

INTERACTIONS OF APPLIED HORMONES IN THE GERMINATION OF *LEPIDIUM VIRGINICUM* SEEDS¹

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Abstract. Tests of the germination response of light sensitive *Lepidium virginicum* seeds to applied gibberellic acid confirmed earlier reports that gibberellic acid can substitute for red light. Further tests indicated that promotion of germination by either red light or gibberellic acid can be nullified by abscisic acid. Dark incubation of the seeds in kinetin alone resulted in only slight promotion of germination. Combinations of suboptimal gibberellic acid plus kinetin did not enhance germination to a level greater than that for kinetin alone. Optimal concentrations of gibberellic acid plus kinetin can reverse inhibition of certain abscisic acid concentrations. A comparison of these responses with those of Grand Rapids variety of lettuce seed was made.

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Seeds of *Lepidium virginicum* L. require light for germination. Red light (660 nm) is most effective in promoting germination, and far-red light is inhibitory (Toole *et al* 1955a, b). Alternating exposures of the seeds to red and far-red light have demonstrated that the germination response is photoreversible and implicates the involvement of the phytochrome system (Toole *et al* 1955a, b). In terms of the photoresponse, therefore, seeds of *Lepidium* resemble the much studied seeds of lettuce (*Lactuca sativa* L. var. Grand Rapids).

The effects of exogenously applied growth regulators on germination have been more thoroughly studied for Grand Rapids lettuce seeds than for *Lepidium*. Germination responses with lettuce have been studied in terms of such growth regulators as gibberellic acid (GA), kinetin, auxin and abscisic acid (ABA) (Ikuma and Thimann 1963; Kahn 1967a, b; Kahn *et al* 1957; Kahn and Tolbert 1965, 1966; Kahn 1968). The relatively limited work involving application of growth regulators to *Lepidium* seeds includes a study by Toole and Cathey (1961), who demonstrated that the red

light requirement for germination can be replaced by applied GA. It is the objective of the present work to further test the germination response of *Lepidium* seeds to applied hormones to determine if these seeds respond in a manner similar to that of lettuce.

METHODS AND MATERIALS

Seeds of *Lepidium virginicum* (obtained through the courtesy of V. K. Toole, Seed Research Laboratory, U.S.D.A., Beltsville, Maryland) were kept in cold storage at -11° to -13°C for approximately 4 years before use in these experiments. Test plates consisted of lots of 50 seeds placed on a single thickness of Whatman #1, 7.0 cm filter paper in pre-sterilized, plastic petri dishes to which the various test solutions, totaling 3 ml in volume, were added. The dishes were wrapped with aluminum foil immediately after the test solutions were added to the seeds. Using a modification of the methods of Toole *et al* (1955a, b), the seeds were first incubated at 15°C for 48 hr. The seeds were then transferred to a 25°C incubator for an additional 48 hr, after which time germination counts were made. Plates of seeds to receive a light treatment were unwrapped in the dark and given the required irradiation at the time of the temperature change, after which they were re-wrapped in foil and transferred to the 25°C incubator.

The red (660 nm) irradiation used in some tests was achieved by passing light from eight 15 watt, cool-white fluorescent tubes through a 12 inch square Carolina Biological Supply red 650 plastic filter. For the far-red (735 nm) irradiation, light from a single 300 watt Sylvania reflector flood light was passed first

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through a water bath and then through a 12 inch square Carolina Biological Supply 750 far-red filter. Irradiance measured at either 660 or 735 nm using an ISCO Model SR spectroradiometer gave a value of $180 \mu\text{w}/\text{cm}^2$ at seed level.

Solutions of ABA (obtained through the courtesy of the Shell Development Co., Modesto, California) and GA (K-salt) were made by dissolving each of these compounds in hot double-distilled water. Kinetin was first dissolved in a few ml of warm 0.05 M NaOH before being brought up to volume with double-distilled water.

