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Additive and Synergistic Effects of Kinetin and Ethrel on Germination, Thermodormancy, and Polyribosome Formation in Lettuce Seeds[1,2]

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ABSTRACT

The inhibition of germination of Grand Rapids lettuce (*Lactuca sativa* L.) seeds at 35 C was removed to a marked extent by kinetin and 2-chloroethylphosphonic acid (ethrel). When both compounds were used together, an additive effect was observed. A synergistic effect was, however, noted when ethrel promoted the kinetin reversal of abscisic acid inhibition of seed germination (light- as well as gibberellic acid-, induced). Both kinetin and ethrel increased the total ribosomal material and the percentage of polyribosomes in lettuce seeds imbibed in the light for 24 hours. A combination of the two compounds showed a synergism in polyribosome formation only at high ethrel concentration. The inability of ethrel to reverse abscisic acid inhibition indicates that kinetin action cannot always be substituted by ethrel. The possible mechanisms involved in the enhanced response by a combination of kinetin and ethrel are discussed.

Plant hormones appear to have designated functions in seed germination (9). For example, the GA_3-induced dark germination of Grand Rapids lettuce seeds is inhibited by ABA and this inhibition is reversed by cytokinins (8). Cytokinins release the dormancy of seeds presumably by counteracting the effects of inhibitors by an as yet unknown mechanism (9). Another feature of cytokinin is its ability to overcome thermodormancy in lettuce (5, 18, 20). There is evidence that this effect of cytokinin may be related to a higher level of ABA in lettuce seeds imbibed at 35 C compared to those imbibed at 25 C (Braun and Khan, unpublished data).

Ethylene and ethrel, an ethylene-releasing compound, also release dormancy in various seeds (3, 6, 7, 21). It has been suggested that cytokinins act by stimulating ethylene production in seeds and other systems (7, 11). Synergistic and additive effects were noted when a combination of kinetin and ethrel was used to release dormancy in cocklebur and Indian rice grass seeds (21). An attempt is made here to determine whether the synergism or the promotion of kinetin effect by ethrel extends to processes in lettuce seeds affected by cytokinins.

MATERIALS AND METHODS

Seed Germination and Incubation. Fifty seeds of lettuce (*Lactuca sativa* L. cv. Grand Rapids) were germinated in the light or dark, in 9-cm Petri dishes on two layers of Whatman No. 1 filter paper moistened with 5 ml of water or appropriate hormone solution. In the dark experiments, Petri dishes were wrapped in aluminum foil, covered with a dark cloth, and transferred to the incubator. Each treatment had at least three replications and was repeated twice.

Polyribosome Isolation. Following 24 hr incubation of seeds in various test solutions, 150 germinating seedlings were frozen in dry ice. The tissue was powdered along with a small piece of dry ice and homogenized in 8 ml of 0.4 M sucrose, 50 mM tris-HCl, pH 8.5, 50 mM KCl, 10 mM $MgCl_2$, and 5 mM mercaptoethanol plus 0.5 ml of 10% Triton X-100. The homogenate was centrifuged at 20,000g for 15 min. The supernatant was layered over a 2.5-ml pad of 1.5 M sucrose solution in a buffer containing 50 mM tris-HCl, pH 7.8, 20 mM KCl, 10 mM magnesium acetate, and 5 mM mercaptoethanol and centrifuged at 139,000g (average) for 2 hr in a Spinco Model 65 fixed rotor. The ribosomal pellet was resuspended in a 1-ml resuspension solution containing the above buffer. The ribosomal resuspension was layered over a 10 to 35% sucrose density gradient containing the buffer and centrifuged for 2 hr at 148,000g in a Spinco Model 41 Ti swinging bucket rotor. The distribution of polyribosomes was determined by recording the absorbance at 254 nm in an ISCO Model 640 density gradient fractionator.

