Literature cited

- 1) Barnes, H. M., Feldmann, J. R. and White, W. V., Jour. Am. Chem. Soc., **72** 4178 (1950).
- 2) Bennet-Clark, T. A. and Kefferd, N. P., Nature, **171** 645 (1953).
- 3) Gordon, S. A., Ann. Rev. Plant Physiol., **5** 341 (1954).
- 4) Hattori, S., Yoshida, S. and Hasegawa, M., Physiol. Plantarum, **7** 283 (1954).
- 5) Hörhammer, L. and Scherm, A., Archiv. Pharmazie, **288/60** 441 (1955).
- 6) Johnson, G. and Shaal, A., Science, **115** 627 (1952).
- 7) Kefferd, N. P., J. Exp. Bot., **6** 129 (1955).
- 8) Konishi, M., Mem. Coll. Agr. Kyoto U., Ser. B., **75** 1 (1956).
- 9) Koshimizu, K. and Mitsui, T., Jour. Agr. Chem. Soc. Jap., **30** 63 (1956).
- 10) Koshimizu, T. and Hiraiwa, K., Jap. Jour. Ecol., **4** 55 (1954).
- 11) Lexander, K., Physiol. Plantarum, **6** 406 (1953).
- 12) Luckwill, L. C., Nature **169** 375 (1952).
- 13) Platt, R. S. Jr. and Thimann, K. V., Science **123** 105 (1956).
- 14) Ruckenbrod, H., Planta **46** 19 (1955).
- 15) Rudkin, G. O. and Nelson, J. M., Jour. Amer. Chem. Soc., **69** 1470 (1947).
- 16) Svendsen, A. B., "Zur Chemie Norwegischer Umbelliferen" Oslo (1954).
- 17) Tang, Y. W. and Bonner, J., Arch. Biochem., **13** 11 (1947).
- 18) Uritani, I. and Matsumura, K., Jour. Agr. Chem. Soc. Jap., **27** 29 (1953).
- 19) Uritani, I. and Miyano, M., *ibid.*, **29** 151 (1955).
- 20) Yamaki, T., Misc. Rep. Res. Inst. Natur. Resour., **17-18** 180 (1950).

Flower Initiation of the Spring Wheat in Total Darkness

by Mamoru SUGINO*

杉野 守*: 全暗黒条件における春小麦の花芽分化

Received September 27, 1957

It has been reported that several plants can initiate flower primordia when cultured in total darkness. To such plants belong not only some short-day plants,^{12 13)} but also many long-day plants such as *Spinacia oleracea*, *Pisum sativum*, *Raphanus sativus*, *Baeria gracilis*, *Rudbeckia bicolor* and *Silene Armeria*.^{6 11 13)}

The present experiments were performed with aims to examine the effects of nutritional conditions upon the growth and development of wheat plants in aseptic culture in test tubes and to test the possibility of flower initiation in total darkness.

Materials and Methods

One of the most early-flowering varieties of spring wheat, Konosu No. 25, was used for the present study on account of its short life cycle. In a preliminary experiment, it was observed that the plant initiates visible ear primordia two weeks after germination under continuous illumination.

The basic culture medium was the modified White's solution. It contains: $\text{Ca}(\text{NO}_3)_2$ -200mg, Mg SO_4 -360mg, Na_2SO_4 -200mg, KNO_3 -80mg, KCl -65mg, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ -16.5mg, MnSO_4 -4.5mg, ZnSO_4 -1.5mg, KI -0.75mg, Fe-citrate-4mg, Ebios-500mg, Agar-8g and distilled water 1000ml. Five groups of the culture media which contained 0, 2, 4, 8 and 10 per cent sucrose of chemical grade respectively were prepared. About 10ml of the culture medium was poured into an 18×150mm test tube and autoclaved

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan. 京都大学農学部応用植物学研究室

at 1.6 atm for 20 minutes. Initial pH was adjusted to 5.6-5.8 with 0.1 N KOH.

