EFFECTS OF NUCLEIC ACID MATERIALS ON FLOWERING I. INFLUENCES OF RIBONUCLEIC ACID ON THE FLOWERING OF RADISH

EFFECTS OF NUCLEIC ACID MATERIALS ON FLOWERING
I. INFLUENCES OF RIBONUCLEIC ACID ON THE FLOWERING OF RADISH

By

Kazuhiko CHIBA and Akio FUJIWARA

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received, September 6, 1964*)

Flowering of plants is controlled by some environmental conditions, especially by the temperature and the daylength which play an important role in regulating their reproductive development, and many evidences have been obtained mainly by studying the responses of the plant to these conditions (1, 2). Furthermore, there are some evidences which suggest that the flowering is affected by the nutritional conditions of the plant itself (3, 4). And these evidences have been of value for agriculture.

The flowering in a plant which induces the initiation of flower primordia instead of producing more leaf primordia is always connected with the changes in the metabolism taking place in the leaves and apical meristems of the plant. But we are almost ignorant of the biochemical mechanisms underlying and preceding the formation of flower buds.

In recent years, the attention of investigators studying the problems of flowering has been devoted to the substances related to nucleic acid, since some of these substances proved to have great influence on the growth and the morphogenetic processes of the plants.

It has been reported by a number of workers that the flowering of some plants was accelerated by the application of ribonucleic acid or other substances related to nucleic acids. For example, Tashima and Imamura (5) showed that the unvernalized seedlings of *Raphanus* could initiate flower primordia by adding ribonucleic acid to the culture medium in the dark, and Kessler, Bak, and Cohen (6) also found with the bean and potato plants that their flowering were promoted by the spray treatment with uracil, xanthine, and caffeine. Furthermore, it was reported by Tomita (7) that the rye diffusate which was obtained in the course of vernalization treatment of seeds was capable of promoting the flowering of winter wheat, and that the effective substances in the diffusate might be uridylic acid.

On the other hand, it has been studied in our laboratory (8) that in the leaves of tobacco grown under short-day or blue light, i.e., the favourable conditions for the flowering, the contents of nucleic acid were higher than in the leaves treated under unfavourable conditions, such as long-day or red light, and also that the contents of nucleic acid in the leaves were increased by the application of nucleic acid materials. Thus, it was suggested that the nucleic acid in the leaves might have some influences on the flowering process.

These data show that the action of ribonucleic acid and the derivatives of nucleic acid metabolism leads to the acceleration of the flowering, and that the nucleic acid contents were increased with the flowering. However, considerably less clear is the role of the nucleic acid in the flowering process.

From these points of views, it is of interest to investigate further the role of ribonucleic acid and substances related to nucleic acid on the flowering. In the present experiments, the effects of ribonucleic acid on the flowering of radish were investigated at the outset to make clear the action of nucleic acid substances on the flowering process in the plant.

Materials and Methods

Radish plant, *Raphanus sativus*, was used as the experimental material. The seeds were soaked overnight in water, and then the swollen seeds were germinated on a moist sandy bed. Three days after the germination, the seedlings were transplanted in each pot filled with 3 kg of an alluvial soil fertilized with 0.5 g of N, P_2O_5, and K_2O, respectively. Then these plants were grown in a greenhouse under natural daylength and temperature.

The experiments were carried out under the natural condition of long-day in June and July, and of short-day in September and October.

With the experiment carried out early in June, the effects of the spray treatment of ribonucleic acid (RNA) were examined. At the spray treatment, 500 p.p.m. aqueous solution of commercial RNA (from yeast) partially decomposed by autoclaving at 20 lb/cm^2 for 30 minutes was used. On all leaves of a plant, the RNA solution was sprayed at intervals of three days during their vegetative development from five days after the germination.

In the experiment initiated in the middle of September, the action on flowering of low temperature treatment, i.e., vernalization, was investigated together with that of the spray treatment with RNA. The seeds were disinfected and soaked overnight in water. The swollen seeds were sowed on two sheets of filter paper in a petri dish, in which distilled water was added sufficiently for the germination. Immediately after the sowing, the seeds were vernalized and germinated by keeping them in a dark cold room at 5± 1°C for 10, 15, and 20 days. Being vernalized for the designated periods, the seedlings were transplanted, grown, and sprayed with RNA solution according to the methods described above.

Among some methods for estimating the acceleration or the retardation of the flowering time, the days reqired until 50 per cent of the bolting rate and the number of leaves on the main stem were recorded here. The number of leaves was also estimated after the complete bolting.

Results and Discussion

The changes in the fresh weight of the tops and roots of the radish plants grown under the natural long-day and short-day conditions are shown in Table 1, and 2, respectively. It is confirmed that the fresh weight of the tops and roots

Table 1. Changes in the fresh weight of the tops and roots of the radish with or without the application of RNA. The plants were grown under the long-day condition. Figures represent g per plant.

