Studies on the Flower Initiation in *Pharbitis* Seedlings III. Influences of X-ray Irradiation

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Abstract

When the seedlings of *Pharbitis nil*, strain "VIOLET", were exposed to a single moderate dose of X-rays (below 1000 R), the growth of seedlings was scarcely affected, but the initiation and development of flower primordia were inhibited considerably. This inhibition was greatest when the irradiation was given 0 to 72 hours after the end of a flower-inductive dark period. Low doses of X-rays (50-150 R) promoted flower initiation to some extent, especially when they were applied before the dark period. The X-ray irradiations applied at different times caused various types of abnormal flower buds. The range within which various abnormal flowers were observed was much wider than that obtained by gamma-ray irradiation. On the whole, however, the present experiments with X-rays confirmed the results obtained by gamma-ray irradiation¹⁾.

Acute gamma-rays below 1300 R did not affect either the elongation or development of vegetative shoots, but severely affected flower initiation when they were applied shortly before and after a flower-inductive dark period¹⁾. In the present investigation similar experiments were carried out using X-rays instead of gamma-rays.

Material and Methods

The material used was the Japanese morning glory, *Pharbitis nil* Chois., strain "VIOLET". All experiments were performed with the seedlings, photoperiodic behaviours of which were reported by Kujirai and Imamura²⁾. The seedlings were grown in an air-conditioned greenhouse kept at 30°, or a room kept at 25-30°. The dark treatments were done at a dark room kept at 30°, or at 25-30°. And the other procedures of the experiments were similar to those already described^{1,3)}. X-ray irradiations were carried out using a Philips deep therapy X-ray machine operated at 200 kV, 19 mA with 1.0 mm Al filter.

Results and Discussion

1) *Influences of low doses of X-rays.* Table 1 shows the flowering responses of *Pharbitis* seedlings which were subjected to a 16-hour dark period (30°), exposed to low doses (49 or 50 R) of acute X-rays at different times, and grown in an air-conditioned greenhouse kept at 30°.

When 50 R of X-ray was given immediately before, in the middle or immediately

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after the dark period, total flowering percentages were decreased considerably (Experiment I in Table 1). This tendency was more obvious in the seedlings which

Table 1. Flowering response of *Pharbitis* seedlings exposed to low dose X-rays at different times before, during, or after a single 16-hour dark period.

		Flowering percentage* at axillary and terminal bud					
		Non-irradiated control	Time of irradiation after the beginning of a 16-hour dark period (30°)				
			−8h	0h	+8h	+16h	+40h
Experiment I (50 R)							
Axillary bud							
1st	0	5.3	0	0	0	0	3.7
2nd	52.9	26.3	25.0	29.4	39.1	13.0	77.8
3rd	88.2	100.0	65.0	88.2	73.9	100.0	100.0
4th	47.1	89.5	35.0	58.8	60.9	78.3	88.9
5th	29.4	78.9	10.0	47.1	43.5	69.6	74.1
Terminal bud	29.4	84.2	10.0	23.5	43.5	65.2	74.1
Total FL %	100.0 (17)	100.0 (19)	80.0 (20)	88.2 (17)	78.3 (23)	100.0 (23)	100.0 (27)
Experiment II (100 R)							
Axillary bud							
1st	0	0	4.5	0	0	0	0
2nd	52.9	50.0	86.4	39.1	31.6	15.0	0
3rd	88.2	95.0	53.6	73.9	63.2	100.0	100.0
4th	47.1	90.0	45.5	26.1	52.6	95.0	93.8
5th	29.4	80.0	27.3	8.7	47.4	95.0	93.8
Terminal bud	29.4	85.0	40.9	4.3	42.1	85.0	93.8
Total FL %	100.0 (17)	100.0 (20)	90.9 (22)	82.6 (23)	63.2 (19)	100.0 (20)	100.0 (20)
Experiment III (150 R)							
Axillary bud							
1st	2.3	0	0	—	0	0	0
2nd	16.3	8.3	13.0	—	6.7	12.0	4.0
3rd	100.0	100.0	91.3	—	93.3	96.0	92.0
4th	93.0	100.0	95.7	—	93.3	88.0	96.0
5th	72.1	91.7	82.6	—	66.7	32.0	32.0
Terminal bud	65.1	79.2	91.3	—	66.7	32.0	32.0
Total FL %	100.0 (43)	100.0 (24)	100.0 (23)	—	100.0 (15)	100.0 (25)	100.0 (25)

* % of plants with flower buds.

Numbers of plants observed are indicated in parentheses.

Experiment I : 50 R=49 R/min×1.02 min.

Experiment II : 100 R=49 R/min×2.02 min.