For the sterilization, seeds were 1) soaked in cresol-water 1:10 solution for 5 minutes, 2) washed in distilled water several times, 3) soaked in 75 per cent alcohol for 5 minutes, 4) soaked in 10 per cent solution of chlorinated lime for 30 minutes and 5) washed in sterilized distilled water several times until the smell of chlorine disappeared in the waste water.

Experiments were started on October 8, 1955. Two grains of the seed were aseptically sown in each test tube and were grown under three light conditions: total darkness, 8-hour short-day and continuous light. The sources of artificial light consisted of four 100 watt incandescent lamps and one 20 watt daylight fluorescent lamp, the luminosity at the plant level being about 1000 lux. In the daytime, they were supplemented with diffused daylight which had about one fiftieth of natural daylight intensity. All cultures were placed in a room kept at $20 \pm 2^\circ\text{C}$.

The observations were carried out 50 days after germination in the long-day series, 56 days in the short-day series and 108 days in the dark series, when the flower primordia were recognizable.

The flowering stage and number of leaves produced on the shoot were observed under a binocular microscope. The former was recorded according to the arbitrary scale given in Table I. The leaf number of tillers, which were often produced in the dark culture, is the sum of the number of the leaves on tiller itself and that on the main shoot up to the node on which the tiller branched.

The dry weights of the plants were measured about one week after harvest when the materials attained constant weight in a desiccator after having been killed in an electric oven at 110°C .

Table I. 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 II. Culture of Konosu No. 25 under continuous illumination, from October 9 to November 28, 1955

Sucrose concns.	0%	2%	4%	8%	10%
No. of plants observed	29	20	14	20	30
No. of plants with flower	29	20	14	20	30
Leaf number	5.27 ± 0.08	5.08 ± 0.05	5.13 ± 0.09	5.17 ± 0.08	5.09 ± 0.06
Flowering stage	4.29 ± 0.11	4.39 ± 0.11	4.90 ± 0.03	4.90 ± 0.06	4.88 ± 0.06
Stem length in mm	117.8 ± 6.05	162.0 ± 4.89	155.7 ± 5.55	167.3 ± 6.39	158.8 ± 5.49
Fresh weight in mg	154.8	151.5	146.4	150.0	145.9
Dry weight in mg	24.2 ± 0.70	35.5 ± 1.65	46.1 ± 1.79	60.3 ± 1.85	60.0 ± 2.31

Table III. Culture of Konosu No. 25 in short days,
from October 9 to December 4, 1955

Sucrose concns.	0%	2%	4%	8%	10%
No. of plants observed	19	25	29	19	24
No. of plants with flower	3	25	29	19	24
Leaf number	8.00*	6.76 $\pm$ 0.12	6.97 $\pm$ 0.11	7.31 $\pm$ 0.15	6.79 $\pm$ 0.15
Flowering stage	0.5 $\pm$ 0.00	2.78 $\pm$ 0.07	3.04 $\pm$ 0.07	2.77 $\pm$ 0.11	2.84 $\pm$ 0.16
Stem length in mm	3.2 $\pm$ 0.67	89.2 $\pm$ 3.81	95.6 $\pm$ 3.15	97.1 $\pm$ 5.38	87.3 $\pm$ 2.45
Fresh weight in mg	111.2	125.0	152.4	161.9	151.3
Dry weight in mg	18.5 $\pm$ 1.05	33.1 $\pm$ 1.38	45.2 $\pm$ 1.53	55.4 $\pm$ 2.15	56.3 $\pm$ 1.69

* The leaf number of plants with flower

Table IV. Culture of Konosu No. 25 in darkness,
from October 9, 1955 to January 25, 1956

