	328.6	34.6
Xylose	16	6.3	0.1	13.9	—	—
Galactose	12	0	0	11.1	210.7	43.2
Glucose	15	66.6	2.2	23.8	282.1	47.6
Fructose	23	100	3.1	29.8	335.6	53.7
Mannose	17	100	3.7	19.1	235.8	55.1
Mannitol	9	11.1	0.7	15.4	179.1	40.8
Sucrose	23	100	4.0	32.0	376.3	62.3
Maltose	19	100	4.4	32.0	—	—
Lactose	20	90.0	4.3	31.3	305.7	48.8
Raffinose	19	100	3.9	35.9	—	—
Starch (soluble 5%)	18	0	0	21.0	—	—

-, not observed.

TABLE 13.

Effect of various sugars sterilized with Seitz-filter on floral initiation. Plants were subjected to continuous illumination at 15° for 30 days.

Sugars (0.15 M)	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
Glucose	15	80.1	3.7	80.1
Fructose	16	87.5	4.2	87.5
Mannose	15	93.4	4.5	93.4
Maltose	16	93.8	4.6	93.8
Sucrose	17	100	4.7	100

autoclaved glucose.

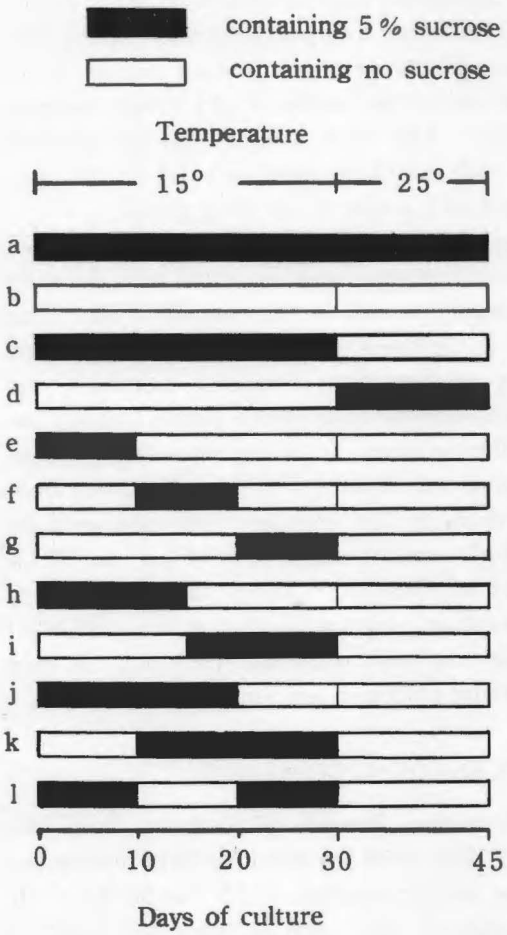
7. *Effect of Time and Duration of Sucrose Supply*

From the experiments mentioned above, it was noticed that sucrose added to the medium was effective in causing floral initiation under continuous illumination at low temperatures. In above experiments, sucrose was supplied to the plants throughout the experimental period. The present experiment is concerned with the effect of sucrose (5%) which is given for various days at different times of the experimental period. The results are presented in Table 14.

In lots a and c, to which 5% sucrose was given during the low temperature treatment, all of the plants initiated floral primordia. However, in lots b and d,

TABLE 14.

Effect of time and duration of sucrose application. Plants were cultured at 15° for 30 days and then at 25° for 15 days under continuous illumination.



	No. of plants	% of plants with flower buds	No. of flower buds per plant
a	21	100	4.0
b	17	0	0
c	22	100	4.8
d	18	0	0
e	19	36.8	1.4
f	22	27.3	1.0
g	18	11.1	0.3
h	20	65.0	2.6
i	17	47.1	2.6
j	21	81.0	3.7
k	21	61.9	2.7
l	20	45.0	1.1

to which sucrose was given after the low temperature treatment or not supplied throughout the experimental period, floral primordia were not initiated. The presence or absence of sucrose after the end of the low temperature treatment had no influence on floral initiation: sucrose was effective for floral initiation only when supplied during the low temperature treatment of 15°.

Comparing the lots c, e, h and j, to which sucrose was given from the start of the experiment for various durations, the flowering percentages increased in the following order: $e < h < j < c$. The percentages of flowering increased with increasing duration of sucrose application. From the comparison among the lots e, f and g, it was found that sucrose given in the earlier phases of the low temperature treatment was more effective than that given in the later phases. Similar effect of sucrose is observed when flowering responses of lots h and i, or

j and k are compared.

8. *Discussion*

Under the present experimental conditions, sucrose and some other sugars applied to the medium promoted floral initiation to a great extent. Maximum flowering responses were observed when plants were cultured on sucrose (4–10 %) media containing 0.75 or 1% agar and on the media of pH values ranging from 4.32 to 5.50 (Tables 5, 6 and 11). One may assume that the reduced flowering response of plants cultured with 0.3 (fluid medium), 1.5 or 2% agar (stiff medium), or at pH 3.59 (fluid medium), is due to the poor growth.

On the medium containing sucrose, floral primordia were initiated without addition of any mineral elements. Some workers have suggested that the ratio of carbohydrate to nitrogen plays an important role in the control of flowering (6, 31, 85). Such may also be the case in the present experiments. The flowering response of the plants increased as concentration of sucrose increased, and 1% sucrose was enough to initiate flower buds on 50% of the plants. However, when 0.05–0.1% KNO_3 was added to the medium, flowering responses were inhibited to some extent, and 2–5% sucrose was required to initiate flower buds on 50% of the plants. Vegetative growth, on the contrary, was promoted by the addition of KNO_3 or $Ca(NO_3)_2$. High concentrations of KNO_3 or $Ca(NO_3)_2$ seem to be unfavorable for the growth of *Pharbitis*. When more than 0.1% KNO_3 or $Ca(NO_3)_2$ was added to the medium, vegetative growth was inhibited considerably, but in this case, flowering responses were not inhibited. It may be concluded that vegetative and flowering responses are antagonistic.

V. EFFECT OF INTENSITY AND QUALITY OF LIGHT

In the experiments of the previous section, *Pharbitis nil* initiated floral primordia under continuous illumination of 1500–2000 lux from daylight fluorescent lamps when the plants were cultured on sucrose medium at 15° for 30 days. It is conceivable, however, that the intensity of light used in the experiments is not strong enough to bring about the inhibitory effect of long day or continuous illumination, which at ordinary temperatures suppresses the flowering response. If enough light intensity is given, it might be probable that continuous illumination has an inhibitory effect on flowering even at low temperatures.

The following experiments were designed to investigate the effect of light intensity, quality and photoperiods on floral initiation of *Pharbitis nil* grown at cool temperatures.

1. *Effect of Light Intensity*

Plants on the sucrose (5%) medium in test tubes were exposed to continuous illumination of various intensities from daylight fluorescent lamps at 15° and 25° from the start of germination for 30 days. All the plants were then transferred to 25° under continuous illumination (1500–2000 lux) for additional 15 days and

TABLE 15.

Flowering responses of *Pharbitis nil* exposed to various intensities of continuous light at different temperatures. The plants were cultured on a medium containing 5% sucrose and exposed to various intensity of light at 15° and 25° for 30 days.

Temperature	Intensity of light in lux	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
15°	0*	22	100	3.1	100
	10	16	87.5	4.3	87.5
	50	20	85.0	3.8	85.0
	100	22	95.5	3.9	95.5
	500	23	100	4.8	100
	1000	21	100	3.7	100
	2000	22	95.5	4.0	95.5
	4000	22	100	5.5	100
	6000	20	100	5.8	100
25°	0*	16	0	0	0
	10	19	0	0	0
	30	21	0	0	0
	50	21	0	0	0
	100	15	0	0	0
	500	22	0	0	0
	1000	20	0	0	0
	3000	15	0	0	0
	6000	18	0	0	0

* cultured under total darkness for 30 days and then subjected to continuous illumination until the observation.

dissected for floral initiation. Results are shown in Table 15. Almost all the plants cultured at 15° initiated floral primordia irrespective of the intensities of light to which the plants were exposed and in total darkness. Plants exposed to 10 to 2000 lux initiated almost the same number of floral primordia, but those kept in total darkness developed less, and those exposed to 4000 and 6000 lux developed more floral primordia. None of the plants cultured at 25° initiated floral primordia irrespective of light intensity.

2. *Effect of Monochromatic Light*

To examine the effect of continuous illumination of monochromatic light, plants were cultured on a medium containing 5% sucrose and subjected to various colored lights of about 2000 erg/cm^2/sec. at 15° for 30 days, and subsequently transferred to 25° under continuous illumination from daylight fluorescent lamps (1500–2000 lux) for 15 days. Far red light was obtained from a 60 watt incandescent lamp filtered with two layers of blue and two layers of red

TABLE 16.

Effect of monochromatic light. *Pharbitis* plants were cultured on a medium containing 5% sucrose and subjected to various colors of light at 15° or 25° for 30 days.

Temperature	Color of light	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
15°	Blue	23	95.7	5.4	95.7
	Green	24	91.7	4.4	91.7
	Red	24	87.5	4.4	87.6
	Far-red	21	85.7	2.6	85.7
	Daylight	19	94.7	5.2	94.7
25°	Blue	16	0	0	0
	Green	16	0	0	0
	Red	15	0	0	0
	Far-red	14	0	0	0
	Daylight	16	0	0	0

cellophane and the energy at test tube surface was about 1000 erg/cm^2/sec. Flowering responses are shown in Table 16. No significant differences were found in flowering percentages of the plants which were cultured under continuous illumination of various colored lights at 15°. The number of floral primordia, however, was somewhat less in the series exposed to far-red light than in others. None of the plants cultured at 25° initiated floral primordia under continuous illumination regardless of the color of light.

It may be said from the results of Tables 15 and 16 that the plants cultured on the sucrose medium initiate floral primordia at 15° irrespective of intensity and quality of light.

3. *Effect of Photoperiod*

Plants were cultured on media containing 5% sucrose or no sucrose and exposed to the following photoperiods at 15° and 25° from the start of germination for 30 days:

D: Total darkness

S: 8-hour illumination of 2000 lux from daylight fluorescent lamps followed by 16-hour dark period.

L: Continuous illumination of 2000 lux from daylight fluorescent lamps.