Each experiment was repeated twice with 4 test plates and 2 double-distilled water control plates per trial, resulting in a total of 600 seeds per trial. The germination percentages for all similar trials were combined and the average determined. There was only slight variation (typically 1-3%) among replications. A seed was considered to have germinated if the radicle emerged through the seed coat.

RESULTS

Preliminary tests confirm the findings of Toole *et al* (1955a, b) that light is essential for *Lepidium* seed germination and that germination is red, far-red photoreversible. In the present study dark-incubated control seeds consistently showed 0% germination; exposure of the seeds to 60 sec of red (660 nm) light resulted in 100% germination, and this effect could be nullified by a subsequent 60 sec exposure to far-red (735 nm) light.

Kinetin Tests. A series of tests was conducted to determine if kinetin could

replace the red light requirement for seed germination. The results (fig. 1) indicated that there was only a minor increase in dark germination with applied kinetin, yielding a maximum germination response of 30% at $1 \mu\text{g}/\text{ml}$ kinetin. Thus with *Lepidium*, applied kinetin cannot completely remove the red light requirement for germination.

Gibberellic Acid Tests. *Lepidium* seeds were incubated in the dark in the presence of various concentrations of GA. The results (fig. 2) confirm the findings of Toole and Cathey (1961) that GA can replace the red light requirement for germination. There is a threshold-type response of *Lepidium* to GA: 0% germination at GA concentrations at or below 10^{-4} M, but 100% germination at 10^{-3} M GA.

Kinetin/Gibberellic Acid Tests. Since kinetin, when applied to dark-grown lettuce seeds, was known to yield very high germination percentages if added along with sub-optimal concentrations of GA (Ikuma and Thimann 1963), a series of tests was conducted to test the possible interaction of kinetin and GA with *Lepidium*. The GA concentration series used in the previous experiment was repeated, but kinetin at $1 \mu\text{g}/\text{ml}$ was included in each plate. It can be seen that there was no promotion or synergistic effect on germination of dark-grown *Lepidium* seeds when kinetin was applied with suboptimal GA concentrations. There was in fact, a slight inhibitory effect of GA at 10^{-6} and 10^{-5} M when present with kinetin (fig. 2). These results then, are in contrast to the promotive effect of kinetin plus suboptimal GA concentrations reported for lettuce seed germination by Ikuma and Thimann (1963).

Abscisic Acid Tests. Effects of ABA at concentrations ranging from 0.001 to $10 \mu\text{g}/\text{ml}$ on germination of *Lepidium* seeds were determined in conjunction with either a 60 sec red light treatment or in total darkness. Germination was 0% in darkness with all ABA concentrations (fig. 3). Red light promotion of germination is prevented by all ABA concentrations greater than $0.1 \mu\text{g}/\text{ml}$, but not by lesser concentrations.

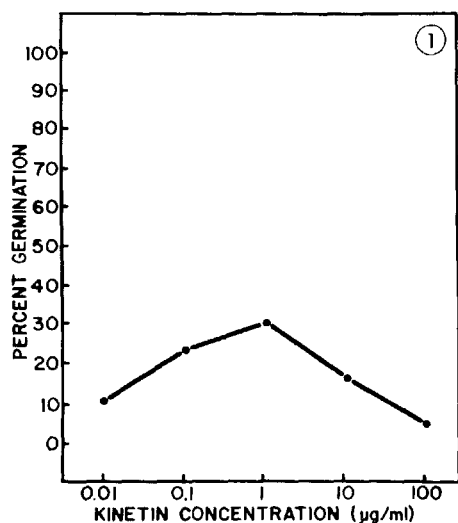


FIGURE 1. Effect of kinetin on the germination of dark-incubated *Lepidium* seeds.

