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EFFECTS OF PHOTOPERIOD AND GIBBERELLIN ON THE GERMINATION OF SEEDS OF *BEGONIA EVANSIANA* ANDR.

MASAYUKI NAGAO, YOJI ESASHI, TERUKO TANAKA, TAKAYOSHI KUMAGAI[1] AND SHITSUE FUKUMOTO

Biological Institute, Tôhoku University, Sendai

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1. Seed germination of *Begonia Evansiana* ANDR. was investigated at 29°C.

2. The germination was induced under long-day conditions, the critical daylength being about 8 hours. Exposure to at least 2 or 3 cycles of long days was necessary for germination. The seeds could germinate under otherwise non-inductive photoperiods, when the dark period was interrupted with a short period of illumination. Thus the photoperiodic behaviour of *Begonia* seeds in germination is similar to that of typical long-day plants in flowering.

3. The application of gibberellin brought about no germination in complete darkness, but markedly reduced the critical daylength for germination, even 1-minute photoperiods being inductive. The germination under continuous light was also favoured by gibberellin application. The action of gibberellin in germination of *Begonia* seeds may be to intensify the light action or to substitute for a part of it.

While studying on the photoperiodism in the formation and sprouting of aerial tubers and in the flowering with *Begonia Evansiana* ANDR., it was found that the germination of the seeds of this species is also controlled by photoperiods. The photoperiodic control of seed germination has been demonstrated in a number of light-responsive seeds since it was reported by ISIKAWA (*1*) and BLACK and WAREING (*2*). Although it seems of interest to compare the photoperiodic phenomenon in germination with that in flowering, there have been few detailed works on the former as yet. In the present paper, the photoperiodic behaviour in the germination of seeds of *B. Evansiana* was studied minutely.

On the other hand, it has been reported that the light requirement for germination of certain light-sensitive seeds is eliminated by the

[1] Present address: Dept. of Botany, Hokkaido University, Sapporo.

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action of gibberellin[1] (*3*), and that the photoperiodic response in flowering is also affected by GA treatment (*4*). It appears therefore interesting to investigate the effect of GA on the photoperiodically-controlled germination of *Begonia* seeds.

MATERIAL AND METHODS

Seeds of *Begonia Evansiana* ANDR. collected in 1956 and 1957 were used for the experiments in 1957 and 1958, respectively.

About 100–250 seeds, which were very small and light, were floated on about 10 ml of distilled water or test solution in glass tubes, ca. 2 cm in diameter and ca. 4 cm in height. Three to four tubes were usually employed for each treatment. The tubes were placed in black boxes made of paper board to keep them dark, the lid of the boxes being taken off for the illumination of seeds. Fluorescent tubes (Mazda "natural daylight") were used as light source, seeds being illuminated at the intensity of about 750 lux. The experiments were carried out at 29°C.

Germination was counted under a dissecting microscope 9 days or more after the start of the experiments, when the germination had practically or completely terminated.

RESULTS

Light Requirement for Germination

Seeds were illuminated for various periods immediately after sowing and then kept in darkness for the remainder of the experimental period. The results of three experiments carried out on separate days are shown in Fig. 1.

Similar effects of illumination were observed in these experiments, although the maximum germination percentages were different among

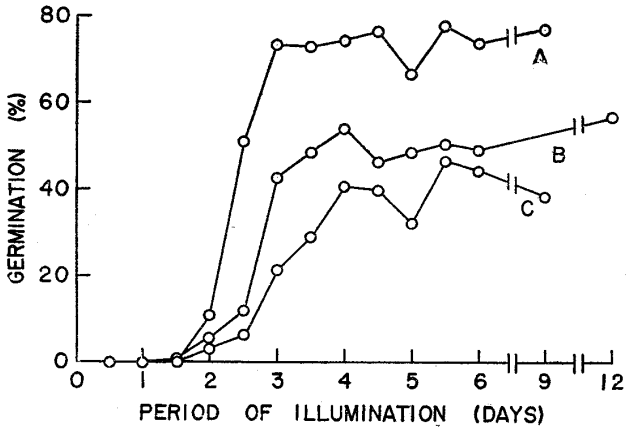

Fig. 1. Germination percentages of seeds exposed to various lengths of illumination. A and C, results after 9 days; B, after 12 days.

[1] The abbreviation GA will be used in this paper.

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them. No seeds germinated unless they were exposed to light at least for 2 days, and illumination of 3 to 4 days' duration was sufficient to give the maximum germination percentage.

