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Promotive Effects of Organic Solvents and Kinetin on Dark Germination of Lettuce Seeds[1, 2]

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ABSTRACT

Significant promotion in dark germination was observed when Grand Rapids lettuce (*Lactuca sativa* L.) seeds were soaked in acetone or dichloromethane, vacuum-dried, and imbibed at 25 C. Permeation of kinetin via these organic solvents further enhanced the dark germination. Those seeds that were affected by acetone and acetone-kinetin treatments and germinated in the dark escaped red-far red photocontrol of germination. Although abscisic acid was not detected in the organic solvent leachates, they did contain other inhibitory substances affecting lettuce seedling growth. In the light, acetone and acetone-kinetin treatments also enhanced the rate of germination and the increased germination by acetone-kinetin treatment was correlated with increased polyribosome formation. The possible mechanisms involved in promotion of lettuce seed germination by organic solvents and kinetin are discussed.

A number of hormones and other chemicals have been used to promote dark germination of light-sensitive lettuce seeds. Whereas GA_3 was shown to substitute for the light requirement in these seeds (12), kinetin was only partially effective in causing germination in the dark (20, 26).

Acetone and DCM[4] have been successfully used as organic solvents to introduce hormones and other chemicals into dry seeds (16, 18, 29) in spite of claims to the contrary (1, 7). The kinetics of penetration of hormones dissolved in acetone into dry seeds show that the amount of chemical penetrating depends on the seed type, penetration time, and the concentration of solution (29). The amounts of chemicals reaching the embryo have been shown to be physiologically active (16, 29).

Treatment of seeds with organic solvent alone increases germination to some extent. Wheat, tomato, and sugar beet seeds germinated better after acetone washing (19). Treatment of Grand Rapids lettuce seeds with DCM resulted in 70% dark germination compared to 50% for untreated seeds after a 5-min exposure to red light following 1 min of imbibition (27). Preliminary studies from this laboratory showed that germination of acetone-treated Grand Rapids lettuce seeds was significantly increased in darkness, and further promotion was observed when kinetin was permeated via acetone (22). Acetone enhanced the rate of germination in light and decreased the requirement of GA_3 for dark germination 10- to 20-fold (17).

The studies reported here were undertaken to examine further the effects which organic solvents and kinetin, alone and in combination, would have on the dark germination and red-far red photomechanism of Grand Rapids lettuce seeds.

MATERIALS AND METHODS

Seeds of lettuce (*Lactuca sativa* L., cvs. Grand Rapids and Parris Island) were stored in airtight containers at 4 C. Three hundred Grand Rapids lettuce seeds were soaked in the dark in 5 ml of redistilled organic solvent (acetone or DMC) or the solvent containing 500 μM kinetin for various periods. The solutions (leachates) were poured off, and the seeds were vacuum-dried for 90 min and used for germination tests. One hundred seeds in each of the three replications were placed on Whatman No. 1 filter paper moistened with 5 ml of H_2O in 9-cm Petri dishes. Untreated seeds were germinated in aqueous solutions of 20 to 200 μM kinetin. Seeds were incubated in the dark at 25 C for 48 hr.

In studies on photocontrol of germination by R and FR light, the untreated, acetone-treated, and acetone-kinetin-permeated seeds were incubated in the dark for 10 hr and then exposed to R (660 nm) for 5 min, followed by FR (730 nm) for 5 min. In some experiments R and FR irradiations ranging from 0.5 to 30 min were used. The red light was obtained by wrapping two layers of du Pont red cellophane around a 40-w white fluorescent tube. Far red light from a 200-w incandescent bulb was filtered through a 2.54-cm-thick running water bath and a Corning filter No. 2600. The energy level of R and FR irradiations was kept at 1150 μw/cm^2. After exposure to R and/or FR, the seeds were returned to the incubator for germination in the dark.

The possible presence of inhibitors in the acetone and DCM leachates (5 ml) of 300 Grand Rapids lettuce seeds, which might be responsible for the dark inhibition of germination, was determined by using photoinsensitive Parris Island lettuce seeds. Parris Island seeds in batches of 100 were soaked in the leachates for 60 min, removed, and vacuum-dried for 90 min. The leachate solution was then transferred to a Petri dish containing two layers of Whatman No. 1 filter paper, the solvent evaporated, and the plate moistened with 5 ml of H_2O. The treated Parris Island seeds were then placed in the Petri dish and incubated in the dark at 25 C. For comparison, untreated seeds were also soaked in acetone or DCM (minus leachate), dried, and incubated in Petri dishes as described above.