After the treatment they were transferred to 25° under continuous illumination and dissected 20 days later. One lot of the plants was kept in total darkness at 15° for 100 days and dissected at the end of the treatment. The results are shown in Table 17.

Plants cultured on the sucrose medium at 15° initiated flower buds irrespective of the photoperiod (total darkness, 8-hour short day and continuous illumina-

TABLE 17.

Effect of photoperiods on floral initiation of *Pharbitis* plant at 15° and 25°. Plants were cultured on media containing 5% sucrose or no sucrose.

i) Sucrose medium

Temperature	Light condition	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
15°	D*	17	100	3.4	100
	D	20	100	3.3	100
	S	21	100	4.7	100
	L	18	100	4.4	100
25°	D	15	0	0	0
	S	16	100	3.8	100
	L	20	0	0	0

ii) Sucrose deficient medium

15°	D	15	0	0	0
	S	18	0	0	0
	L	14	0	0	0
25°	D	—*	—*	—*	—*
	S	16	100	4.1	100
	L	23	0	0	0

D*: cultured under total darkness for 100 days and then observed immediately.

D: Total darkness for 30 days.

S: 8-hour illumination of 2000 lux from daylight fluorescent lamps followed by a 16-hour period for 30 days.

L: Continuous illumination of 2000 lux from daylight fluorescent lamps. *All plants had withered and died.

tion). On the other hands, all of the plants cultured on media containing no sucrose at 15° remained vegetative under all photoperiods used in the present experiment. The plants cultured at 25° initiated floral primordia only under short day conditions on the both media containing sucrose and no sucrose, but did not initiate under continuous illumination and in total darkness. This indicated that floral initiation of the plants cultured at 25° was controlled by photoperiod, but not by sucrose added to the medium. On the other hand, floral initiation of the plants cultured at 15° was controlled by sucrose supply, but not by photoperiod. Takimoto (73) reported that *Pharbitis* plants cultured on sucroseless medium at 10° initiated flower buds under short day condition, but not under continuous illmination. In this experiment, the plants were exposed to short days for 90 days, but in the present experiment for only 30 days. *Pharbitis* plants cultured on sucroseless medium grew very slowly at low temperature. Very young seedlings are not sensitive to photoperiod (42), and when the plants

are grown at low temperature, it seems to take long durations for them to get photoperiodic sensitivity. In the present experiment, the plants were exposed to short days from the start of germination; therefore, the short days given during the early stages are believed to be ineffective. If the plants cultured on sucroseless medium were exposed to short days for a much longer period, they might initiate flower buds even at low temperature.

4. *Differences in Flowering Behavior Induced by Short Photoperiod and by Low Temperature*

In the experiments mentioned above, the plants cultured on sucrose medium at 15° for 30 days initiated floral primordia irrespective of the light conditions, i.e., colors and intensities of light and photoperiods (total darkness, 8-hour short days and continuous illumination). It is interesting that all of the plants that initiated flower buds at 15° had always terminal flower, and that in all the treatments shown in Tables 15–17, the values in percentage of flowering plants are the same as those in percentage of plants with terminal flower. Usually *Pharbitis* plants subjected to short days initiate axillary flower buds without initiating the terminal flower when the photoperiodic stimulus is weak. Terminal flower buds were developed only when the plants received a strong flowering stimulus.

To compare the flowering behavior induced by short days at ordinary tem-

TABLE 18.

Flowering response under short days at 25°. Plants were cultured on a medium containing 5% sucrose and kept under continuous illumination at 25° for 5 days after the germination, and then subjected to 8-hours short photoperiod.

Duration of short day treatment in days	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	15	33.3	0.3	0
3	14	85.7	2.5	7.1
5	17	94.2	4.1	58.8

TABLE 19.

Flowering response under continuous illumination at 15°. Plants were cultured on a medium containing 5% sucrose and subjected to continuous illumination at 15° immediately after the germination.

Duration of cool treatment in days	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
15	15	33.3	0.9	33.3
20	12	83.3	3.4	83.3
30	15	100	4.0	100

perature with that induced by low temperature, following experiments were made. Plants were cultured on a medium containing 5% sucrose. One group of the plants was cultured at 25° and exposed to continuous illumination for 5 days after germination. Subsequently they were subjected to 8-hour short days for 1, 3 and 5 days. After the short day treatment they were kept under continuous illumination at 25°. Another group was cultured at 15° under continuous illumination from the start of germination for 15, 20 and 30 days. After the end of the treatment they were transferred to 25° and exposed to continuous illumination. Table 18 shows that flowering percentage of the plants exposed to 3 short days was 85.7%, but only 7.1% of the plants initiated terminal flower. On the contrary, when plants were exposed to cool temperatures, percentage of flowering plants and percentage of the plants with terminal flower buds showed the same values in each treatment (Table 19).

When plants were exposed to 15° for 15 days, only 33.3% of them initiated flower buds, but all of the flowering plants had the terminal flower. Differences in position of the flower buds induced by short photoperiod and low temperature are shown shematically in Fig. 2. These differences in position of flower primordia owing to temperature may be due to a difference in the activity of lateral and terminal growing point, which can be differentially influenced by temperature.

Generally, with *Pharbitis* seedlings a single 16-hour dark period is enough to induce a maximum flowering response at 25° when planted in clay pots containing garden soil (20, 74). In vitro, however, *Pharbitis* seedlings were induced to flower only to a slight extent when subjected to one short day at 25° (a 16-

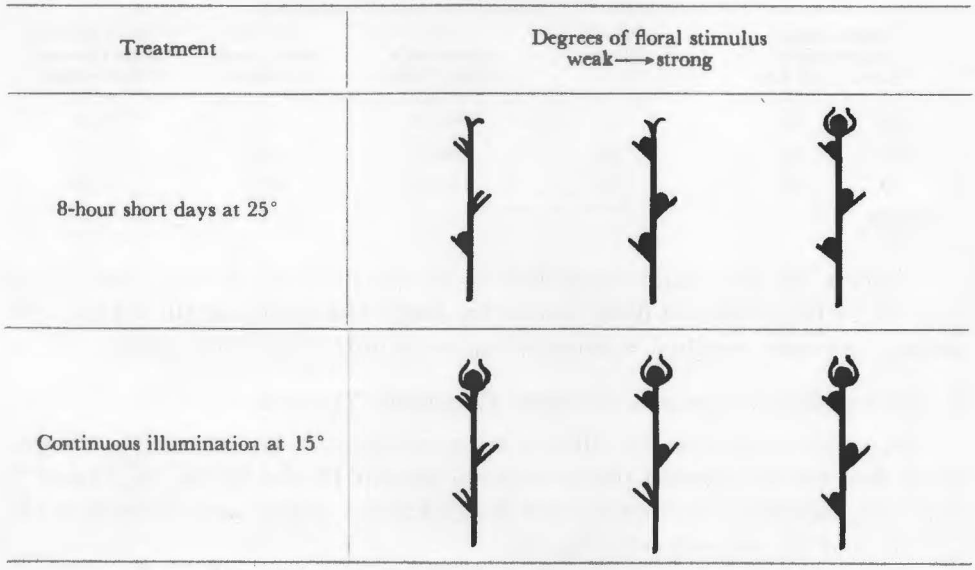


Fig. 2. Differences in position of flower buds developed under short days at 25° and under continuous illumination at 15°

hour dark period), and 5 such short days were not enough for a maximum flowering response. The difference in photoperiodic sensitivity may be due to the cultural condition, but the details remain to be investigated.

Results mentioned in the present chapter suggested that the flowering response of *Pharbitis* plants subjected to continuous illumination at 15° is different from the photoperiodic response, and the low temperature participates in the flowering process independently of light.

VI. INFLUENCE OF HIGH TEMPERATURE ON LOW TEMPERATURE EFFECT

In the present experiments, the effect of high temperatures given after or during low temperature treatment was examined on floral initiation in *Pharbitis nil* under continuous illumination.

1. *Effect of High Temperature in Alternating Temperature Treatment*

As was mentioned in the above experiment (Chapter III), daily alternations of 10° for 16 hours and 25° for 8 hours resulted in floral initiation under continuous illumination. In the following experiment, plants were exposed to various alternating temperatures (i.e., 10° for 16 hours and 20°, 25° or 30° for 8 hours) for 30 days and then transferred to 25° under continuous illumination (Table 20).

TABLE 20.

Flowering response of *Pharbitis* plants subjected to continuous illumination at various alternating temperatures (10° for 16 hours and 20°, 25°, 30° for 8 hours) for 30 days.

Alternating temperature 16 hrs.	8 hrs.	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
10°	20°	15	93.3	4.2	93.3
10°	25°	18	94.5	4.3	94.5
10°	30°	16	25.0	0.6	25.0

Almost all the plants subjected to 10° (16 hrs.)–20° (8 hrs.) and 10° (16 hrs.)–25° (8 hrs.) initiated floral primordia. Daily alternation of 10° (16 hrs.)–30° (8 hrs.), however, resulted in floral initiation in only 25% of the plants.

2. *Effect of High Temperature after Low Temperature Treatment*

In order to examine the effect of temperature after low temperature treatment, five lots of *Pharbitis* plants were cultured at 15° for 10, 15, 20, 25 and 30 days and thereafter each group was divided into 4 groups and cultured at 20°, 25°, 30° and 35°, respectively (Fig. 3).

When the plants were cultured at 15° for 20 days or less, flowering response was greatly influenced by subsequent cultural temperatures. It decreased with

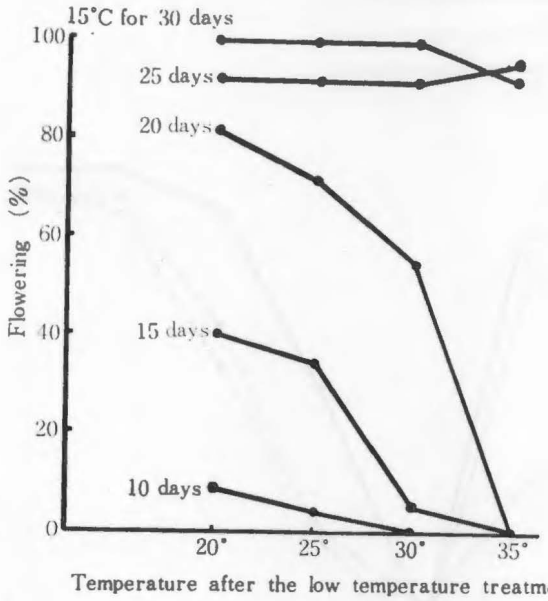


Fig. 3. The effect of high temperature after the low temperature treatment at 15° for various durations.

increasing subsequent temperatures from 20° to 35°. Even the plants cultured at 15° for 20 days did not initiate floral primordia when transferred to 35° after chilling. On the contrary, flowering percentages of plants which were cultured at 15° for 25 and 30 days were not influenced by the subsequent temperatures. It is noteworthy that when *Pharbitis* plants received low temperature treatments for sufficient duration, i.e., 25 days or more, floral initiation was not inhibited by subsequent high temperature. Flowering response of plants which were subjected to low temperature of 15° for 20 days or less seems to remain in an unstable state.