Sucrose concns.	0%	2%	4%	8%	10%
No. of plants observed	10	29	32	22	30
No. of plants survived	0	(2)	20	20	23
Survival percentage	0	(6.9)	62.5	90.9	76.6
No. of plants with flower	—	0	7	16	17
% of flowering plants against survivals	—	0	35	80	77.3
Leaf number in flowering stem	—	—	10.42 $\pm$ 0.20	10.38 $\pm$ 0.15	10.35 $\pm$ 0.26
Flowering stage	—	—	0.50 $\pm$ 0.00	0.69 $\pm$ 0.06	0.76 $\pm$ 0.09
No. of tillers developed per survival	0	0	0.9	1.0	1.0
No. of leaves per live stem	—	(11.0)	10.1	10.3	10.3
dead stem	5.5	6.6	7.4	7.0	7.2
Length of live stem	—	(545.0)	207.7 $\pm$ 20.0	156.0 $\pm$ 9.6	196.1 $\pm$ 16.4
dead stem	180.0 $\pm$ 17.9	313.4 $\pm$ 14.0	267.0 $\pm$ 10.3	222.8 $\pm$ 14.6	218.5 $\pm$ 13.2
Fresh weight of live plant	—	(209.0)	176.5	146.6	134.0
Dry weight of live plant	—	(41.0)	46.5 $\pm$ 2.20	48.7 $\pm$ 2.99	54.4 $\pm$ 3.67
dead plant	15.6	30.2	32.5	29.0	34.0

(): Plants were partly imbedded in agar medium.

Results

The results are represented in Tables II, III, IV and V.

1) Generally, the wheat plants developed in the test tube were very small in size as compared with the normal plants grown in the field. This apparently "adaptive" growth is probably due to the limited supply of food materials, since in sand culture without fertilizer the plant developed also much smaller size than the normal.

The growth of the wheat plant in the test tube depended largely on light conditions and also on sucrose content of the culture medium. Under continuous illumination, all plants grew fairly well and developed flowers even on the medium without sucrose, whereas in short days all plants grew meagerly and only 3 out of 19 plants could initiate flower primordia on the sugarless medium. In the dark, no

plants could survive without sugar, and on the medium containing the lowest concentration (2%) of sucrose, were alive only two plants whose shoots were partly imbedded in the medium.

2) Stem lengths of the plants cultured under continuous light or short-day conditions were nearly the same irrespective of the sucrose concentrations ranging from 2 to 10 per cent in the media, although those cultured on the medium without sucrose showed markedly small values (Tables II and III).

Under continuous darkness, stems were elongated markedly and showed an etiolated appearance, but their growth seems to be restricted to a certain limit (Table IV). The tops of main axes in the dark-grown plants were almost dead after having reached this limit, and on the media containing more than 4 per cent sucrose, tillers developed. On sugarless media, the shoots elongated up to 180 mm and were all dead, probably due to the exhaustion of reserve materials. In the presence of sucrose in the medium, further but limited stem elongation could occur, a maximum value of 313 mm being attained on the 2 per cent sucrose medium. The length and the number of dead stems and dry weight of dead plants had nearly the same limited values except the lower value on the sugarless medium.

3) Fresh weight was not varied remarkably with sucrose concentrations in long days, but it showed relatively lower values on media without and with 2 per cent sucrose in short days. It is remarkable that the fresh weight of the dark grown plants decreased with increasing sucrose concentrations.

Dry weight was greatly affected by the sucrose added. With increasing sucrose concentrations, the dry weight of plants increased under all light conditions.

4) Number of leaves formed on the axis before flowering was not so much affected by sucrose concentrations either in long or short days, although a somewhat higher value was obtained on the sugarless medium. In the darkgrown plants, the leaf numbers were nearly equal on the media with more than 4 per cent sucrose.

5) Flowering stage was not varied by sucrose concentrations in long days. In short days somewhat lower value was obtained in the series without sucrose. In the dark culture, although the development of flower primordia was slow and poor, a gradual increase in flowering stages was observed with increasing sucrose concentrations. The details of flower development in darkness are represented in Table V.

