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Floral Induction by Dark Pre-Treatment in Non-Vernalized Winter Barley

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Dark pre-treatment at early growth stage was found to induce the flowering of non-vernalized barley varieties irrespective their different thermal and photoperiodic responsibility. The possibility that the promotive effect of dark pre-treatment and of vernalization is based on the different mechanism was suggested.

INTRODUCTION

It was reported previously that in certain winter barley varieties dark treatment preceded to long day condition can replace, nearly quantitatively, the vernalization effect (Inouye and Ito, 1968; Adachi *et al., 1970).*

In the present experiment, aseptic culture of barley plants was examined to compare the flowering induced by dark pre-treatment and vernalization.

MATERIALS AND METHODS

In most experiments *Hordeum vulgare cv.* Hashirihadaka was used since short day treatment can hardly replace the effect of vernalization (Hirano and Suge, 1963) although it requires 45 days of vernalization treatment under aseptic cultural condition (Adachi *et al.,* 1970). This may be convenient to distinguish the dark effect from so called "short-day vernalization".

The basal culture medium was a modified White's solution. The medium consisted of 360 mg $MgSO_4$, 200 mg $Ca(NO_3)_2$, 200 mg Na_2SO_4, 80 mg KNO_3, 65 mg NaH_2PO_4, 1.5 mg $ZnSO_4$, 1.5 mg H_3BO_3, 1.5 mg KI, 4 mg Fe-citrate, 10 g sucrose, 7 g agar and 1,000 ml deionized water. The 16 x 250 mm test tubes which contain 10 ml of the culture medium were nutoclaved at 1.0 kg/cm² for 20minutes.

Well matured seeds in medium size were sterilized by immersing in 75 % alcohol for 3 minutes then in 10 % freshly prepared calcium hypochlorite solution for 20 minutes, and finally in 3 % hydrogen peroxide for 20 minutes. Immediately after sterilization, two seeds were sown in each tube and the tubes were covered with paper to prevent the medium from drying. The tubes were kept at 25°C under darkness for 2 days before subjecting to various

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treatments. For dark treatment, the tubes were wrapped with light proof black papers. Vernalization was held at 5°C. Dark pre-treatment and short day treatment (8 hours light and 16 hours dark) was carried out at 25°C. After these treatment, plants were grown in the continuous artificial light at 25°C. The light was obtained from fluorescent tubes, mercury lamps and incandescent lamps, and the light intensity was about 6,000 lux at plant level.

The number of leaves formed before the initiation of floral primordia, was used as a measure of the readiness to flower. Floral primordia was examined with a binocular microscope about four months after seeding. Each treatment consisted of about 20 tubes.

RESULTS AND DISCUSSION

Effect of dark p-e-treatment on floral induction of different barley varieties

Four barley varieties, Kinai No. 5, Akashinriki, Hashirihadaka and Kobinkatagi, with different thermal and photoperiodic hehaviours (Suge, 1961), were used. Seeds were planted on the medium and prcincubated at 25°C under darkness for 2 clays before incubated at 5°C or 25°C under darkness for 30 days in spring barlcys and for 45 days in winter barleys, and then subsequently grown under continuous light at 25°C for about three months (Table 1).

Floral initiation was promoted not only by vernalization (5°C) but also by dark pre-treatment (25°C) in all varieties, although the promoting effect was less in the latter. It is notable that not only Hashirihadaka but also Kobinkatagi responded to the dark pre-treatment although these two varieties are known to have quite different vernalized ability induced by short day, but have almost same vernalizing response induced by low temperature (Hirano

Table 1. Effects of vernalization and dark pre-treatment on floral induction of four barley varieties.