Analysis of Abscisic Acid. The ABA content in acetone leachate was determined as described previously (6). The leachate was dried with a stream of N_2 and taken up in H_2O, pH adjusted to 8.3 with 10% NH_4OH, and partitioned three times with DCM. After readjusting the pH of the aqueous phase to 2.6 with 10% HCl, it was again partitioned three times with DCM. ABA was analyzed by electron capture GLC as described by Seeley and Powell (25).

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[4] Abbreviations: DCM: dichloromethane; R: red light; FR: far red light.

Polyribosome Isolation. Treated seeds were incubated for 12 hr in light or dark. Polyribosomes were isolated as described previously (23). The ribosomal resuspension was layered over a 10 to 35% sucrose density gradient and centrifuged for 2 hr at 148,000*g* in a Spinco Model SW 41 Ti rotor. The distribution of polyribosomes was determined by recording the absence at 254 nm in an ISCO Model 640 density gradient fractionator.

RESULTS

Promotion of Dark Germination of Seeds Treated with Organic Solvents and Organic Solvent-Kinetin Solutions. The dark germination percentages of Grand Rapids lettuce seeds treated with organic solvents or organic solvent-kinetic solutions are shown in Table I. Germination of untreated seeds was promoted when incubated in aqueous solutions of kinetin. Soaking the seeds in acetone or DCM for up to 12 hr also increased the germination. Further promotion of germination occurred when kinetin 500 μM was permeated via organic solvents into the seeds. Similar promotion was noted when solvent-treated seeds were germinated in presence of 200 μM aqueous solutions of kinetin (data not shown). Acetone and kinetin appeared to have an additive effect. Prolonged soaking (24 hr) of lettuce seeds in acetone had a deleterious effect on germination. This effect was not apparent when the seeds were permeated with acetone-kinetin. Both acetone and DCM, alone and in combination with kinetin, appeared to be equally effective in enhancing dark germination.

ABA Content of Leached and Unleached Seeds. In order to determine if the increased germination of Grand Rapids lettuce seeds in the dark by treatments with organic solvents is related to the removal of ABA, the ABA contents of untreated and acetone-treated seeds and that of acetone leachate were determined. Although ABA was detectable in both acetone-treated (10.3 ng/100 mg) and untreated (10.7 ng/100 mg) seeds, the difference in ABA content in these two treatments was not significant. This level of ABA was much lower than that required for inhibition of germination of lettuce seeds (6). ABA was not detectable in the acetone leachate of the seeds.

Because the extractable amount of ABA was low in Grand Rapids lettuce seeds, and because acetone treatment did not decrease the ABA to a significant extent, the possibility was tested that the acetone and DCM leachates of Grand Rapids lettuce seeds might remove inhibitor(s) other than ABA. A bioassay method using light-insensitive Parris Island lettuce seeds was used on the premise that the inhibitor being assayed might be light-sensitive. Parris Island seeds germinated (24 hr) equally well in presence or absence of the organic solvent leachate of Grand Rapids seeds. Subsequent growth (4 days) of the seedlings was significantly inhibited in presence of the leachate (Table II). DCM appeared to leach out inhibitors(s) to a greater extent than acetone. Increasing the period of soaking from 10 min to 2 hr increased the inhibitor content of the leachates. Both acetone and DCM leachates caused greater inhibition of root growth compared to shoot growth.

Table I. *Promotion of Dark Germination of Grand Rapids Lettuce Seeds Treated with Organic Solvents and Organic Solvent-Kinetin Solutions.*

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

Treatment	Period of soaking	% Germination, 48 hr in dark - KIN	% Germination, 48 hr in dark + KIN*
Untreated control	-	10^{c}	23 - 26^{b}
Acetone	10 min	26^{ab}	54^{a}
"	1 hr	29^{ab}	55^{a}
"	12 hr	27^{ab}	56^{a}
"	24 hr	16^{bc}	55^{a}
DCM	10 min	30^{ab}	60^{a}
"	1 hr	34^{a}	55^{a}
"	12 hr	33^{a}	55^{a}

* Kinetin concentration in organic solvents was 500 μM. In untreated control kinetin concentration (in the aqueous incubation medium) ranged from 20 μM to 200 μM.

Table II. *Inhibition of Shoot and Root Growth of Parris Island Seedlings by Acetone and Dichloromethane Leachates of Grand Rapids Lettuce Seeds.*

Grand Rapids lettuce seeds were soaked for 10 min and 2 hr in acetone and dichloromethane. See "Materials and Methods" for further details. Values in each column followed by the same letter are not significantly different at the 5% level.