Treatments	Days after germination				
	22	25	28	31	34
			Top		
None	0.31	0.93	1.46	2.98	4.66
RNA-spray	0.32	1.04	2.16	3.31	5.47
			Root		
None	0.06	0.19	0.74	2.66	4.88
RNA-spray	0.06	0.25	1.16	2.70	5.45

Table 2. Changes in the fresh weight of the tops and roots of the radish plants treated with RNA-spray and vernalization, grown under the short-day condition. The plants were vernalized by keeping at 5± 1°C for 10 days. Figures represent g per plant.

Treatments	Days after germination							
	14	17	20	24	27	29	34	39
				Top				
None	0.53	1.05	1.54	2.39	—	4.67	5.84	7.52
RNA-spray	0.53	1.15	2.11	3.17	—	4.72	6.80	8.45
Vernalization	0.16	0.43	0.97	1.51	3.12	—	5.30	—
Vernalization and RNA-spray	0.19	0.46	0.90	1.45	3.20	—	5.33	—
				Root				
None	0.11	0.26	0.54	2.02	—	5.95	9.08	18.60
RNA-spray	0.11	0.28	0.73	2.62	—	6.30	11.09	23.60
Vernalization	0.04	0.06	0.11	0.24	1.20	—	6.55	—
Vernalization and RNA-spray	0.04	0.06	0.12	0.27	1.23	—	3.75	—

of the radish, except the case of the vernalized ones, increases to some extent by the spray treatment with RNA in either daylength condition. Thus, it is supposed that the vegetative growth is accelerated by the application of RNA.

However, with the plants vernalized during the germination period and induced in the direction of the reproductive development, the growth was little affected by treating with RNA, as shown in Table 2 which shows only the result of 10 days' exposure. The results obtained with the treatment of 15 or 20 days' exposure are not shown here, because they have the same trend as that with 10 days' exposure.

Since the radish used as the experimental material is a long-day plant, its flower is induced under long-day condition, and not under short-day condition. However, when vernalized by treating the germinating seedlings with low temperature, they are induced to flower independent of the daylength conditions. In the present study, when exposed to low temperature during the germination period, the plants were completely vernalized by 20 days' exposure, and incompletely by the exposure of 10 and 15 days (Fig. 1-2).

As shown in Fig. 1-1 the spray treatment with RNA had no effect on the bolting rate of the unvernalized plants growing under the long-day condition. In addition, no significiant difference was observed in the number of leaves on the main stem of the sprayed plants and of the control. Consequently, the RNA

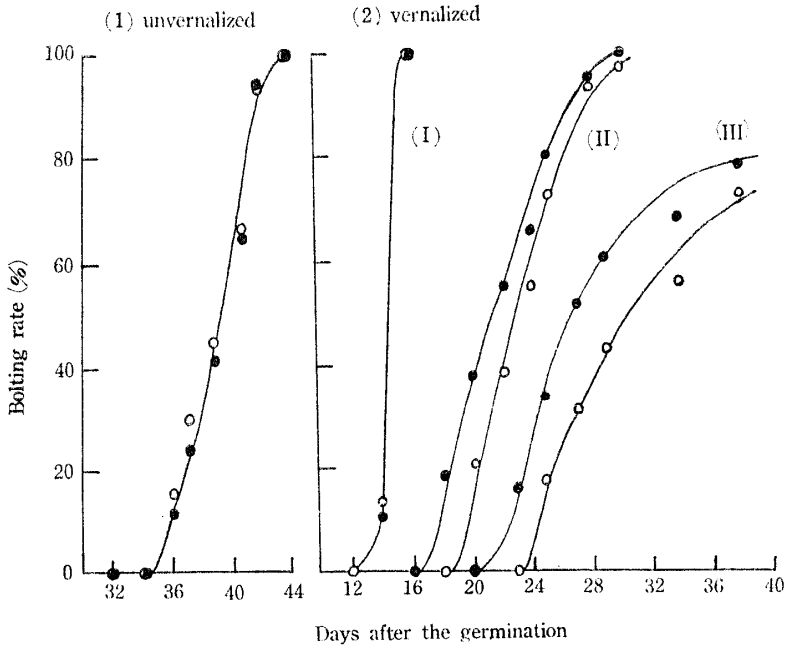

Fig. 1. Accumulation curves of the bolting rate of (1) unvernalized radish plants grown under the long-day condition, and (2) ones vernalized for 20 (I), 15 (II), and 10 (III) days and grown under the short-day condition. Closed circles; with the spray treatment of RNA, and open circles; without the RNA treatment.

sprayed on the leaves seems to be effective in promoting the growth (Table 1), but not in promoting the flowering.

The effects of RNA on the bolting of the radish plants vernalized for 10, 15, and 20 days are summarized in Fig. 1-2. With the plants exposed to low temperature insufficiently for complete vernalization, the initiation of bolting was hastened by the application of RNA, while the bolting of the completely vernalized plants was not affected by its application.

