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SUCCESSIVE PROCESSES INVOLVED IN THE GERMINATION RESPONSE OF *NASTURTIUM* SEEDS

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1. The seeds of *Nasturtium palustre* DC. do not germinate, either in the light or darkness, at various constant temperatures, but require for their full germination a certain period of a low temperature (5°) applied immediately after light irradiation. These results indicate the existance of at least two processes, a light-dependent process and a low temperature-requiring process, in the initiation of germination of *Nasturtium* seeds. Experimental evidence indicated further that the light exposure causes two different processes in the seed germination.

2. When a dark period at 23° was inserted between the light irradiation and the low temperature treatment the germination was suppressed. The inhibitory effect of the inserted dark period at 23° was eliminated by a short irradiation during the darkness (light-break).

3. Prolonged exposure of *Nasturtium* seeds to any concentration of gibberellin brought about no germination when exposure was given in complete darkness. The germination was promoted only when light irradiation was applied to the seeds. A short application of gibberellin at a fairly high concentration was, however, remarkably effective for the germination even in the darkness, and the germination was inhibited as the gibberellin application was lengthened. It was considered that gibberellin could substitute for the combined effect of light irradiation and low temperature treatment to induce the germination of *Nasturtium* seeds, and that gibberellin was inhibitive toward the reactions following the above treatments which induced the germination.

In recent years the effects of gibberellin treatment on plants have received much attention. Various aspects of these problems were summarized by BRIAN (*1*), and STOWE and YAMAKI (*2, 3*) in their recent reviews. There are many seeds which require cold temperature treatment, exposure to light, or some other factor(s) for their germination. It has

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been known that these requirements are eliminated or replaced in certain cases by the action of gibberellin (*4, 8*).

FUJII *et al.* (*5*) have shown that a fairly high concentration of gibberellin was favorable for the induction of germination in *Sedum* seeds, but unfavorable for the development following the induction of germination.

On the other hand, many workers investigated the interaction between light and temperature on seed germination (*9, 11*). The data reported hitherto show that the pattern of interaction differs according to the kind of seeds used. Seed germination of some *Rumex* was greatly stimulated by changing only the incubation-temperature from 20° to 30° or 20° to 5° (*10*). ISIKAWA and ISHIKAWA (*12*) reported that in the seeds of *Elsholtzia* germination was not induced at various constant temperatures, irrespective of whether they were kept in the light or darkness. It was shown that, for their full germination, a certain period of low temperature (5°) immediately after the light irradiation is necessary. These studies seem to open a new avenue of approach to the elucidation of the process of germination.

The present study aims at analyzing the process of germination into its intermediate processes. It is hoped that these experiments will demonstrate some of the details of the interaction between light and temperature in the mechanism of seed germination. The effect of gibberellin upon these intermediate processes also forms a subject of this study.

MATERIAL AND METHODS

The experiments were carried out with the seeds of *Nasturtium palustre* DC. collected in October 1959 at Kohriyama, Fukushima Prefecture. The sample of gibberellin was generously supplied by Dr. Y. MURAKAMI of the National Institute of Agricultural Science.

The methods used were similar to those previously described for *Nigella* seeds by ISIKAWA (*9*). As light source, a standard cool white flourescent lamp was used. A definite intensity of irradiation was obtained by regulating the distance from the light source to the irradiated PETRI dish. Temperature was usually controlled at 23° unless otherwise described.

A definite amount of gibberellin was added to the autoclaved agar solution just before solidification. On 0.7—0.8% agar-bed, in which gibberellin was dissolved in various concentrations, a filter paper wet with corresponding gibberellin solution was placed directly, and the seeds were disseminated on it.

When short exposures to gibberellin were made in the germination process, the following procedures were taken. A sheet of filter paper, on which the seeds had been pre-soaked with water, was placed between four sheets of dried filter paper to remove the humidity surrounding the seeds, and then in darkness the former paper with seeds was transferred onto a PETRI dish containig the indicated concentration of gibberellin, and exposed to gibberellin for various time periods. After the gibberellin treatment, the seeds were washed in running water for 5 minutes under a blue light (fluorescent light filtered through six layers of dark blue cellophane), and then allowed to germinate on a non-gibberellin-containing agar-bed in darkness. In all experiments, two lots, each containing 100 seeds, were tested. Under our experimental conditions, all the seeds employed were found to germinate uniformly.