Effect of Daily Photoperiod on Germination

As shown in the above experiments, light is required for the germination of *Begonia* seeds. It was found in the following experiments that the germination is photoperiodically controlled. In one experiment, the seeds were exposed to various lengths of daily photoperiod for 10 days

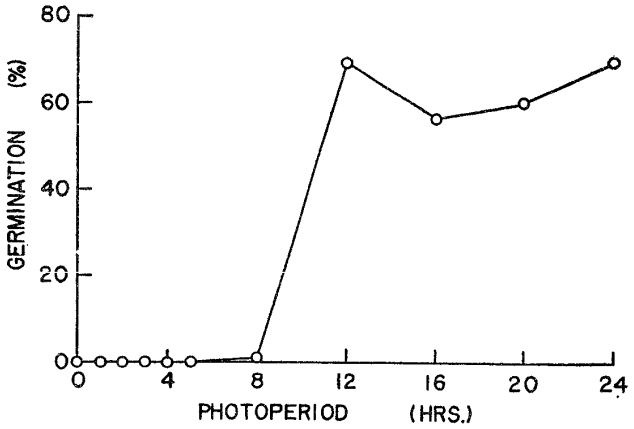

Fig. 2. Germination percentages of seeds exposed to various lengths of photoperiod for 10 days.

(Fig. 2), and in another, they were subjected to 6 cycles of photoperiodic treatments and then maintained in the dark for 7 days (Fig. 3).

Germination was observable only when the photoperiod exceeded about 8 hours and increased with the lengths of photoperiods. Photoperiods of 12 hours or more gave nearly constant germination percentages. Thus the results suggest that the photoperiodic response in germination of *Begonia* seeds corresponds to that in flowering of long-day plants.

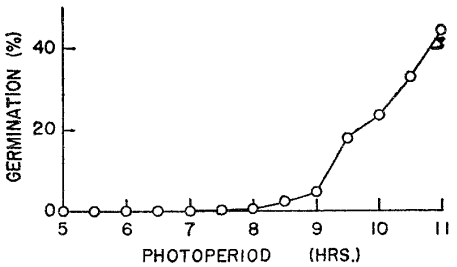

Fig. 3. Germination percentages of seeds exposed to various lengths of photoperiod for 6 days and then kept in darkness for 7 days.

Effect of Light-Interruption of Dark Periods

Flowering of long-day plants can be induced under usually non-inductive photoperiods, if the long dark period is interrupted with light. *Begonia* seeds were also induced to germinate even under 4-hour photoperiods when a light-break of one hour was applied in the middle of the

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dark period, 6.2 and 15.0 per cent germination being obtained after 10 and 12 days, respectively. In the next experiment, the seeds were exposed to 6 cycles of 7-hour photoperiod and subsequent 17-hour dark period which was interrupted in the middle with various lengths of illumination. The seeds were then kept in the dark for 7 days. The results are shown in Fig. 4. A light-break of only 5 minutes was effective.

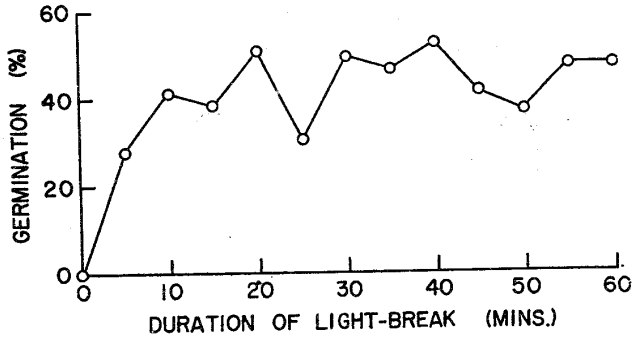

Fig. 4. Effect on germination of various lengths of light-interruptions given in the middle of 17-hour dark period.

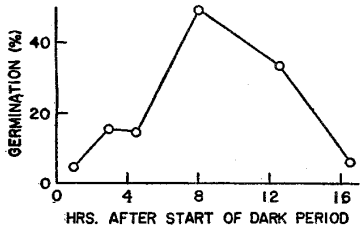

Fig. 5. Effect on germination of a 30-minute light-break given at various times in the 17-hour dark period of each of 6 cycles.

The following experiment was then carried out to determine the effect of the light-break in relation to the time at which it is given. The experimental conditions were similar to the above except that a 30-minute light-break was given at various times in the dark period of each cycle. As shown in Fig. 5, the light-break was most effective when given about the middle of the dark period.

The present results clearly indicate that the germination of *Begonia* seeds is affected in a similar way to the flowering of long-day plants by the light-break given during the dark period.

Minimum Number of Cycles Required for Germination

Seeds were exposed to 1 to 6 cycles of various photoperiods, and then kept in darkness for the remainder of the experimental period. The results are given in Fig. 6.