Experiment III : 150 R=50 R/min×3 min.

received 100 R of X-ray (Experiment II in Table 1). In the seedlings which received 50 R of X-ray, considerable differences in flowering responses were observed between each axillary bud and terminal bud. In the second axillary buds flower inhibition occurred whenever the X-ray was applied within the experimental duration, except when it was given 64 hours after the beginning of the dark period. On the other hand, in the fourth and the fifth axillary buds and also in the terminal buds, flower initiation was significantly promoted by 50 R of X-ray applied 8 hours before, 40 hours and 64 hours respectively, after the beginning of the flower-inductive dark period (Experiment I in Table 1). Similar results were obtained with 100 R of X-ray, with some irregularities (Experiment II in Table 1).

The results of other experiments in which the seedlings were exposed to 150 R of X-ray at different times before, during or after a single dark period are shown in

Table 2. Flowering response of *Pharbitis* seedlings exposed to low dose X-rays at different times before, during, or after a single dark period of 10 and 12 hours.

Experiment I	Flowering percentage* at axillary and terminal bud					
	Non-irradiated control	Time of irradiation (150 R**) after the beginning of a 12-hour dark period (25-30°)				
		-8h	0h	+12h	+24h	+40h
Axillary bud						
1st	0	0	0	0	0	0
2nd	2.4	5.9	0	20.8	8.0	10.0
3rd	46.3	94.1	72.7	58.3	88.0	95.0
4th	61.0	58.8	31.8	41.7	76.0	10.0
5th	0	11.8	0	0	8.0	0
Terminal bud	0	0	0	0	0	0
Total FL %	100.0 (41)	100.0 (17)	90.9 (22)	91.7 (24)	100.0 (25)	100.0 (20)
Experiment II	Flowering percentage* at axillary and terminal bud					
	Non-irradiated control	Time of irradiation (150 R**) after the beginning of a 10-hour dark period (25-30°)				
		-8h	0h	+10h	+24h	+40h
Axillary bud						
1st	0	0	0	0	0	0
2nd	0	0	0	0	0	0
3rd	6.4	16.0	15.4	0	3.8	0
4th	0	0	3.8	0	0	0
5th	0	0	0	0	0	0
Terminal bud	0	0	0	0	0	0
Total FL %	6.4 (47)	16.0 (25)	19.2 (26)	0 (24)	3.8 (26)	0 (25)

* % of plants with flower buds.

** 150 R = 50 R/min $\times$ 3 min.

Numbers of plants observed are indicated in parentheses.

Table 1 (Experiment III), Tables 2 and 3. Durations of the dark period were 16 (Experiment III in Table 1), 12 (Experiment I in Table 2), and 10 hours (Experiment II in Table 2 and Table 3).

In Experiment III shown in Table 1, flower initiation was inhibited by X-ray irradiation in almost all the buds, but a slight promotion was observed when the seedlings were irradiated 8 hours before the dark period. Table 2 shows that flower initiation was promoted slightly by the irradiation with low doses of acute X-rays. Table 3 shows an example of relatively strong promotion of flower initiation caused by low doses of X-rays. In this case, X-ray irradiation given 12 hours before a 10-hour dark period (25-30°) induced a terminal flower primordium (2.9%). The plant used in the present study has never initiated a terminal flower when subjected to a single 10-hour dark period without X-ray irradiation.

It is certain that low doses of acute X-rays from 50 to 150 R promote flower initiation, especially when they are given before the dark treatment.

Table 3. Flowering response of *Pharbitis* seedlings exposed to low dose X-rays before or after a 10-hour dark period.

	Non-irradiated	Time of irradiation (150 R*) after the beginning of a 10-hour dark period					
	control	-24h	-12h	0h	+10h	+18h	+35h
Flowering percentage	9.1	74.3	85.3	40.6	6.8	55.2	71.8
Number of flower buds per plant	0.09	0.86	1.03	0.44	0.14	0.55	0.79
Percentage of plants with terminal bud	0	0	2.9	0	0	0	0
Number of observed plants	22	35	34	32	29	29	39

* 150 R=50 R/min × 3 min.

Table 4. Inhibition of flower initiation by high dose of X-ray.**

Experiment	Treatment	Flowering plants Plants observed	Flowering percentage	Number of flower buds Plants observed	Percentage of plants with terminal flower buds
I	Non-irradiated control	17/17	100.0	2.6	29.4
	Irradiated (1000 R)				
	24h after 16hd*	0/20	0	0	0
	48h after 16hd*	6/16	37.5	0.8	12.5
	72h after 16hd*	13/15	86.7	1.7	60.0
II	Non-irradiated control	43/43	100.0	2.8	2.3
	Irradiated (1000 R)				
	0h after 16hd*	0/37	0	0	0
	24h after 16hd*	0/41	0	0	0

16hd*: A single 16-hour dark period at 25° in Experiment I, and at 25-30° in Experiment II.