RESULTS

Sensitivity of seeds to light followed by low temperature

In a preliminary experiment not reproduced here it was confirmed that not only the light but also a low temperature-treatment immediately after irradiation is necessary for inducing the germination of *Nasturtium palustre* DC.

To observe the sensitivity to light immediately followed by a low temperature (5°), the seeds were first irradiated for 24 *hr* with the light of 1500 lux after various times of imbibition in darkness, and exposed to 5° in darkness for 24 *hr* immediately after the irradiations. The percentage of germination increased with the increase of imbibition-time prior to irradiation until it reached a maximum value (80%) after about 4 days of imbibition, and decreased gradually after 7 days with further increase in the imbibition time. Consequently, the subsequent experiments were performed on seeds with 5 day imbibition.

Interaction between light irradiation and low temperature treatment

To observe the interaction between light irradiation and low temperature treatment, the following experiments were performed. After 5 days of dark imbibition at 23°, the seeds were irradiated with light of 1500 lux for various periods of time and then were kept at 5° for various durations. The seeds were allowed to germinate in darkness at 23° for 5 days after the treatments. The effectiveness of the light period, after low temperature-treatment for 6 *hr*, increased markedly with the length of irradiation time up to 24 *hr*, and then decreased as the time of light exposure was further lengthened (Curve A in Fig. 1). This decrease of percentage of germination due to the prolonged exposure to light, however, was eliminated with longer application of low temperature (5°). When the low temperature-treatment was applied for 24 *hr*, the maximum germination percentage (92%) was maintained for 72 *hr* (Curve C in Fig. 1). The experimental results outlined above may be taken as indicating that the process of germination of *Nasturtium* seeds involves the following successive phases:

——→Light-dependent process——→Low temperature-requiring process——
——→(Reactions)——→Germination

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In the next experiment, various durations of darkness at 23° were inserted between light (1500 lux) and low temperature (5°) treatments. Several lots of *Nasturtium* seeds were irradiated for 24 *hr* with light of 1500 lux after 5 days of dark imbibition and were returned to darkness at 23°. After dark periods of various durations at 23°, they were exposed to 5° for 6 or 24 *hr*. It was found that as the duration of darkness between the light irradiation and low temperature treatment was increased, the promoting effect of low-temperature treatment decreased linearly to disappear eventually (Fig. 2). These data indicate that the darkness at 23° inserted between the light and low temperature treatments was inhibitory to germination, an effect which might be compared with that of the dark period in the flowering of long-day plants.

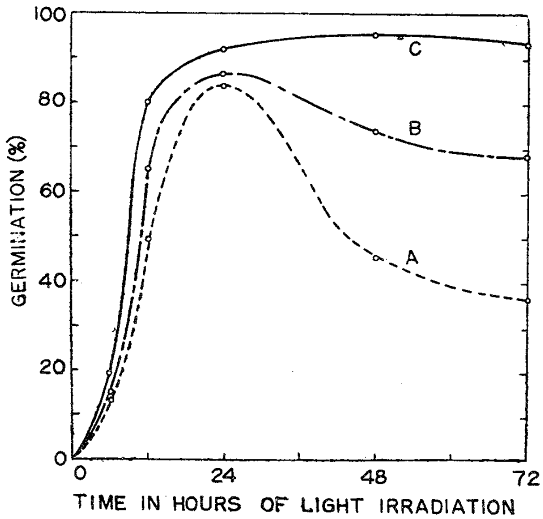

Fig. 1. Effect on germination of irradiation (with 1500 lux light) of various durations followed by low temperature treatments lasting for 6 *hr* (curve A), 12 *hr* (curve B,) and 24 *hr* (curve C.).