Germination medium in addition to water	% Inhibition of Fr. Wt. after 4 days: Shoot	Root
None	0^{c}	0^{c}
Dried acetone	6^{c}	5^{c}
Dried acetone leachate (10 min)	29^{b}	55^{b}
Dried acetone leachate (2 hr)	34^{b}	61^{ab}
Dried DCM	11^{c}	11^{c}
Dried DCM leachate (10 min)	38^{ab}	66^{ab}
Dried DCM leachate (2 hr)	50^{a}	74^{a}

R-FR Photoreversible Phytochrome System in Relation to Acetone and Acetone-Kinetin Treatments of Lettuce Seeds. The possibility was examined that acetone and acetone-kinetin treatments might have affected the phytochrome system which controls the reversible R-FR photoreaction. The various treatments are shown in Table III. Untreated seeds responded to R by germinating close to 100% and the effect was reversed by FR. Kinetin promoted germination of untreated seeds in the dark to some extent, and the level of promotion remained unaltered by FR given after R exposure. The promotion of dark germination by acetone and further promotion by acetone-kinetin also remained unaltered by FR given after R. In all cases exposure to R alone or subsequent to FR gave nearly 100% germination.

Effect of Different Lengths of R and FR Exposures on Germination of Lettuce Seeds. Time of R exposure required to obtain about 100% germination in various treatments is shown in Figure 1. Acetone-kinetin treatment, which gave 52% germination in the dark required only 0.5 min of R exposure for maximum germination. The length of R exposure for the same level of germination in acetone-treated seeds, which gave 19% germination in the dark, was 1 min. In untreated seeds with a 5% dark germination, the time of R exposure required for 100% germination was 5 min. In all treatments, nearly 50% of the seeds germinated in response to 0.5 min of R.

The promotion of germination by 1 min of R exposure in untreated, acetone-treated, and acetone-kinetin permeated seeds was reversed by 1 min of FR to nearly the levels of germination obtained in the dark (Table III). No further reduc-

Table III. *Effect of Acetone and Acetone-Kinetin on Photoreversibility of Phytochrome in Grand Rapids Lettuce Seed*

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

	% Germination, 48 hr			
	Untreated		Acetone-permeated (1 hr)	
	- KIN	+ KIN (100 μM)	- KIN	+ KIN (500 μM)
Dark control	7[b]	18[b]	23[b]	50[c]
R	97[a]	99[a]	97[a]	98[a]
R-FR	7[b]	24[b]	25[b]	59[bc]
R-FR-R	95[a]	98[a]	97[a]	98[a]
R-FR-R-FR	8[b]	32[b]	23[b]	66[b]
R-FR-R-FR-R	98[a]	99[a]	98[a]	97[a]

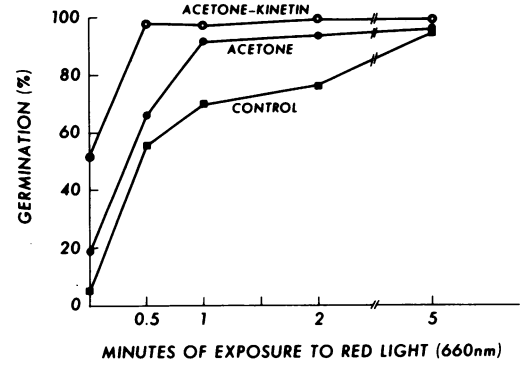

FIG. 1. Germination response of untreated, acetone-treated, and acetone-kinetin permeated Grand Rapids lettuce seeds to various periods of R exposure. Seeds were soaked in acetone and acetone-kinetin solutions for 1 hr.

tion in germination occurred in acetone-treated and acetone-kinetin permeated seeds by increasing the period of FR exposure up to 30 min.

Increased Polyribosome Formation by Acetone-Kinetin Treatment. Acetone and acetone-kinetin treatments not only promoted germination of Grand Rapids lettuce seeds in the dark (Table I), but also enhanced the rate of germination in the light. Differences in germination were observed in untreated, acetone-treated, and acetone-kinetin treated seeds 12 hr after imbibition in the light and these differences were reflected also at the level of polyribosome formation, particularly in the case of acetone-kinetin treated seeds (Fig. 2). There was no germination in any of the treatments in the dark at 12 hr and no differences were noted in the level of polyribosomes (data not shown).

DISCUSSION

Promotion of dark germination in Grand Rapids lettuce seeds by organic solvents such as acetone and DCM, and further promotion as a result of kinetin permeation, could be related to the removal of inhibitor and/or the restraining influence of the two-cell-layered endosperm on embryo growth.

There is good evidence for the mechanical or permeability barrier regulating germination of light-sensitive seeds (10, 24). The two-cell-layered endosperm of lettuce seeds (5, 11) is lipoidal in nature (21) and has considerable tensile strength (2). The treatment of plant cells with acetone has been shown to cause a complete disruption of the integrity of the membrane (28). Evidence has recently been presented that GA_3 profoundly alters the metabolism of the endosperm and that the requirement of GA_3 for dark germination of lettuce seeds is reduced 10- to 20-fold as a consequence of acetone treatment (15, 17). Thus acetone and DCM treatments could conceivably lead to the dissolution or weakening of the endosperm. The possibility that organic solvent might also leach out some inhibitory substances from the seeds other than ABA was indicated from the present study.

