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Mode of Action of Gibberellic Acid and Light on Lettuce Seed Germination[1, 2]

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ABSTRACT

The seeds of lettuce (*Lactuca sativa* L. cv. Grand Rapids) germinate in darkness at 25 C when treated by gibberellic acid (GA_3) for 1 hour following 2 hours of imbibition. The time of GA_3 application influences the rate and the final percentage of seeds that germinate. In contrast, red light illumination given at different times affects only the rate and not the final germination percentage. The early process(es) of germination initiated by GA_3 or light treatment can be arrested by subjecting the treated seeds to a nongerminative temperature of 35 C. The results suggest differences in the mode of action of light and GA_3 during germination. They indicate that different kinds of processes are involved in the biochemical control of germination.

Gibberellins are known to replace the effect of light on seed germination at moderate temperatures (2-6, 12). In apple seeds light was shown to increase endogenous levels of GA (10). The response of GA_3-treated lettuce seeds to different regimes of red and far red light failed to support the thesis that light acted through an increase in GA level (2). It has been demonstrated that the requirement for GA and Pfr decrease with weakening or removal of a germination barrier in lettuce seeds by organic solvents or by mechanical means (7, 9, 11). Thus, GA_3 and light actions might be related to changes in some membrane properties or to weakening of the endosperm layer.

In previous studies, Black (2) attempted to elucidate the mechanism of light and GA_3 action in germination of lettuce seeds by varying the time of light treatment in the presence of GA. The aim of this work was to obtain new data by varying the duration of both GA_3 and light treatments.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa* L. cv. Grand Rapids 1974 harvest) achenes (seeds) were used throughout this study. Seeds were dry-stored at 5 C until used.

All seeds were soaked at the rate of 1 ml of water/100 seeds for 2 hr at 5 C in the dark prior to soaking at 25 or 35 C. Inbibed seeds were transferred in lots of 50 seeds to 9-cm Petri dishes containing two layers of Whatman No. 1 paper moistened with 5 ml of water or appropriate GA_3 (Eastman Kodak Co., Rochester, N.Y.) solutions. The plates were wrapped in aluminum foil and covered with dark cloth prior to incubation. Seeds were incubated at 25 C (a germinative temperature) and 35 C (a nongerminative temperature). In these seeds thermodormancy is not induced by a prior imbibition of up to 18 hr at 35 C as determined by the time taken to initiate germination as well as by total germination occurring in 24 hr at 25 C. In the experiments reported here the time at 35 C before transfer to 25 C never exceeded 12 hr. At various times (beginning with imbibition at 5 C) germination was counted under dim green safelight. Protrusion of the radicle was taken as the criterion of germination.

In experiments requiring short duration GA_3 treatments, seeds were removed after 1 hr from GA_3 solutions and washed 10 times with 10-ml portions of water before returning to Petri plates containing only water. For short red light exposures (5 min), two 15 w day-light fluorescent tubes wrapped in two layers of Du Pont red cellophane were used to provide a light intensity of approximately 450 μw/cm^2.

Each treatment was replicated at least three times, and each experiment was repeated twice. The data are average of three or more replicates.

RESULTS AND DISCUSSION

An initial 1-hr treatment with GA_3 at 25 C stimulated seed germination. Both rate and final percentage of germinated seeds were concentration-dependent (Fig. 1A). When GA_3 was present continuously in the medium at 25 C a slightly greater enhancement was observed at the same concentrations (Fig. 1B). These data indicate that continuous presence of GA is not essential for germination and point to the adequacy of short treatment for the time-related action of GA_3. We cannot rule out the possibility that some GA_3 remained in the seed tissues following washing. This does not appear likely, however, as another plant hormone, ABA, has been shown to be removed rather easily from lettuce seeds by washing in water (8).

Differences in final germination were also noted when GA_3 was applied for 1 hr at different times at 25 C (Fig. 2, A and B). The final germination reached 100% when 10^{-3} M GA_3 was administered during the initial 1 hr of soaking at 25 C (Fig. 2A). When GA_3 was applied at later times the rate and final percentage of germination decreased progressively. The start of germination was, however, not affected by the time of application of GA_3. The time-dependent response of GA_3 was shown more clearly at 10^{-5} M concentration of the hormones (Fig. 2B). When the hormone was applied at 11 hr an inhibition of germination occurred. These results indicate that GA_3-mediated germination is both concentration- and time-dependent (Figs. 1 and 2).

