

## Flower initiation in total darkness in long-day plant, *Hordeum vulgare* L. var. nudum Hook. f., winter naked barley

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Flower initiation in total darkness in long-day plant,  
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winter naked barley

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Recently it was reported that two winter naked barley varieties which require vernalization about 15 days for the flower initiation in long day condition initiated flower primordia in total darkness." In these barley plants, temperature effect on the flower initiation was not similar in dark and long day condition, i.e., vernalization seemed not to be indispensable for the flower initiation in darkness, but in long day condition.

By using winter naked barley varieties which require about 30 days of chilling for the flower initiation, the present experiment was designed to study the effect of temperature on the flower initiation in darkness.

### Materials and methods

The plant materials used in the experiment were two winter naked barley varieties, Hashiri-hadaka and Hinode-hadaka. The grade of low temperature requirement for the flower initiation of these varieties are known to be V in the former and to be VI in the latter. In this grading used in Japan the highest requirement is referred to VII, and the lowest one to I in wheat and barley varieties.<sup>2)</sup>

The culture medium was of the following composition: 360 mg Mg-SO<sub>4</sub>, 200 mg Na<sub>2</sub>SO<sub>4</sub>, 200 mg Ca(NO<sub>3</sub>)<sub>2</sub>, 80 mg KNO<sub>3</sub>, 65 mg KCl, 16.5 mg NaH<sub>2</sub>PO<sub>4</sub>, 4.5 mg MnSO<sub>4</sub>, 1.5 mg ZnSO<sub>4</sub>, 1.5 mg H<sub>3</sub>BO<sub>3</sub>, 0.75 mg KI, 10 mg Fe-citrate, 100 g sucrose, 6 g agar and 1,000 ml redistilled water. Test tubes, 16x250 mm, containing about 10 ml of the culture medium were autoclaved at 1.0 kg/cm<sup>2</sup> for 20 minutes.

To obtain uniform growth, well matured seeds were selected. For the sterilization, seeds were soaked in 80 per cent alcohol for 3 minutes, in 10 per cent solution of chlorinated lime for 20 minutes and finally

in 3 per cent hydrogen peroxide for 20 minutes. Two seeds were sown in each tube. Each experimental lot consisted of about 20 tubes.

For dark cultures, the tubes were wrapped with light-proof paper, and for long day (continuous light) cultures, the upper portion of the tubes was covered with paper to prevent the medium from drying. As the light source, a combination of forty-eight 200-w fluorescent lamps, four 400-w mercury lamps, four 200-w reflector lamps, two 15-w black light lamps and two 100-w incandescent lamps were used. The intensity was about 6,000 lux at plant level.

The position of the flower primordia on the main culm, as expressed by the number of leaves produced by the time of flowering, was used as a measure of the readiness to flower. The observation of flower primordia was carried out with a binocular microscope.

## Results and discussion

### *1. Effect of duration of vernalization on flower initiation under long day condition.*

The seeds were germinated at 25°C for 48 hours in the dark, and subjected to 5°C for 0, 10, 15 or 30 days in the dark. Then, they were transferred to 25°C under long day condition. The plants were examined for flower initiation 150 days after seeding. The results are given in Table 1.

The plants which received vernalization for 0 and 10 days did not initiate flower primordia. In the lots which received for 15 days of vernalization, only one of Hashiri-hadaka plants initiated flowers at 11.0 th node, but Hinode-hadaka plants did not initiate flowers. In the lots which received vernalization for 30 days, Hinode-hadaka plants initiated flowers at 8.5 th node and Hashiri-hadaka plants did flowers at 9.2 th node.

Table 1. Effect of duration of vernalization on flower initiation under long day condition at 25°C.

a) Hashiri-hadaka

Duration of vernalization at 5°C in days	Number of plants	Number of flowering plants (%)	Number of leaves of flowering plants <sup>a</sup>
0	20	0	(6.2±0.65)
10	20	0	(7.0±0.00)
15	20	5	11.0±0.00
30	20	30	9.2±0.41
45	26	85	8.7±0.64

## b) Hinode-hadaka

Duration of vernalization at 5°C in days	Number of plants	Number of flowering plants (%)	Number of leaves of flowering plants*
0	20	0	(6.8±0.41)
10	20	0	(7.0±0.00)
15	20	0	(7.8±0.70)
30	20	65	8.5±0.51
45	40	97	8.6±0.78

\* The number of leaves of plants without flower primordia is enclosed in parentheses.

For flower initiation under long day condition, these naked barley varieties require vernalization about 30 days and it seems that Hashiri-hadaka may be more sensitive to vernalization than Hinode-hadaka.