Materials	Pre-treatment[1]	No. of plants	Flowering (%)	No. of leaves[2]
Kinai No. 5	5°C in dark	23	96	7.9 (6.0)
	25°C in dark	20	85	8.6 (6.7)
	25°C in light	20	10	13.5 (6.5)
			93	
Akashinriki	5°C in dark	27	80	8.7.72 (6.0)
	25°C in dark	25		(5.2)
	25°C in light	22	14	10.7 (6.1)
Hashirihadaka	5°C in dark	41	95 71	10.59.2 (7.0)
	25°C in dark	34		(7.5)
	25°C in light	25	0	(7.4)
Kobinkatagi	5°C in dark	32	84	10.4 (8.2)
	25°C in dark	26	54	12.3 (8.5)
	25°C in light	26	0	(8.5)

[1] Duration of each treatment was 30 days in Kinai No. 5 and Akashinriki, and 45 days in Hashirihadaka and Kobinkatagi. [2] The number of leaves in plants without floral primordia is shown in parentheses.

and Suge, 1963). The different degree of promotion between vernalization and dark pre-treatment was less in spring varieties than winter ones. Untreated control plants received no vernalization and no dark pre-treatment could not initiate floral primordia in winter varieties although about 15 % of plants initiated floral primordia in spring varieties. These results suggest dark pre-treatment is able to replace the vernalization in barley plants.

Effect of sucrose on floral induction in dark pre-treatment

Sufficient amount of sucrose in medium is indispensable for floral initiation of wheat and barley under total darkness (Sugino, 1957 ; Inouye and Ito, 1968). To examine the effect of different sucrose concentrations on floral induction, Hashirihadaka barley was cultured on media containing 0, 1, 3, 5 and 10 % of sucrose, and subjected to 5°C or 25°C under darkness for 45 days. All plants were then grown under continuous light at 25°C (Table 2).

No difference was detected in all sucrose concentrations except vernalization induced by low temperature appeared to be more effective than dark pre-treatment. When plants were grown in medium' containing no sucrose, 20 % of vernalized plants initiated flowers at about 10 th node whereas all plants received only the dark pre-treatment died after about 5 leaves had developed. Control plants without vernalization or dark pre-treatment failed to initiate floral primordia irrespective of sucrose concentration. It is suggested that sucrose may play a role as an energy source for the growth during the dark pre-treatment but can not replace the triggering effect on floral induction.

Effect of durations of dark pre-treatment. short day and vernalization on floral induction

Effect of low temperature on flowering can be replaced by short day treat-

Table 2. Effect of sucrose on floral induction of Hashirihadaka winter barley in dark pre-treatment.

Prc-treatment"	Sucrose conc. (%)	No. of plants	Flowering (%)	No. of leaves[2]
5°C in dark	0	23	26	10.3 (6.8)
	1	37	92	9.6 (6.7)
	3	37	89	9.7 (6.5)
	5	34	94	9.6 (7.0)
	10	41	95	9.5 (8.0)
25°C in dark	0	22	0	(5.2)
	1	28	57	9.8 (7.6)
	3	38	74	10.0 (7.5)
	5	26	50	10.2 (6.9)
	10	26	77	10.1 (6.2)
25°C in light	0	16	0	(7.1)
	1	32	0	(8.3)
	3	17	0	(8.6)
	5	21	0	(8.0)
	10	9	0	(8.7)

[1] Duration of each treatment was 45 days. [2] The number of leaves in plants without floral primordia is shown in parentheses.

Table 3. Effect of durations of dark pre-treatment, short day and vernalization on floral induction of Hashirihadaka winter barley.

Pre-treatment	Duration in days	No. of plants	Flowering (%)	No. of leaves[2)]
5°C in dark	15	30	43	10.2 (7.5)
	30	40	98	9.2 (7.0)
	45	34	94	8.7 (7.0)
25°C in dark	15	23	9	12.0 (7.8)
	30	30	57	11.5 (8.5)
	45	30	80	10.5 (7.5)
Short day at 25°C[1)]	15	28	0	(7.8)
	30	24	0	(7.4)
	45	25	0	(6.8)

[1)] 8 hours light and 16 hours dark. [2)] The number of leaves in plants without floral primordia is shown in parentheses.

ment in certain winter barley varieties (Suge, 1961 ; Chujo, 1962, 1975). Seeds of Hashirihadaka barley were sown on the medium and subjected to 5°C and 25°C under darkness or short day condition at 25°C for 15, 30 and 45 days. Thereafter, they were grown under continuous light at 25°C (Table 3).

