Studies on the Flower Initiation of Spring Wheat in Sterile Culture

I. Effects of Photoperiods and Photo-sensitive Growth Stage

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Received November 10, 1960

Recently many studies on flower initiation have been carried out, using the plants cultured aseptically in test tubes containing the artificial culture medium. It is known that many plants initiate flower primordia in total darkness^{1,2,4,5}), and even at cool temperature³). Sugino⁶) reported that the spring wheat, Konosu No. 25, initiated flower primordia under continuous illumination, 8-hour short days, and complete darkness.

The present experiments were performed with the aim to examine the effects of photoperiods upon flower initiation and growth of spring wheat cultured in test tubes.

Material and Methods

One of the most early-flowering varieties of spring wheat, Konosu No. 25, was used in the present experiments. This plant requires no chilling and has a very short life cycle and initiates visible ear primordia two weeks after germination under continuous illumination.

For seed sterilization, well-matured, and medium-sized seeds were selected. They were 1) washed with a germicidal soap, 2) sucked with vacuum pump in 70 per cent ethyl alcohol solution for 2 minutes, 3) immersed in 10 per cent solution of chlorinated lime for 30 minutes, and then 4) washed with distilled and sterilized water several times. The sterilized seeds were sown aseptically, one grain per test tube containing the modified White's medium consisting of 200 mg. $\text{Ca}(\text{NO}_3)_2$, 360 mg. MgSO_4 , 200 mg. Na_2SO_4 , 80 mg. KNO_3 , 65 mg. KCl , 16.5 mg. KH_2PO_4 , 4.5 mg. MnSO_4 , 1.5 mg. ZnSO_4 , 1.5 mg. H_3BO_3 , 0.75 mg. KI , 2.5 mg. Fe-citrate , 40 g. sucrose, 8 g. agar and 1,000 ml. distilled water.

The light conditions were as follows: continuous illumination, 8-hour short days, and total darkness. The plants were grown under various combinations of these three light conditions. In the experiments started on October 7, 1955, and February 20, 1956, the source of artificial light consisted of four 100 watt incandescent lamps and one 20 watt daylight fluorescent lamp, and the luminosity at the plant level was about 1,000 lux. In the daytime, they were supplemented with diffused daylight which had about one fiftieth of natural daylight intensity. In the experiment started on January 10, 1958, only the artificial light was used, and the luminosity at the plant level was about 2,000–3,000 lux.

All experiments were performed in an air-conditioned room at $20 \pm 2^\circ$. In the experiments mentioned above, the observations were carried out 35–50 days after germination. And, in the experiment started on December 11, 1956, in which the plants were cultured only in total darkness, the observations were carried out 110–160 days after germination. The flowering stage and the number of leaves produced

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on the main stem (leaf number) were observed under a dissecting microscope. The value of flowering stage was recorded according to the arbitrary scale shown in Table 1.

Table 1. Values assigned to stages in development of the flower primordia.

Values	Description of the shoot apex
0	Vegetative, smooth dome
0.5	Slightly elongated apex
1	Elongated phalloid apex
2	Double ridge
3	Formation of side spikelet
4	Anther initials visible
5	Heading

Table 2. Culture of Konosu No. 25 under various photoperiodic treatments, from October 7 to November 10, 1955.

L (The continuous illumination): 24-hour photoperiod+no dark period.

S (The short day): 8-hour photoperiod+16-hour dark period.

Photoperiodic treatment (days)	Flowering percentage	Position of flower, indicated by leaf number	Flowering stage	Height of plants (mm.)	Number of plants observed
35 L + 0 S	100	5.1	4.2	99	11
20 L + 15 S	100	5.2	4.1	93	15
10 L + 25 S	100	5.2	3.7	74	24
5 L + 30 S	100	6.0	3.2	68	19
10 S + 25 L	100	6.0	3.9	79	19
20 S + 15 L	100	6.5	2.8	38	13
35 S + 0 L	100	7.0	2.3	25	8

Results

Depression of flowering and growth under prolonged short day treatment and darkness: All plants, subjected to continuous illumination, 8-hour short photoperiod, or both light conditions with various combinations, initiated flower primordia. With increasing exposure to 8-hour short days, however, the values of flowering stages and the heights of plants decreased, and the leaf number, which indicated the position of the node from which the plant entered into reproductive phase, increased gradually (Table 2). With increasing duration of dark culture, the values of flowering stages and fresh- and dry-weights decreased. The leaf number increased with increasing duration of dark culture. Although the heights of plants increased to considerable extent in the prolonged darkness, this increase may be due to etiolation (Tables 3, 4). Thus flowering and growth were depressed under the prolonged short days, and especially in darkness.