## 2. Effects of temperature and duration of vernalization on flower initiation in total darkness

After 48 hours incubation at 25°C, the seeds were treated at 5°C for 0, 10, 15 or 30 days in the dark. Thereafter, they were kept to constant temperatures of 5, 10, 15, 20, 25 or 30°C in total darkness. The plants were investigated for flower initiation 170 days from start of the experiment. The results are shown in Table 2.

As shown in Table 2, the plants of these varieties initiated flower primordia in total darkness and temperature effect was very similar. In these materials, effects of temperature and duration of vernalization on the flowering were identical with that of previous paper, i.e., no significant difference in flowering response was obtained by the temperature conditions (5-30°C) applied in darkness.

In view of the above facts, it seems that the winter naked barley varieties did not require the vernalization for flower initiation in darkness.

## 3. Effect of preceding dark treatment on flower initiation under long day condition at 25°C

After 48 hours incubation at 25°C, the tubes were placed in constant temperatures of 5, 10, 15, 20, 25 or 30°C for 10, 15, 30 or 45 days in total darkness. In an experiment, vernalization at 5°C was carried out under continuous light (about 100 lux) for 15, 30 or 45 days. Thereafter, the tubes were transferred to long day at 25°C. The plants were determined at 100 days after seeding. The results are presented in Table 3.

Effect of preceding dark treatment on flower initiation was very

Table 2. Effects of temperature and duration of vernalization on flower initiation in total darkness.

a) Hashiri-hadaka

Temperature (°C)	Duration of vernalization at 5°C in days	Number of plants	No. of flowering plants(%)	No. of leaves of flowering plants	No. of leaves of non-flowering plants
5	30	15	60	9.1±0.93	7.0±0.00
10	0	33	75	10.4±1.02	7.0±0.00
	10	20	65	10.2±0.97	7.0±0.00
	15	25	67	10.4±0.73	7.0±0.00
	30	13	60	10.7±1.29	7.0±0.00
15	0	20	65	10.9±1.08	7.0±0.00
	10	15	60	10.5±0.68	7.0±0.00
	15	21	61	10.9±1.25	7.0±0.00
	30	16	62	10.3±0.54	7.0±0.00
20	0	15	67	9.7±1.20	7.0±0.00
	10	16	50	9.0±0.00	7.0±0.00
	15	15	60	9.9±0.60	8.0±0.00
	30	16	62	10.0±0.89	8.0±0.00
25	0	18	56	9.8±0.23	8.0±0.00
	10	17	47	9.8±0.60	5.7±0.50
	15	14	50	9.3±1.25	6.4±0.71
	30	25	60	9.5±0.91	6.4±0.65
30	0	14	7	13.0±0.00	7.0±0.00
	10	15	0	—	5.7±0.65
	15	15	0	—	5.9±0.59
	30	14	50	10.1±0.38	6.1±0.38

similar in these varieties. The barley plants of these varieties, Hashiri-hadaka and Hinode-hadaka, which received the preceding dark treatment for 10 days did not initiate flowers, regardless of temperature conditions. In the experimental lots which received for 15 days of dark treatment at 15°C, both Hinode-hadaka and Hashiri-hadaka plants had only one flowering plant; the former initiated at 8.0 th node and the latter did at 9.0 th node, respectively. At 10°C, Hinode-hadaka plants could not initiate flowers, but Hashiri-hadaka plants did at 10.0 th node. On the other hand, the plants received vernalization at 5°C under continuous light for 15 days could not initiate flowers. The plants received the preceding dark treatment for 30 or 45 days initiated flowers, regardless of temperature conditions,

## b) Hinode-hadaka

Temperature (°C)	Duration of vernalization at 5°C in days	Number of plants	No. of flowering plants(%)	No. of leaves of flowering plants	No. of leaves of non-flower- ing plants
5	30	23	65	8.9±0.75	7.0±0.00
10	0	28	60	10.7±0.75	7.0±0.00
	10	35	86	9.8±0.88	7.0±0.00
	15	32	72	9.8±0.64	7.0±0.00
	30	36	83	9.4±0.64	7.0±0.00
15	0	33	67	10.2±1.12	7.0±0.00
	10	38	71	10.7±0.96	7.0±0.00
	15	33	67	10.2±0.90	7.0±0.00
	30	20	60	10.2±0.97	7.0±0.00
20	0	25	64	9.8±1.07	7.0±0.00
	10	37	70	9.8±0.91	7.0±0.00
	15	15	60	9.8±1.30	7.0±0.00
	30	27	67	9.3±0.48	7.0±0.00
25	0	12	25	10.3±1.18	6.3±0.50
	10	32	45	9.9±1.28	6.3±0.52
	15	15	60	9.5±0.72	6.3±0.49
	30	24	50	10.3±0.78	6.6±1.09
30	0	15	20	10.3±0.58	6.7±0.65
	10	16	6	11.0±0.00	6.3±0.91
	15	18	7	10.5±0.71	6.1±0.57
	30	19	53	8.8±0.60	6.0±0.00

From the above results, it seems that these naked barley varieties likely require the preceding dark treatment as same duration as vernalization, about 30 days.

