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The Effect of Growth Retardants on Phytochrome-Induced Lettuce Seed Germination

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Abstract. Experiments were carried out to explore the involvement of the plant hormone gibberellin (GA) in the light-induced germination of lettuce seeds. Three growth retardants known to be inhibitors of GA biosynthesis were tested for their effect on red-light-induced germination. Chlormequat chloride (CCC) and AMO-1618 had no effect, but ancymidol was strongly inhibitory. Moreover, the inhibition caused by ancymidol was completely overcome by GA_3. CCC and AMO-1618 inhibit the formation of *ent*-kaurene, while ancymidol blocks the oxidation of *ent*-kaurene to *ent*-kaurenoic acid. Ancymidol also was found to inhibit GA-induced dark germination of lettuce seeds, and this inhibition was partially reversed by higher levels of GA. Therefore, the results suggest two possibilities for the relationship between phytochrome and GA in this system: first, the rate-limiting step in the germination of light-sensitive lettuce seeds, that which is regulated by phytochrome, is the oxidation of *ent*-kaurene to *ent*-kaurenoic acid. Alternatively, red-light treatment may result in the release of active GA-like substances which, in turn, induce germination. In either case the results presented here support the view that phytochrome exerts its effect on lettuce seed germination by means of GA rather than via an independent pathway.

Abbreviations: CCC, chlormequat chloride; AMO-1618, 2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl piperidine-1-carboxylate; GA, gibberellin; Pfr, the far-red-light-absorbing form of phytochrome; ABA, abscisic acid.

Control of germination of light-sensitive lettuce seeds by phytochrome and by endogenous plant growth regulators has been an object of study for many years. The observation that treatment with GA in the dark can substitute for the red-light requirement suggested the possibility that phytochrome acts by inducing the production of GA, which, in turn, stimulates germination. (See Khan 1977 for review.) However, the synergistic promotion of germination by suboptimal levels of Pfr (the far-red absorbing form of phytochrome) and GA observed by Bewley et al. (1968) was indicative of independent action of the two promoters, and this view dominated the literature for many years. Recently, Carpita and Nabors (1981) resolved this apparent paradox by demonstrating that the effects of suboptimal Pfr and suboptimal GA are additive on the growth of the embryonic axis, suggesting that the synergism in the germination response may be explained by a "threshold growth potential" necessary for the embryo to break through the seed coat. Furthermore, Bianco and Bulard (1981) have recently shown that red light causes a significant increase in free GA_9 within 30 min.

The present experiments were carried out to test another approach to the question of the involvement of GA in phytochrome-induced lettuce seed germination. If induction of GA synthesis is a necessary intermediate stage in the response to red light, then inhibition of GA biosynthesis should prevent phytochrome-induced germination. Thus, three growth retardants thought to be inhibitors of GA biosynthesis were tested for their effects on red-light-induced lettuce seed germination—chlormequat chloride (CCC), AMO-1618, and ancymidol.

Materials and Methods

Seeds (achenes) of lettuce (*Lactuca sativa*, cv. Grand Rapids, Lot No. 11110-18639, 1980) were purchased from Ferry-Morse Seed Company, Mountain View, California, USA. For each experimental point, 50 seeds were placed on one piece of Whatman No. 1 filter paper, 9.0 cm in diameter, in a 100-mm diameter polystyrene Petri dish. Three milliliters of test solution was added under dim green light, and the seeds were allowed to imbibe the solution for 16 h in the dark at 25°C. The dishes were then irradiated with red light for 5 min or with red light for 5 min followed by far-red light for 10 min as indicated, and the seeds were returned to the dark. After an additional 48 h at 25°C, germination was measured. The results were repeated on at least three separate occasions.

Red light was obtained from a Leitz Prado slide projector (high setting) through a Corion 660 nm interference filter directed at an angle of 45° to the Petri dish. Far-red light was obtained with the same projector and a Corion 730 nm interference filter. The integrated photon flux of the red source at the surface of the Petri dish was 15.6 μmol m^{-2} s^{-1}, as measured with an Optronic Model 741-V Scanning Spectroradiometer. The photon flux of the far-red source was 5.6 μmol m^{-2} s^{-1}.

