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Reversal of Induced Dormancy in Lettuce by Ethylene, Kinetin, and Gibberellic Acid

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ABSTRACT

The germination of lettuce seeds (*Lactuca sativa* L., cv. Premier Great Lakes) was significantly inhibited by high temperature (32 C), 0.1 mM abscisic acid or 0.4 M mannitol. Ethylene (16 μl/1 of air) partially reversed the dormancy induced by all three inhibitors but only in the presence of 1 mM gibberellic acid (GA) or light. Neither ethylene plus GA nor ethylene plus light were able to promote germination when thermal inhibition was imposed at 36 C. Addition of 0.01 mM kinetin to the ethylene plus GA or light reversed thermodormancy at 36 C. The dormancy imposed by abscisic acid was also reversed by kinetin. Kinetin was unable to reverse the osmotic dormancy imposed by mannitol. The reversal of osmotic dormancy by ethylene or ethylene plus GA was actually inhibited by kinetin but only in the light. Kinetin apparently stimulates cotyledonary growth in the presence of light, and this growth may compete for certain metabolites critical to radicle growth and subsequent germination. Kinetin and ethylene, as demonstrated primarily in the thermodormancy at 36 C and in osmotic dormancy, appear to regulate a common event(s) leading to germination but through mechanisms unique to each respective growth regulator. The regulation of germination by ethylene is absolutely dependent upon an interaction with GA and/or light.

The germination of lettuce seeds is reportedly dependent upon cellular expansion initiated within the embryo (5). Cellular expansion and subsequent germination are blocked by imposing thermodormancy at supraoptimal incubation temperatures (4). Cellular expansion and germination are also blocked in the presence of a strong osmoticum (5). More detailed studies have revealed localized areas of cellular compression and expansion within the embryos of dormant seeds (4). However, the growth of excised embryos is not blocked at temperatures that induce thermodormancy (2). The seed coat apparently presents a physical barrier to cellular expansion within the embryo which is a prerequisite to radicle elongation and subsequent germination. Certain varieties of lettuce (Grand Rapids) do not germinate without a prior exposure to red light (7). Seeds that do not germinate in the dark are considered to be photodormant. The growth of excised embryos obtained from photodormant lettuce seeds is not blocked in the dark (7). Therefore, thermodormancy and photodormancy are maintained by the intact seed coat. Pavlista (11) has also described a visual deterioration of the seed coat in lettuce that occurs prior to germination. The evidence presented to date strongly suggests that germination in lettuce is regulated through a mechanism(s) responsible for destroying the seed coat.

Endogenous phytohormones have been assigned significant regulatory functions in the germination of lettuce (1, 3, 12). Photodormancy in lettuce can be reversed through a phytochrome reaction activated by red light (8). Gibberellic acid will substitute for the red light requirement and promote the dark germination of photodormant seeds (6). Thermodormancy in lettuce is partially reversed by ethylene or kinetin (3, 8, 12). However, the reversal of thermodormancy by ethylene occurs only in the presence of light (visible or red) or GA (3, 8). The test seeds incubated at optimal temperatures germinate equally well in the light or dark (3). Kinetin does not require light to reverse thermodormancy at 32 C (3). We have recently found that thermodormancy is not reversed by ethylene plus GA or light at 36 C. Yet, seeds incubated at 36 C for 36 hr will germinate when subsequently incubated at 22 C (3). The lack of induced germination at 36 C cannot be attributed to decreased viability within the seeds. Consequently, we have postulated the existence of an additional regulatory process in germination that is blocked at 36 C. The data reported here indicate that kinetin is an absolute requirement for germination at 36 C.

It was our intention to define further the roles and interactions of ethylene, kinetin, GA, and light in lettuce seed germination. This objective was accomplished by testing the indicated growth regulators against various treatments or conditions known block germination in lettuce. Darkness (1), ABA (1, 12), mannitol (9), and high temperatures (3, 8) were four divergent and well documented inhibitors selected for these experiments. The reversal of induced dormancy imposed by these inhibitors provides additional information with respect to the regulation of germination via seed coat destruction and/or cellular expansion.

MATERIALS AND METHODS

Germination. Lettuce seeds (*Lactuca sativa* L. cv. Premier Great Lakes) were incubated as previously described (3). Germination was recorded at the indicated time intervals when the radicle had visibly protruded through the seed coat. All manipulations involving dark treatments were performed in a darkroom under a green safelight that did not promote germination.