RESULTS

Additive Effect of Kinetin-Ethrel in Release of Thermodormancy. The effect of various temperatures on germination is shown in Table I. A 35 C temperature completely inhibits germination, and the optimum temperature for germination is 25 C. This thermodormancy in lettuce seeds is released to a marked extent in the presence of kinetin or ethrel (Table II). An additive effect in the release of thermodormancy was observed when the two compounds were used simultaneously. No such effect was noted when a combination of GA_3 and kinetin or GA_3 and ethrel was used, suggesting that kinetin-ethrel effect on germination is rather specific.

Additive Effect of Kinetin and Ethrel on Germination. Although kinetin and ethrel separately had very little effect on lettuce seed

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Table I. *Effect of Temperature Variation on Germination of Grand Rapids Lettuce Seed in Light*

Temperature	Percentage Germination		
	15 hr	18 hr	24 hr
17	0[c]	0[b]	80[c]
25	42[a]	76[a]	96[ab]
30	26[b]	62[a]	84[bc]
35	0[c]	2[b]	2[d]

[1] Values in each column followed by the same letter are not significantly different at the 5% level.

Table II. *Additive Effect of Kinetin and Ethrel in Germination of Grand Rapids Lettuce Seed at High Temperature*

Treatment	Percentage Germination at 35 C[1]		
	18 hr	24 hr	48 hr
Water	2[d]	2[e]	8[e]
GA_3, 30 μM	8[d]	12[e]	20[e]
Kinetin, 50 μM	32[bc]	60[c]	88[a]
Ethrel, 350 μM	22[c]	43[cd]	52[d]
GA_3, 30 μM + kinetin, 50 μM	20[c]	44[d]	50[b]
GA_3, 30 μM + ethrel, 350 μM	25[c]	50[cd]	56[b]
Kinetin, 50 μM + ethrel, 350 μM	48[a]	88[a]	100[a]
GA_3, 30 μM + kinetin, 50 μM + ethrel, 350 μM	44[ab]	82[ab]	92[a]

[1] Values in each column followed by the same letter are not significantly different at the 5% level.

Table III. *Additive and Synergistic Effects of Kinetin and Ethrel on Seed Germination and Reversal of ABA Inhibition, Respectively*

Treatment	Percentage Germination at 25 C[1]			
	15 hr	18 hr	20 hr	22 hr
Water	44[bc]	80[b]	98[ab]	98[a]
ABA, 40 μM	0[e]	2[e]	6[d]	8[e]
Kinetin, 50 μM	50[b]	85[ab]	98[ab]	100[a]
Ethrel, 350 μM	48[b]	84[ab]	96[ab]	98[a]
ABA, 40 μM + kinetin, 50 μM	16[d]	36[d]	60[c]	80[b]
ABA, 40 μM + ethrel, 350 μM	0[e]	0[e]	0[d]	0[e]
Kinetin, 50 μM + ethrel, 350 μM	66[a]	96[a]	100[a]	100[a]
ABA, 40 μM + kinetin, 50 μM + ethrel, 350 μM	32[c]	54[c]	85[b]	92[ab]

[1] Values in each column followed by the same letter are not significantly different at the 5% level.

germination at 25 C, a combination of these two compounds enhanced the germination (15 hr, 18 hr) (Table III).

Promotion of Kinetin Effect by Ethrel in Reversing ABA Inhibition of Germination. In view of the additive effects of the kinetin-ethrel combination, it was considered desirable to determine whether such an effect also exists in another cytokinin-mediated process, namely cytokinin reversal of ABA inhibition of lettuce seed germination. Although kinetin was markedly effective in reversing ABA inhibition of germination, especially after 20 and 22 hr, ethrel was ineffective (Table III). However, a combination of kinetin and ethrel was more effective than kinetin alone in reversing the ABA inhibition. This effect was synergistic and can be clearly seen throughout the course of germination.

The promotion of kinetin effect by ethrel in reversing ABA inhibition was not limited to germination in light alone but also occurred in GA_3-induced germination in the dark (Table IV). The response to various hormones was similar in light and dark, except that germination in the dark required the presence of GA_3 (compare Tables III and IV).