The authors wish to express their thanks to Shikoku Agricultural Experiment Station, which kindly provided the naked barley seeds.

### Summary

By using an aseptic culture of winter naked barley, Hashiri-hadaka and Hinode-hadaka, effect of culture temperature on the flower initiation in total darkness was investigated.

1. For the flower initiation under long day condition at 25°C, these two

Table 3. Effect of preceding dark treatment on flower initiation under long day condition at 25°C.

a) Hashiri-hadaka

Temperature ("C)	Duration in dark treatment	Number of plants	No. of flowering plants(%)	No. of leaves of flowering plants	No. of leaves of non-flowering plants
5	10	12	0	—	6.5±0.57
	15	17	0	—	7.2±1.27
	15*	13	0		7.7±1.56
	30	24	79	8.7±0.67	7.0±0.00
	30*	24	96	8.2±0.38	6.0±0.00
	45	26	85	8.7±0.64	7.0±0.00
	45*	26	97	8.4±0.50	7.0±0.00
10	10	12	0	—	6.3±0.78
	15	15	13	10.0±0.00	7.0±0.00
	30	19	53	9.5±0.52	7.0±0.00
	45	27	85	10.1±0.84	7.0±0.00
15	10	13	0		7.3±0.95
	15	15	7	9.0±0.00	7.0±0.00
	30	22	63	9.4±0.81	7.0±0.00
	45	24	87	9.9±0.76	7.0±0.00
20	10	12	0	—	7.0±1.41
	15	15	0		7.0±0.70
	30	15	30	10.0±1.00	7.0±0.00
	45	21	72	9.9±0.70	7.0±0.00
25	0* (Control)	12	0	—	6.4±0.53
	10	14	0		7.0±1.06
	15	12	0	—	6.5±0.70
	30	16	69	9.7±1.25	6.0±0.00
	45	19	89	9.4±1.09	6.0±0.00
30	10	12	0		6.0±0.00
	15	10	0	—	5.7±0.50
	30	11	0		6.0±0.00
	45	10	20	11.0±1.73	7.0±0.00

varieties required about 30 days of vernalization. In total darkness, however, these materials which received no vernalization initiated the flower primordia at 25°C. Moreover, Hashiri-hadaka plants which received no vernalization did at temperature even as high as 30°C.

2, In agreement with result of the varieties somewhat low in the

## b) Hinode-hadaka

Temperature (°C)	Duration in dark treatment	Number of plants	No of flowering plants(%)	No. of leaves of flowering plants	No. of leaves of non-flowering plants
5	10	15	0	—	5.6±0.54
	15	14	0	—	6.8±0.89
	15*	23	0	—	6.4±0.50
	30	33	82	8.6±0.23	6.0±0.00
	30*	32	a7	8.0±0.54	6.0±0.00
	45	40	97	8.6±0.78	7.0±0.00
	45"	32	96	8.0±0.51	6.0±0.00
10	10	21	0	—	6.0±0.83
	15	23	0	—	7.2±1.00
	30	34	91	8.8±0.57	6.0±0.00
	45	35	97	9.2±0.87	7.0±0.00
15	10	22	0	—	6.0±0.95
	15	20	15	8.0±0.00	6.0±0.00
	30	24	a7	10.3±1.27	7.0±0.00
	45	33	a5	9.5±0.79	7.0±0.00
20	10	19	0	—	6.4±1.12
	15	17	0	—	6.2±1.23
	30	16	56	9.0±1.09	7.0±0.00
	45	24	67	8.9±0.80	7.0±0.00
25	(Control)	17	0	—	5.9±0.66
	10	14	0	—	7.7±1.22
	15	12	0	—	6.8±0.94
	30	13	77	9.3±0.75	6.0±0.00
	45	18	67	9.3±0.89	6.0±0.00
30	10	11	0	—	6.6±0.72
	15	12	0	—	6.0±1.22
	30	11	0	—	6.0±1.00
	45	15	14	9.0±0.00	6.0±0.00

\* Seeds were germinated in continuous light.

vernalization requirement for the flower initiation, no significant difference in flowering response was obtained by the temperature conditions (5–30°C) applied.

3. In these varieties, effect of the dark treatment at the early stage of growth on flowering was same as that of the vernalization.



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