When treatments were given for 15 days, 43 % of plants initiated floral primordia after vernalization whereas only 9 % of plants initiated floral primordia after dark pre-treatment. Almost all plants received 30 or 45 days vernalization initiated floral primordia after having about 9 leaves. In the dark pre-treatment, flowering response increased gradually as the periods of the treatment were increased, and 80 % of plants initiated floral primordia after having 10.5 leaves in the treatment of 45 days. However, floral initiation did not occur when the plants were subjected to short day treatment immediately after germination. It indicates the promotive effect of dark pre-treatment on floral induction is differed from the effect of short day treatment.

Effect of seedling age prior to dark pre-treatment or vernalization on floral induction

It is well known that vernalization of winter cereals can be advanced not only in immature seed stage on the mother plant or in germinating stage but also in early seedling stage (Purvis, 1961 ; Napp-Zinn, 1973). It is reported in winter wheat that the most favorable temperature for vernalization does not differ among different ages of seedling at the start of the treatment, although vernalizing effect decreased with increasing seedling age (Chujo, 1975). Seeds of Hashirihadaka were planted on medium and incubated at 25°C under darkness for 2 days. The germinated seeds were cultured first under continuous light at 25°C for 0, 1, 2, 5 and 10 days, and subsequently received vernalization under continuous light about 100 lux or dark pre-treatment for 45 days, respectively. Thereafter, they were grown under continuous light at 25°C for three months (Table 4).

All of vernalized plants initiated floral primordia irrespective of cultural

Table 4. Effect of seedling age prior to dark pre-treatment or vernalization on floral induction of Hashirihadaka winter barley.

Pre-treatment	Number of days[1]	No. of plants	Flowering (%)	No. of leaves[2]
5°C in dark		17		7.5
	1	24	100	8.0
	2	20	100	8.5
	5			
	10	16 14	100 100	8.6 9.6
25°C in dark	0	28	57	9.8 (7.6)
	1	15	47	10.3 (6.9)
	2	18	33	10.0 (6.4)
	5	14	36	10.4 (6.3)
	10	14	14	10.5 (6.7)

[1] Days of cultivation at 25°C under continuous light prior to dark pre-treatment or vernalization. [2] The number of leaves in plants without floral primordia is shown in parentheses.

durations under continuous light prior to vernalization, although the number of leaves before initiation of floral primordia increased with increasing cultural durations prior to vernalization. On the other hand, flowering response in dark pre-treatment decreased gradually with increasing cultural durations prior to dark pre-treatment, and only 14% of plants initiated floral primordia in 10 days of culturing prior to dark pre-treatment. The difference in the promotive effects between vernalization and dark pre-treatment increased gradually as the cultural durations under continuous light before vernalization or dark pre-treatment were prolonged.

Effect of alternating treatment of vernalization and dark pre-treatment on floral induction

As shown in the above result, the dark pre-treatment was effective for floral initiation in non-vernalized plants, but flowering response was always less than that induced by vernalization. Therefore, alternative treatments of vernalization and dark pre-treatment on Hashirihadaka prior to continuous light were set up in order to examine the possible mechanism of floral induction caused by these two treatments (Table 5).

Maximum flowering response was obtained by vernalization under continuous light and followed by vernalization under darkness; next responses were obtained in the order that alternative vernalization in darkness and dark pre-treatment, dark pre-treatment, and untreated control. These results indicate that the dark pre-treatment can not fully replace vernalization in promoting floral induction. Furthermore, continuous light given during low temperature promoted floral induction under high temperature condition. The results presented here support that the floral induction caused by dark pre-treatment and by vernalization is based on the different mechanisms. In dark condition, irrespective of temperature, barley plants may flower by their natural tendency to flower.

Table 5. Effect of alternating treatment of vernalization and dark pre-treatment on floral induction of Hashirihadaka winter barley.

Days of pre-treatment 0 15 30 45	No. of plants	Flowering (%)	No. of leaves[1)]
	17	100	7. 5
	15	53	8. 8 (7. 0)
	22	91	9. 7 (7. 5)
	26	92	9.7 (7. 0)
	20	60	9.8 (6.4)
	23	0	(6. 2)

5°C under continuous light ; 5°C in darkness ; 25°C in darkness ; and 25°C under continuous light (untreated control). [1)] The number of leaves in plants without floral primordia is shown in parentheses.

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