** : 1000 R=100 R/min × 10 min.

2) *Influences of high doses of X-rays.* About 1000 R of acute gamma-rays given after a flower-inductive dark period inhibited flower initiation strikingly. To know whether this is also the case with X-rays, the seedlings were exposed to the same dose of X-rays at different times after a single 16-hour dark period (25-30°), and flowering responses were examined. Table 4 shows that 1000 R of X-ray was very inhibitory for flowering response, and that the inhibitory effect was decreased by delaying irradiation.

3) *Relation between the dose rate and the duration of a medium X-ray irradiation.* The dosage of 600 R at the level of the plumule was given immediately after the dark period in three ways, i. e., for four minutes at a dose rate of 150 R per minute, for 12 minutes at 50 R per minute, and for 36 minutes at 16.7 R per minute. The results are shown in Table 5. This shows that flowering responses are affected

Table 5. Effect of dose rate and duration of X-ray irradiation on flowering response.

	Non-irradiated control	Dose rate × Duration irradiated* (Total dosage=600 R)		
		150 R/min × 4 min.	50 R/min × 12 min.	16.7 R/min × 36 min.
Flowering percentage	100.0	35.3	21.1	7.1
Number of flower buds per plant	3.73	0.59	0.37	0.07
Percentage of plants with terminal flower buds	52.6	17.6	5.3	0
Number of plants observed	19	17	19	14

* X-rays were irradiated immediately after a 16-hour dark period.

Table 6. Effect of filtered X-ray (450 R) on flowering response of *Pharbitis* seedlings.

Filter	Time of irradiation after the beginning of a 16-hour dark period	Flowering plants	Flowering percentage	No. of flower buds	Percentage of plants with terminal flower buds
		No. of plants observed		No. of plants observed	
Non-filter	0h	14/16	87.5	3.7	87.5
	8h	17/29	58.6	1.2	31.0
	16h	4/15	26.7	0.5	6.7
1.0 mm Al	0h	26/26	100.0	4.0	88.5
	8h	22/38	57.9	1.9	39.5
	16h	19/26	73.1	1.6	7.7
1.0 mm Al + 0.5 mm Cu	0h	25/25	100.0	3.6	64.0
	8h	23/25	92.0	4.2	84.0
	16h	17/29	58.6	1.2	6.9
Non-irradiated control		28/28	100.0	5.1	92.9

more strongly by prolonging the duration of X-ray irradiation than by increasing the dose rate.

4) *Influences of filtration of X-rays on flowering responses.* Seedlings were subjected to a 16-hour dark period (30°), exposed to X-rays filtered with a 1.0 mm Al plate or a 1.0 mm Al+0.5 mm Cu plate at different times during the dark treatment, and grown in an air-conditioned greenhouse kept at 30°. Control plants were exposed to non-filtered X-rays. The dosage of these X-rays was 450 R at the level of the plumule. Flowering responses are shown in Table 6. No significant differences in the flowering responses were observed between the plants exposed to filtered and non-filtered X-rays. But, non-filtered X-rays produced slightly stronger inhibitory effects. In the following experiments either a 1.0 mm Al filter or a 1.0 mm Al+0.5 mm Cu filter was used, except when strong X-ray irradiation was needed.

Table 7. Flowering response of *Pharbitis* seedlings exposed to X-ray (450 R) at different times before, during, or after a 16-hour dark period.

	Non-irradiated	Time of irradiation after the beginning of a 16 ^h dark period						
	control	-72 ^h	-48 ^h	-24 ^h	0 ^h	+8 ^h	+16 ^h	+40 ^h
Flowering plants Plants observed	41/41	25/25	22/23	22/23	21/22	12/23	1/19	14/26
Flowering percentage	100.0	100.0	95.7	95.7	95.5	52.2	5.3	53.8
No. of flower buds plants observed	2.5	3.9	4.5	3.3	3.7	0.9	0.1	0.5
Percentage of plants with terminal flower buds	22.0	88.0	91.3	65.1	81.8	17.4	0	0

Fig. 1. Flowering response of *Pharbitis* seedlings exposed to gamma- or X-rays at different times before, during, or after a 16-hour dark period.