Treatments. Incubation temperatures of 32 or 36 C were achieved with a serological water bath. Solutions of 0.01 mM kinetin, 0.1 mM (±) *cis-trans*-ABA and 0.4 M mannitol were made by dissolving the materials in glass-distilled H_2O. Each solution was then adjusted to a pH of 6.5 with dilute NaOH. Distilled H_2O treated in the same manner was used as the control. Fifty seeds were incubated in 50-ml flasks containing 1.5 ml of each test solution and germination recorded at the indicated times. Each experiment was conducted with three flasks/individual treatment. The experiments were then repeated at least three times. Seeds were treated with ethylene by injecting sufficient dilutions of the gas into a closed incubation system to give a final concentration of 16 μl/1 of air.

RESULTS AND DISCUSSION

Premier Great Lakes lettuce seeds did not germinate when incubated at 32 C (Table I). Full germination occurred within 24 hr when the thermodormant seeds were subsequently incubated at 22 C. Therefore, thermodormancy had been imposed at 32 C. Thermodormancy was partially reversed by incubating the seeds under continuous light in 16 μl of ethylene/liter of air (Table I). Negm *et al.* (10) have shown that the response to ethylene is essentially saturated at 1 to 5 μl of ethylene/liter of air. They found that concentrations of ethylene up to 16 μl/liter air were not inhibitory to germination.

Table I shows that germination occurred equally well in either the light or dark at 22 C. This variety of lettuce did not become photodormant when incubated in the dark at optimal temperatures. The reversal of thermodormancy by ethylene occurred only when the seeds were incubated in the light (Table I); thus, photodormancy was induced at 32 C and superimposed upon thermodormancy. GA partially reversed the induced photodormancy when applied simultaneously with ethylene (Table I). Ethylene-induced germination was also increased under continuous light by the addition of GA. The ethylene-GA data can be interpreted in the following manner: (*a*) ethylene must interact with light or GA to promote germination at 32 C; (*b*) GA and light apparently regulate a common event in germination but through different mechanisms; (*c*) the dependence of ethylene-induced germination upon light manifests itself as photodormancy at 32 C.

Kinetin, a synthetic cytokinin, is also capable of reversing thermodormancy in lettuce (Table I). Reversal by kinetin was not dependent upon the presence of light and/or GA. Kinetin-induced germination was significantly increased in the light or dark by the addition of ethylene and GA to the treatment. Neither kinetin nor ethylene reversed thermodormancy when the incubation temperature was increased to 36 C. Germination occurred only at 36 C if kinetin and ethylene were applied simultaneously. Reversal of thermodormancy at 36 C by kinetin-ethylene also required either light or GA. Therefore, kinetin and ethylene are directing the synthesis or activation of discrete processes (substances) thus must interact to promote germination at 36 C. A secondary interaction also exists between light and/or GA and ethylene/kinetin.

In order to test the universality of the kinetin-ethylene-light/GA interactions, the same experiments were repeated at 22 C in the presence of two other inhibitors, ABA and mannitol. A dose response study was conducted for each inhibitor to enable the selection of a concentration that significantly inhibited germination throughout the experimental time interval.

Germination was completely inhibited by 0.1 mM ABA in the dark (Table II). A partial reversal of inhibition was attained under continuous light. Germination in the light was progressively increased by treatment with GA, ethylene, or both. Neither ethylene nor GA reversed ABA-imposed dormancy in the dark. As previously observed in thermodormancy, ethylene only promoted dark germination in the presence of GA. ABA apparently interferes with developmental processes requiring both light and/or GA in conjunction with ethylene. Germination may occur when the collective progress of these interactions reaches a certain threshold level within the seed. This assumption would permit an excess of one regulatory component to compensate for the deficiencies of another within biological limits. The partial additivity observed within multicomponent treatments could be explained by this hypothesis.

Kinetin partially reversed the dormancy imposed by ABA in both the light or dark (Table II). The reversal by kinetin was always enhanced by the addition of GA. There did not appear to be any significant interaction between kinetin and ethylene unless GA was also included in the treatment. Enhancement of the kinetin-ethylene treatment by GA was observed in the light or dark. The additive increases in germination imply that each phytohormone regulates a unique system which contributes to the total germination process.