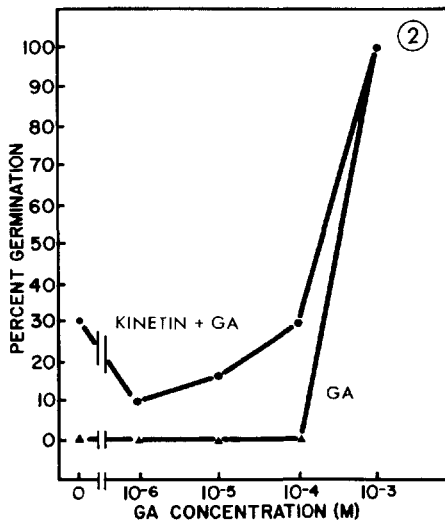


FIGURE 2. Effect of GA on the germination of *Lepidium* seeds incubated in the dark with (●) or without (▲) 1 µg/ml kinetin.

ABA/GA Tests. Since GA is a strong promoter and ABA a strong inhibitor of *Lepidium* germination, further tests were conducted to determine the interaction of GA and ABA when applied together to dark grown seeds. GA, at the concentration that had previously been shown to result in 100% dark germination (10⁻³ M), was applied to all seeds in

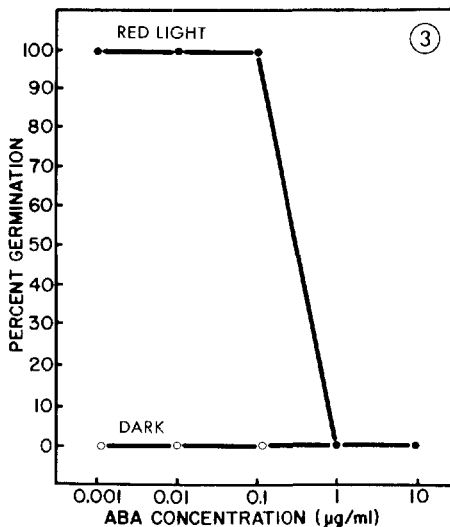


FIGURE 3. Effect of ABA on the germination of *Lepidium* seeds after incubation in the dark with (●) or without (○) a 60 sec red light interruption.

the test in conjunction with ABA in a series of concentrations from 0.001 to 10 µg/ml. It is evident that ABA, at concentrations of 1 µg/ml or greater, can completely nullify the promotive effect of GA on dark germination of *Lepidium* seeds (fig. 4). At concentrations less than 1 µg/ml, ABA is slightly less inhibitory.

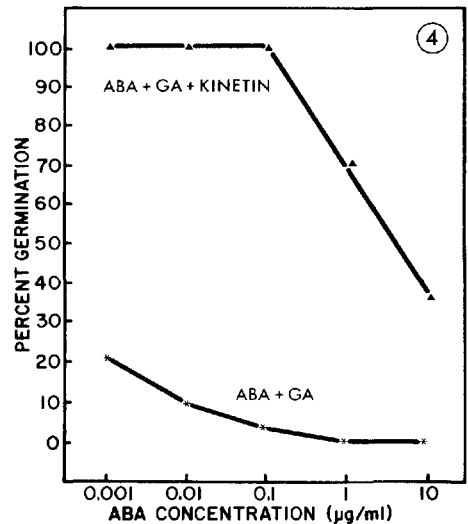


FIGURE 4. Effect of ABA in combination with GA at 10⁻³ M and either 0 (●) or 1 (▲) µg/ml kinetin on the germination of dark-incubated *Lepidium* seeds.

GA/ABA/Kinetin Tests. It has been shown that GA at 10⁻³ M can completely replace the red light requirement in *Lepidium* seed germination (fig. 2). It has also been shown that this promotive effect of GA can be greatly or entirely nullified by various concentrations of ABA (fig. 4). One question which still remained was whether the application of kinetin, in combination with GA, could reverse ABA inhibition of germination, as was known to be the case with lettuce (Kahn 1968).

Seeds were incubated with optimal concentrations of both GA (10⁻³ M) and kinetin (1 µg/ml). In addition, ABA was added to the series of plates at concentrations from 0.001 to 10.0 µg/ml. The addition of kinetin with GA can completely nullify the inhibitory effect of ABA at low and moderate concentrations of ABA, and can partially nullify

this effect at higher concentrations of ABA (fig. 4).