It can be argued that the responsiveness of the earlier times of GA_3 applications could be due to a continuing imbibition early but only exchange and diffusion into the seeds later. This does not appear to be the case as during 2 hr imbibition at 5 C, the weight of seeds increased by about 55%, and further imbibition at 25 C for 1, 2, 4, and 6 hr (or 3, 4, 6, and 8 hr

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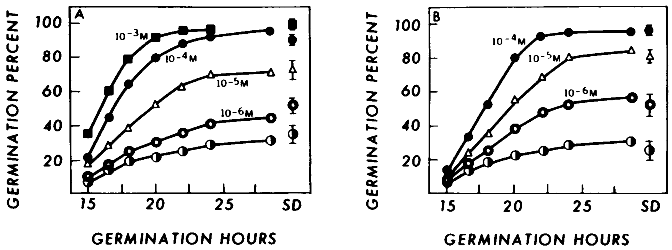

FIG. 1. Germination of Grand Rapids lettuce seeds treated with different concentrations of GA_3 for 1 hr (A) or continuously (B). Seeds were soaked for 2 hr in water at 5 C, treated with GA_3 solutions for 1 hr at 25 C, and then transferred to water at 25 C (A). Seeds were soaked for 2 hr in water at 5 C and then treated continuously with GA_3 solutions at 25 C. (B). ◑: untreated; SD: vertical bars denote standard deviation.

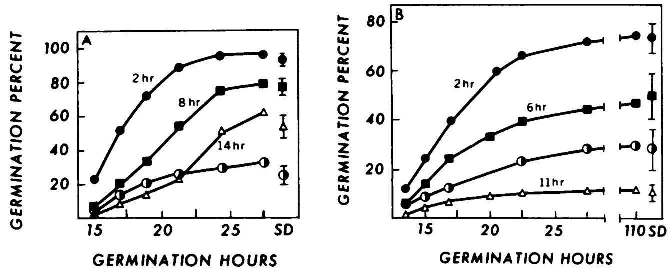

FIG. 2. Effect of 1 hr of GA_3 treatment at various times on seed germination. A: 10^{-3} M; B: 10^{-5} M. Seeds were soaked during 2 hr in water at 5 C, transferred to 25 C and treated with GA_3 for 1 hr after times indicated from the beginning of soaking. ◑: untreated with GA_3.

total imbibition) increased the weight to 59, 63, 71, and 77%, respectively. After 12 and 24 hr, the weight increased to 81 and 83%, respectively (data not shown). Thus, it is obvious that imbibition continued at a rather constant rate after the rapid initial uptake. It is not likely, therefore, that the amount of GA_3 available for action, at least during initial hours, was influenced to any considerable extent by rate of imbibition or water influx.

In order to confirm whether GA_3 affects a process soon after imbibition and during initial hours of soaking, a series of experiments was performed. When seeds were treated during the 1st hr at 25 C with 10^{-3} M GA_3 and transferred to 35 C at various times, complete germination required at least 15 hr soaking (2 hr at 5 C + 1 hr treatment with GA_3 at 25 C + 12 hr at 25 C minus GA_3) (Table I). Thus, the results indicate that the hormone-mediated processes are sensitive to 35 C prior to radicle protrusion.

The results of an opposite experiment involving treatment of seeds for 1 hr at 35 C followed by various times at this temperature prior to transfer at 25 C are shown in Figure 3. Germination was not affected if the transfer was made before 6 hr (2 hr at 5 C + 1 hr GA_3 treatment at 35 C + 3 hr at 35 C minus GA_3). The germination of seeds transferred after 6 hr was delayed. The time required for the start of germination shifted by the same period as the additional time the seeds were kept at 35 C above the initial period. The delayed germination did not cause a noticeable decrease in the final germination percentage. Thus, the high temperature-induced changes in germination curves have a qualitative rather than a quantitative character.

