

## Short Communication

Inhibition of Gibberellic Acid-induced Germination by Abscisic Acid and Reversal by Cytokinins<sup>1, 2</sup>

A. A. Khan

New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456

Received June 17, 1968.

The germination of seed can be controlled by application of exogenous hormones at physiological concentrations. It is therefore possible that the natural control of germination involves an interplay of hormones, both promoters and inhibitors. We have shown that cytokinins overcome the inhibitory action of several natural inhibitors in the germination of the photosensitive Grand Rapids lettuce seed (4, 5). There are other reports of cytokinin-inhibitor antagonism in germination and dormancy (6, 7, 10, 12, 14). Chrispeels and Varner have shown that gibberellic acid ( $GA_3$ ) induced  $\alpha$ -amylase synthesis in the excised barley aleurone is inhibited by abscisic acid and reversed only partially by excess  $GA_3$  (2). In the intact barley seed, likewise,  $GA_3$  fails to reverse abscisic acid inhibited  $\alpha$ -amylase production and coleoptile growth (8, 9). Cytokinins, such as kinetin and benzyladenine, on the other hand, nearly completely reverse the abscisic acid inhibition of  $\alpha$ -amylase synthesis and coleoptile growth in the seed (9).

This communication will show that another  $GA_3$  induced process, namely dark germination of Grand Rapids lettuce seed, is inhibited by abscisic acid and is reversed by the cytokinin, kinetin but not by excess  $GA_3$ .

Lettuce seeds (*Lactuca sativa* var. Grand Rapids. Ferry Morse Seed Company, 1967) were germinated at 20° and 25° in total darkness in 5 cm Petri plates on 2 layers of filter paper soaked in 2 ml of test solution (4 replicates of 50 seeds each). Germination was recorded after 48 hours. Emergence of the radicle was taken as criterion of germination.

The effect of abscisic acid and kinetin on  $GA_3$  induced dark germination was examined at 20° (fig 1) and 25° (fig 2). A concentration of 0.05 mM  $GA_3$  was sufficient for complete induction of germination at 20° (fig 1, curve B) while at 25° much higher concentration of  $GA_3$  (5 mM) was

required for complete induction (fig 2, curve B). Kinetin at 0.05 mM caused a slight increase in germination over water (curve A, 0 conc of  $GA_3$ ) and had little effect on  $GA_3$  induced germination (curve A).  $GA_3$  induced germination was completely inhibited by abscisic acid at 25° and appreciably at 20° (curve E). This inhibition of  $GA_3$  induced germination was reversed to a large extent by kinetin (curves C, D). A progressively increased reversal of abscisic acid (0.04 mM) inhibition by kinetin (0.05 mM) was obtained in presence of increasing concentrations of  $GA_3$ .  $GA_3$  by itself, even at high concentrations, failed to reverse abscisic acid inhibition of germination to more than 20% (figs 1, 2, curve E). At a fixed concentration of  $GA_3$ , kinetin at 0.05 mM was more active (curve C) than at 0.5 mM (curve D) in reversing abscisic acid inhibition of germination. Similar results were obtained with <sup>6</sup>N-benzyladenine (data not shown).

The results presented here show that  $GA_3$  induced germination in the photosensitive Grand Rapids lettuce seed in the dark is blocked by abscisic acid. This block is removed to a large extent by kinetin but not by excess  $GA_3$  (5 mM). These data when considered together with kinetin reversal of abscisic acid inhibition of germination in light (4, 5) would suggest that light and  $GA_3$  induced germination may be controlled by the same hormonal mechanism(s). The mechanism(s) controlling light and  $GA_3$  induced lettuce seed germination, however, is not yet fully understood.

Data in this report and those of Ikuma and Thimann (3) suggest that  $GA_3$  and cytokinins act at different sites. Unlike  $GA_3$ , cytokinins do not induce  $\alpha$ -amylase synthesis in barley endosperm (1, 9) nor do they induce dark germination of lettuce seed to any marked extent as shown in the data presented here and elsewhere (3, 11). Furthermore, a progressively increased reversal of abscisic acid inhibition of germination by a fixed amount of kinetin and increasing concentrations of  $GA_3$  would suggest that  $GA_3$  may be the primary stimulus for lettuce seed germination in the dark and the action of kinetin is perhaps limited to the site(s) of inhibition. The possibility that kinetin may be acting at other site(s) as well or that its action may be

<sup>1</sup> Supported in part by the American Seed Research Foundation.

<sup>2</sup> Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1650.