3. *Effect of High Temperature during Low Temperature Treatment*

The present experiment is concerned with the effect of high temperature which was given before, during or after the low temperature treatment. Plants were subjected to 15° for 30 days in total, which were interrupted by 5 warm days of 20°, 25° and 30° at various times (Fig. 4).

High temperature treatment for 5 days before low temperature treatment of 15° reduced flowering responses, and the flowering percentage decreased as temperature rose from 20° to 30°. As the high temperature interruption of chilling was delayed, the inhibitory effect became much stronger, and the interruption given from 10th to 15th days after the start of cold treatment reduced flowering responses most strikingly. When the high temperature interruption was begun 25 or 30 days after the start of low temperature treatment, the flowering response was not inhibited and almost all the plants initiated floral primordia.

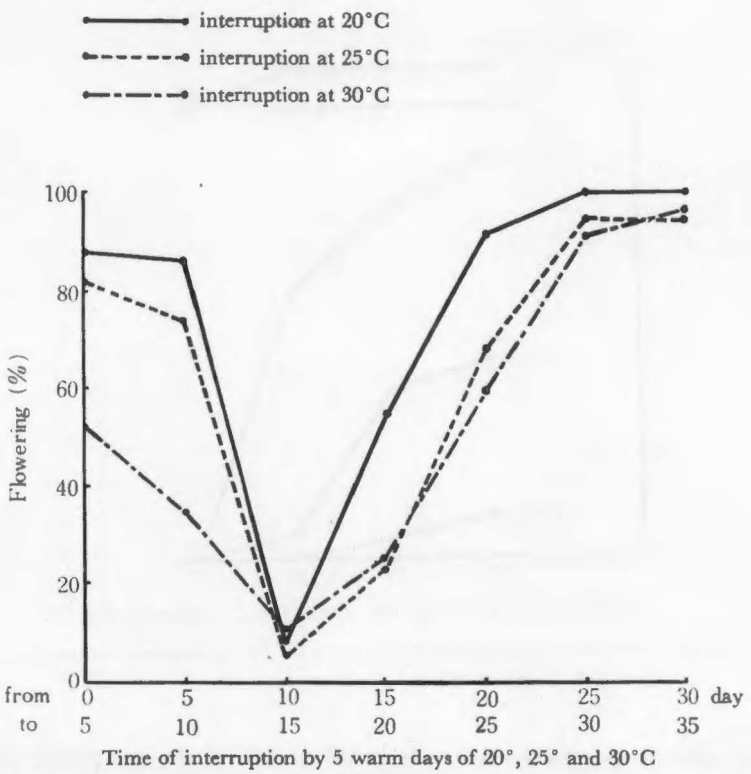


Fig. 4. The effect of interruption by 5 warm days of 20°, 25° and 30° which were given at various times during the low-temperature treatment of 15°.

4. *Discussion*

To obtain the maximum flowering response, *Parbitis* plants must be exposed to low temperature for 25–30 days or more. High temperature (35°) given after the 25–30 days of cold treatment does not influence the flowering responses. As was mentioned in Chapter III floral primordia seemed to be formed during the 25–30 days of cold treatment (For details see Chapter X). After the floral primordia have been formed, high temperature may not have any effect on flowering response. However, if the plants were exposed to low temperature for an insufficient duration (10–20 days), flowering responses were suppressed by subsequent exposure to high temperature, and the higher the subsequent temperature, the more striking was the flower inhibition. In this case, the floral primordia may not be formed during the low temperature treatment, and as long as the primordia are not established the following high temperature may inhibit flowering responses.

Furthermore, when the low temperature treatment was interrupted by 5 days of high temperature from 11th to 15th days of the cold treatment, flowering responses were reduced strikingly. These results indicate that the effect of the first phase of cold treatment is unstable and that the chilling must be

given continuously to bring about a maximum flowering response.

In contrast to the case of *Pharbitis*, in vernalization the effect of cold treatment is reduced by subsequent exposure to high temperature even if the plants were vernalized sufficiently (4, 36, 47, 59). Moreover, an interrupted cold treatment is effective for vernalization, i.e., the effects of cold treatments interrupted by ordinary temperature are additive; but such is not the case in *Pharbitis*. Therefore, effect of low temperature on floral initiation of *Pharbitis* may be different from that in vernalization. The details of this problem will be discussed later (cf. General consideration).

VII. EFFECT OF AGE OF SEEDLINGS AT THE START OF LOW TEMPERATURE TREATMENT

In the experiments mentioned in the previous chapters, floral primordia were initiated under continuous illumination when *Pharbitis* plants were subjected to low temperature immediately after germination. In the present experiment, plants were cultured first at warm temperature under continuous illumination or in darkness for several days and subsequently subjected to cold temperature under continuous illumination for 30 days. Observations were made 50 days after germination.

1. *Effect of Age of Seedling Grown under Continuous Illumination*

(1) Seeds were planted on media containing 5% sucrose or no sucrose and were kept at 25° under continuous illumination for 0, 2, 4, 8, 12 and 16 days, and subsequently exposed to continuous illumination at 15° for 30 days. The results shown in Table 21 indicate that the flowering responses to low temperature treatment differ greatly owing to the age of the seedlings at the start of cold treatment.

On sucrose deficient media floral primordia were not initiated when plants were exposed to 15° immediately after germination, whereas plants cultured at 25° for several days before cold treatment initiated some floral primordia. Almost all the plants that were exposed to 25° for 4 days initiated floral primordia. With the plants exposed to 25° for 8 days or more, the flowering percentages decreased gradually as the durations of treatment at 25° before the cold treatment were prolonged.

On sucrose medium, all the plants initiated floral primordia when they were exposed to 15° immediately after planting. Flowering responses decreased with increasing duration of pretreatment at 25°. Plants cultured at 25° for 12 days did not initiate floral primordia.

(2) Another experiment repeated gave entirely similar results as shown in Table 22 and Fig. 5. Eight groups of plants were cultured at 25° for 0, 2, 4, 6, 8, 10, 15, 20 and 25 days respectively, and thereafter they were exposed to continuous illumination at 15° for 30 days.

On sucrose deficient media, flowering responses increased with increasing culture duration at 25° from 0 to 6 days and decreased with further increase

TABLE 21.

The effect on floral initiation of a pretreatment at 25° prior to low temperature treatment. Plants were cultured at 25° under continuous illumination for various days and subsequently subjected to continuous illumination at 15° for 30 days.

Sucrose conc. (%)	Days of pretreatment at 25°	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
0	0	10	0	0	0
	2	16	50.0	1.2	43.8
	4	13	92.4	4.3	92.4
	8	11	63.6	1.5	54.5
	12	13	46.2	1.2	23.0
	16	16	37.5	0.8	0
5	0	12	100	3.8	100
	2	15	100	3.9	100
	4	18	94.5	4.3	94.5
	8	19	10.5	2.6	0
	12	20	0	0	0
	16	19	0	0	0

TABLE 22.

The effect on floral initiation of a pretreatment at 25° prior to low temperature treatment. Plants were cultured at 25° under continuous illumination for various days and subsequently subjected to continuous illumination at 15° for 30 days.

Sucrose conc. (%)	Days of pretreatment at 25°	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
0	0	18	0	0	0
	2	13	23.1	0.7	23.1
	4	20	50.0	1.5	40.0
	6	21	100	3.9	100
	8	17	88.3	3.4	88.3
	10	24	70.8	2.4	33.3
	15	18	44.4	1.2	22.2
	20	23	34.8	1.3	13.1
	25	22	13.6	0.1	0
5	0	18	100	4.1	100
	2	18	100	3.9	100
	4	17	82.4	3.4	82.4
	6	22	22.8	0.9	13.4
	8	18	11.1	0.2	7.7
	10	19	0	0	0
	15	22	0	0	0
	20	24	0	0	0
	25	23	0	0	0

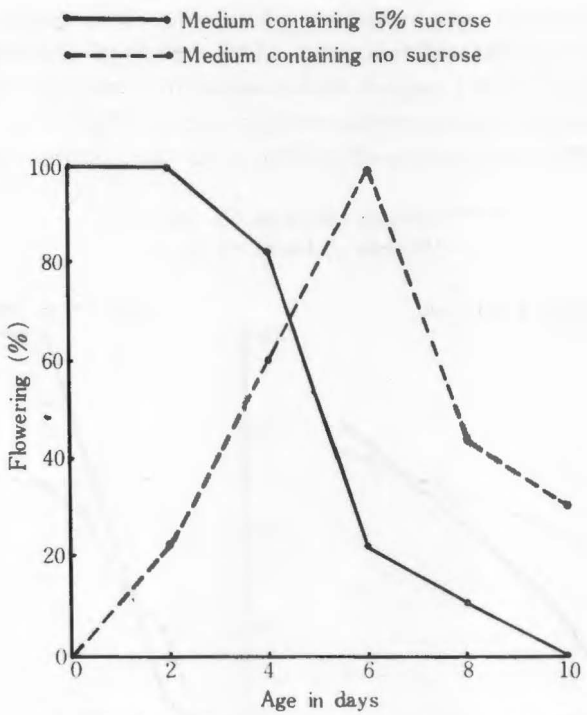


Fig. 5. The effect of age of seedlings to low temperatures effect. Plants were cultured at 25° under continuous illumination for various days and then subjected to continuous illumination at 15° for 30 days.

in culture duration at 25°. The plants cultured at 25° for 6 days showed a maximum flowering response. On sucrose media, all the plants exposed to low temperature immediately or two days after the planting initiated floral primordia. Flowering responses decreased with increasing pretreatment at 25°, and the plants cultured at 25° for 10 days or more did not initiate floral primordia at all. The maximum flowering responses were brought about when the plants were exposed to low temperature at 15° immediately after germination.