In another experiment, the seeds were irradiated with light of 1500 lux for 24 *hr* and, after the intervening dark periods at 23° of various

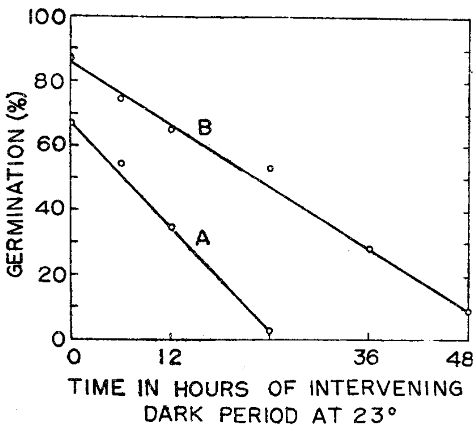

Fig. 2. Effect of insertion of dark incubation at 23° between a 24 *hr* light period and low temperature treatment lasting for 6 *hr* (A) and 24 *hr* (B).

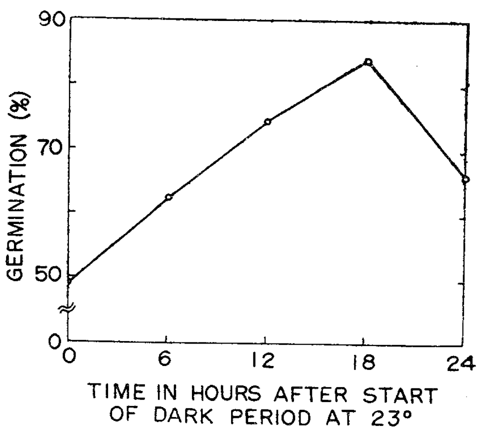

Fig. 3. Effect on germination of a 10 *min* light-break given at various times in the 24 *hr* dark period at 23° inserted between light and low temperature treatments.

durations which were interrupted with light, the seeds were exposed to the low temperature for 24 *hr*. A 10-minute light-break was given at various times during the dark period. The results obtained showed that the germination of *Nasturtium* seeds was affected by the light-beak during the dark period in a way similar to that observed for the germination of *Begonia* seeds and for the flowering of long-day plants. The light-break was most effective when given at about the 3/4 point of the dark period (Figs. 3 and 4)

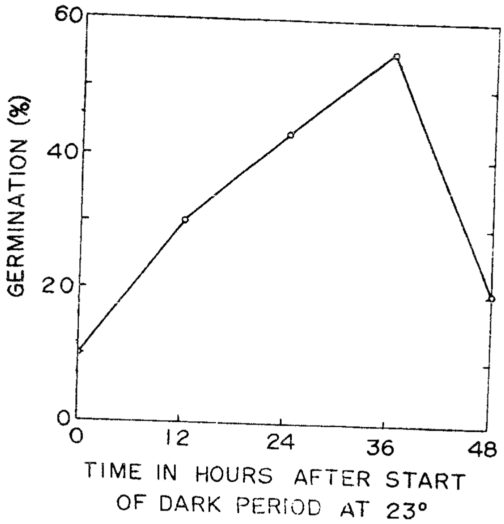

Fig. 4. Effect on germination of a 10 *min* light-break given at various times in the 48 *hr* dark period at 23° inserted between light and low temperature treatments.

Effect of gibberellin on the germination

To make clear the interaction between light irradiation and gibberellin treatment, the following experiments were performed. After 5 days of imbibition, seeds were irradiated for 24 *hr* in the presence of various concentrations of gibberellin. The experimental results obtained are shown in Fig. 5.

Gibberellin brought about no germination of *Nasturtium* seeds in complete darkness. The germination was promoted only when the seeds

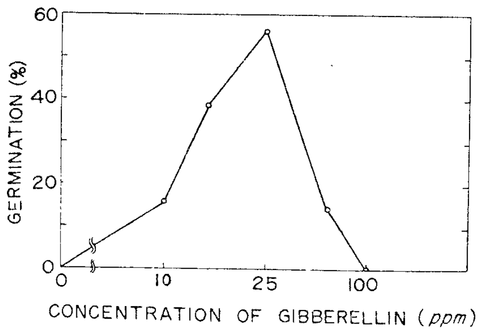

Fig. 5. Effect of concentra tion of gibberellin on germination of seeds under the light irradiation for 24 *hr*.