Gibberellic acid (GA_3) and abscisic acid (ABA) were obtained from Sigma Chemical Company. AMO-1618 (2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl piperidine-1-carboxylate) was purchased from Calbio-

Table 1. Effect of growth retardants on phytochrome-induced lettuce seed germination[a]

Compound	Light treatment	% Germination	
		$-GA_3$	$+GA_3$
Control	Dark	4	98
Control	5'R	100	—
Control	5'R + 10'FR	4	—
CCC	5'R	96	—
AMO-1618	5'R	92	—
Ancymidol	5'R	8	100
ABA	5'R	0	0

[a] Lots of 50 seeds each were imbibed in the dark in 3.0 ml of the test solution for 16 h, irradiated as indicated, and returned to the dark at 25°C for 48 h. Gibberellic acid was at 0.25 mM; all other compounds were at 0.1 mM; all solutions, including controls, contained 0.1% ethanol.

chem-Behring Corp., and chlormequat chloride (CCC) was purchased from Chem Service, West Chester, Pennsylvania, USA. Ancymidol (A-Rest, EL-531, α-cyclopropyl-α-[*p*-methoxyphenyl]-5-pyrimidine methyl alcohol) was obtained from Eli Lily and Co. ABA and ancymidol were prepared at 0.1 *M* in ethanol and diluted to the final concentration with water. Aqueous stock solutions at 0.05 *M* were prepared for CCC and AMO-1618. In the test solutions, GA_3 was used at 0.25 mM and all of the other compounds were used at 0.1 mM. All test solutions contained 0.1% ethanol.

Results and Discussion

The results are shown in Table 1. This seed lot showed low dark germination, good promotion by red light, and complete photoreversibility by far-red. CCC and AMO-1618 had no effect on phytochrome-induced lettuce seed germination. However, ancymidol showed strong inhibition of red-light-induced germination. Moreover, the inhibition caused by ancymidol was completely overcome by GA_3.

These results can be explained on the basis of our understanding of the mode of action of the growth retardants. CCC and AMO-1618 are thought to inhibit the cyclization reaction from geranylgeranyl pyrophosphate to *ent*-kaurene catalyzed by kaurene synthetase (see Sembdner et al. 1980 for review). Ancymidol acts at the next steps in GA biosynthesis, the oxidation of *ent*-kaurene to *ent*-kaurenol (Coolbaugh and Hamilton 1976) and the further oxidation to *ent*-kaurenal and *ent*-kaurenoic acid (Coolbaugh et al 1978). Thus, the data presented here are consistent with the hypothesis that the rate-limiting step in the germination of light-sensitive lettuce seeds, that which is regulated by phytochrome, is in the oxidation of *ent*-kaurene to *ent*-kaurenoic acid. If so, one would expect *ent*-kaurene to have no effect on lettuce seed germination, while *ent*-kaurenoic acid should overcome both the light requirement and ancymidol inhibition. Kaurene is without effect (Bewley et al. 1968), but no experiments with kaurenoic acid have been reported. Preliminary experiments

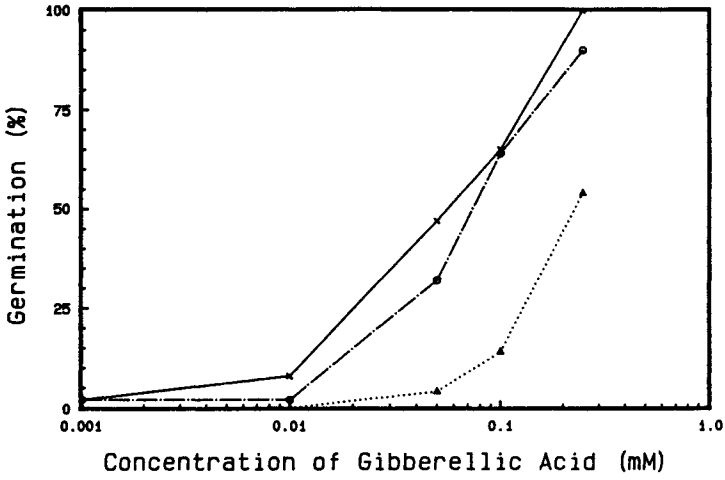

Fig. 1. Effect of ancymidol on gibberellin-induced dark germination. Lettuce seeds were imbibed in 0.1% ethanol (X), 0.01 mM ancymidol (○), or 0.1 mM ancymidol (△) and the indicated concentration of gibberellic acid. Germination was recorded after 64 h in the dark.

in the course of the present study indicated no effect of *ent*-kaurenoic acid (results not shown); however, it was not determined whether the compound penetrated into the seed.

An alternative explanation for the effect of ancymidol is that this growth retardant may be acting as a GA antagonist rather than as an inhibitor of biosynthesis. Leopold (1971) demonstrated that ancymidol inhibits GA-induced lettuce hypocotyl growth and that this inhibition can be overcome by higher concentrations of GA. Similar results for GA-induced lettuce seed germination (in the dark) are shown in Fig. 1. Ancymidol at 0.1 mM inhibits germination induced by 0.05 mM GA_3, and this inhibition is at least partially reversed by increasing the GA_3 concentration to 0.25 mM. Thus, it is possible that red-light treatment of lettuce seeds results in the release of active GA-like substances from a subcellular compartment (Cooke et al. 1975, Evans and Smith 1976) or in the interconversion of inactive GAs to more active forms (Cooke and Kendrick 1976). It is not possible at this time to rule out either inhibition of biosynthesis or antagonism of released GA as the mechanism of ancymidol action on lettuce seed germination. However, both mechanisms are consistent with the view that phytochrome exerts its effect by controlling the level of active GA rather than by an independent pathway.