2. *Age and Growth*

In connection with the flowering response mentioned above, growth pattern of the plants was observed. Plants were cultured on media containing sucrose or no sucrose under continuous illumination at 25°.

As shown in Fig. 6-a, shoot length increased rapidly during the culture period. Somewhat higher values were obtained on sucrose deficient medium as compared with those on media containing 5% sucrose.

Fresh weight increased and attained maximum values about 10 days after the planting on both media (Fig. 6-b). However, the maximum value of the fresh weight was greater on the sucrose medium than on the sucrose deficient medium.

Dry weight was greatly increased by the sucrose added and increased rapidly during the first 2–10 days after sowing. Dry weight of 23.6 mg at the start of planting increased to 70.1 mg on media containing sucrose after 30 days, but increased only to 32.4 mg on media without sucrose (Fig. 6–c).

Chlorophyll content increased rapidly at 25° and showed maximum values

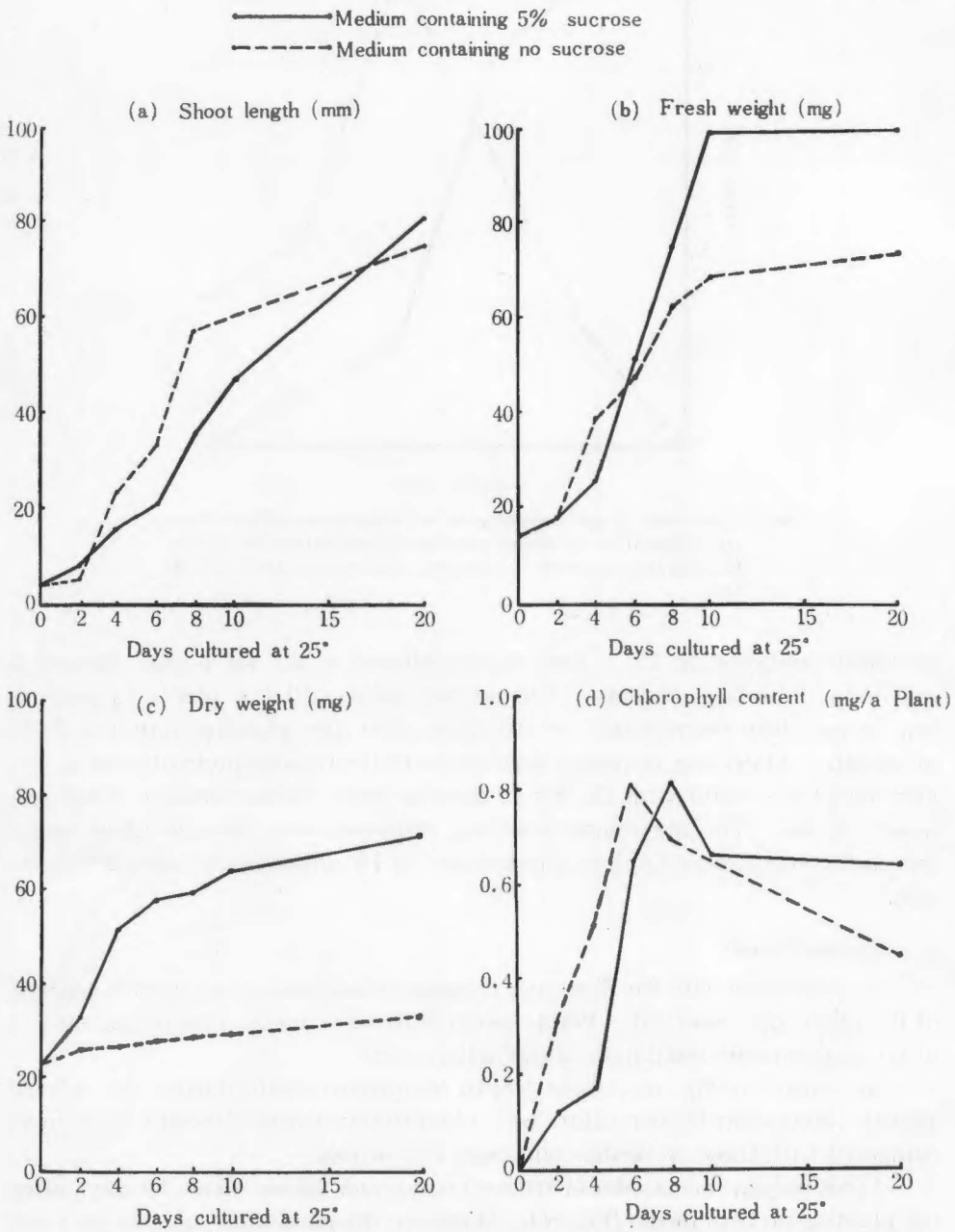


Fig. 6. Growth of *Pharbitis* plant subjected to continuous light (2000 lux) at 25° in vitro.

6–8 days after the planting. Thereafter it decreased gradually. At an earlier age, chlorophyll content of plants which grew on sucrose deficient media increased more rapidly than on media with sucrose, attaining its maximum 2 days earlier on the former than on the latter (Fig. 6-d).

3. *Effect of Age of Seedling Grown under Darkness*

Fig. 5 and Fig. 6-d show that on sucrose deficient media flowering response to low temperature and chlorophyll content reach their maxima 6 days after planting, indicating that there may exist a correlation between them. To examine this possibility, plants were cultured in darkness for various periods prior to low temperature treatment of 15°. Plants were cultured for 0, 2, 4, 6, 8 and 10 days in darkness at 25°, and etiolated seedlings were exposed to continuous illumination at 15° for 30 days. As shown in Fig. 7, the results

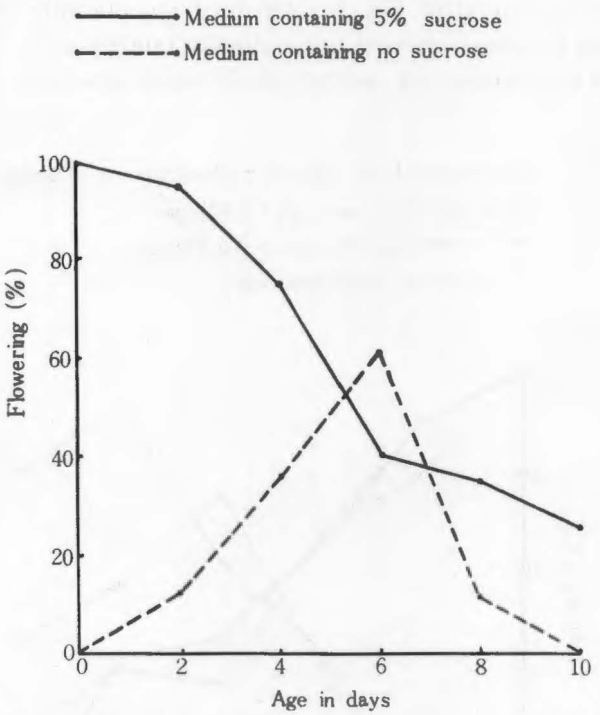


Fig. 7. The effect of age of seedlings to low temperature effect. Plants were cultured at 25° under darkness for various days and then subjected to continuous illumination at 15° for 30 days.

remained similar to those in Fig. 5. Flowering responses have the same tendency irrespective of the light condition before low temperature treatment. On media containing no sucrose floral initiation did not occur when the plants were subjected to low temperature immediately after germination. Plants kept in darkness for 6 days at 25° prior to the cold treatment brought about a

maximum flowering response. However, the flowering responses were weaker as compared with the plants grown in continuous light before the cold treatment. On sucrose medium, flowering responses decreased with increasing duration in darkness at 25° as well as in continuous light. Almost all the plants that were cultured in darkness at 25° for 10 days or more died when exposed subsequently to continuous light at 15°, so that the data were omitted in Figure.

4. *Effect of Nutritive Components*

In order to examine the effect of nutritive components and age of seedlings on the flowering response at low temperature, plants were cultured on the following series of culture media: A, 0.8% plain agar; MA, minerals+0.8% agar; SA, 5% sucrose+0.8% agar; MSA, minerals+5% sucrose+0.8% agar. They were exposed to continuous illumination at 25° for various days and subsequently subjected to low temperature (15°) for 30 days. The results of the experiments are presented in Fig. 8. On media containing sucrose (SA and MSA), flowering responses showed quite similar tendencies to those in Fig. 5. On sucrose deficient media too, similar results were obtained, but on medium

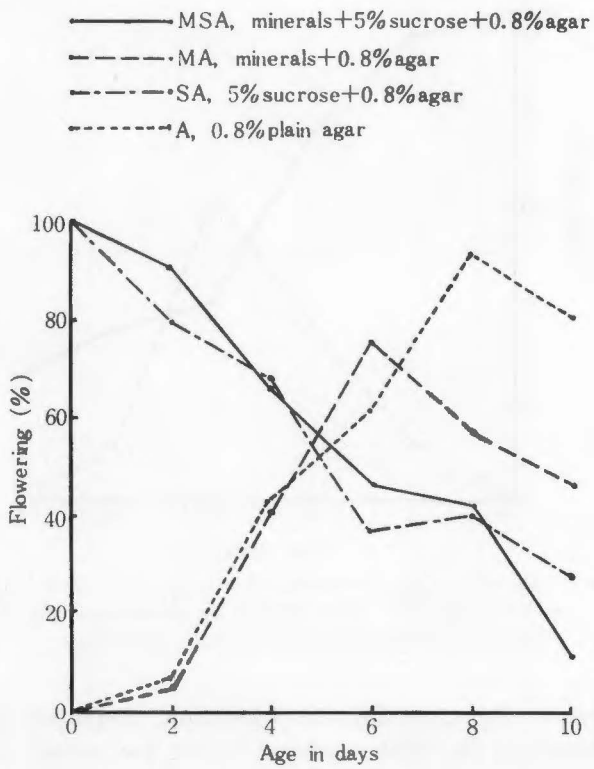


Fig. 8. The effect on floral initiation of ages and nutritive components. Plants were cultured at 25° under continuous illumination and then subjected to continuous illumination at 15° for 30 days.

