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Release of Heat Pretreatment-Induced Dormancy in Lettuce Seeds by Ethylene or Cytokinin in Relation to the Production of Ethylene and the Synthesis of 1-Aminocyclopropane-1-carboxylic Acid during Germination

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Abstract. The germination of lettuce (*Lactuca sativa* L.) seeds was greatly reduced when the seeds were heated at 97°C for 30 h prior to imbibition. This dormancy was effectively released when ethylene (1–100 ppm) or benzyladenine (BA) (0.005–0.05 mM) was applied during the imbibition period. Ethylene was not required during the early part of imbibition, but was essential during the period immediately prior to radicle protrusion. Treatment with 1-aminocyclopropane-1-carboxylic acid (ACC) (0.1–10 mM) stimulated germination, but was not as effective as ethylene or cytokinin treatment. During the germination of nondormant lettuce seeds, ethylene production increased rapidly and reached a peak at 24 h, which coincided with the emergence of the radicle, and then declined; the level of ACC increased as ethylene production rate increased, but remained at a high level after radicle protrusion. In heat-pretreated dormant lettuce seeds, the increases in percent germination, ethylene production, and ACC levels were all delayed and lower than those of nondormant seeds, and these increases were accelerated by treatment with ethylene or cytokinin.

Lettuce seeds are dormant when imbibed at supraoptimal temperatures. Such thermodormancy is found in the light-sensitive (e.g. Grand Rapids) and the light-insensitive (e.g. Great Lakes) types of lettuce seeds when imbibition oc-

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curs at 35°C. Ethylene or kinetin partially reversed this dormancy. When combined, a synergistic effect of ethylene and kinetin was noted, and germination was almost complete (Dunlap and Morgan 1977, Keys et al. 1975, Negm et al. 1973, Rao et al. 1975). A combination of C_2H_4 and CO_2 also overcame dormancy caused by high imbibition temperature (Keys et al. 1975, Negm et al. 1972, Negm et al. 1973). Even when imbibition is carried out at an optimal temperature of 25°C, dormancy can also occur when the dry seeds are preheated at 97°C for an extended period; this inhibitory effect of heat pretreatment can also be reversed by the addition of ethylene during imbibition (Burdett and Vidaver 1971, Stewart and Freebairn 1969). The thermodormancy caused by high imbibition temperature has been studied extensively, but there are only a few studies dealing with heat-pretreatment dormancy. In this investigation, we characterized the action of exogenous ethylene on release of dormancy induced by high temperature pretreatment and studied the accompanying changes in endogenous ethylene production and the endogenous level of ACC, the immediate precursor of ethylene (Adams and Yang 1979). Moreover, we have examined the effect of cytokinin on this dormancy, since cytokinin induces ethylene production and releases the thermodormancy caused by high imbibition temperatures (Keys et al. 1975, Rao et al. 1975, Dunlap and Morgan 1977).

Materials and Methods

Plant Materials and Germination Conditions

Seeds of lettuce (*Lactuca sativa* L. cv. Great Lakes 659-700) were purchased from Asgrow Seed Company. Seeds were incubated on filter paper moistened with water or designated solutions in Petri dishes enclosed in a 1-liter jar or enclosed in Erlenmeyer flasks. To absorb ethylene released by the seeds, a center well containing a paper wick moistened with 0.25 ml of 0.25 M mercuric oxide in 2 N perchloric acid was hung in the flask. To trap CO_2, KOH solution was similarly used. To expose seeds to various ethylene concentrations, a predetermined amount of ethylene was injected into the container to achieve the desired concentration, which was verified by gas chromatography as described below. For the germination rate, 50 seeds were used in each lot, and each treatment had at least four replications and was repeated at least twice. Germination, as indicated by radicle protrusion, was scored at the indicated time. Dormancy was induced by pretreating seeds at 97°C for 30 h and then imbibing them at 30°C in the dark.

Ethylene Measurement

For measurement of ethylene production, 0.5 g of seeds was used in each lot. At the end of the incubation, a 1-ml gas sample was withdrawn from the sealed flask with a hypodermic syringe, and C_2H_4 was assayed with a gas chromatograph equipped with an activated alumina column and a flame-ionization detector.

Table 1. The effect of C_2H_4 and CO_2 on the release of dormancy induced by preheating treatment.