Inability of GA_3 to overcome the inhibition by abscisic acid indicates that ABA is not acting on GA biosynthesis. This observation confirms that of Poggi-Pellegrin and Bulard (1976), who showed that ABA inhibition of red-light-induced lettuce seed germination was not reversible by GA at ABA concentrations higher than 8 μM.

There have been few reported attempts to determine the effects of growth retardants on light-induced lettuce seed germination. Black (1969) described experiments with AMO-1618, CCC, and Phosphon D. CCC was inactive, but AMO-1618 (1.2 mg/ml) and Phospon D inhibited the promotive effect of red light, and the inhibition was overcome with GA_3. The difference in response between AMO-1618 and CCC was not addressed, but the source of the dis-

crepancy between the activity of AMO-1618 seen there and its inactivity in the present experiments may be the much higher concentrations used by Black. In an additional experiment not shown here, seeds were punctured through the seed coat and cotyledons prior to imbibition, simulating conditions used in the experiments reported by Black. AMO-1618 still had no effect on red-light-induced germination at 0.1 mM.

Recently, Leung and Bewley (1981) have provided new evidence by studying the enzyme α-galactosidase, associated with light-induced lettuce seed germination. This enzyme is induced by both GA and red light, but the lag period for increased activity is shorter with GA (1.0 h) than with light (2.5 h). Red-irradiated isolated axes will cause an increase of the enzyme in the cotyledons of de-tipped seeds, indicating the production of a diffusible factor, and GA_3 can substitute for the irradiated axes. In view of the experiments reported here, it would be interesting to determine the effect of ancymidol on the production of this diffusible factor.

References

Bewley, JD, Negbi M, Black M (1968) Immediate phytochrome action in lettuce seeds and its interaction with gibberellins and other germination promoters. Planta 78:351–357

Bianco J, Bulard C (1981) Influence of light treatment on gibberellin (GA_4, GA_7, and GA_9) content of *Lactuca sativa* L. cv. Grand Rapids achenes. Z Pflanzenphysiol 101:189–194

Black M (1969) Light-controlled germination in seeds. Symp Soc Exp Biol 23:193–217

Carpita NC, Nabors MW (1981) Growth physics and water relations of red-light-induced germination in lettuce seeds. V. Promotion of elongation in the embryonic axes by gibberellins and phytochrome. Planta 152:131–136

Cooke RJ, Kendrick RE (1976) Phytochrome controlled gibberellin metabolism in etioplast envelopes. Planta 131:303–307

Cooke RJ, Saunders PF, Kendrick RE (1975) Red light induced production of gibberellin-like substances in homogenates of etiolated wheat leaves and in suspensions of intact etioplasts. Planta 124:319–328

Coolbaugh RC, Hamilton R (1976) Inhibition of *ent*-kaurene oxidation and growth by α-cyclopropyl-α-(*p*-methoxyphenyl)-5-pyrimidine methyl alcohol. Plant Physiol 57:245–248

Coolbaugh RC, Hirano SS, West CA (1978) Studies on the specificity and site of action of α-cyclopropyl-α-[*p*-methoxyphenyl]-5-pyrimidine methyl alcohol (ancymidol), a plant growth regulator. Plant Physiol 62:571–576

Evans A, Smith H (1976) Localization of phytochrome in etioplasts and its regulation *in vitro* of gibberellin levels. Proc Nat Acad Sci USA 73:138–142

Khan AA (ed) (1977) The physiology and biochemistry of seed germination and dormancy. North-Holland Publishing Company, Amsterdam.

Leopold AC (1971) Antagonism of some gibberellin actions by a substituted pyrimidine. Plant Physiol 48:537–540

Leung DWM, Bewley JD (1981) Red-light- and gibberellic acid-enhanced α-galactosidase activity in germinating lettuce seeds, cv. Grand Rapids: Control by the axis. Planta 152:436–441

Poggi-Pellegrin M-C, Bulard C (1976) Interactions between abscisic acid, gibberellins and cytokinins in Grand Rapids lettuce seeds germination. Physiol Plant 36:40–46

Sembdner G, Gross D, Liebisch H-W, Schneider G (1980) Biosynthesis and metabolism of plant hormones. In: MacMillan J (ed) Encyclopedia of plant physiology, New series, 9. Springer-Verlag, Berlin, pp 281–444.