A, a maximum flowering response was brought about in plants which were exposed to 25° for 8 days instead of 6 days. Another experiment similar to that mentioned above was carried out, but in this experiment plants were kept in darkness at 25° for various days prior to the cold treatment. The results shown

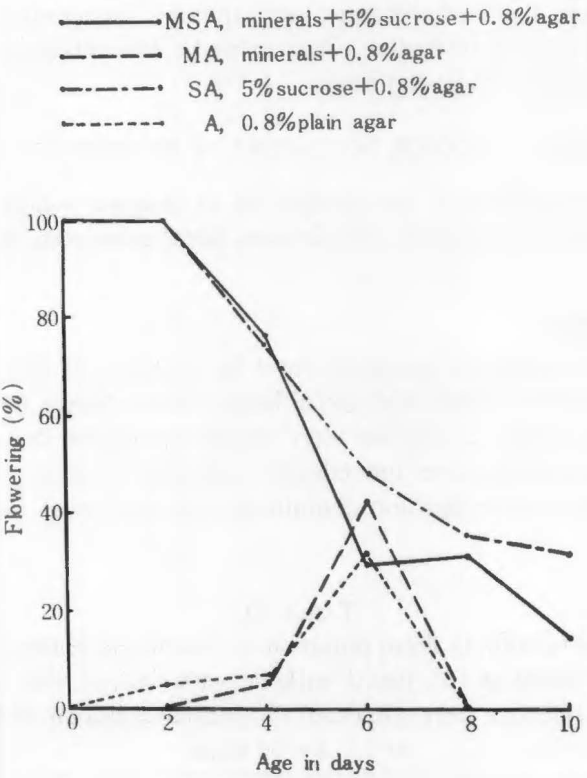


Fig. 9. The effect on floral initiation of age and nutritive components. Plants were cultured at 25° under darkness and then subjected to continuous illumination at 15° for 30 days.

in Fig. 9 are quite similar to those shown in Fig. 8. On media containing no sucrose (A and MA), however, flowering responses showed lower values than when plants were cultured in light before the cold treatment.

5. *Discussion*

Influence of age upon the effect of low temperature in *Pharbitis nil* was greatly affected by the presence or absence of sucrose in culture medium. The maximum flowering response was observed when treatmentt with low temperature of 15° were started immediately after germination on sucrose medium. On sucrose deficient medium, however, 6–8 day old plants were the most sensitive to the cold treatment. As shown in Fig. 6-d, chlorophyll content of the seedlings showed the maximum value 6–8 days after germination. However,

as was shown in Fig. 7, plants cultured on sucrose deficient media showed the maximum floral response when they were cultured in darkness for 6 days at 25° before cold treatment. They had no chlorophyll at the start of the cold treatment. Therefore, the sensitivity to low temperature may not have direct connection with the increase of chlorophyll content.

It seems that the carbohydrate, age and low temperature have a close relationship with floral initiation. But to make the relationship clear, more detailed experiments will be required.

VIII. FLORAL INITIATION IN CULTURE OF DECOTYLATED EMBRYO

The present experiment was carried out to examine whether the plumule deprived of cotyledon is capable of initiating floral primordia under continuous illumination.

1. *Effect of Cotyledon*

Seeds were swollen in sterilized water for 24 hours at 25°. Then the seed coats were removed by hand, and apical halves of cotyledons in one group and both cotyledons entirely in another were cut off aseptically by scissors. These plants and intact plants were immediately exposed to continuous illumination at 15° on White's culture medium containing 5% sucrose or no sucrose for 30 days (Table 23).

TABLE 23.

Effect of cotyledon in floral initiation of *Pharbitis nil* under continuous illumination at 15°. Plants without, with half, or with intact cotyledons were subjected to continuous illumination at 15° for 30 days.

Sucrose conc. (%)	Condition of cotyledon	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm
0	None	—	—	—	—
	Half	—	—	—	—
	Intact	8	0	0	21.5
5	None	15	0	0	15.9
	Half	10	30.0	0.5	25.9
	Intact	14	100	3.4	27.0

On sucrose deficient medium, the plants whose cotyledons were reduced or cut off were all dead. Intact plants did not initiate floral primordia as in the previous experiments. On sucrose medium, plants without cotyledon grew meagerly and could not initiate floral primordia. With the plants with reduced and intact cotyledons the flowering percentages were 30 and 100, respectively. Cotyledons may be necessary for floral initiation in *Pharbitis* plants under con-

tinuous illumination at 15°.

2. *Effect of Alternating Temperature Treatment*

In the above experiment, the plants entirely deprived of cotyledon grew poorly at 15° as compared with intact plants. As the low temperature treatment for 30 days seemed to be too short for optimal flowering, the excised plumules were kept at 15°, 10° (16 hrs.)-25° (8 hrs.), 10° (18 hrs.)-25° (6 hrs.) and 25° for 70 days. As shown in Table 24, no plants initiated floral primordia.

TABLE 24.

Culture of decotylated embryo at various temperatures. Embryo axes cultured on a medium containing 5% sucrose were exposed to continuous illumination at various temperatures for 30 and 70 days.

Temperature treatment	Duration of culture in days	No. of plants	% of plants with flower buds	Shoot length in mm
15°	30	12	0	17.5
	70	8	0	26.1
10° (16 hrs.) −25° (8 hrs.)	30	14	0	18.3
	70	13	0	25.6
10° (18 hrs.) −25° (6 hrs.)	30	12	0	17.9
	70	14	0	24.3
25°	70	14	0	33.3

3. *Effect of Some Substances Added to the Medium*

In the above experiments, the plants could not initiate floral primordia without cotyledons. In an attempt to search for a substitute of cotyledon, several substances were added to the basal medium containing 5% sucrose. Ebios*, casein hydrolysate, IAA, gibberellin and kinetin were tested. The plumules on these media were exposed to continuous illumination at 15° for 30 days. The results are shown in Table 25. None of these substances used had any effect on floral initiation. Shoot length showed higher values on media containing Ebios (0.1, 1%), casein hydrolysate (0.1, 0.5%), IAA (1 ppm), gibberellin (0.1, 1 ppm) or kinetin (1 ppm) as compared with those on media containing sucrose only.

4. *Floral Initiation in Culture of Decotylated Embryo*

(1) In the above experiments, plumules were exposed to 15° immediately after cotyledon were cut off. Shoot lengths of plants were markedly small at the end of the experiment. Embryo axis of *Pharbitis* from the swollen seeds was 4.9

* Dried brewer's yeast manufactured by the Dainippon Vitamin Seiyaku K. K., Tokyo

TABLE 25.

Influences of some substances on flowering response and shoot length in culture of embryo axis at 15° for 30 days.

Components of culture medium	No. of plants	% of plants with flower buds	Shoot length in mm
Sucrose 5%	17	0	17.8
〃 7.5%	12	0	17.0
〃 10%	18	0	15.2
Sucrose 5% + Ebios 0.1%	15	0	20.0
〃 + 〃 1%	15	0	19.6
Sucrose 5% + Casein hydrolysate 0.1%	18	0	19.3
〃 + 〃 〃 0.5%	16	0	24.4
Sucrose 5% + IAA 0.1 ppm	14	0	17.5
〃 + 〃 1 ppm	15	0	24.6
Sucrose 5% + Gibberellin 0.1 ppm	13	0	21.0
〃 + 〃 1 ppm	12	0	20.9
Sucrose 5% + Kinetin 0.1 ppm	8	0	14.8
〃 + 〃 1 ppm	12	0	19.1

mm in length and became 15 mm at the end of the low temperature treatment of 15° for 30 days. Epicotyl grew scarcely.

In the next experiments, decotylated embryos on media containing 5% sucrose were exposed to continuous illumination at 25° for 2, 5 and 8 days before cold treatment. Thereafter they were subjected to continuous illumination at 15° for 30 days (Table 26). In the plants pretreated at 25° for 8 days, hypocotyls grew to 15 mm in length before the start of the cold treatment, but epicotyls were not visible. The plants exposed to 25° for 0 or 8 days did not initiate floral

TABLE 26.

The effect of various pretreatment at 25° in culture of decotylated embryo. Embryo axes on a medium containing 5% sucrose were cultured under continuous illumination at 25° for several days and then exposed to continuous illumination at 15° for 30 days.

Ages of plant when treated at 15°	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm
0	17	0	0	15.9
2	20	5.0	0.1	20.8
5	21	33.3	0.9	36.7
8	22	0	0	43.4

primordia by the subsequent exposure to 15°. A few plants which were exposed to 25° for 2 or 5 days prior to the cold treatment initiated floral primordia. The percentages of flowering plants were 5.0 and 33.3%, respectively.

(2) To know the effect of nutrients the above experiment was repeated with different media. Decotylated embryos were cultured on basal medium containing 5% sucrose, 8% sucrose and 5% sucrose+0.5% Ebios, and exposed to 15° for 50 days under continuous illumination (Table 27).

TABLE 27.
The effect of various pretreatment at 25° in culture of decotylated embryo. Embryo axes on various media were cultured under continuous illumination at 25° for various days and then exposed to continuous illumination at 15° for 50 days.

Components of media	Ages of plants when treated at 15°	No. of plants	% of plants with flower buds	No. of flower buds per plant	Shoot length in mm
5% sucrose	0	12	0	0	16.5
	2	24	41.6	1.1	19.8
	5	24	33.3	0.6	23.1
	8	16	6.3	0.1	27.5
8% sucrose	0	9	0	0	16.7
	2	9	0	0	18.6
	5	12	50.0	1.4	20.8
	8	9	33.3	0.7	22.2
5% sucrose + 0.5% Ebios	0	14	0	0	18.7
	2	19	36.8	1.0	22.8
	5	23	43.5	1.2	33.3
	8	17	11.7	0.3	37.5

When the plants were cultured on 5% sucrose medium, the maximum flowering percentage, 41.6%, was obtained in the lot which was pre-cultured at 25° for 2 days. However, on media containing 8% sucrose or 5% sucrose+0.5% Ebios, the maximum flowering percentages, 50.0% or 43.3% respectively, were obtained when the plants were pre-cultured at 25° for 5 days.