At least 2 cycles of longer photoperiods or 3 cycles of shorter photoperiods were necessary for germination. The germination percentage increased with the number of cycles given and nearly reached the maximum by the exposure to 5 cycles. The fact that at least 2 days' exposure was needed for germination in the previous experiments in which the seeds were exposed to single photoperiods (Fig. 1), is consistent with the present results.

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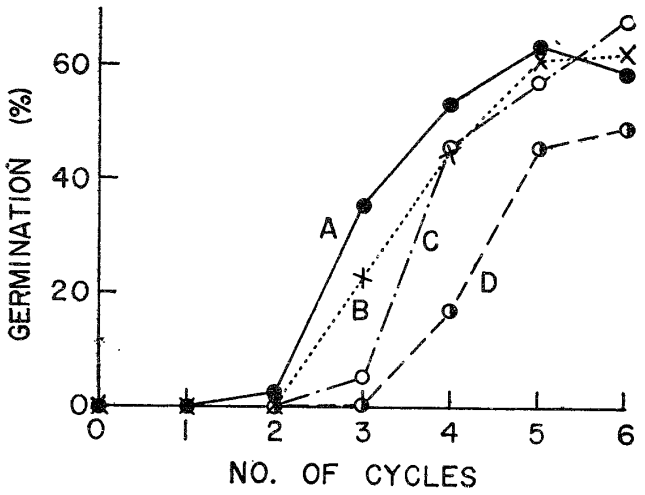

Fig. 6. Relation of germination to the number of cycles of various photoperiods. Results of separate experiments. Photoperiods, (A) 20 hours; (B) 16 hours; (C) 12 hours; (D) 7 hours, dark period being interrupted in the middle for 15 minutes. Germination after 10 days (A to C) and 13 days (D).

Effect of Gibberellin on Seed Germination under Various Daily Photoperiods

The effect of GA[3] on germination in complete darkness was examined first. No seeds germinated in the dark during 10 days in GA solutions at concentrations ranging from 0.01 to 1000 ppm. This indicated that GA did not serve as a substitute for light.

Then the experiments were carried out to determine the effect of GA on the germination of seeds receiving various photoperiods. In the presence of GA at various concentrations, the seeds were illuminated continuously for 10 days, or they were given 7 cycles of 8-, 5- or 2-hour

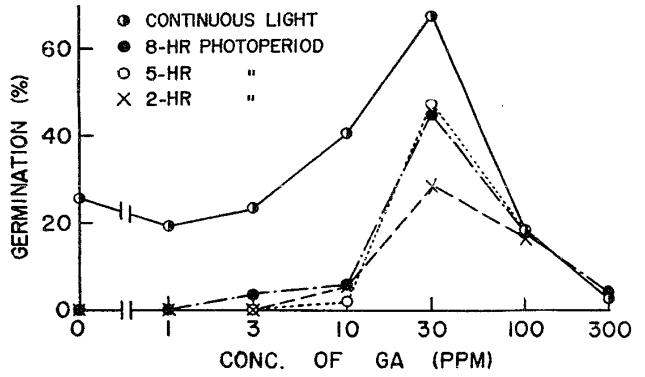

Fig. 7. Effect of gibberellin on germination of seeds under various photoperiods.

[3] Gibberellin was kindly supplied by the Kyowa Fermentation Industries, Tokyo.

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photoperiods and then maintained in the dark for 3 days. The results of the experiments carried out separately are summarized in Fig. 7.

Germination under continuous light was increased by GA application, the concentration of 30 ppm being the optimum. It is to be noted that, in GA solutions, the seeds could germinate under the photoperiods which otherwise induced no germination, the optimum concentration being 30 ppm also in this case. The result is compared with the fact that many long-day plants are induced to flower even under short-day conditions when they are treated with GA (*4*).

Since it was found that the critical daylength for germination is considerably reduced by GA application, germination in 30 ppm GA under the photoperiods shorter than 2 hours was tested in the same way as the above. In this experiment, however, the seeds had been allowed to imbibe water in darkness for 6 hours before the photoperiodic treatments were begun. (This procedure was adopted in all the subsequent experiments.) Table I shows that the seeds germinate well even under 1-minute photoperiods.