Treatment	$-C_2H_4$	Air	$+C_2H_4$	$+C_2H_4$ $+CO_2$	$+C_2H_4$ $-CO_2$
% germination	0	18 ± 5.1	64 ± 3.6	65 ± 4.3	64 ± 1.8

All seeds were preheated at 97°C for 30 h. Germination was carried out at 30°C and scored at 42 h. For $-C_2H_4$ and $-CO_2$ treatments, $Hg(C10_4)_2$ solution and KOH solution were included, respectively, in the germination flasks. For $+C_2H_4$ and $+CO_2$ treatments, the concentrations employed were 16 µl/l and 0.5%, respectively. Each treatment was performed in quadruplicate and the mean value ± SD is presented.

Table 2. Effect of C_2H_4 treatment during various imbibition periods upon germination of dormant lettuce seeds.

	Periods during imbibition when C_2H_4 was given (h)				
Treatment	none	0–12	12–24	24–36	0–48
% germination	5 ± 3.7	6 ± 1.4	5 ± 4.2	78 ± 2.1	68 ± 2.1

Seeds were pretreated for 30 h at 97°C and imbibed at 30°C in air or in the presence of C_2H_4 (16 µl/l) for 12 h or 48 h during the 48 h imbibition period. Germination was scored at the end of 48 h imbibition. Each treatment was performed in quadruplicate and the mean ± SD is presented.

Assay of ACC

Seed samples weighing 0.5 g were extracted for ACC determination after imbibition for various periods. ACC was assayed according to Lizada and Yang (1979).

Results and Discussion

Effects of Ethylene, BA, and ACC on Releasing Dormancy

Untreated lettuce seeds used in this study readily germinated at a rate of 95% or higher at 25°C or 63% at 30°C in the light or darkness after 48 h of imbibition. However, when the lettuce seeds were pretreated at 97°C for 30 h and imbibed at 30°C, germination was reduced to 0–20% after 48 h of imbibition. When the seeds were treated with ethylene during imbibition at 30°C, germination increased to approximately 60–70% within 48 h and reached 80–90% after 72 h (Tables 1 and 2). Thus, ethylene treatment is effective in restoring the germination capability lost due to high temperature pretreatment. As reported by others (Keys et al. 1975, Negm et al. 1972, 1973) and confirmed in the present seed lots, the presence of both CO_2 and C_2H_4 is required for the release of thermodormancy induced by high imbibition temperature. However, in the present heat-pretreated seeds, CO_2 is not required for releasing this dormancy, as shown in Table 1. Thus, the release of high-imbibition temperature dormancy and heat-pretreatment dormancy showed different requirements for CO_2.

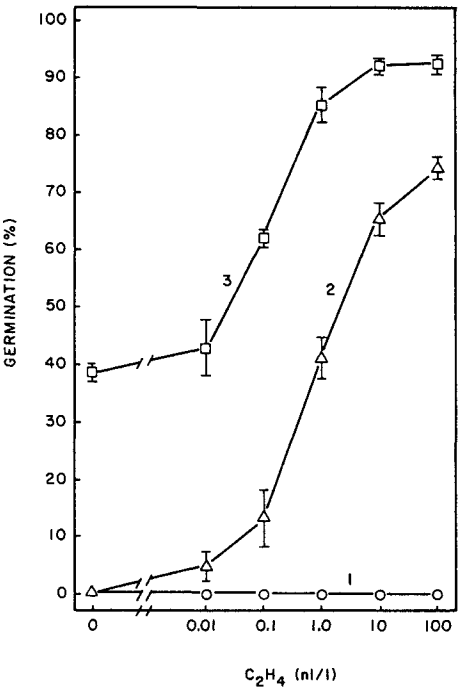

Fig. 1. Effect of different concentrations of C_2H_4 on releasing dormancy in heat-treated lettuce seeds. Lettuce seeds were preheated at 97°C for 30 h and imbibed at 30°C in the presence of various concentrations of ethylene. Germination rates were scored at 24 h (curve 1), 48 h (curve 2), and 72 h (curve 3). Each treatment was performed in triplicate and the mean value ± SD (bar) is presented.