In the present experiments, the plants from the excised embryo are scarcely developed leaves during the low temperature treatment. At the end of low temperature treatment of 15° for 50 days, only two or three rudimentary leaves of about 4 mm in length were visible. They nevertheless initiated floral primordia to some extent at relatively low temperature. Therefore plumule itself seems to be susceptible to low temperature as in the case of vernalization (4, 10, 11, 23, 28, 56, 57, 58, 59, 77, 86, 87, 88, 89).

IX. EXPERIMENTS WITH OTHER STRAINS OF *PHARBITIS NIL* AND RELATED PLANTS

In the present experiments, effect of low temperature upon floral initiation was examined with several strains of *Pharbitis* and *Quamoclit* species. The materials used were as follows: strain "Africa"*, "Purple"**, "Scarlet"***, "Kidachi", "Tendan", "Violet" of *Pharbitis nil* and *Quamoclit pennata* and *Q. angulata*. The basic culture medium was a modified White's solution containing 5% sucrose and 0.8% agar. All the plants were grown under continuous illumination from daylight fluorescent lamps, the luminosity of which was from 1500 to 2000 lux at the plant level. They were kept at 15° for 30 days, and cultured at 25° for additional 20 days, at the end of which time they were dissected for the observation. Control plants were exposed to continuous illumination at 25° and dissected 50 days after germination.

TABLE 28.

Floral initiation in various strains of *Pharbitis nil*, *Quamoclit pennata* and *Q. angulata*. Plants were subjected to continuous illumination at 15° for 30 days. Control plants were exposed to continuous illumination at 25° for 50 days.

a) Cultures at 15°

Strains	No. of plants	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
Pharbitis nil "Africa"	16	0	0	0
"Kidachi"	15	33.3	0.7	0
"Purple"	23	83.7	4.0	78.4
"Scarlet"	16	43.7	2.2	37.5
"Tendan"	22	81.8	2.6	81.8
"Violet"	23	100	4.7	100
Quamoclit pennata	9	33.3	0.7	0
// *angulata*	30	53.4	0.8	0

b) Cultures at 25°

Pharbitis nil "Africa"	16	0	0	0
"Kidachi"	16	0	0	0
"Purple"	16	0	0	0
"Scarlet"	15	0	0	0
"Tendan"	16	0	0	0
"Violet"	24	0	0	0
Quamoclit pennata	15	0	0	0
// *angulata*	17	0	0	0

* Collected 1960 by Dr. Kazuo Furusato in Central Sudan.

** "Purple flower with white margin" from Dr. S. Nakayama.

*** Secured by the courtesy of late Mr. T. Megata.

As shown in Table 28, almost all the strains of *Pharbitis* and *Quamoclit* plants initiated floral primordia under continuous illumination at 15° except strain "Africa". The flowering percentages of "Purple" and "Tendan" showed similar values as "Violet". *Quamoclit* also initiated floral primordia to some extent under continuous illumination at 15°. But control plants cultured under continuous illumination at 25° did not initiate floral primordia in any strains of *Pharbitis* and *Quamoclit* plants.

X. GROWTH RATE AND FLOWERING

As shown in the foregoing experiments, *Pharbitis nil* initiated floral primordia under continuous illumination at 15°, but did not at 18° or higher temperature. Maximum flowering was observed when plants were cultured on sucrose medium at 15° for 30 days. Plants cultured at 15° grew poorly as compared with those at 25° and it seems that floral initiation in *Pharbitis nil* exposed to continuous illumination at 15° is associated with the poor vegetative growth.

In the present experiment, observations have been made on the vegetative growth at various temperatures. Plants were cultured on White's media containing 5% sucrose or no sucrose and exposed to continuous illumination at 10°, 15°, 20°, 25° and 30° for 30 days. The luminosity of light was from 1500 to 2000 lux from daylight fluorescent lamps at the plant level. Every 10 days through the experiments of 30 days, 10 plants were sampled at randam to examine shoot length, fresh weight, dry weight, chlorophyll content and the number of leaves differentiated. Chlorophyll content was determined by the method proposed by Koski (30).

(1) *Shoot Length* (Fig. 10)

The mean length of embryo axes from the swollen seeds was 4.9 mm. On sucrose deficient media, shoots increased almost linearly in length throughout the experimental period and no significant difference was found between the plants cultured at 20°, 25° and 30°. At 15°, the increase in shoot length was strongly suppressed. On sucrose medium, shoot length increased rapidly at 20° or higher temperatures for the first 10 days after planting, but thereafter its growth rate reduced gradually. Shoot length at 30° nearly attained its maximum value at 10th days. The temperature of 20°–25° was favorable for the shoot elongation. Shoot length of plants cultured at 15° showed considerably lower values as compared with those at 20° or higher. At the end of the experiment of 30 days, plants were shorter on sucrose medium than those on sucrose deficient medium. At 10°, shoots scarcely elongated on both media during 30 days.

(2) *Fresh Weight* (Fig. 11)

The fresh weight of plants at the start was 74.8 mg on the average. On sucrose deficient media, fresh weight of seedlings showed higher values at 20° and 25°, and relatively lower values at 30°. At 15°, considerably lower value

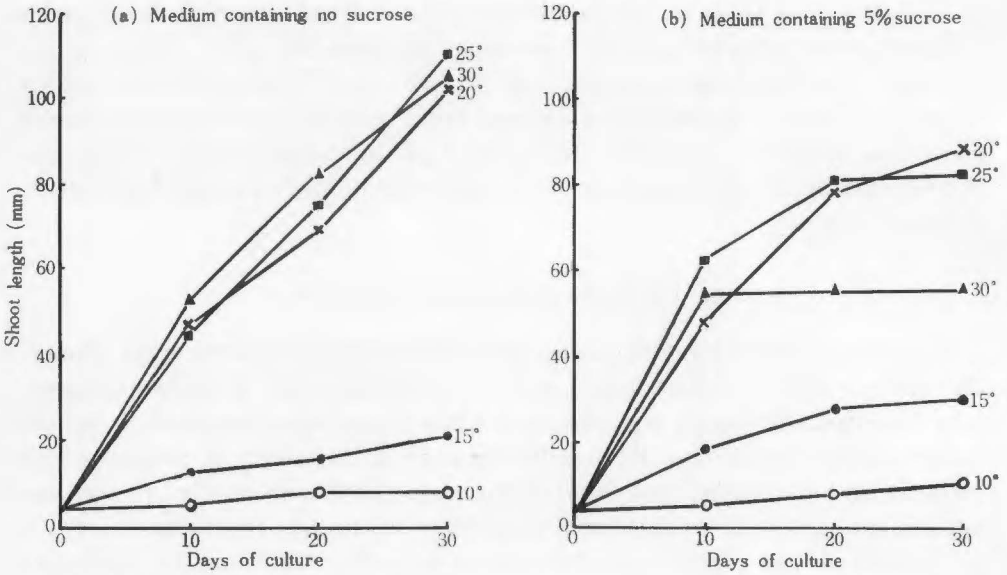


Fig. 10. Shoot length of *Pharbitis nil* cultured under continuous illumination in vitro.

was obtained. On sucrose media, fresh weight increased rapidly at 20° or higher temperatures during the first 10 days often decreased later. At 20° or higher temperatures the maximum values were obtained in 10 days. This was caused by the withering or death of cotyledons. At 15°, cotyledons did not die, but fresh weight was smaller. At the end of the experiment at 10° for 30 days, fresh weight of the plant was 110 mg on sucrose medium and only 99.8 mg on sucrose deficient medium.

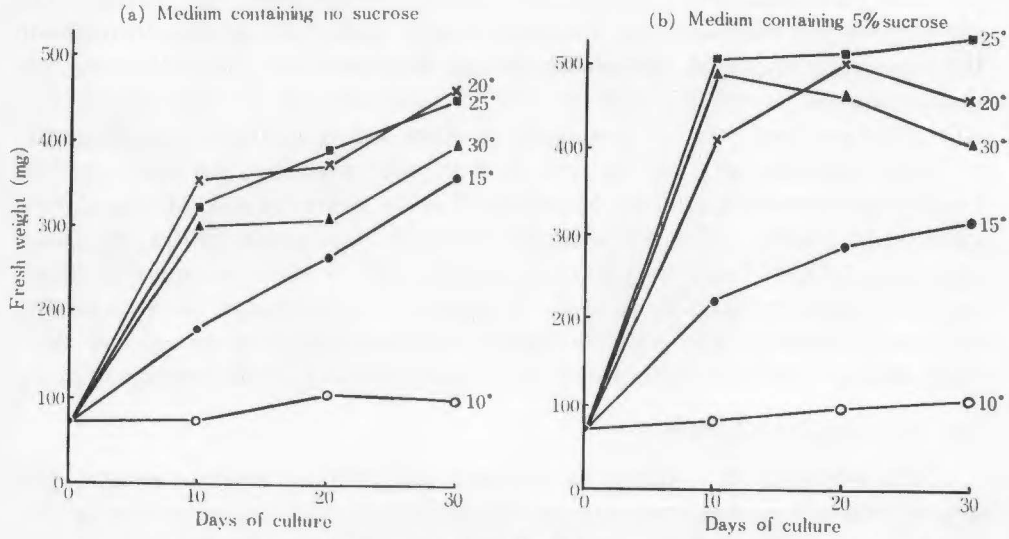


Fig. 11. Fresh weight of *Pharbitis nil* cultured under continuous light (2000 lux) in vitrro.

(3) *Dry Weight* (Fig. 12)

The dry weight of plants at the start was 23.6 mg on the average. There was a marked difference in the dry weight between the seedlings grown on sucrose and sucrose deficient media.

On sucrose deficient medium, only small increase in dry weight occurred at various temperatures during the first 30 days and no significant differences in dry weight were found between the plants cultured at 15° and 30°. On sucrose medium, dry weight increased considerably. The increase, however, was hardly influenced by the temperature in the range of 15° to 30°. At 10°, dry weight scarcely increased on sucrose deficient medium, while on sucrose medium it increased to a slight extent with increasing culture duration.