TABLE I

Percentage Germination of Seeds under Short Photoperiods in the Presence of 30 ppm Gibberellin

Experiment	Photoperiod (minutes)								
	0	1	3	5	10	30	60	90	120
I	0	19.1	13.5	27.3	22.7	11.1	21.9	21.4	12.0
II	–	15.4	–	17.5	–	16.6	12.9	–	19.7

Effect of Gibberellin on Germinaton of Seeds Given a Single Light Exposure of Various Durations

As already mentioned, at least 2 days' illumination is required for germination when the light is applied continuously. On the other hand, it was shown that the critical daylength for germination is markedly reduced by the action of GA. Therefore the following experiment was made to test whether the duration of single light exposure needed for

TABLE II

Percentage Germination of Seeds Exposed to Single Photoperiods in the Presence or Absence of 30 ppm Gibberellin

Gibberellin (ppm)	Duration of light exposure						
	7 mins	1 hr	5 hrs	24 hrs	42 hrs	48 hrs	72 hrs
30	0	0	0	0	0	7.2	43.0
0	–	–	–	0	0	0	14.4

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germination is shortened by GA application. Seeds were given single photoperiods of 7 minutes to 72 hours in the presence or absence of 30 ppm GA, and then maintained in darkness. The results obtained 10 days after the start of the experiment are shown in Table II.

While the germination percentage of seeds illuminated for 72 hours was considerably increased by GA application, no seeds germinated even in the presence of GA unless they were exposed to light for 48 hours or more. The results suggest that GA does not seem to reduce the length of illumination period required for germination, although GA increases the germination percentage if the seeds are given a light exposure of necessary duration.

Minimum Number of Cycles of 1-Minute Photoperiods Required for Germination in the Presence of Gibberellin

Seeds were exposed in the presence of 30 ppm GA to 1 to 7 cycles of 1-minute photoperiods and then kept in darkness. Germination was counted 10 days after sowing. Germination began in the seeds subjected to 3 cycles of the photoperiodic treatment (Table III).

TABLE III

Percentage Germination of Seeds Exposed to Various Cycles of 1-Minute Photoperiods in the Presence of 30 ppm Gibberellin

No. of cycles	1	2	3	4	5	6	7
% germination	0	0	0.5	0.3	1.7	5.1	10.3

DISCUSSION AND CONCLUSIONS

While the aerial tuber (*5*) and the flower (*6*) of *Begonia Evansiana* are formed under short-day conditions, the seed of it germinates in response to long days. Exposure to cycles including a certain minimum photoperiod is required for germination. The germination percentage increases with the length of photoperiod until it reaches the maximum, which is maintained under still longer photoperiods and continuous light. The seeds can be induced to germinate under usually non-inductive photoperiods, if the long dark period is interrupted with a short period of illumination. The light-break is most effective when given near the middle of the dark period. All these facts indicate that the photoperiodic behaviour of *Begonia* seeds in the germination resembles to that of long-day plants in the flowering. This is also true with the effect of GA to be discussed later.

Among many instances of photoperiodic control of germination so far recorded, those studied minutely are only few. BLACK and WAREING (*2*) and BÜNSOW and VON BREDOW (*7*) have investigated in detail the photoperiodism in germination of seeds of *Betula pubescens* and *Kalanchoë*

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blossfeldiana, respectively, and demonstrated that the photoperiodic behaviour of these seeds in germination is similar to that of long-day plants in flowering. Recently, ISIKAWA and YOKOHAMA (*8*) also reported some light-sensitive seeds of this type.

The photoperiodic response of *Begonia* seeds was markedly modified by GA application. After the experiments were finished, EÜNSOW and VON BREDOW (*7*, *9*) reported similar results with *Kalanchoë blossfeldiana*. The GA treatment not only increased the germination of *Begonia* seeds under continuous light, but also reduced considerably the critical daylength for germination. However, no seeds germinated in complete darkness even in the presence of GA, and the duration of a single light exposure required for germination was not shortened by GA treatment, at least 2 days' illumination being necessary.

The above results show that GA promotes the germination only when photoperiodic treatments are applied to the seeds. Thus the effect of GA may be to intensify in some way the action of light given periodically. However, assuming that, in *Begonia* seeds, light initiates some process(es), which proceeds under light and finally leads to germination, the action of GA may substitute for the latter part of the light action. Since it has been known that the light requirement of certain seeds such as of lettuce and tobacco is eliminated by the action of GA (*3*), it may be natural to suppose that the course of germination of *Begonia* seeds also involves some process whose light requirement can be replaced by GA. However, the process caused by GA may probably not be the same as that caused by light, as suggested by EÜNSOW and VON BREDOW (*7*) and POLJAKOFF-MAYBER et al. (*10*), for the germination under continuous light is also increased by GA application.