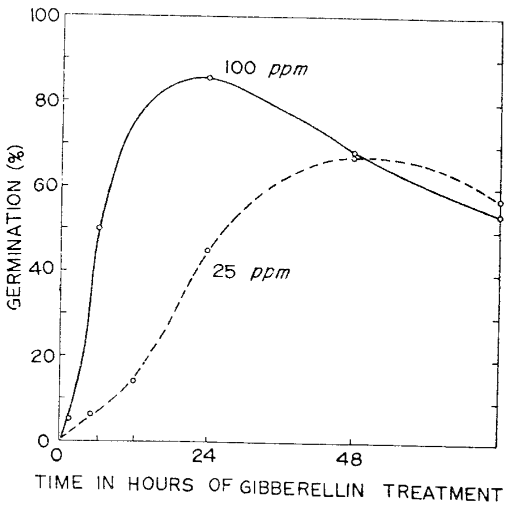

Fig. 6. Effect on germination of gibberellin treatment immediately after the irradiation.

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were subjected to light irradiation. A similar phemonenon has already been reported for *Kalanchoë* (*13*) and *Begonia* seeds (*14*). The effectiveness of gibberellin on germination response of *Nasturtium* seeds was highest at 25 *ppm*, but it was not observed at 100 *ppm* (Fig. 5). The fact that gibberellin induced the germination of *Nasturtium* seeds only when the light irradiation was applied to the seeds indicates that gibberellin might act as a substitute for the low temperature treatment (5°). Support was given to this inference also by the experiment, in which the seeds, after 5 days of dark imbibition, were irradiated for 24 *hr* and immediately thereafter exposed to 25 or 100 *ppm* gibberellin for various durations at 23°. The effectiveness of gibberellin treatment in inducing germination increased at first and then gradually decreased as the time of gibberellin application was lengthened (Fig. 6).

As will be seen in the figure, the maximum percentages of germination in the treatment with 25 and 100 *ppm* gibberellin were 67.5% with 48 *hr* exposure and 85% with 24 *hr* exposure. The decrease in germination percentage with longer applications of gibberellin may be due to the inhibition of reactions following the treatment which induces the germination, a phenomenon which has also been reported for *Sedum* seeds (*5*).

To observe the interaction between light irradiation and a short application of gibberellin, the seeds were irradiated, on the 5th day of dark imbibition, with the light of 1500 lux for various period and then exposed to 100 *ppm* gibberellin for various durations (Fig. 7). In the case of gibberellin treatment lasting for 6 *hr*, the effectiveness of the light period on germination increased with the increase of irradiation time up to 24 *hr*, and then decreased as the irradiation was further lengthened (Fig. 7). This decrease of germination percentage, however, was not observed in the seeds which were treated with gibberellin of 100 *ppm* for 24 *hr*. The maximum germination percentage (85.5%) which was attained by irradiating for 24 *hr*, was maintained for the experimental period which lasted for 72 *hr*.

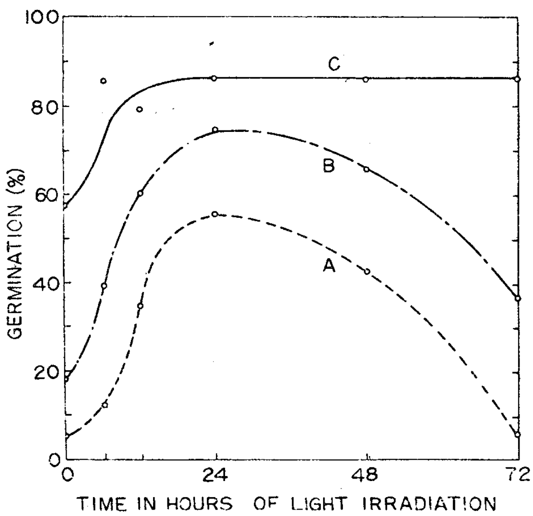

Fig. 7. Effects of various lengths of light irradiation before gibberellin treatments lasting for 6 *hr* (curve A), 12 *hr* (curve B) and 24 *hr* (curve C).