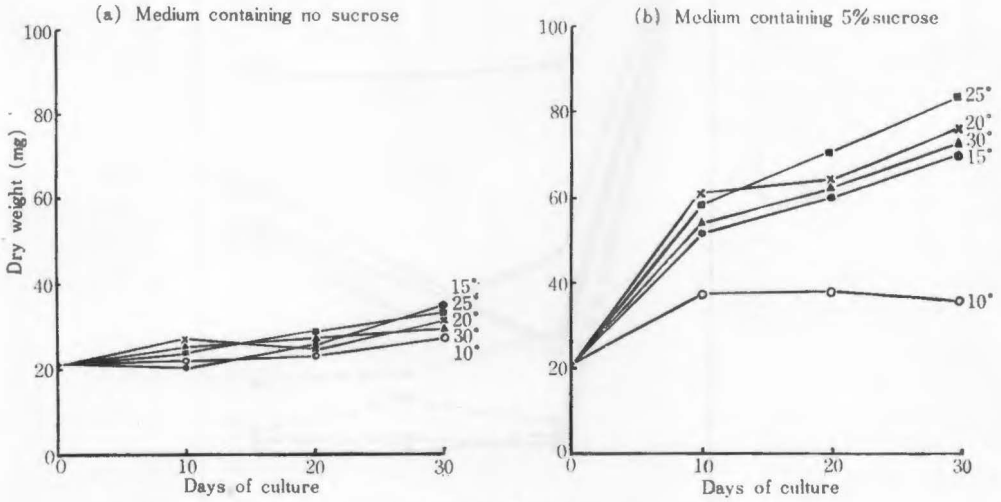


Fig. 12. Dry weight of *Pharbitis nil* cultured under continuous light (2000 lux) in vitro.

(4) *Ratio of Dry Weight to Fresh Weight* (Fig. 13)

The average percentage of dry weight to fresh weight at the start was 31.5. Generally speaking, the ratio of dry weight on media containing 5% sucrose showed higher values than those on media containing no sucrose. It is interesting that on sucrose media the ratio at 15° was considerably larger than at any other temperatures, except at 10°. At 10°, the plants scarcely grew, and the ratio remained high for long durations. However, it decreased gradually on sucrose deficient medium, and increased slightly during the first 20 days on sucrose medium.

(5) *The Number of Leaves Differentiated* (Fig. 14).

Two leaf primordia were present in the swollen seed. At the end of experiment for 30 days, about 8 leaves were differentiated at 20° or higher temperatures. At 15°, about 6 leaves were differentiated on sucrose deficient media, but only 4.5 leaves on sucrose media. On sucrose medium, small floral

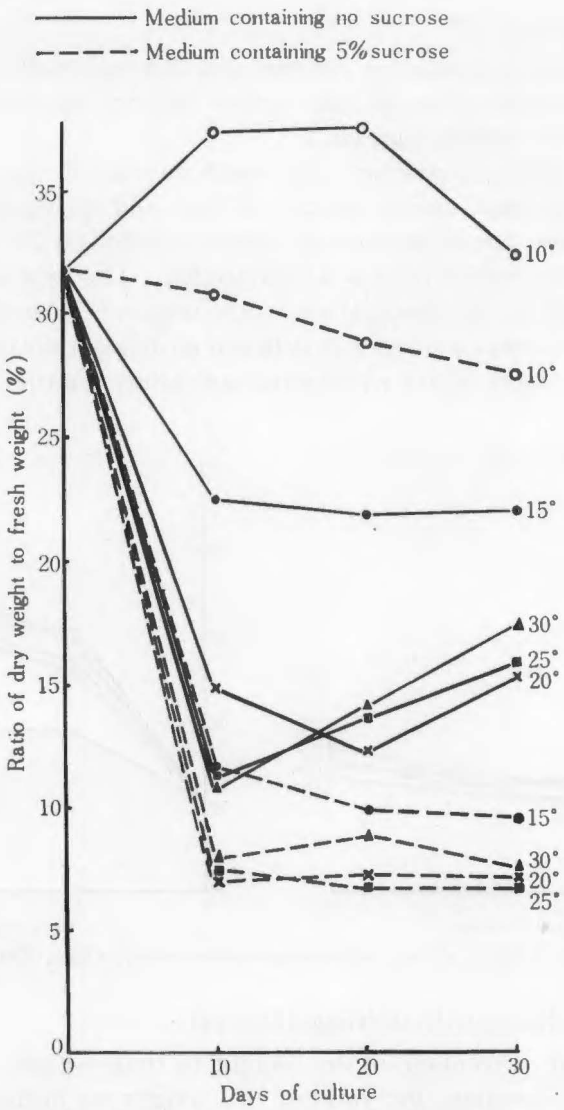


Fig. 13. Ratio of dry weight to fresh weight.

primordia were already developed at the end of the 30-day cold treatment, and all the plants developed terminal flower buds after they had produced 4 or 5 leaves, so that further differentiation of leaves was impossible. At 10°, new leaf primordia scarcely differentiated on both media containing 5% sucrose or no sucrose during the first 30 days.

(6) *Chlorophyll Content* (Fig. 15)

At the temperatures of 20° or higher, chlorophyll (a+b) content attained the maximum on 10th day on both media and subsequently decreased gradually. At 15°, however, chlorophyll content increased gradually throughout the

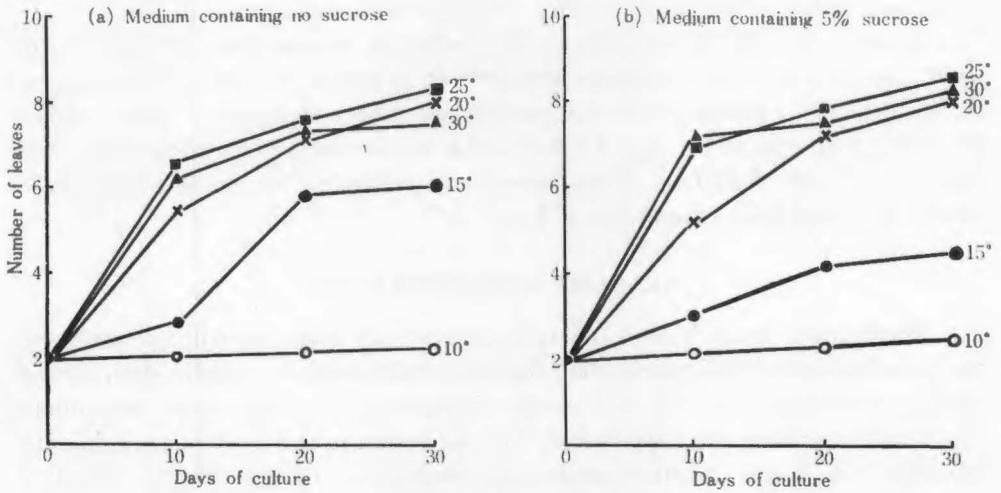


Fig. 14. Number of leaves differentiated.

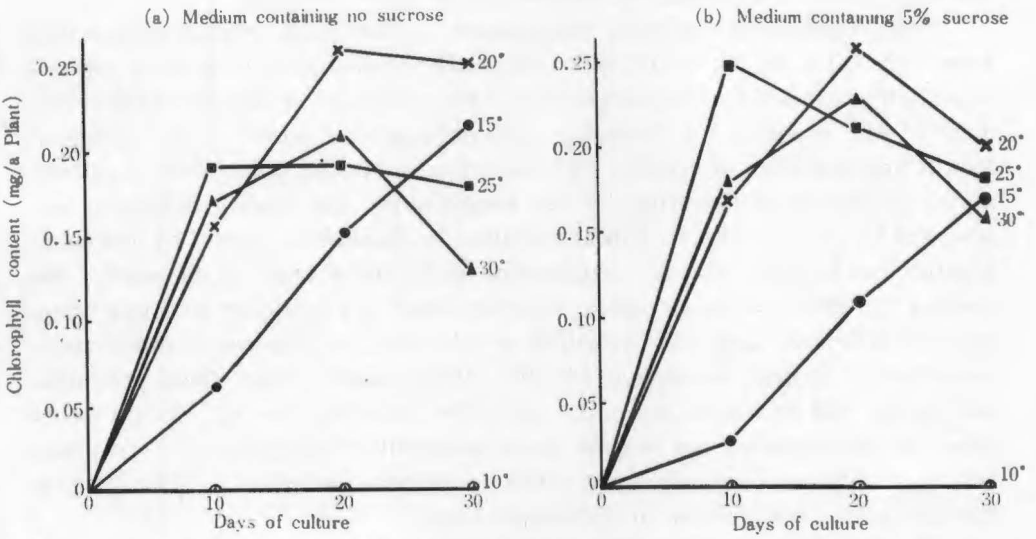


Fig. 15. Chlorophyll content of *Pharbitis* plant cultured under continuous light (2000 lux) in vitro.

experimental duration. At 10°, plants did not produce a measurable amount of chlorophyll.

(7) *Discussion*

Considerable differences in the vegetative growth, i.e., shoot length, fresh weight and number of leaves, were observed between the plants cultured at 15° and 20°. But the difference in dry weight was not so remarkable. This was probably caused by the lower respiration at low temperature. On sucrose medium, the percentage of dry weight to fresh weight was significantly high

at 15° as compared with that at other temperatures used, except at 10°.

It seems that floral initiation at relatively low temperature irrespective of light condition may be related to poor growth or the high ratio of dry weight. At 10°, however, plants grow very poorly and flower sparingly. The reduced flowering response at 10° may be due to the low activity of growing point, and therefore longer durations of treatment are necessary for floral initiation as shown in Takimoto's experiment (73).

XI. GENERAL CONSIDERATION

Pharbitis nil, strain Violet, is a typical short day plant at ordinary temperature, and initiates floral primordia after the application of a single dark period of adequate length (20, 21). It remains vegetative for months under continuous illumination or long photoperiods at 25°. As shown in the present experiments, however, the plants cultured on sucrose medium at 15° for 30 days initiated floral primordia irrespective of light conditions, i.e., total darkness, 8-hour photoperiod and continuous illumination. The low temperature of 15° promotes flowering process in *Pharbitis nil* irrespective of light conditions.

