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Inhibition of Cottonseed Germination with Abscisic Acid and Its Reversal

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ABSTRACT

Germination of cottonseed (*Gossypium hirsutum* L.) was inhibited by abscisic acid. Inhibition was greater when seeds were soaked in abscisic acid for 5 hours and dried prior to germination than when abscisic acid was applied in the germination medium. (2-Chloroethyl)phosphonic acid, gibberellic acid, and kinetin partially overcame the inhibitory action of abscisic acid. Combinations of (2-chloroethyl)phosphonic acid with gibberellic acid or kinetin were more effective than the individual substances. Germination also was partially restored by removal of seed coats. Fusicoccin completely restored germination of abscisic acid-treated seeds.

ABA inhibits seed germination of many species (1, 7). Ihle and Dure (2, 3) demonstrated that ABA inhibits precocious germination of cotton embryos. While 1 μM ABA inhibited germination of excised immature embryos (3), 38 μM ABA (10 μg/ml) did not inhibit germination of mature embryos (2).

Growth regulators, particularly GA_3, counteract many effects of ABA (1, 7). Recently, Lado *et al.* (4) showed that fusicoccin, a metabolite of *Fusicoccum amygdali* Del., counteracts the inhibitory effects of ABA on germination of radish and lettuce seeds. This paper reports inhibition of germination of mature cottonseed by ABA, and reversal of this inhibition by growth regulators and by removal of seed coats.

MATERIALS AND METHODS

Field-grown cotton bolls (*Gossypium hirsutum,* Stoneville 213) were picked on the day they became fully opened, and their seeds were dried and delinted with H_2SO_4. Any seeds that were visibly damaged, immature, or which floated on water were discarded. Germination of these selected seeds was 100%. Embryos were obtained by removing the seed coats with a razor blade; damaged embryos were discarded.

To effect germination, each 50 seeds or embryos were rolled in a 10 x 46-cm strip of Whatman No. 3 filter paper.[1] This was moistened with 16 ml of water or growth regulator solution and incubated at 30 C. "Presoaked" seeds were placed in ABA solutions for 5 hr, air-dried to less than 10% seed moisture, and then germinated in water or growth regulator solutions. The growth regulators used were ABA, IAA, 6-(furfurylamino)purine (kinetin), GA_3, CEPA[2] and fusicoccin.

Germination percentages were determined from 400 seeds, and seedling lengths are expressed as mean values for seeds that germinated.

RESULTS AND DISCUSSION

ABA inhibited germination and seedling elongation in both cottonseed and embryos (Table I). Inhibition of elongation occurred at lower concentrations than inhibition of germination. Presoaking seeds with 0.32 mM ABA completely inhibited germination, whereas 1 mM ABA was required in the germination solution to completely inhibit germination of embryos or seeds. Presoaking seeds in water, rather than ABA, had no effect on germination of seeds or their response to ABA.

Ihle and Dure (2) previously faled to inhibit germination of mature cotton embryos with 38 μM ABA (10 μg/ml). My results show that concentrations above 0.1 mM are necessary.

Several growth regulators counteracted ABA-induced inhibition of cottonseed germination (Table II). Fusicoccin was most effective. Other substances incompletely restored germination when used individually. Combinations of CEPA and GA_3, or CEPA and kinetin restored a percentage of germination, but seeds germinated more slowly than those treated with fusicoccin. Concentrations of growth regulators less than 0.32 mM stimulated germination of ABA-treated seeds to a lesser extent. In similar studies with radish and lettuce seeds, Lado *et al.* (4) found that fusicoccin was more effective than benzyladenine or GA_3 in counteracting ABA.

Table I. *Effect of ABA Concentration on Cottonseed and Embryo Germination and Seedling Elongation*

Seeds were either presoaked in ABA solutions for 5 hr and air-dried for 40 hr prior to germination, or they were germinated in the presence of ABA solutions. Embryos were germinated in the presence of aqueous ABA solutions. Germination at 30 C for 48 hr.

ABA concentration	Germinated in ABA Solutions: Embryos Germ.	Embryos Lgth.	Seeds Germ.	Seeds Lgth.	Presoaked Seeds Germ.	Presoaked Seeds Lgth.
	(%)	(mm)	(%)	(mm)	(%)	(mm)
0 (Control)	100	78	100	60	100	62
3.2 µM	100	83	100	64	100	60
10.0 µM	100	72	100	63	100	61
32.0 µM	98	54	100	58	100	50
0.1 mM	81	31	100	46	49	29
0.32 mM	41	19	78	19	0	0
1.0 mM	0	0	0	0	0	0

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2] Abbreviation: CEPA: (2-chloroethyl)phosphonic acid (Ethephon).

Table II. *Interaction of ABA and Other Growth-regulating Substances on Germination of Cottonseed*

Seeds were presoaked in either water or 0.32 mM ABA for 5 hr, air-dried for 48 hr, and then germinated in paper rolls at 30 C. Growth regulators were applied directly to the rolls as 0.32 mM solutions in 0.05 M Na citrate buffer, pH 4.7.

Presoak	Growth Regulators[1]	Cumulative Germination (%)			
		1 day	2 days	4 days	9 days
Water	Buffer	89	99	100	
Water	CEPA	97	100		
Water	K	73	99	99	99
Water	GA_3	97	100		
Water	IAA	21	100		
Water	FC	92	100		
ABA	Buffer	3	7	15	34
ABA	CEPA	7	25	48	75
ABA	K	4	31	41	66
ABA	GA_3	1	15	39	78
ABA	IAA	1	10	15	33
ABA	CEPA + K	7	59	88	99
ABA	CEPA + GA_3	9	64	91	96
ABA	CEPA + IAA	3	27	42	88
ABA	K + GA_3	2	25	45	96
ABA	K + IAA	0	14	19	74
ABA	GA_3 + IAA	0	11	17	81
ABA	CEPA + K + GA_3	7	65	99	99
ABA	CEPA + K + IAA	1	39	67	95
ABA	CEPA + GA_3 + IAA	4	48	92	96
ABA	K + GA_3 + IAA	0	9	21	95
ABA	CEPA + K + GA_3 + IAA	1	53	89	97
ABA	FC	88	93	97	99

[1] Abbreviations used: K: kinetin; FC: fusicoccin.

IAA at 0.32 mM, either alone or in combination with other growth regulators, was inhibitory to seed germination. This inhibition was probably due to the herbicide-like effect of IAA (5). Seedlings germinating in IAA solutions were stunted and had distorted, swollen radicles and transition zones.

Radicle distortion and transition zone swelling similar to that observed in IAA treatments was observed in fusicoccin-treated seedlings. Fusicoccin has been reported to mimic the effects of IAA in stimulating cell enlargement (6). Fusicoccin stimulated both the rate and percentage of germination of ABA-inhibited cottonseed, whereas IAA had no effect.

Removal of seed coats from ABA-presoaked seeds and gentle rinsing of embryos in distilled H_2O partially counteracted the effects of ABA. At 4 days following rinsing, germination of individual lots of embryos ranged from 44 to 100%. Reversal of the ABA effect was probably due to removal of the ABA by leaching; however, reasons for the inconsistency of results are not known.

Ihle and Dure (3) showed that ABA inhibited production of a protease and isocitratase during precocious germination of cotton embryos. This was due to inhibition of translation of preexisting mRNA. They proposed that these enzymes were representative of a group of germination enzymes whose synthesis could be controlled in immature embryos by ABA. This paper demonstrates that germination of mature cottonseed is inhibited by ABA and that this inhibition is counteracted by fusicoccin. ABA and fusicoccin may prove useful in studies of cottonseed metabolism.

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