Synergistic Effect of Kinetin and Ethrel on Polyribosome Formation. As part of an effort to determine the role of hormones at the molecular level, the effects of kinetin and ethrel on the formation of polyribosomes in lettuce seeds imbibed at 25 C (in the light) for 24 hr were studied. Kinetin (50 μM) or ethrel (70 and 350 μM) alone promoted polyribosome formation (Fig. 1). The promotion by ethrel at 70 μM was greater than at 350 μM. A combination of kinetin at 50 μM and ethrel at 350 μM synergistically promoted polyribosome formation and the total ribosomal material. When ethrel was used at 70 μM, such synergism with kinetin was not observed. The reason for this is not known. Polyribosomes when expressed as percentage of total ribosomal material, showed an increase by kinetin and ethrel and a further promotion by a combination of the two compounds (Table V).

The promotion of polyribosome formation by kinetin and ethrel and further promotion when they were used together was independent of germination or the length of the radicle (Table VI). However, the effects of these compounds on polyribosome formation were directly correlated with their effects on the extent of cotyledon expansion at 24 hr (compare Table VI and Fig. 1).

DISCUSSION

In the present study it is demonstrated that kinetin is capable of overcoming the inhibition of germination in Grand Rapids lettuce seeds by high temperature, thus supporting earlier reports (5, 18, 20). In addition, ethrel also released thermodormancy but not to the same extent as kinetin. A combination of kinetin and ethrel increased both the rate and the percentage of germination. Similar results were also obtained with other lettuce varieties (19).

Unlike kinetin, ethrel was unable to reverse the ABA inhibition of germination in the dark (GA_3-induced) as well as in the light. This result suggests that ethrel cannot substitute for kinetin in overcoming ABA inhibition. The response to ethrel (ethylene) probably depends upon the removal of ABA block to germina-

Table IV. *Synergistic Promotion of Kinetin Effect by Ethrel in Reversal of ABA Inhibition of GA_3-induced Germination*

The seeds were germinated in total darkness at 25 C.

Treatment	Percentage Germination (48 hr)[1]
GA_3, 30 μM + water	88[ab]
GA_3, 30 μM + ABA, 40 μM	6[d]
GA_3, 30 μM + kinetin, 50 μM	96[a]
GA_3, 30 μM + ethrel, 350 μM	90[a]
GA_3, 30 μM + ABA, 40 μM + kinetin, 50 μM	39[c]
GA_3, 30 μM + ABA, 40 μM + ethrel, 350 μM	8[d]
GA_3, 30 μM + kinetin, 50 μM + ethrel, 350 μM	100[a]
GA_3, 30 μM + ABA, 40 μM + kinetin, 50 μM + ethrel, 350 μM	75[b]

[1] Values in the column followed by the same letter are not significantly different at the 5% level.