In the next experiment, various durations of darkness at 23° were inserted between light irradiation and a short application of gibberellin.

The experiments were performed in the same way as with the low temperature treatment. Several lots of *Nasturtium* seeds were irradiated for 24 *hr* with light of 1500 lux. After dark periods of various durations, they were exposed to 100 *ppm* gibberellin for 6 *hr*. As the duration of darkneess between the light irradiation and the gibberellin treatment was increased, the effect of gibberellin in promoting germination decreased linearly and finally became zero (Fig. 8). The complete loss of gibberellin-effect occurred when the intervening dark period lasted about 50 *hr*. These experimental results, together with those of the similar experiments with the low temperature treatment, may be assumed to indicate that gibberellin can substitute for low temperature treatment (5°) after the light irradiation.

Noteworthy was the fact that even a short application of gibberellin at a fairly high concentration was remarkably effective for the germination in darkness. In fact, when the seeds were exposed for various durations to gibberellin at various concentrations after 4 days of imbibition, the effectiveness of gibberellin treatment increased at first, and then gradually decreased with the increase of exposure time (Curve A in Fig. 9). These results indicate that gibberellin could substitute also for light to induce the germination of *Nasturtium* seeds, and that the higher the concentration the more pronounced was the gibberellin effect.

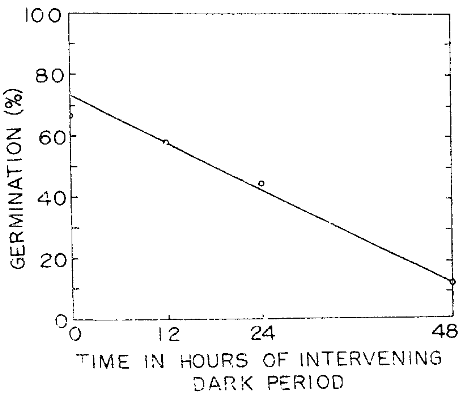

Fig. 8. Effect of various durations of dark incubation at 23° inserted between irradiation and gibberellin treatment lasting for 6 *hr*.

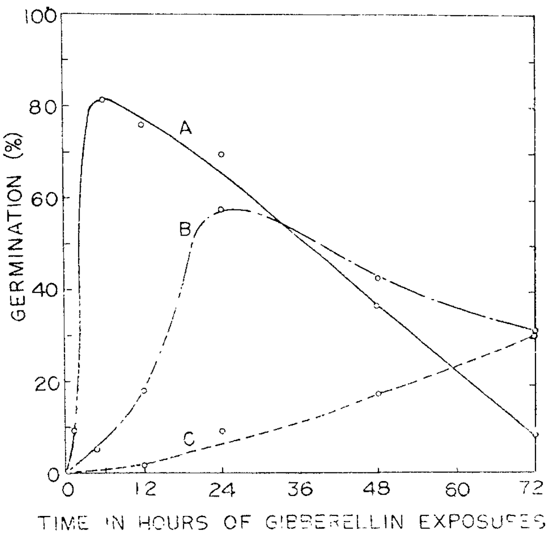

Fig. 9. Effect on germination of a short application of gibberellin at concentrations of 500 (curve A), 100 (curve B), and 25 *ppm* (curve C)

As may be seen from Fig. 9, gibberellin at a concentration of 25 *ppm* gave very low germination percentages, even if the exposure time was

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lengthened. It may be noticed, however, that the effect of gibberellin was remarkably enhanced when the seeds were irradiated with light before the gibberellin treatments (Figs. 6 and 9).

Similar results were obtained in the case of gibberellin exposure before light irradiation. Although the seeds, which had been exposed to gibberellin of 100 *ppm* for 4 days of imbibition, gave only a small

TABLE I

Effect on germination of light irradiation after gibberellin treatment

Light irradiation was given on the 4th day of imbibition in the presence of 100 *ppm* gibberellin.