Promoting effects of photoperiods inserted in continuous darkness on flowering and growth: In order to examine the photoperiodic sensitivity of the dark-grown plants at various growth stages, the plants were cultured in darkness, and exposed to 5 or 10 day continuous illumination 0, 5, 10, 20 days after the germination (Tables 3, 4).

With the light interception for 10 days, all plants initiated flower primordia, irrespective of the growth stage during which the light was given. When the light was given 10 or 20 days after germination, the values of flowering stages, fresh- and dry-weights of plants decreased, and the leaf number increased as compared with those exposed to the light at earlier growth stages. When the 5 day light interception was given at the beginning of the sixth day after germination, flowering percentages showed higher values (95 and 83%) as compared with those given to the other growth stages, and the values of flowering stages also increased (Tables 3, 4).

Thus the results show that 1) the photoperiods inserted in continuous darkness

Table 3. Culture of Konosu No. 25 under various photoperiodic treatments, from February 20 to April 11, 1956.

L: continuous illumination. D: continuous darkness.

Photoperiodic treatment (days)	Flowering percentage	Position of flower, indicated by leaf number	Flowering stage	Height of plants (mm.)	Fresh weight of plants (mg.)	Dry weight of plants (mg.)	Number of plants observed
50 L + 0 D	100	5.1	5.0	116	362	51	15
20 L + 30 D	100	5.0	4.6	182	318	59	24
10 L + 40 D	100	5.4	2.5	112	250	50	22
5 L + 45 D	89	7.3	1.0	95	260	37	26
5 D + 5 L + 40 D	95	6.7	1.6	90	228	39	22
10 D + 5 L + 35 D	57	7.6	1.9	101	215	39	28
5 D + 45 L	100	5.4	4.2	112	314	39	19
10 D + 40 L	100	6.1	2.6	73	248	40	28
20 D + 30 L	100	7.2	1.5	148	251	35	18
50 D + 0 L	0	7.0*	0.0	182	193	28	27

* The number of leaves produced in vegetative growth.

Table 4. Culture of Konosu No. 25 under various photoperiodic treatments, from January 10 to March 3, 1958.

L: continuous illumination. D: continuous darkness.

Photoperiodic treatment (days)	Flowering percentage	Position of flower, indicated by leaf number	Flowering stage	Height of plants (mm.)	Fresh weight of plants (mg.)	Dry weight of plants (mg.)	Number of plants observed
50 L	100	5.0	5.0	101	447	139	25
50 D	0	7.5*	0.0	216	171	37	30
5 L + 45 D	80	7.3	1.3	139	187	45	25
5 D + 5 L + 40 D	83	7.5	1.8	176	231	52	35
10 D + 5 L + 35 D	79	8.4	1.5	146	154	38	35
15 D + 5 L + 30 D	75	8.5	1.4	155	167	42	35
20 D + 5 L + 25 D	77	8.9	1.4	180	170	40	35
10 D + 10 L + 30 D	100	6.7	3.2	172	199	56	35
20 D + 10 L + 20 D	100	7.2	3.0	187	196	59	35

* The number of leaves produced in vegetative growth.

promote flowering and growth, 2) the light period of 5 days is not enough for flower promotion but that of 10 days is sufficient, and 3) the growth stage from the sixth day to the tenth day after germination is the most sensitive one to photoperiodic treatment.

Discussion

The study on the flowering of spring wheat, Konosu No. 25, cultured *in vitro* was published by Sugino⁶⁾ in 1957. He reported that this plant initiated flower primordia under three light conditions (total darkness, 8-hour short days, and continuous illumination), and that sucrose, more than 4 per cent, must be added to the medium in dark culture, 2 per cent sucrose in short days culture, and no sucrose in continuous illumination. He concluded that "....Especially, in dark culture, the amount of sugar added to the medium has a very significant influence not only upon the survival of plants but also upon the flowering in wheat....".