Table V. Development of flower primordia in the dark culture
a. Observation 103 days after inoculation

% of sucrose in media	Values* ¹								Mean
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	
0	—	—	—	—	—	—	—	—	—
2	0	0	0	0	0	0	0	0	0
4	7	0	0	0	0	0	0	0	0.5±0.00
8	11	4	1	0	0	0	0	0	0.69±0.06
10	11	3	2	1	0	0	0	0	0.76±0.09

b. Observation 135 days after germination*2

10	9	8	2	0	0	2	2	0	1.29±0.23
10*3	3	0	5	2	8	2	1	0	2.05±0.19

*1 See Table I

*2 Experiment performed with the aims to test the effects of phosphate on flower initiation.

*3 Medium contained 5 times phosphate in excess.

Discussion and Conclusion

The growth and development of wheat plants are accelerated by light.¹⁾⁻⁴⁾ The longer the day length is, the faster the plant grows. This is probably due, at least in part, to the increased photosynthesis as pointed out by Pohjakallio.⁷⁾

Sucrose added to the culture medium increases the dry weight with increasing concentrations, but no significant differences in the fresh weight, stem elongation and flowering stage are found on the media with more than 2 per cent sucrose under long- and short-day conditions.

In the dark culture, the stem elongation and fresh weight are somewhat decreased with increasing sucrose concentrations, although the dry weight and flowering stages are increased. These results indicate that light affects the growth of plants not only by the production of carbohydrates, but also by other influences on some metabolic processes as suggested by Went.¹⁴⁾

For the successful culture of higher plants under “heterotrophic” conditions, some practical difficulties must be overcome: The quality and quantity of the nutrients which are essential to the normal development of the plant must be clarified. A suitable method of supplying nutrients must be contrived. And last, the abnormal elongation or etiolation which may alter the physiological activities, must be inhibited by some means.

One of the examples of success in culturing higher plants heterotrophically to maturity was given by Spoehr¹⁰⁾. He cultured albino maize by feeding with sucrose given to the cut ends of the leaves under diffuse light. The plants grown under this condition, free from etiolation, were sturdy and developed to pistillation. But the exclusion of light is preferable for “heterotrophic” culture, since the light may have other biochemical effects besides photosynthesis.

Gorham has also succeeded in culturing *Lemna* under absolute darkness.⁵⁾ In this case the plants were fed with mineral nutrients containing sucrose, organic nitrogen (e.g., amino acids) and other growth factors (e.g., yeast extract) through the fronds.

In the present work the wheat, Konosu No. 25, grew at least for 135 days after germination and developed to the flowering stage 4 (see Table I) on the mineral medium containing 10 per cent sucrose in absolute darkness. The plants showed the typical symptoms of etiolation. The elongated main axes were almost dead after a limited growth and further growth and development was succeeded by the

new tiller, which was brought about by adding the higher concentrations of sucrose. The most vigorous growth, however, was observed when some parts of shoots had been imbedded in agar medium and the nutrients were supplied through the leaves.

For the initiation of flower primordia and the development up to anther formation at least, light is not indispensable in the wheat plant, but the number of leaves formed on the axes before flowering—the number is directly related to the development⁸⁾—is influenced by light period. Before flowering, Konosu No. 25 develops about 5 leaves in the long day, 7 in the short day and 10 in darkness. The results are interesting as compared with those obtained in other plants by previous authors (Table VI).

Table VI. Number of leaves on flowering shoots

Materials	Photoperiodic conditions			Researchers
	Long day	Short day	Darkness	
Alaska pea	7	12	7	Leopold, A. C. ⁶⁾
<i>Raphanus sativus</i> L.	8.7 ^{*1} ±1.3	11±1.0	8.2±1.1	Tashima, Y. ¹¹⁾
<i>Baeria gracilis</i>	4.8 ^{*1} ±0.15	7.1±0.23	6.2±0.16	Takimoto, A. ^{*2}
Wheat (Konosu No. 25)	5 ^{*1}	7	10	

*1 Continuous light

*2 Report presented in the 20th General Meeting of the Botanical Society of Japan at Hiroshima, 1955.