5) *Effects of X-ray irradiation given at different times before or after a flower-inductive dark period.* The previous experiments on acute gamma-rays showed that there was a definite relation between the stage of flower development at the time when gamma-ray irradiation was given and the production of various abnormal flower buds, and it was suggested that the early stage of flower initiation can be divided into some developmental substages¹⁾. To investigate whether or not X-ray irradiation also affects the flower initiation in a similar manner as gamma-rays, the seedlings were irradiated by medium X-rays at different times, before, in the middle, or after a 16-hour dark period (30°). The seedlings were grown in an air-conditioned greenhouse kept at 30° throughout the experimental period. The results are shown in Table 7 and Fig. 1. An acute X-ray irradiation of 450 R applied before a 16-hour dark period slightly lowered the flowering percentages. But the numbers of flower buds per plant and the percentages of plants with terminal flower buds showed an increase over those of the control group. Maximum flowering response was observed when X-ray was given 48 hours before the dark period. On the other hand, the flowering responses were inhibited heavily when the seedlings were irradiated in the

Table 8. Flowering response of *Pharbitis* seedlings exposed to X-ray (1000 R= 100 R/min×10 min) at different times before and after a single 16-hour dark period (16^{hd})

	Non-irradiated control	Before 16 ^{hd}				Time of irradiation After 16 ^{hd}					
		24h	0h	0h	12h	24h	36h	48h	72h	96h	120h
Flowering plants Plants observed	43/43	15/30	0/31	0/37	0/35	0/41	2/35	23/37	32/34	30/30	26/26
Flowering percentage	100.0	50.0	0	0	0	0	5.7	62.2	94.1	100.0	100.0
No. of flower buds per plant	2.8	0.6	0	0	0	0	0.1	0.6	1.2	2.2	2.5
Distribution of abortive flower buds*											
Axillary bud											
1st	0/43	0/30	0/31	1/37	3/35	2/41	4/35	1/37	0/34	0/30	0/26
2nd	0/43	0/30	0/31	4/37	4/35	21/41	25/35	3/37	0/34	0/30	0/26
3rd	0/43	0/30	0/31	2/37	1/35	0/41	0/35	7/37	6/34	4/30	2/26
4th	0/43	0/30	0/31	0/37	0/35	0/41	0/35	0/37	0/34	0/30	2/26
Distribution of abnormal flower buds**											
Axillary bud											
1st	0	0	0	0	0	0	0	0	0	2/30	0
2nd	0	0	0	0	0	0	2/35	14/37	3/34	0	1/26
3rd	0	0	0	0	0	0	1/35	3/37	14/34	6/30	0
4th	0	0	0	0	0	0	0	0	0	2/30	5/26

* Number of abortive flower buds/Number of buds observed.

** Number of abnormal flower buds/Number of buds observed.

middle or after the 16-hour dark period. The maximum inhibition was observed at the end of the dark period. Thus, an acute X-ray irradiation given before the dark period promoted flowering, whereas that applied in the middle or after the dark period inhibited it strikingly.

Using a relatively high dose of X-ray (1000 R), a similar experiment was carried out (Table 8). Throughout the experimental period, the plants were grown in an air-conditioned greenhouse kept at 25–30°. Although 450 R of X-ray applied 48 hours after the end of a 16-hour dark period (25–30°) scarcely affected the flowering responses, the high dose of X-ray used at that time inhibited flowering considerably.

As in the case of gamma-ray irradiation, some abortive flower buds which had two to four bract-like leaves without any other floral organs, and some abnormal flower buds having three to four bracts with other normal floral organs, were observed when an acute X-ray irradiation was given within 120 hours after a 16-hour dark period. The distribution of such abortive or abnormal flower buds in the first to the fourth axillary buds is shown in Table 8. The X-ray irradiations applied at different times caused various types of abnormal flower buds. The range within which various abnormal flowers were observed was much wider than that obtained by gamma-ray irradiations (cf., Tables 7 and 8 in the previous paper¹⁾). Moreover, in order to produce the abnormalities similar to those caused by gamma-rays, a higher dose of X-ray had to be used. Using such strong X-rays, substages of the early stage of the initiation and development of flower primordia could not be recognized so sharply as when gamma-rays were used. On the whole, however, the present experiments with X-rays confirmed the results obtained by gamma-ray irradiation.

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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 III.

X 線照射の影響

アサガオ（紫）の幼植物の花芽分化にたいする電離放射線の影響をしらべる一連の研究の一部として、ガンマー線につづいて X 線の acute irradiation の影響をしらべる実験をおこなった。その結果、X 線は花芽分化にかんしてガンマー線と同様な影響をおよぼすことが明らかであった。すなわち、栄養生長に顕著な影響をおよぼさない線量（1000 R）以下を短日処理後 72 時間以内に照射すると花芽分化は著しく抑制され、照射時期によりさまざまな奇形花を生じた。さらに、低線量の X 線（50～150 R）を暗処理前に照射することにより、花芽分化の促進が見られたのは注目すべき結果である。（静岡大学理学部生物学教室）