In the present experiments, the photoperiods given to dark culture showed the promoting effect on flowering and growth of the plant. On the other hand, it was shown that flowering and growth were depressed with the prolonged short days and darkness. These depression may be due to a shortage of the photosynthetic products, probably of sugars.

With regard to leaf number, the following facts were found. In the cases in which continuous darkness was intercepted with the continuous illumination for 10 days or more, the number of leaves produced on the main axis before flower initiation were almost equal to those observed at the bending of light interception, i.e. the plant seems to cease differentiating new leaf primordia on the shoot apex immediately after exposure to light and to begin initiating flower primordia.

In the case in which continuous darkness was intercepted with the continuous illumination for 5 days, the growth stage from the fifth to the tenth day after germination was most sensitive to photoperiods, and a gradual decrease in sensitivity was observed with delaying exposure to the light. The decrease in sensitivity may be due to a decrease in total metabolic activity caused by an insufficient supply of the nutrients in the prolonged dark culture.

Plants cultured in darkness for 50 days did not initiate flower buds on the main axes and the tillers which had little developed on account of the apical dominant

Table 5. Culture of Konosu No. 25 in total darkness, from December 11, 1956, to March 31 and to May 21, 1957.

Duration of dark culture in days	110	160
Main axis		
Flower initiation*	1/11	2/16
Flowering stage	1.0	1.5
Tiller		
Flower initiation**	0/8	5/23
Flowering stage	0.0	2.4

* Denominator: number of plants dissected.

Numerator: number of main axes with flower primordia.

** Denominator: number of tillers dissected.

Numerator: number of tillers with flower primordia.

growth of the main axis. But those cultured for 160 days initiated flower primordia on the tillers to some extent, and rarely on the main axes (Table 5).

Summary

- 1) The spring wheat, Konosu No. 25, was cultured aseptically in test tube containing the modified White's medium under various light conditions.
- 2) The flowering and growth of spring wheat were depressed under the prolonged short days, and especially in darkness.
- 3) The photoperiods inserted in continuous darkness promoted flowering and growth. The photoperiod of 5 days was not enough for flowering, but that of 10 days was sufficient.
- 4) The early growth stage from the sixth to the tenth day after germination was most sensitive in photoperiodic response. The sensitivity decreased gradually with proceeding growth stages in dark culture.
- 5) The plants cultured in darkness for 50 days did not initiate flower primordia, but those cultured for 160 days initiated on the tillers and rarely on the main axes.

I am much indebted to Prof. S. Imamura of Kyoto University for his much criticisms for this paper.

References

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摘 要

和田清美：無菌培養における春まき小麦の花芽形成について

I. 明期の効果と感光期

春まきコムギ 鴻巣 25 号)を試験管内で無菌培養を行ない、種々の光条件下、特に暗黒下での培養に与えられた明期の花芽形成と生長におよぼす影響を調べた。(1) この植物の花芽形成および生長は、連光下で促進され、短日下で低下し、暗黒化では特に抑えられる。しかし、全暗黒下でも花芽形成能を有することが示された。たとえば、連光下では発芽後 20 日にして出穂を始めるが、暗黒下での培養では、50 日にも発芽がまったく見られず、まれではあるが 160 日でようやく主軸(茎頂)に花芽をみる。またこの時期には分けつした枝にやや多くの花芽を見ることができる。(2) 暗黒下での培養の途中で明期を与えた場合、(生育時期にかかわらず)、5 日間連続する明期は花芽形成に不十分であったが、10 日間の明期は十分であることを見た。(3) 暗黒下での培養の各生育時期に与えられた 5 日間連続する明期の花芽形成におよぼす効果は、それが与えられる生育時期で異なる。その結果からみて発芽後 5~10 日が明期に対してもっとも強く反応する生育時期と考えられる。そして、暗黒下での培養が長びくにつれて、明期の効果は減ずることが観察された。(静岡大学文理学部生物学教室)