When the imbibition temperature was lowered to 25°C, germination of heat-pretreated seeds in the absence of ethylene increased to 33%. Thus, the effect of ethylene in these heat-pretreated seeds was more pronounced when seeds were imbibed at 30°C as compared to 25°C. It should be noted that the role of ethylene in promoting percent germination under the present experimental conditions can be attributed to release of thermodormancy caused by both heat pretreatment and by high imbibition temperature. However, the ethylene effect in releasing heat-pretreatment dormancy is considered to be the major one, because the dormancy caused by imbibition at 30°C (resulting in 63% germination) was small compared with that caused by heat pretreatment (resulting in 33% germination). Moreover, in heat-pretreated seeds imbibed at 30°C, ethylene treatment was effective in completely restoring the germination from 18% to 65%, which was the level found in those seeds receiving no heat pretreatment.

The effect of different concentrations of ethylene (0.01–100 μl/l) upon germination rate of lettuce seeds is shown in Fig. 1. By 48 h after imbibition, continuous exposure to 0.01 μl/l C_2H_4 elicited some small effect, but the highest rate was observed at 100 μl/l. The concentration of C_2H_4 giving half maximal activity was about 1 μl/l. When seed were scored 3 days after imbibition, 1 to 100 μl/l C_2H_4 had the same effect on releasing dormancy.

BA was also capable of releasing dormancy due to heat pretreatment of the seeds (Fig. 2A). The optimal concentrations ranged form 0.005–0.05 mM. Thus, both BA and ethylene were capable of releasing dormancy, but no syn-

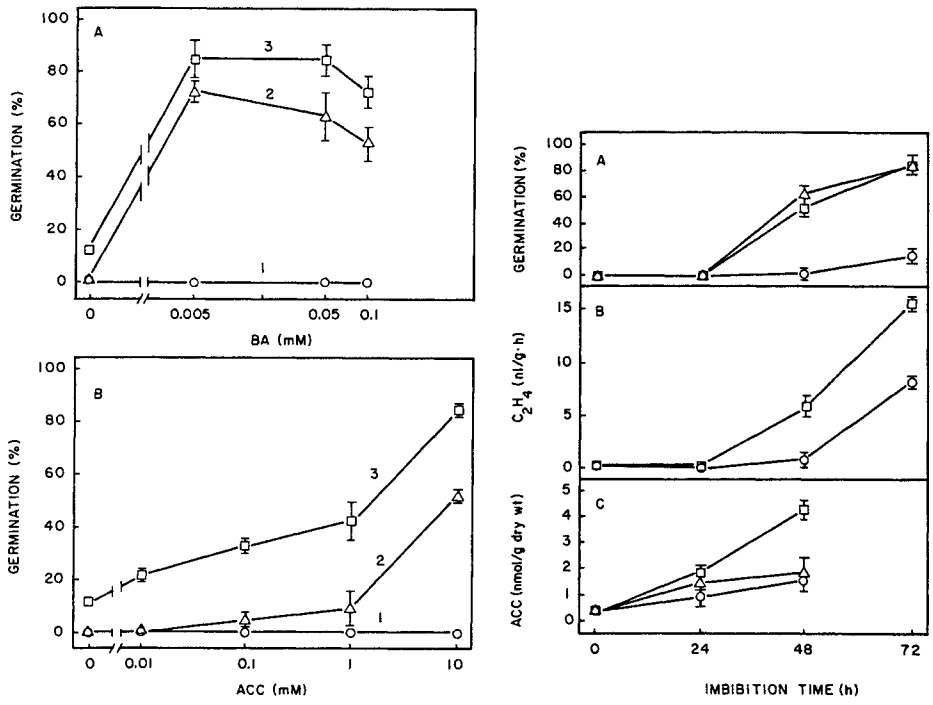

Fig. 2. Effect of different concentrations of BA (A) or ACC (B) on releasing dormancy. Seeds were imbibed at 30°C, and germination rates were scored at 24 h (curve 1), 48 h (curve 2) and 72 h (curve 3). Each treatment was performed in triplicate, and the mean value ± SD (bar) is presented. **Fig. 3.** The changes in % germination (A), ethylene production rates (B), and ACC levels (C) in heat-pretreated lettuce seeds under different treatments: (○), no treatment; (△) treated with 100 μl/l C_2H_4; (□), treated with 0.05 mM BA. Each treatment was performed in triplicate, and the mean value ± SD (bar) is presented. In panels B and C, the values were expressed per g of initial dry seeds.

