Mode of Action of Gibberellic Acid and Light on Lettuce Seed Germination\textsuperscript{1, 2}

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ABSTRACT

The seeds of lettuce (\textit{Lactuca sativa} L. cv. Grand Rapids) germinate in darkness at 25 C when treated by gibberellic acid (GA\textsubscript{3}) for 1 hour following 2 hours of imbibition. The time of GA\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2}-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\textsubscript{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\textsubscript{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\textsubscript{3} and light treatments.

MATERIALS AND METHODS

Lettuce (\textit{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. Inhibited 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\textsubscript{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 thermomancy 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\textsubscript{3} treatments, seeds were removed after 1 hr from GA\textsubscript{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 \(\mu W/cm^2\).

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\textsubscript{3} at 25 C stimulated seed germination. Both rate and final percentage of germinated seeds were concentration-dependent (Fig. 1A). When GA\textsubscript{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\textsubscript{3}. We cannot rule out the possibility that some GA\textsubscript{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\textsubscript{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\textsubscript{3} was administered during the initial 1 hr of soaking at 25 C (Fig. 2A). When GA\textsubscript{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\textsubscript{3}. The time-dependent response of GA\textsubscript{3} was shown more clearly at \(10^{-3} M\) concentration of the hormones (Fig. 2B). When the hormone was applied at 11 hr an inhibition of germination occurred. These results indicate that GA\textsubscript{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\textsubscript{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...
deviation. soaked for imbibition) and influx. After 12 and that imbibition continued of initial influenced to radicle the hormone-mediated germination curves delayed. hr (2 prior temperature kept at 25 C) minus illumination of Grand C. The illumination of GA3 for 1 hr at 25 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 25 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>
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<th>Time (hr)</th>
<th>Hours</th>
<th>Germination, 35 C</th>
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<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
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<tr>
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<td>7</td>
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<tr>
<td>15</td>
<td>12</td>
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<td>16</td>
<td>13</td>
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</tr>
<tr>
<td>17</td>
<td>14</td>
<td>90.0 ± 1.5</td>
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Fig. 3. Effect of different intervening periods at 35 C following GA3 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 GA3 for 1 hr at 25 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 GA3, 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.
and the temperature than for 35°C between the transfer caused quantitative changes by GA3 (Figs. 2 and 4). (a) Light-stimulated germination begins about 3 to 4 hr earlier than the germination initiated by GA3 (Figs. 2 and 4). (b) A delay in GA3 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 GA3-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 GA3-treated seeds than for light-treated ones (Figs. 3 and 5).

These differences between light and GA3 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 GA3 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, GA3, 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 GA3 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 GA3, 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 GA3 and light but not affected by high temperature; (b) those affected by light and high temperature but not by GA3; (c) the processes affected by 35°C, but not by GA3 or by light.

Although GA3 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 GA3 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 GA3 and light action are related to change in the membrane properties.

LITERATURE CITED