DISCUSSION

The removal of the red light requirement for germination by applied GA, as previously established for *Lepidium* by Toole and Cathey (1961), was reconfirmed in this study. Our response with *Lepidium* differed from that reported for lettuce (Kahn *et al* 1957; Toole and Cathey 1961) in that the lettuce showed a gradual increase in germination percentage with increase in GA concentration whereas the *Lepidium* response showed a threshold concentration effect. Optimal concentrations of GA can, however, completely substitute for red light in both *Lepidium* and lettuce seed germination. These results strengthen the hypothesis that GA plays a primary role in the germination of light sensitive seeds.

The application of kinetin caused a slight increase in germination of *Lepidium*, as it does in lettuce (Ikuma and Thimann 1963). The response by *Lepidium* differed, however, from that of lettuce with regard to effects of applied kinetin under conditions of sub-optimal concentrations of GA. With lettuce there is generally an enhancement of germination with the application of kinetin under these conditions (Ikuma and Thimann 1963), but with *Lepidium* there was no enhancement of germination above the level obtained with kinetin alone. In fact, the slight promotion of germination caused by applied kinetin was somewhat nullified when sub-optimal GA was included.

Application of ABA strongly inhibits germination of both lettuce (Kahn 1967a, b, 1968) and *Lepidium*. Likewise, the promotion of dark germination by GA can be reversed by ABA with both types of seed. Our tests indicate that a combination of kinetin and GA at optimum concentrations can remove the inhibitory response to certain concentrations of ABA. This is similar to the response reported for lettuce (Kahn and Tolbert 1965; Kahn 1968). Kahn (1971) refers to this as the "permissive role of kinetin" in lettuce seed germination.

The total germination responses of *Lepidium* and lettuce to applied hor-

mones are thus very similar. One apparent difference may be involved in the response of each to GA. The lettuce germination response involves an intermediate germination percentage at sub-optimal GA concentrations. The percentage germination at this intermediate level can be increased either by increasing the GA concentration or by adding kinetin. In terms of the concentrations used in this study, a comparable intermediate germination level for *Lepidium* at sub-optimal GA concentrations appeared to be lacking, as did any promotion of germination by kinetin applied with low concentrations of GA. In fact, low concentrations of GA tended to reduce the slight enhancement of germination normally associated with application of kinetin alone. These results suggest that although GA appears to be a primary factor in the germination of both of these light sensitive seeds, the series of events in which GA triggers germination might be slightly different in lettuce and *Lepidium*.

LITERATURE CITED

- Ikuma, H. and K. V. Thimann 1963 Action of kinetin on photosensitive germination of lettuce seed as compared with that of gibberellic acid. *Plant Cell Physiol.* 4: 113-128.
- Kahn, A. A. 1967a Antagonism between cytokinins and germination inhibitors. *Nature* 216: 166-167.
- 1967b Antagonism between dormin and kinetin in seed germination and dormancy. *Amer. J. Bot.* (suppl.) 54: 639.
- 1968 Inhibition of gibberellic acid-induced germination by abscisic acid and reversal by cytokinins. *Plant Physiol.* 43: 1463-1465.
- 1971 Cytokinins: Permissive role in seed germination. *Science* 171: 853-859.
- , J. A. Goss and D. E. Smith 1957 Effect of gibberellin on germination of lettuce seed. *Science* 125: 645-646.
- and N. E. Tolbert 1965 Reversal of inhibitors of seed germination by red light plus kinetin. *Physiol. Plant.* 18:41-43.
- and ——— 1966 Inhibition of lettuce seed germination and root elongation by derivatives of auxin and reversal by derivatives of cycocel. *Physiol. Plant.* 19: 81-86.
- S. B. Hendricks 1955a Photocontrol of *Lepidium* seed germination. *Plant Physiol.* 30: 15-21.
- 1955b Interactions of temperature and light in germination of seeds. *Plant Physiol.* 30: 473-478.
- Toole, V. K. and H. M. Cathey 1961 Responses to gibberellin of light-requiring seeds of lettuce and *Lepidium virginicum*. *Plant Physiol.* 36: 663-671.