It is noteworthy that GA treatment at a concentration of 100 ppm was considerably inhibitory to the germination of *Begonia* seeds under continuous light, since it has been known in a number of species that GA does not inhibit the seed germination even at fairly high concentrations (*3*, *11*). This appears of interest when considered in connection with the fact that GA, which is often effective in the breaking of various kinds of dormancy, seems to induce or prolong the dormancy in the case of aerial tubers of *Begonia* (*12*).

REFERENCES

(*1*) S. ISIKAWA. 1954. Light sensitivity against germination. I. "Photoperiodism" of seeds. *Bot. Mag. Tokyo*, **67**, 51-56.

(*2*) M. BLACK and P. F. WAREING. 1954. Photoperiodic control of germination in seed of birch (*Betula pubescens* EHRH.). *Nature*, **174**, 705; M. BLACK and P. F. WAREING. 1955. Growth studies in woody species VII. Photoperiodic control of germination in *Betula pubescens* EHRH. *Physiol. Plant.*, **8**, 300-316.

(3) F. LONA. 1956. L'acido gibberellico determina la germinazione dei semi di *Lactuca scariola* in fase di scoto-inhibizione. *Ateneo parmense*, **27**, 641-644 (*Ber. wiss. Biol.*, **117**, 320 (1958)); A. KAHN, J. A. GOSS and D. E. SMITH. 1957. Effect of gibberellin on germination of lettuce seed. *Science*, **125**, 645-646; K. OGAWARA and K. ONO. 1957. Effect of gibberellin on germination of light-sensitive tobacco seeds. *Proc. 1st Japanese Gibberellin Symposium*, 9-10 (In Japanese); S. M. ALCORN and E. B. KURTZ. JR., 1959. Some factors affecting the germination of seed of the saguaro cactus (*Carnegiea gigantea*). *Amer. Jour. Bot.*, **46**, 526-529.

(4) B. B. STOWE and T. YAMAKI. 1957. The history and physiological action of the gibberellins. *Ann. Rev. Plant Physiol.*, **8**, 181-216; B. B. STOWE and T. YAMAKI. 1959. Gibberellins: stimulants of plant growth. *Science*, **129**, 807-816; P.W. BRIAN. 1959. Effects of gibberellins on plant growth and development. *Biol. Rev. Cambridge Phil. Soc.*, **34**, 37-84.

(5) Y. ESASHI and M. NAGAO. 1958. Studies on the formation and sprouting of aerial tubers in *Begonia Evansiana* ANDR. I. Photoperiodic conditions for tuberization. *Sci. Rep. Tôhoku Univ.* 4th Ser. (Biol.), **24**, 81-88.

(6) Unpublished data.

(7) R. BÜNSOW and K. VON BREDOW. 1958. Wirkung von Licht und Gibberellin auf die Samenkeimung der Kurztagpflanze *Kalanchoë blossfeldiana*. *Biol. Zbl.*, **77**, 132-141.

(8) S. ISIKAWA and Y. YOKOHAMA. 1959. On the photoperiodic phenomenon in seed germination. Abstracts of Papers Presented at the 24th Annual Meeting of the Botanical Society of Japan, 38 (In Japanese).

(9) R. BÜNSOW and K. VON BREDOW. 1958. Einfluss der Gibberelline auf die Tageslängenabhängigkeit der Samenkeimung von *Kalanchoë*. *Naturwiss.*, **45**, 95-96.

(10) A. POLJAKOFF-MAYBER, M. EVENARI and G. NEUMANN. 1958. Effect of red light and gibberellic acid on the temperature-inhibited germination of lettuce seeds. *Bull. Res. Counc. Israel*,, D, **7**, 99-102 (*Ber. wiss. Biol.*, **132**, 63 (1959)).

(11) C.W. DONOHO, JR. and D. R. WALKER. 1957. Effect of gibberellic acid on breaking of rest period in Elberta peach. *Science*, **126**, 1178-1179; E. YAMADA. 1957. Effect of gibberellin on the germination of some dormant seeds. *Proc. 1st Japanese Gibberellin Symposium*, 42 (In Japanese); M. GOO and H. TUTIHASI. 1958. Hastening the germination of *Pinus densiflora* seeds by gibberellin. *Jour. Japanese Forestry Soc.*, **40**, 509-511 (In Japanese with English abstract); S. NAKAMURA. 1959. Promoting effect of gibberellin on seed germination in egg-plants, *Perilla* plants and other crops. *Agr. and Hort.*, **34**, 1277-1278 (In Japanese).

(12) M. NAGAO and E. MITSUI. 1959. Studies on the formation and sprouting of aerial tubers in *Begonia Evansiana* ANDR. III. Effect of gibberellin on the dormancy of aerial tubers. *Sci. Rep. Tôhoku Univ.* 4th Ser. (Biol.), **25**, 199-205.