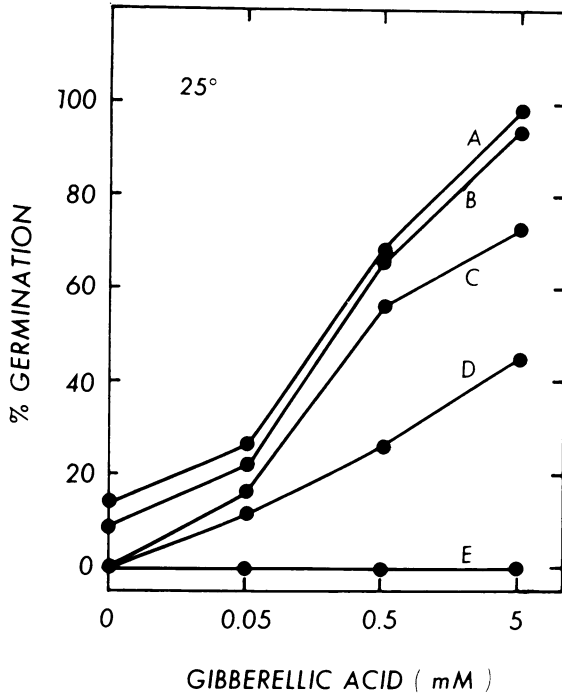


Fig. 2

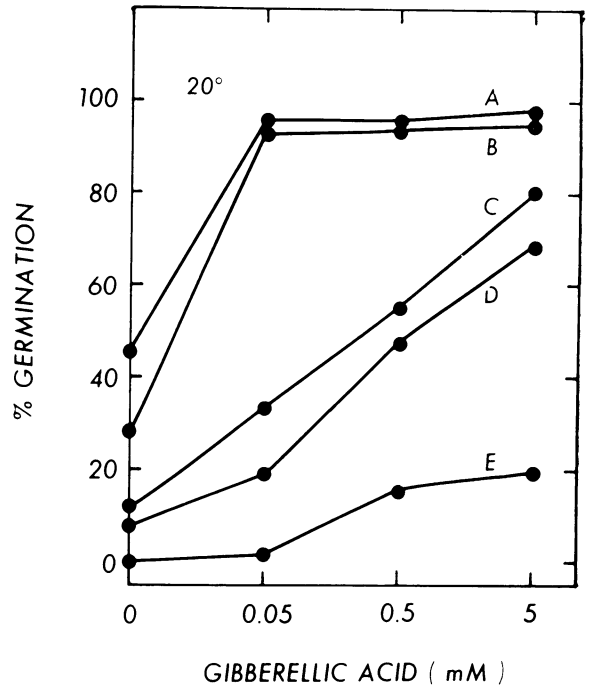


Fig. 1

FIG. 1. (right) The effect of abscisic acid and kinetin on gibberellic acid induced dark germination of lettuce seed at 20°. A) kinetin (0.05 mM); B) water; C) kinetin (0.05 mM) plus abscisic acid (0.04 mM); D) kinetin (0.5 mM) plus abscisic acid (0.04 mM); E) abscisic acid (0.04 mM).

FIG. 2. (left) The effect of abscisic acid and kinetin on gibberellic acid induced dark germination of lettuce seed at 25°. Designation of curves same as in figure 1.

indirect cannot be ruled out, however. Similar conclusions were arrived at by Ikuma and Thimann (3).

The participation of more than 1 hormone in biological systems is not uncommon. An interplay of hormones may result in synergistic, antagonistic, additive, and permissive effects (13). In the present instance the effect of kinetin may be regarded both as antagonistic and permissive—antagonistic to the action of the inhibitor, abscisic acid and permissive to the action of the hormone, G.A.<sub>3</sub>. The question as to how these hormones work await further investigation.

### Acknowledgments

I am indebted to Dr. Y. Yeo, Woodstock Agricultural Research Center, for a generous donation of the synthetic racemic abscisic acid (abscisic II) and Merck and Company for a sample of gibberellic acid.

### Literature Cited

- BRIGGS, D. E. 1963. Biochemistry of barley germination: Action of gibberellic acid on barley endosperm. *J. Inst. Brew.* 69: 13-19.
- CHRISPEELS, M. J. AND J. E. VARNER. 1967. Hormonal control of enzyme synthesis: On the mode of action of gibberellic acid and abscisic acid in aleurone layer of barley. *Plant Physiol.* 42: 1008-16.
- IKUMA, H. AND K. V. THIMANN. 1963. Action of kinetin on photosensitive germination of lettuce seed as compared with that of gibberellic acid. *Plant Cell Physiol.* 4: 113-28.
- KHAN, A. A. AND N. E. TOLBERT. 1965. Reversal of inhibitors of seed germination by red light plus kinetin. *Physiol. Plantarum* 18: 41-43.
- KHAN, A. A. 1967. Antagonism between cytokinins and germination inhibitors. *Nature* 216: 166-67.
- KHAN, A. A. 1967. Antagonism between dormin and kinetin in seed germination and dormancy. *Am. J. Botany (suppl.)* 54: 639.
- KHAN, A. A. 1966. Breaking of dormancy in *Xanthium* seeds by kinetin mediated by light and DNA-dependent RNA synthesis. *Physiol. Plantarum.* 19: 869-74.
- KHAN, A. A. AND R. D. DOWNING. 1968. Cytokinin reversal of dormin (abscisic II) inhibition of growth and  $\alpha$ -amylase synthesis in barley seed. *Physiol. Plantarum.* In press.
- KHAN, A. A. 1969. Cytokinin-inhibitor antagonism in the hormonal control of  $\alpha$ -amylase synthesis and growth in barley seed. *Physiol. Plantarum.* In press.
- KNYPL, J. S. 1966. Synergistic inhibition of kale seed germination by coumarin and (2-chloroethyl) trimethylammonium chloride and its reversal by kinetin and gibberellic acid. *Planta* 72: 292-96.

11. MILLER, C. O. 1958. The relationship of the kinetin and red light promotions of lettuce seed germination. *Plant Physiol.* 33: 115-17.
12. SANKHLA, N. AND D. SANKHLA. 1968. Reversal of ( $\pm$ )-abscisin II induced inhibition of lettuce seed germination and seedling growth by kinetin. *Physiol. Plantarum* 21: 190-95.
13. TATA, J. R. 1966. Hormones and the synthesis and utilization of ribonucleic acid. In: *Progress in Nucleic Acid Research and Molecular Biology*. J. N. Davidson and W. E. Cohn, eds. Academic Press, Inc., New York. p 191-250.
14. VAN OVERBEEK, J., J. E. LOEFFLER, AND M. I. R. MASON. 1967. Dormin (abscisin II), inhibitor of plant DNA synthesis? *Science* 156: 1497-99.