ergistic effect was observed (data not shown). The relationship between cytokinins and ethylene in releasing dormancy in the present system is not clear. Both cytokinins and ethylene are known to be involved in the regulation of seed dormancy in a number of systems. Ketring and Morgan (1971) suggested that cytokinins release peanut dormancy primarily through stimulation of ethylene synthesis. However, in the release of lettuce seed thermodormancy caused by high-imbibition temperature, synergistic and additive effects between ethylene and cytokinins have been noted, suggesting that they act at different sites (Dunlap and Morgan 1977, Rao et al. 1975). ACC was also capable of stimulating the germination of the dormant seeds particularly at high concentration (10 mM); ACC treatment was, however, less effective than ethylene or BA treatment (Fig. 2B). It should be noted that ACC treatment caused no increase in ethylene production at 24 h, when there was no germination, but caused a surge in ethylene production at 48 h, when 40% of the seeds had germinated (data not shown). Thus, a significant conversion of ACC to ethylene occurred only after the seeds had germinated. This may explain why

ACC greatly stimulated ethylene production but exerted less effect on germination than did BA or ethylene treatment. IAA at concentrations ranging from 0.01–1.0 mM exhibited no promoting effect.

Effect of Ethylene on Various Imbibition Periods

To determine during which phase of imbibition ethylene is most effective in promoting germination, seeds were exposed to ethylene for 12 h at different periods, and the respective germination percentages were compared to those that received no ethylene or continuous ethylene. As shown in Table 2, lettuce seeds did not respond to ethylene when the ethylene treatments occurred during the first 12 h of imbibition, and the maximal effect of ethylene was exerted during the 24–36 h imbibition period. Exposure of dormant seeds to ethylene for 24–36 h resulted in germination percentages that were even higher than the germination percentage of seeds receiving ethylene during the entire 48 h imbibition period. These results indicate that ethylene exerts its effect on releasing dormancy during the imbibition phase immediately before the emergence of the radicle, and that the early imbibition phase does not require the presence of ethylene.

Patterns of Ethylene Production and Changes in ACC Content During Germination in Nondormant and Dormant Lettuce Seeds

No ethylene production was detected by dry lettuce seeds. However, ethylene production by the nondormant seeds imbibed at 25°C was detectable after 6 h and reached a peak about 24 h after the start of imbibition, at which time germination reached 86%. Subsequently, ethylene production declined, but germination increased to 95% at 36 h (data not shown). Thus, during the 12–24 h imbibition period, both ethylene production and germination increased markedly. This is expected, since maximum ethylene production by germinating seeds occurs at the stage of radicle protrusion (Burg and Burg 1968, Takayanagi and Harrington 1971). At 36 h or more after the start of imbibition, ethylene production declined, while germination increased gradually because most of the germinated seeds were at the post-protrusion stages in which ethylene production was lower than that at the protrusion stage. The amount of free ACC in the dry seeds was quite low (about 0.3 nmol/g dry seed), but increased gradually with time throughout the entire imbibition period. In nondormant seeds, ethylene production had declined by 48 h, whereas ACC level had increased 10-fold.

The effects of C_2H_4 and BA treatments on releasing dormancy in relation to the changes in ACC levels and endogenous ethylene production are shown in Fig. 3. BA treatment stimulated germination that was accompanied by an increase in ethylene production and in endogenous ACC level. Ethylene treatment promoted germination as effectively as BA treatment, but ACC levels under ethylene treatment were not much higher than that of the control and were significantly lower than that of BA treatment. Since ethylene is known

to cause autoinhibition of ethylene production by lowering the levels of ACC (Riov and Yang 1982), the suppressed ACC levels in ethylene-treated seeds could have resulted from the autoinhibitory effect of ethylene.

Discussion

The present study shows that heat pretreatment impaired the ability of lettuce seeds to germinate and to synthesize ACC and ethylene, and that BA or ethylene treatment effectively restored this ability. The role of cytokinin in releasing dormancy caused by high imbibition temperature has been thought to be related in part to the enhancement of ethylene production. The role of ethylene in releasing various types of dormancy (Ketring 1977) or in improving the vigor of aged seeds (Takayanagi and Harrington 1971) has been well established. Nevertheless, the role of endogenous ethylene in germinating nondormant seeds is not clear. As much as application of aminoethoxyvinylglycine, a potent ethylene biosynthesis inhibitor, effectively inhibits ethylene production without affecting the germination process (De Greef and De Proft 1981, Hoffman et al. 1983), ethylene synthesis appears to be a result rather than a requirement of the germination process.

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