The percentage of "atypical" germination in the presence of ABA was very high. Normal germination is characterized by the protrusion of the radical through the seed coat followed by expansion of the cotyledons. In "atypical" germination the cotyledons expand and rupture the seed coat prior to radicle elongation. We postulate that development within the radicle, cellular expansion and elongation, was partially blocked in the presence of ABA. Cotyledonary expansion, initiated at some stage during incubation, eventually ruptures the seed coat and permits further embryonic growth. This particular stage of cotyledonary development is not inhibited by ABA.

Seeds incubated in mannitol under continuous light for 4 days at 22 C did not germinate (Table III). The incubation imposed by mannitol was completely reversed by rinsing the seeds for 5 min in distilled H_2O. Therefore, the osmotic inhibition of germination was reversible and constituted a form of dormancy. The reversal of osmotic dormancy reportedly decreases upon prolonged incubation in the dark (9). This loss was attributed to the progressive decay of an initial photoinductive exposure to red

Table I. Percent germination after 36 hr of incubation under the indicated conditions.

Treatments[1] at 22 C	Dark	Light
Water	84±4	86±6
Treatments[1] at 32 C	**Dark**	**Light**
Water	2±2	6±3
GA	4±2	8±3
Ethylene	4±2	60±4
Ethylene + GA	38±3	80±4
Kinetin	64±3	74±6
Kinetin + GA	68±4	68±4
Kinetin + Ethylene	64±2	86±2
Kinetin + GA + Ethylene	88±3	94±4
Treatments[1] at 36 C	**Dark**	**Light**
Water	0	0
GA	0	0
Ethylene	0	0
Ethylene + GA	0	0
Kinetin	0	0
Kinetin + GA	0	0
Kinetin + Ethylene	0	88±4
Kinetin + GA + Ethylene	62±4	100

1/ All treatments used 16 µl ethylene/l of air, 1 mM GA and 0.01 mM kinetin.

Table II. Time course for germination of seeds incubated in 0.1 mM ABA at 22 C under the indicated conditions.

Lights Treatments[1]	36 hr	48 hr	60 hr
ABA	4±2	20±4	38±2
ABA + GA	16±4	26±2	62±4
ABA + Ethylene	12±2	52±4 (24±2)[2]	94±4 (44±4)[2]
ABA + GA + Ethylene	40±4 (16±2)[2]	90±4 (30±6)[2]	
ABA + Kinetin	36±6	92±2 (18±2)[2]	
ABA + Kinetin + GA	78±10 (24±2)[2]	96±4 (40±6)[2]	
ABA + Kinetin + Ethylene	16±6	78±4 (32±2)[2]	96±6 (44±6)[2]
ABA + Kinetin + GA + Ethylene	84±6 (30±4)[2]	96±2 (50±10)[2]	
Dark Treatments[1]	**36 hr**	**48 hr**	**60 hr**
ABA	0	0	0
ABA + GA	0	0	0
ABA + Ethylene	0	0	8±2
ABA + GA + Ethylene	10±4	26±6 (4±2)[2]	91±4 (22±2)[2]
ABA + Kinetin	0	18±8	86±6 (8±4)[2]
ABA + Kinetin + GA	14±4 (10±6)[2]	90±4 (14±2)[2]	
ABA + Kinetin + Ethylene	0	38±6 (16±2)[2]	78±2 (34±4)[2]
ABA + Kinetin + GA + Ethylene	48±4 (8±2)[2]	96±2 (44±4)[2]	

1/ All treatments used 0.1 mM ABA, 16 µl ethylene/l of air, 1 mM GA and 0.01 mM kinetin

2/ Percent of "atypical" germination (see text for explanation).

Table III. Time course for germination of seeds incubated in 0.4 M mannitol at 22 C under the indicated conditions.