The results of experiments similar to those described in Figure 2 but using a 5-min red light illumination are shown in Figure 4. The illumination was effective at all times during soaking at 25 C. The shape of the germination curve was affected depending upon the time when the treatment was given. The illumination applied after 2 and 5 hr did not influence the start of germination, but an application after 8 hr markedly delayed the germination. Light given anytime in the first 5 hr was equally effective but after this time any delay in its application delayed some critical step in germination. It seems that at least a 3-hr period is needed between the action of light and the start of processes affecting germination. The light-treated seeds in all cases achieved 100% germination. Thus, light has only a qualitative influence on germination in contrast to GA_3 which shows a quantitative effect (compare Figs. 2 and 4). Furthermore, it was noticed that light-induced germination was always 3 to 4 hr earlier than the germination induced by GA_3 (*cf.* Figs. 2-5). This could be due to a slower uptake of GA_3 to the site of action.

A 5-min illumination at the nongerminative temperature (35 C) following imbibition at 5 C was effective promoting germination of seeds on subsequent transfer to the germinative temperature (25 C) (Fig. 5). If the transfer was made earlier than 6 hr (2 hr imbibition at 5 C + 5 min red light exposure at 35 C + 2 hr and 55 min at 35 C) the high temperature treatment did not affect the time of the start of germination; later transfers after 10 and 13 hr delayed the start of germination in a fashion similar to that caused by belated illumination of 25 C (Fig. 4).

Our results illustrate several differences between stimulation

Table I. Effects of different intervening periods at 25 C following GA_3 treatment on subsequent germination at 35 C

The seeds were soaked in water at 5 C for 2 hr, treated with 10^{-3}M GA_3 for 1 hr at 25 C, returned to water at 25 C and then after different times transferred to 35 C. Germination values are means ± standard deviation of at least 3 replicates.

Total hours of soaking before transfer to 35 C	Hours after GA_3 treatment	Per cent germination, 35 C 24 hr	48 hr
3	0	0	0
6	3	0	0
10	7	4.6 ± 1.5	7.3 ± 2.0
15	12	61.6 ± 2.3	76.2 ± 9.8
16	13	91.8 ± 1.3	96.0 ± 1.1
17	14	90.0 ± 1.5	95.2 ± 6.1

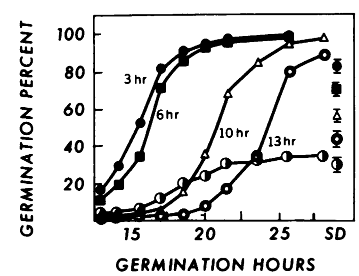

FIG. 3. Effect of different intervening periods at 35 C following GA_3 treatment (1 hr) on subsequent germination at 25 C. Seeds were soaked in water during 2 hr at 5 C, treated with 10^{-3} M GA_3 for 1 hr at 35 C, kept for various times at this temperature in water, and transferred to 25 C after times indicated from the beginning of soaking. ◑: untreated with GA_3, untreated at 35 C.

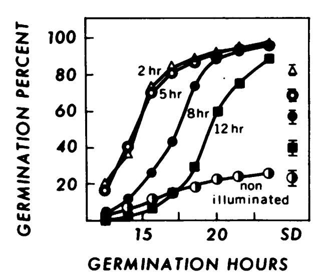

FIG. 4. Effect of short red light exposure at different times on germination. Seeds were soaked in water for 2 hr at 5 C, and illuminated for 5 min with red light at 25 C after times indicated from the beginning of soaking. ◑: unilluminated.

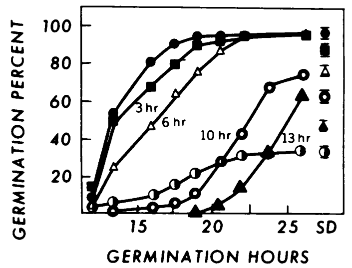

FIG. 5. Effect of various intervening periods at 35 C following red light exposure on subsequent germination at 25 C. Seeds were successively soaked in water for 2 hr at 5 C, illuminated for 5 min at 35 C, kept for various times in the dark at this temperature, and transferred to 25 C after times indicated from the beginning of soaking. ◑: unilluminated and untreated at 35 C and; ●: illuminated and untreated at 35 C.