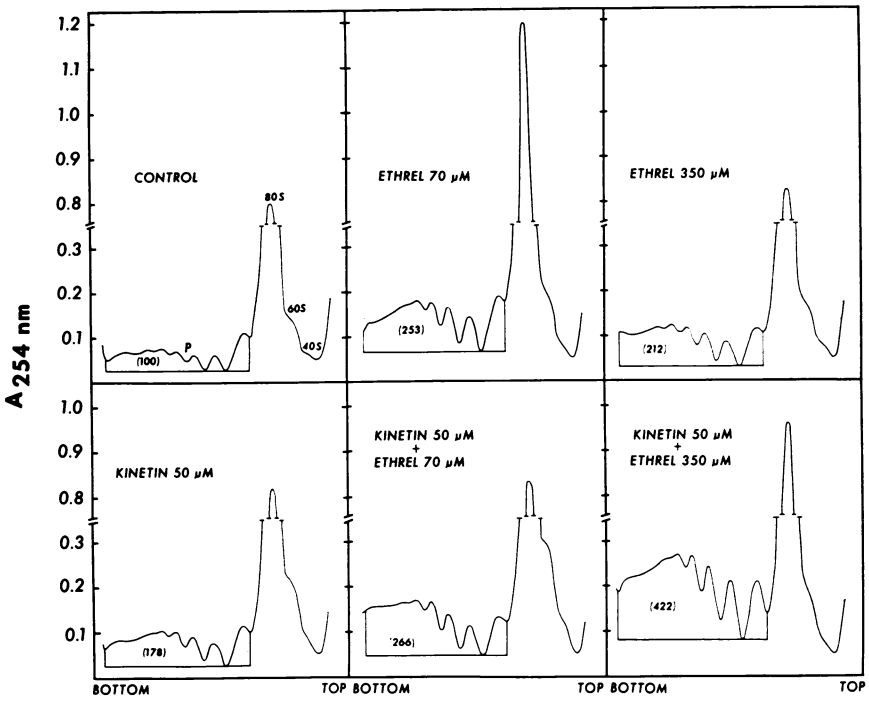

FIG. 1. Effect of kinetin and ethrel alone and in combination on polyribosome formation. Grand Rapids lettuce seeds were germinated in light at 25 C for 24 hr. Polyribosomes were isolated from 150 germinating seeds. The numbers in parentheses indicate the percentages of polyribosome content (P) as compared to control.

Table V. *Effect of Kinetin and Ethrel on Polyribosome Formation*

The seeds were germinated in the light at 25 C for 24 hr and polyribosomes isolated.

Treatments	Polyribosomes as % of Total Ribosomal Material (P/M + P × 100)[1]
Water	25
Kinetin, 50 μM	35
Ethrel, 70 μM	35
Ethrel, 350 μM	39
Kinetin, 50 μM + ethrel, 70 μM	44
Kinetin, 50 μM + ethrel, 350 μM	56

[1] The contents of monoribosome (M) and polyribosomes (P) were measured by tracing and cutting out the area and weighing it.

Table VI. *Growth and Germination Characteristics of Lettuce Seeds*

Seeds were soaked for 24 hr in solutions of 50 μM kinetin and 350 μM ethrel. Ten seeds were picked randomly for measurements on radicle length and szie of cotyledons.

Treatment	Germination	Length of Radicle, (35×)[1]	Area of Cotyledon (35×)[2]
	%	*mm*	*cm²*
Water	96	139[a]	14.8[c]
Kinetin	98	141[a]	19.0[ab]
Ethrel	98	147[a]	16.2[bc]
Kinetin + ethrel	98	146[a]	18.4[a]

[1] Radicle length at 35× magnification.

[2] Cotyledon magnified 35×, the area traced on a graph paper. cut, and weighed. The area was then determined (1 cm^2 = 7.7 mg).

tion by kinetin. The increased response by ethrel in the presence of kinetin could result from the removal of the clock to ethylene action and/or production. Ethylene production has been correlated with the extent of germination (7), and kinetin has been reported to act by producing ethylene in seeds (7) and other systems (1, 4, 10). These observations are consistent with the permissive role of cytokinins in the control of seed dormancy and germination proposed earlier (9). The dormancy-releasing action of kinetin and other cytokinins is well known (9). Ethrel (7, 21) and ethylene (3, 6, 17) have also been shown to release dormancy. Presumably the release of dormancy in these seeds by ethylene is not a result of removal of inhibition by ABA-like compounds.

The synergism or an enhancement of kinetic effect by ethrel suggests that the two growth regulators interact with each other (14). It is also possible that kinetin and ethrel enhance each other's action by some other mechanism. Recently, it has been suggested that kinetin acts synergistically with auxin to stimulate ethylene production (2, 4, 10, 12). Whether auxin is, in fact, involved in the action of kinetin during germination is not known.

Synergistic and additive effects of kinetin and ethrel on polyribosome formation was also noted in seeds soaked for 24 hr. The effect of kinetin and ethrel appears to be related to the growth (expansion) of cotyledons. Cytokinins have been reported to cause expansion of cotyledons (13). An increase in the percentage of polyribosomes by kinetin and ethrel is presumably due to an increase in mRNA-ribosome attachment. Earlier work from this laboratory (21) showed a synergistic effect of the two compounds on ATP synthesis during dormancy release in *Xanthium* seeds. A requirement of ATP for attachment of ribosome to mRNA has been suggested (15). A similar increase in the synthesis of ribosomes by ethrel and ethylene has been observed in fruit tissue (16).

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