Light Treatments[1]	48 hr	72 hr	96 hr
Mannitol	0	0	0
Mannitol + GA	0	0	0
Mannitol + Ethylene	0	16±4	40±6
Mannitol + GA + Ethylene	12±4	42±6	88±6
Mannitol + Kinetin	0	0	0
Mannitol + Kinetin + GA	0	0	0
Mannitol + Kinetin + Ethylene	0	4±2	12±2
Mannitol + Kinetin + GA + Ethylene	2±1	12±4	26±8
Dark Treatments[1]	**48 hr**	**72 hr**	**96 hr**
Mannitol	0	0	0
Mannitol + GA	0	0	0
Mannitol + Ethylene	0	0	6±2
Mannitol + GA + Ethylene	6±2	54±6	92±4
Mannitol + Kinetin	0	0	0
Mannitol + Kinetin + GA	0	0	0
Mannitol + Kinetin + Ethylene	0	10±2	26±2
Mannitol + Kinetin + GA + Ethylene	4±0	34±5	78±4

1/ All treatments used 0.4 M mannitol, 16 µl ethylene/l of air, 1 mM GA and 0.01 mM kinetin

light. We did not observe such a decrease in the germination potential of our dark-incubated seeds.

Ethylene partially reversed the osmotic dormancy imposed by mannitol, but only in the light (Table III). The ability of ethylene to promote dark germination was again dependent upon the addition of GA. However, ethylene-induced germination was maximized in the simultaneous presence of both light and GA.

Kinetin, up to this point in our study, had been the most effective single treatment for reversing induced dormancy. However, kinetin was unable to overcome osmotic dormancy (Table III). In fact, kinetin reduced the germination induced by ethylene or ethylene and GA under continuous light. The reduction was almost nonexistent in the dark. The combination of ethylene and kinetin actually promoted dark germination over the ethylene alone.

We noted that ungerminated seeds treated with kinetin in the presence of light always exhibited cotyledonary greening. Cotyledonary greening did not occur when the same treatments were conducted in darkness. Ungerminated controls incubated only in mannitol under continuous light did not initiate cotyledonary greening. The cotyledons of ungerminated seeds apparently become sensitive to kinetin at some time during the incubation period (6). The cotyledons respond to kinetin by initiating Chl synthesis but only in the presence of light. We hypothesize that kinetin inhibited germination somewhat in the light via the greening reaction. The greening also indicates the initiation increased metabolic activity within the cotyledons. The possible induction of a metabolic sink within the cotyledons leads to competition with the radicle for critical metabolites. The lack of necessary substrates within the radicle decreases its growth potential and reduces germination.

The data clearly demonstrate a requirement for cytokinins, gibberellins, and ethylene in lettuce seed germination. The function of ethylene is absolutely dependent upon the presence of gibberellin or light. GA and light are not directly interchangeable and, therefore, mediate functions in germination independent of ethylene. The function of the cytokinin, kinetin, apparently occurs independent of light or GA. However, there are instances (thermodormancy at 36 C, ABA treatments) where interactions with ethylene, light, or GA were observed.

Some of the inconsistencies observed between promoters and inhibitors, *i.e.* higher rates of "atypical" germination in the presence of ABA and inhibitory properties of kinetin within osmotic dormancy, can be explained in the following manner. Assume that growth of the embryo is initiated within the radicle. The radicle normally expands and protrudes through the seed coat which has undergone a progressive deterioration. Within a certain time interval, the cotyledons begin growth independent of radicle elongation. The cotyledons may require a longer incubation time to develop a response mechanism receptive to the endogenous or exogenous phytohormones. Thus, inhibition of radicle development by ABA gives rise to increases in "atypical" germination via subsequent unrestricted growth within the cotyledons.

The hormonal control of germination could occur through embryonic growth and/or destruction of the seed coat. The target tissues, *i.e.* endosperm radicle, may differ for each phytohormone. Therefore, additive effects upon germination would result from the simultaneous activation of several events each of which could independently result in germination. The induction of germination by simultaneous application of several phytohormones is abnormal and results from a destruction of the normal compartmentalization of phytohormones within the seed. Induced germination is also a function of the mechanism of inhibition. An inhibitor may selectively block only one of the mechanisms by which germination may occur. For instance, ABA inhibits the growth of excised embryos (1) while supraoptimal temperatures block destruction of the seed coat (5). Under these conditions, the possibility of reversal of dormancy increases with multiple hormone treatments covering a broader range of target tissues. Consequently, germination has often been characterized by responses to phytohormones that were obtained within an abnormally long incubation period (3, 8, 12). During prolonged dormancy, developmental sequences within the seed may not be uniformly delayed. The hormonal control of germination should now be investigated by defining the critical events within more discrete time frames.

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