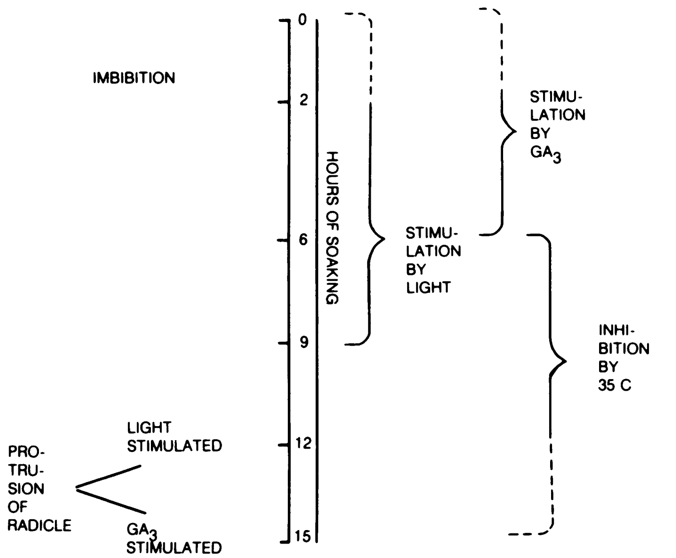

FIG. 6. Proposed scheme of regulation of germination of Grand Rapids lettuce seeds by external factors.

of germination of Grand Rapids lettuce seeds by short red light illumination and 1 hr GA_3 treatment. (*a*) Light-stimulated germination begins about 3 to 4 hr earlier than the germination initiated by GA_3 (Figs. 2 and 4). (*b*) A delay in GA_3 application caused quantitative changes in the rate and percentage of seeds germinated, whereas a delayed illumination results in only qualitative changes manifested by a shift in the time of start of germination, the final germination percentage remaining about the same (Figs. 2 and 4). (*c*) The GA_3-initiated processes are inhibited to a lesser degree by the nongerminative (35 C) temperature than the light-initiated processes. The period of time between the transfer to germinative temperature (25 C) and the start of germination is shorter for GA_3-treated seeds than for light-treated ones (Figs. 3 and 5).

These differences between light and GA_3 effects, coupled with the fact that AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride), an inhibitor of GA biosynthesis, did not affect the light-induced Grand Rapids seed germination (data not presented), indicate that both light and GA_3 are acting on different, probably parallel, chains of events leading to germination.

Based on the results presented here, an attempt is made to present schematically (Fig. 6) the various components of germination (or radicle protrusion) as influenced by light, GA_3, and supraoptimal temperature.

The imbibition, a purely physical process, appears to be independent of temperature changes (1). The metabolic processes of germination presumably start when the hydration of seed colloids attains a sufficiently high level. In lettuce seeds the early metabolic processes do not appear to be influenced by the nongerminative temperature of 35 C (Figs. 3 and 5). In photoblastic seeds such as Grand Rapids, these early processes are under light and GA_3 control (Figs. 2 and 4). The temperature-sensitive processes in the seeds lie between the 6th hr from the start of imbibition and the time of radicle protrusion (Figs. 3 and 5). The temperature-sensitive processes are affected by red light but not by GA_3, however illumination given only during the first 3 hr is effective.

This scheme (Fig. 6) allows us to distinguish three types of processes during the germination: (*a*) those mediated by GA_3 and light but not affected by high temperature; (*b*) those affected by light and high temperature but not by GA_3; (*c*) the processes affected by 35 C, but not by GA_3 or by light.

Although GA_3 and light showed distinctly different effects in the control of germination, the results obtained do not provide any proof of the nature of their site(s). A brief treatment needed for GA_3 to stimulate germination seems to indicate that this hormone might act as a trigger switching on some processes in a fashion similar to that of red light. It seems possible that early GA_3 and light action are related to change in the membrane properties.

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