With regard to the effects of light on flowering, three cases were found.

- 1) Short day treatment retards flower initiation as compared with long-day and dark treatments: Pea and radish.
- 2) Increasing duration of light accelerates flower initiation: wheat.
- 3) Long-day treatment accelerates and short-day treatment retards the initiation of flower primordium as compared with dark treatment: *Baeria*.

In a recent paper Sen and Leopold,⁹⁾ citing the works of Tashima¹¹⁾ and others on the dark cultures of radish and other plants, have recognized the physiological significance of sugar added to the medium in flower initiation. As shown in the present work (Tables II, III, IV), under short days and 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. The flowering percentage and flowering stages increase with increasing sugar concentrations. Under continuous illumination, however, sugar has, if any, a very slight influence on flowering. The lengthening of light period seems to compensate for the absence of sugar in the culture medium. This may be interpreted from the nutritional standpoint.

But in all plant in Table VI, with exception of wheat, flower initiation occurs earlier in dark culture than in short-day condition, suggesting that short days retard flower initiation. This indicates that the alternation of light and darkness is not only nutritionally but also morphogenetically of a decisive importance in flower initiation.

In contrast to the above plants, wheat initiates ear primordia earlier* in short days than in total darkness, suggesting that the nutritional factor plays a more dominant role in the initiation of flowers in the wheat than in the other plants such as pea, radish and *Baeria*.

Summary

1) The spring wheat, Konosu No. 25, was cultured on media containing White's mineral nutrients and sucrose of various concentrations under three photoperiodic conditions: continuous illumination, short days and total darkness.

2) The effects of sucrose concentrations on the growth and development of the wheat plant were investigated. For the normal development of the plants, more than 4 per cent sucrose must be added in darkness, 2 per cent in short days and no sucrose in long days. On dry matter production, effects of sucrose were appreciable under all light conditions.

3) In darkness the wheat plants could sustain their growth at least for 135 days and initiate flowers on the media containing more than 4 per cent sucrose.

4) The number of leaves formed on the axes before flower initiation was 5 in long days, 7 in short days and 10 in darkness.

I wish to express my deep gratitude to Prof. S. Imamura, Laboratory of Applied Botany in Kyoto University, and Assist. Prof. Y. Tashima of Kagoshima University for their helpful suggestion and criticism through the investigation.

Literature cited

- 1) Chinoy, J. J., *Nature* **104**: 887 (1949). 2) ——— and Nanda, K. K., *Physiol. Plantarum* **4**: 209 (1951). 3) ——— and ———, *ibid.* **4**: 427 (1951). 4) ——— and ———, *ibid.* **4**: 575 (1951). 5) Gorham, P. L., *Canad. Jour. Res.* **28**: 356 (1950). 6) Leopold, A. C., *Plant Physiol.* **24**: 530 (1949). 7) Pohjakallio, O., *Physiol. Plantarum* **5**: 409 (1952). 8) Purvis, O. N., *Ann. Bot. N. S.* **48**: 919 (1934). 9) Sen, S. P. and Leopold, A. C., *Plant Physiol.* **31**: 323 (1956). 10) Spoehr H. A., *Plant Physiol.* **17**: 397 (1942). 11) Tashima, Y., *Proc. Japan Acad.* **29**: 271 (1953). 12) ———, *Memoirs Fac. Agric. Kagoshima Univ.* **2**: 1 (1956). 13) Tashima, Y. and Imamura, S., *Proc. Japan. Acad.* **29**: 591 (1953). 14) Went, F. W., *Dynamics of Growth Process*, ed. Boell, E. J., Princeton (1954).

* In the wheat the main axis dies almost entirely in the dark and ear formation occurs on tillers. It is doubtful, therefore, whether the leaf number of wheat, which includes the leaves produced on tiller and main axis before flowering, can be directly compared with that of other plants in the Table.