Furthermore, to investigate whether the action of the sprayed RNA leads to the promotion of bolting alone, or the acceleration of the formation of flower buds too, the number of leaves on the main stem bolted was examined after the complete bolting. By this examination, it is supposed to be capable of estimating the progress of the initiation of flower buds, for the number of leaves of a plant with corymb, such as *Raphanus*, ought to be limited in consequence of the initiation of flower buds on the apical meristem. The results given in Table 3

Table 3. Effects of RNA spray treatment on the days required until 50 per cent of the bolting rate and the number of leaves of the radish plant vernalized at 5±1°C for 10, 15, and 20 days. The plants were grown under the short-day condition.

Treatments	Days treated with low temperature	Days required for 50% bolting rate	Number of leaves on the main stem
Vernalization	10	34	24.9 ± 1.3
Vernalization and RNA-spray	10	27	21.1 ± 1.4
Vernalization	15	24	14.5 ± 1.8
Vernalization and RNA-spray	15	21	10.1 ± 1.1
Vernalization	20	15	9.9 ± 0.6
Vernalization and RNA-spray	20	15	9.6 ± 0.6

show that the initiation of flower buds was accelerated by treating the incompletely vernalized plants with RNA, because significant differences were obtained in the number of leaves on the main stem between the vernalized plants and the vernalized-RNA sprayed ones. The days required 50 per cent of the bolting rate was also reduced by a week with 10 days' exposure and three days with 15 days' exposure in consequence of the spray treatment with RNA. In the case of 20 days' exposure, it may be inferred that there were no effects of RNA on the bolting and the flowering because the flower buds initiated in a short period as a result of the complete vernalization.

From the present results, it is suggested that with the radish plants vernalized

and induced to reproductive development, the flowering process is accelerated by the external application of RNA.

Recently, several evidences were found indicating RNA or nucleic acid materials to be effective in promoting flowering of cold requiring plants. Tashima and Imamura (5) found that the application of RNA was effective in promoting flowering of unvernalized *Raphanus sativus*. Suge and Yamada **(9)** examined the effects of chemicals such as uracil, uridylic acid, α-naphthaleneacetic acid, gibberellin, and kinetin on the flowering of the excised embryos of winter wheat which were partially vernalized, and found that uracil and uridylic acid were most effective in promoting the flowering. On the other hand, it has been reported (10, 11, 12) that the flowering of certain plants which do not require vernalization was promoted by the application of some materials related to nucleic acid, and it was inhibited by the treatment with antimetabolites of nucleic acid, such as 5-fluorouracil, thiouracil, and 8-azaguanine. These results together with the results of our study, indicate that metabolism of nucleic acid or its constituents may be involved in the process of flowering.

In the course of the present study, the possibility that the action of RNA in the promotion of flowering may be due to some substances derived from its decomposition was suggested. We will report on this subject in the following paper.

Summary

(1) The effects of RNA on the flowering of the radish plant were investigated, by means of spray treatment on the surface of the leaves.

(2) The plants were grown under the natural long-day or short-day conditions, and also with and without the application of RNA which was decomposed by autoclaving. In either daylength, the RNA sprayed on the leaves seems to be effective in promoting the growth, but not in promoting the flowering.

(3) When exposed to 5 ± 1°C during the germination period, the plants were completely vernalized by 20 days' exposure and incompletely by the exposure of 10 and 15 days. With the plants vernalized incompletely, the initiation of flower buds was accelerated by spraying with RNA solution. Because the days required until 50 per cent of the bolting rate was shortened and the number of leaves on the main stem was reduced in comparison with those of the plants grown without the application of RNA.

(4) It is suggested that with the radish plants vernalized and induced to the reproductive development, the flowering process is accelerated by the external application of RNA.

References

1) Lang A. (1952). Ann. Rev. Plant Physiol. **3**, 265.
2) Chourad P. (1960). *ibid.*, **11**, 191.

3) Eguchi, T., T. Matsumura, and M. Ashizawa (1958). Proc. Amer. Soc. Hort. Sci. **72**, 343.
4) Wittwer, S. H. and F. G. Teubner (1957). Amer. J. Bot. **44**, 125.
5) Tashima, Y. and S. Imamura (1954). Bot. Mag. Tokyo **67**, 281.
6) Kessler, B., R. Bak., and A. Cohen (1959). Plant Physiol. **34**, 604.
7) Tomita T. (1959). Tohoku J. Agr. Res. **10**, 1.
8) Fujiwara, A. and M. Kurosawa (1963). J. Sci. Soil & Manure, Japan, **32**, 319.
9) Suge, H. and N. Yamada (1963). Proc. Crop Sci. Japan, **32**, 77.
10) Marushige, K. and Y. Marushige (1962). Bot. Mag. Tokyo, **75**, 270.
11) Bonner, J. and Jan A. D. Zeevaart (1962). Plant Physiol. **37**, 43.
12) Hess D. (1961). Planta **57**, 13.