Planta (Berl.) 102, 368–371 (1972)

Short Communication

A Distinction between the Actions of Abscisic Acid, Gibberellic Acid and Cytokinins in Light-Sensitive Lettuce Seed

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Received September 27, 1971

Summary. Using isolated lettuce seed embryos it can be shown that the inhibitory effect of abscisic acid upon germination is reversed solely by cytokinin. In the intact seed, however, gibberellic acid is also required for this reversal to be manifested in germination.

Stimulation to germination of dark-imbibed light-sensitive lettuce seeds by gibberellic acid (GA_3) has been widely documented (since Lona, 1956). More recently the observation has been made that this promotion can be reversed by abscisic acid (ABA) (Khan, 1968). Khan found that increasing the concentration of gibberellin, however, does not overcome the inhibitory effect of ABA, although this can be reversed by cytokinins in the presence of low concentrations of GA_3. Furthermore, he has demonstrated that there is a progressively increasing reversal of ABA inhibition by increasing concentrations of GA_3 in the presence of a fixed amount of cytokinin (kinetin). This led him to speculate that the gibberellin and the cytokinin are acting at different sites, GA_3 acting as a stimulus to germination at a promotive site and cytokinin action being limited to the site(s) of inhibition (Khan, 1968; Khan and Waters, 1969). This hypothesis has been borne out with our approach using isloated lettuce seed embryos.

In Table 1 we compare the actions of GA_3, ABA and the cytokinins benzyladenine (BA) and kinetin on the germination of intact light-sensitive Grand Rapids lettuce seeds (*Lactuca sativa* var. Grand Rapids, Ferry Morse Seed Co., 1970). The large stimulation to germination by GA_3 (0.29 mM, 100 μg/ml) and small stimulation by cytokinin (BA or

* This research was supported by NRC of Canada Trust No. A6352 to J. D. Bewley.

** Holder of an NRC of Canada Postgraduate Scholarship.

Table 1. *Effect of ABA, cytokinin and gibberellin on the germination of intact lettuce seed*

Treatment	% germination
GA_3 0.29 mM	99 ± 1
BA 0.1 mM	25 ± 9
Kinetin 0.1 mM	29 ± 6
ABA 0.04 mM	4 ± 1
GA_3 0.29 mM + ABA 0.04 mM	16 ± 10
GA_3 2.9 mM + ABA 0.04 mM	11 ± 4
BA 0.1 mM + ABA 0.04 mM	7 ± 0
Kinetin 0.1 mM + ABA 0.04 mM	3 ± 1
GA_3 0.29 mM + ABA 0.04 mM + BA 0.1 mM	69 ± 4
GA_3 0.29 mM + ABA 0.04 mM + Kinetin 0.15 mM	27 ± 10
Water	12 ± 3

Seeds were counted after an imbibition time of 48 hours in darkness. Approximately 100 seeds were used per treatment, with a minimum of 2 replicates

kinetin, 0.1 mM) is reversed by ABA (0.04 mM). This inhibition cannot be reversed by increasing the concentration of GA_3 alone to 2.9 mM, or of cytokinin alone to 0.5 mM (see Table 1 and also Khan, 1968). If, however, BA and GA_3 are added together in the presence of ABA the inhibition is removed, although kinetin and GA_3 together are ineffective (cf. Khan, 1968). The possible reason for this latter observation is discussed later. On the basis of Khan's hypothesis, therefore, it is likely that, in intact seeds, BA is acting to reverse the ABA inhibition, but since BA alone is unable to stimulate germination of these seeds in darkness (see Table 1) the additional presence of GA_3 is necessary for this promotion. Isolated lettuce seed embryos were used to confirm this interpretation.

When embryos are removed from their restrictive endosperm and outer seed coat layers, germination occurs in darkness (see water control in Table 2). This germination can be completely inhibited by ABA at 0.03 mM and, again, GA_3 at a concentration of 0.29 mM will not reverse this inhibition, and reverses it only partially at the supra-optimal concentration of 1.45 mM (500 µg/ml). On the other hand, both BA and kinetin at 0.01 mM concentration can completely overcome the ABA effect. The fact that kinetin is effective in reversing the ABA inhibition of isolated embryos but not of intact seeds is interesting when one considers the great variability between seed batches in their response to saturating concentrations of kinetin. Thus, Leff (1964) obtained 80–90% germination in darkness with the 1957 harvest, Khan (1968)

Table 2. *Effect of ABA, cytokinin and gibberellin on the germination of isolated lettuce seed embryos*

Treatment	% germination
Water control	100 (10, 10)
ABA 0.02 mM	5 (1,0)
ABA 0.03 mM	0 (0,0)
ABA 0.02 mM + GA_3 0.29 mM	0 (0,0)
ABA 0.02 mM + GA_3 1.45 mM	30 (3,3)
ABA 0.03 mM + BA 0.01 mM	100 (10, 10)
ABA 0.03 mM + Kinetin 0.01 mM	100 (10, 10)

Embryos were dissected in green light from seeds imbibed in darkness on water for 2 hours. Two replicates of 10 embryos were used per treatment (actual numbers germinated per replicate indicated in brackets)

obtained 45% germination with the 1967 harvest, but Bewley *et al.* (1968) obtained only 2% with the 1964 harvest from the same source. It is quite plausible that the response to kinetin could be a function of a variable permeability barrier set up by the endosperm layer. As far as the 1970 harvest is concerned the action of kinetin must be restricted since it has little promotive effect on the intact seeds, in the presence of GA_3, but is completely effective at much lower concentrations on the isolated embryos.

It is relevant to note that the dark germination of two varieties of non-light-sensitive lettuce seed, in the intact state, can be inhibited by ABA. This inhibition can be relieved by kinetin alone and somewhat by gibberellin at low concentrations (10 mg/l; 2.9×10^{-5} M) (Sankhla and Sankhla, 1968). This concentration, however, is well below the optimal concentration necessary for promotion to germination of other lettuce seed varieties (light-sensitive and non-light-sensitive) (e.g. see Toole and Cathey, 1961, var. Great Lakes; Ikuma and Thimann, 1960, var. Grand Rapids; Haber and Luippold, 1960, var. New York). At present it cannot be determined whether higher concentrations of GA_3 might have relieved the ABA inhibtion of these non-light-sensitive varieties.

In conclusion, using the technique of isolating embryos from light-sensitive lettuce seed, we have been able to demonstrate the action of cytokinin as exclusively reversing the inhibitory action of ABA. The promotive action of GA_3 is, therefore, occurring at a site independent of that of the other two hormones. These results also tend to disfavor the idea that ABA is a natural inhibitor in lettuce seed coats, because gibberellin alone will stimulate the germination of these seeds in darkness, without a requirement for exogenously supplied cytokinins.

Furthermore, there is no evidence for the production of endogenous cytokinins prior to the onset of visible germination (Barzilai and Mayer, 1964).

We are grateful to Hoffmann-La Roche Ltd. for their generous gift of abscisic acid.

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