With regard to the effect of temperature on flowering, vernalization is well known (2, 4, 19, 33, 39, 47, 67, 90). Originally vernalization means a physiological effect caused by low temperature which "induces or hastens the development of the capacity for flowering, and although its action is not visible at first, it appears as an aftereffect" (4). Vernalization in the broad sense is defined as any promotion of flowering by low temperature, and such a definition was accepted by some workers. Floral initiation in *Pharbitis nil* caused by low temperature can be referred to as vernalization in the broad sense of the word. Concerning the effects of temperature, however, there is a tendency to divide them into two different categories according to whether the response is direct (non-inductive) or indirect (inductive) (67, 90). If the plants initiate floral primordia during the cold treatment, not after, the effect is called direct. On the other hand, if the plants do not initiate floral primordia during the cold treatment but do so later as after-effect, the effect is indirect (inductive). The latter is usually called vernalization in the narrow sense.

Pharbitis plants must be kept at 15° for 30 days to obtain the maximum flowering response. At the end of the treatment for 30 days, all the plants have already developed floral primordia (cf. Chapter III and X). Therefore the effect of low temperature on flowering of *Pharbitis* is considered to be a direct one, and not vernalization in the narrow sense.

Effect of low temperature on flowering response of *Pharbitis* is similar to that of vernalization in some respects (4, 10, 11, 12, 28, 56, 57, 58, 59, 60, 77, 86, 87, 88, 89).

1) Shoot apex seems to be a respective organ to chilling.

2) Effect of low temperature of insufficient duration is reduced by subsequent exposure to high temperature.

3) When embryos separated from the rest of seeds are vernalized, sugars, e.g., sucrose promote flowering. Flowering response of *Pharbitis nil* at low temperature is also promoted by the sugar supply.

4) The sucrose is more effective for the promotion of flowering when given during the early portion than given during the later portion of the cold treatment.

On the contrary, however, one may find some differences between the effects of low temperatures on flowering of *Pharbitis* and on vernalization.

1) Effect of low temperature on flowering response of *Pharbitis* is a direct effect (non-inductive), whereas the effect of low temperature in vernalization is indirect (inductive).

2) A constant temperature of 15° is optimal for floral initiation of *Pharbitis*, but in vernalization the optimum temperature is much lower (2, 4, 33, 39, 47).

3) Effect of low temperature is not reduced by subsequent exposure to high temperature if the cold treatment was given for an enough duration.

4) Effect of intermittent low temperature treatment is additive in vernalization (61), but not in the flowering of *Pharbitis*.

Pharbitis plants initiate floral primordia not after but during the cold treatment. In other words, the plants have to grow to differentiate flower buds during the cold treatment. At 10° or lower temperatures, plants grow so slowly, and 30 days may not be enough to develop flower buds; but if the duration of cold treatment is prolonged much longer, they may flower even at 10° or lower (73). At 15°, plants grow very slowly, but sufficiently to differentiate flower buds during the first 30 days. At higher temperatures, plants grow rapidly, showing a strikingly difference in vegetative growth between 15° and 20°. They flower at 15° but not at 20°. These results suggested that vegetative growth and reproductive growth is antagonistic. If the vegetative growth is suppressed by some environmental or experimental conditions, plants might flower (3, 13, 18, 29, 38, 62). The effect of KNO_3 added to the medium may be interpreted in this way: the intrate promoted vegetative growth at 15°, but suppressed flowering response to a slight extent.

At ordinary temperature, however, *Pharbitis* plants initiate floral primordia very easily by being subjected to one long dark period (16 hours), without showing any reduction in vegetative growth. Many investigators believe the existence of floral hormone which is produced by favorable photoperiod (2, 15, 33, 39, 44, 71, 82, 83). Others, on the contrary, have an opinion that flowering is controlled by inhibitors which are produced by unfavorable photoperiod (34, 81). Many data have been presented to support either one of these hypotheses. However, from the well established fact that many plants which are sensitive to photoperiod initiate flower buds in total darkness (7, 16, 24, 37, 72, 76, 78, 79), it may be reasonable to consider that the plants have a natural disposition to flower, and this may be suppressed or pormoted by photoperiodic condition.

At low temperatures, photoperiodic effect may be suppressed greatly, and

the plants may flower by their own natural tendency to flower. In this case, however, vegetative growth and reproductive growth seem to be antagonistic. In the present experiment, sucrose which does not suppress vegetative growth was very effective for floral initiation. It might have some effect for promoting natural tendency to flower.

Many data presented in this paper suggest that the floral initiation caused by low temperature and by photoperiodic treatment is based on the different mechanisms. Photoperiodic stimulus is received by leaves, and flowering hormone (florigen) produced in the leaves is transferred to the growing point. As was shown in Chapter VIII *Pharbitis* seedlings deprived of cotyledons initiated floral primordia at low temperatures. They had no leaves which were required to receive the photoperiodic stimulus. Nevertheless, they initiated flower buds indicating that the effect of low temperature for floral initiation seemed to be received at growing point. Was florigen produced at growing point at low temperature? If such be the case, it may follow that the effect of low temperature is an inductive (indirect) effect, because floral initiation may be established after the florigen is produced. Why must low temperature be given continuously until the floral primordium is initiated?

If one define the "florigen" as a hormone which is produced in leaves by photoperiod, it may be concluded that *Pharbitis* plants are able to initiate floral primordia at low temperature without participation of florigen. It is highly probable that there are many links in the chain of reactions which lead to the floral initiation. One of the links may be conducted by florigen which is produced in leaves by photoperiodic stimulus, and others may proceed only in the growing point and this may be started by some environmental conditions without the participation of the florigen. Metabolic reaction system at growing point may be changed to reproductive system either by florigen or by other environmental conditions. Floral initiation of *Pharbitis nil* at low temperature is considered to be caused by the latter reaction system rather than by florigen.

XII. SUMMARY

Pharbitis nil initiates floral primordia under continuous illumination when cultured in vitro at relatively low temperatures. Studies on the influence of environmental factors upon floral initiation in *Pharbitis nil* brought about the following results.

1) *Pharbitis* plants initiate floral primordia under continuous illumination at low temperatures when 2–10% sucrose was added to the medium. Among constant temperature treatments used, 15° was most favorable for floral initiation. However, 30 days' exposure to cold temperature was required to cause maximum flowering. Constant temperatures above 18° or below 5° did not cause flowering.

Floral primordia also developed when plants were exposed to alternating temperatures. Daily alternations of 10° (16hrs.)—25° (8hrs.), 10° (20hrs.)—25°

(4hrs.) and 15° (20hrs.)—25° (4hrs.) brought about the initiation of floral primordia in almost all the plants grown on sucrose media. Daily alternating temperatures of 10° and 25° were effective in causing floral initiation when the plants were exposed to 10° for more than 12 hours. Daily alternations of 1° and 25° or 5° and 25° were effective for causing floral initiation to some extent when the plants were subjected to these low temperatures for 12–16 hours. Daily alternation of 20° and 25° was not effective for floral initiation. When exposed to alternating temperatures of 5° (16 hrs.)—25° (20 hrs.) on sucrose deficient media, a few plants initiated floral primordia.

2) The flowering behavior of *Pharbitis nil* was studied under various nutritional conditions. Plants were grown under continuous illumination at a temperature of 15°. Optimal flowering responses were observed when the plants were cultured on sucrose (4–10%) media containing 0.75 or 1% agar and with pH values ranging from 4.32 to 5.50. Minerals of White's solution added to the medium had little influence on floral initiation; the plants initiated floral primordia on agar medium containing sucrose without minerals. If 0.05–0.1% KNO_3 or $Ca(NO_3)_2$ was added in sucrose media, the flowering response was slightly suppressed. However, these media promoted vegetative growth.

Glucose, fructose, mannose, maltose, lactose and raffinose were as effective as sucrose in causing floral initiation, but xylose, galactose and mannitol were less effective.

Sucrose was more effective for promoting floral initiation when applied during the early part than when applied during the later of the low temperature period.

3) Plants cultured at 15° initiated floral primordia irrespective of the light conditions; intensity, color and photoperiod. Plants cultured at 25° did not initiate floral primordia under any light conditions, except under short photoperiods. *Pharbitis* plants subjected to short days developed only axillary buds at ordinary temperature when the photoperiodic stimulus was not strong enough. Terminal flower buds developed only when the plants received a strong flowering stimulus. At 15°, however, all the plants that initiated floral primordia developed a terminal flower, even when they received a weak stimulus.

4) Alternating temperatures of 10° for 16 hours and 20°–25° for 8 hours caused floral initiation in more than 90% of the plants. But alternating temperature of 10° for 16 hours and 30° for 8 hours caused floral initiation in only 25% of them. When the plants cultured at 15° for 10–20 days were transferred to various temperatures ranging from 20° to 35°, flowering percentages decreased with increasing temperature. In particular, the flowering responses were inhibited strikingly when exposed to 30° or 35° after the insufficient cold treatment. When the plants were exposed to 15° for 25 or 30 days, subsequent high-temperature had no inhibitory effect on flowering response.

When low temperature treatment was interrupted by warm temperature at the middle of a 30-day cold treatment, the flowering response was inhibited

strikingly. The flowering response may be in a labile state 10 to 15 days after the start of low temperature treatment.

5) The influence of age on low-temperature effect was greatly affected by the presence or absence of sucrose in the medium. On sucrose deficient media, floral primordia did not initiate when plants were exposed to 15° immediately after the germination. But flowering response increased with increasing period of pretreatment at 25°, and the plants kept at 25° for 6 days before cold treatment showed a maximum value. If the plants were cultured at 25° for more than 6 days before cold treatment, flowering responses gradually decreased with increasing culture durations at 25°. On sucrose medium, the maximum flowering responses were brought about when plants were exposed to low temperature at 15° immediately after the germination. Floral responses decreased with increasing duration of the pretreatment at 25°. Plants cultured at 25° for 10 days or more did not initiate floral primordia at all. Influence on flowering response of age at the beginning of the cold treatment depends upon the presence or absence of sucrose contained in the media.

6) When the embryo axes were cultured at 15° immediately after the cotyledons were cut off from the swollen seeds, the plants did not initiate floral primordia. When plants were cultured under continuous illumination at 25° for several days, they initiated floral primordia to some extent by subsequent cold treatment.

7) Some strains of *Pharbitis nil*, "Kidachi", "Scarlet", "Tenden", "Purple", and *Quamoclit* plants also initiated floral primordia under continuous illumination on sucrose medium at 15°.

8) The effect of low temperature on floral initiation in *Pharbitis nil* is considered to be a direct (non-inductive) one. It does not seem to be an aftereffect and may not be vernalization.

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