Length of light irradiation (*hr.*)	Lengths of gibberellin exposure (days)	Germination (%)
0	1	57.0
	2	36.5
	3	28.5
	4	14.0
6	1	71.0
	2	73.0
	3	66.0
	4	67.0
24	1	55.0
	2	53.5
	3	64.0
	4	59.0

germination percentage in the dark (about 14%), the percentage increased remarkably when the light was applied immediately after the gibberellin treatment (Table I).

DISCUSSION

The interaction between light and temperature on seed germination has been investigated by many workers (*8-11*). The results obtained were multifarious, indicating the diversity of the patterns of interaction in different kinds of seeds. The germination of *Nasturtium* seeds was not induced at various constant temperatures, irrespective of whether they were kept in the light or in darkness. It was shown that, for their full germination, a certain period of low temperature immediately after the light irradiation is necessary. These results show the existence of at least two steps (a light-dependent process and a low temperature-requiring process) in the germination response of *Nasturtium* seeds. The effectiveness of the light application rapidly increases at first and then decreases with the prolongation of irradiation time, the decrease being, however, eliminated with longer application of low temperature.

On the other hand, a dark incubation at 23° inserted between the light irradiation and low temperature treatment is inhibitory to the germination. The germination percentage, however, is increased if the dark period is interrupted with a short period of irradiation. Thus, the processes occurring during the inserted dark periods resembles those in the flowering of long-day plants.

In analogy to the mechanism assumed for the flowering process of long-day plants, the germination mechanism of *Nasturtium* seeds may be pictured as follows: a dark period at 23° tends to destroy the changes which have taken place in the preceding light period, but if the light period is extended beyond a certain limit, further changes take place, which are different from the dark-period-effect at 23°.

From the analysis of the germination response of *Nasturtium* seeds, we can distinguish four interrelated processes: (I) a light-dependent process; (II) a low-temperature-requiring process; (III) a dark process antagonistic to the light process; and (IV) a low-intensity light process antagonistic to the dark process. If the antagonistic relationships are expressed by dotted lines, the sequence of the processes in *Nasturtium* seeds can be illustrated, in analogy to those assumed for flowering (*15*), by the following scheme:

$$\longrightarrow \text{I} \longrightarrow \text{II} \longrightarrow (\text{Reactions}) \longrightarrow \text{Germination}$$

$$\text{I} \overset{\cdots}{\longleftarrow} \text{III} \overset{\cdots}{\longleftarrow} \text{IV}$$

Naturtium seeds share with *Kalanchoë* (*13*) and *Begonia* seeds (*14*) a property of being unable to germinate in complete darkness after prolonged exposure to gibberellin of any concentration; germination occurring only when the seeds are irradiated with light. NAGAO *et al.* (*14*) have suggested that the action of gibberellin might substitute for the latter part of the light action. Gibberellin is most effective for the germination of *Nasturtium* seeds at 25 *ppm*, but inhibitory at a concentration of 100 *ppm*. Thus, the effect of gibberellin upon germination has two aspects, inhibiting and stimulating. A short application of gibberellin at a fairly high concentration is remarkably effective for the germination even in darkness, but the germination is inhibited as the time of gibberellin application is lengthened. It is considered that gibberellin can substitute for the coupled effect of light irradiation and low temperature treatment to induce the germination of *Nasturtium* seeds and is inhibitive to the reactions following the treatments which induce the germination.

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On prolonged treatment with gibberellin in the dark, the inhibitive effect of gibberellin on the development outweighs the promotive one on the induction of germination, with a result that no germination is brought about at any gibberellin concentration. It is, however, conceivable that, if the seeds are irradiated with light, they are induced to germinate because the promotive effect of gibberellin of 25 *ppm* is enhanced by light. The fact that 100 *ppm* gibberellin does not induce germination even under the condition of light irradiation may be interpreted as being due to its strong inhibitory effect upon the development following the germination-inductive stage.

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