Cytokinins are known to promote germination of light-sensitive lettuce seeds in the dark (20, 26). The additive effect of acetone and kinetin in promoting dark germination in the present study suggests that these two factors affect germination by different mechanisms. Cytokinins have been shown to oppose

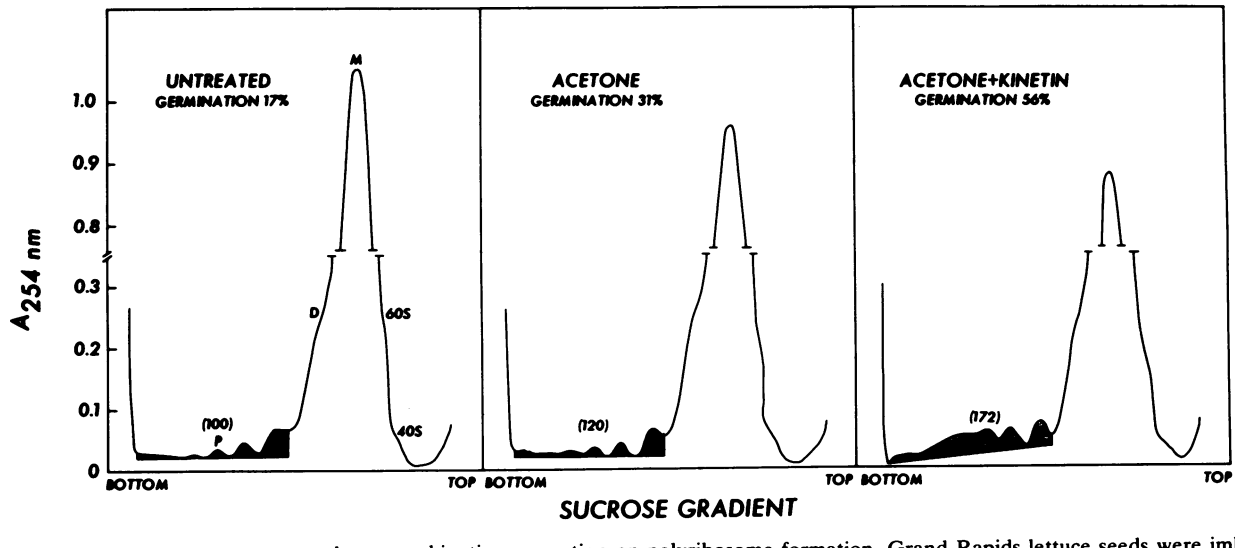

FIG. 2. Effect of acetone treatment and acetone-kinetin permeation on polyribosome formation. Grand Rapids lettuce seeds were imbibed in light at 25 C for 12 hr. Polyribosomes were isolated from 150 imbibed seeds. The numbers in parentheses are polyribosome contents (P) as per cent of control. M: monomer; D: dimer.

the inhibitory effects of natural inhibitors of seed germination and this was suggested to be one of the mechanisms of natural control of germination (13, 14). The involvement of inhibitors in the light-sensitive seeds is suggested from several studies (8, 15). Thus, it is possible that the action of acetone and DCM is largely limited to weakening of the endosperm barrier, whereas the action of kinetin is related to alleviation of the inhibitor effect.

The present data show that the amount of Pfr (active form of phytochrome) required for germination decreases by acetone treatment and a further decrease in such requirement is found when kinetin is permeated into lettuce seeds. The Pfr required for promotion of germination in light-sensitive lettuce seeds appears to depend upon the conditions of stress imposed upon the embryo (9). Recent studies by Speer (27) show that puncturing of the pericarp and endosperm as well as treatment of seeds with DCM decrease the amount of R exposure required for germination. It has been suggested that the low level of Pfr present in light-sensitive lettuce seeds in the dark causes bypasses of the light requirement (3, 24) when these seeds are treated with kinetin (20), thiourea (30), and chloramphenicol (4).

The promotive effects observed in this study of acetone and acetone-kinetin treatments on germination were unaffected by either R or FR exposures. Far red has been shown to be ineffective in reversing R effect in lettuce seeds imbibed in aqueous solution of kinetin (20); in fact FR promoted germination in presence of kinetin (20, 26). A germination path independent of R-FR photocontrol appears to have been created by these treatments.

The increase in germination in light in acetone and acetone-kinetin treatments was directly correlated with the level of polyribosomes. This could be related to removal of mechanical restraints and/or inhibitor(s). Protein synthesis and possibly other processes could be controlled to some extent by treatment of seeds with organic solvents and organic solvent-hormone solutions.

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