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Abscission: Role of Abscisic Acid

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Abstract. The effect of abscisic acid on cotton (*Gossypium hirsutum* L. cv. Acala 4-42) and bean (*Phaseolus vulgaris* L. cv. Red Kidney) explants was 2-fold. It increased ethylene production from the explants, which was found to account for some of its ability to accelerate abscission. Abscisic acid also increased the activity of cellulase. Increased synthesis of cellulase was not due to an increase in aging of the explants but rather was an effect of abscisic acid on the processes that lead to cellulase synthesis or activity.

The ability of a diffusible substance from cotton fruit to accelerate the abscission of cotton explants ultimately led to the discovery of what is now known as abscisic acid, a plant hormone capable of regulating a number of plant processes (9, 16). The role of abscisic acid in abscission however, remains uncertain. The substance promotes the abscission of explants, but then so do a large number of other compounds, many of which are not normal constituents of leaves (1). Evidence both for (9) and against (15) a role for this hormone in abscission has been presented. However the work of Dale and Milford (15) has been criticized because chromotographic behavior of their compound differed from the β-inhibitor and did not promote abscission of young cotton fruit.

This paper presents a series of experiments designed to elucidate the role of abscisic acid in the abscission of isolated abscission zone explants, taking advantage of improved techniques for the measurement of cell separation (14) and the production of cell wall degrading enzymes (4).

Materials and Methods

Plant Material. The methods used to grow bean (*Phaseolus vulgaris* L. cv. Red Kidney) and cotton (*Gossypium hirsutum* L. cv. Acala 4-42) plants, and to prepare and store explants, have been described earlier (1, 6, 7). Each experimental datum with bean explants represents experiments repeated on 3 different occasions with 3 sets of 10 explants. Cotton experiments were repeated twice, but since there are 2 separation layers per explant, each experimental datum represents a total of 120 observations.

Application of Chemicals. Indole-3-acetic acid (IAA) and abscisic acid were applied by placing a 50 μl drop of 1.5% agar containing the IAA or abscisic acid on the distal cut surface of an explant. IAA concentration was 5×10^{-5} M and abscisic acid, except for some preliminary concentration curve experiments, was 5×10^{-4} M. The abscisic acid used in these experiments was a gift of the R. J. Reynolds Tobacco Company and consisted of 47.3 % *d,l-cis,trans* abscisic acid (the natural isomer) and 52.3 % of the *d,l-trans-trans* isomer. All concentrations were based on the *cis-trans* isomer. Samples of *d l-cis,trans* isomer (SD 16108) were a gift of the Shell Development Company, and a number of preliminary experiments with this material indicated that there was no essential difference in the properties of the 2 preparations.

Earlier papers from this laboratory have described the treatment of explants with ethylene and CO_2 in desiccators and gas collection bottles (4), the measurement of ethylene by gas chromatography (8), the injection of actinomycin D (1 μg/μl water) into the separation layers of explants (6), the determination of cellulase activity by the loss of viscosity of sodium carboxymethyl cellulose (CMC) (4), and break strength measurements by a recording abscissor (14).

Results

The effect of abscisic acid on break strength and ethylene production from explants is summarized in table I. The data are in agreement with earlier observations (1) that increasing amounts of abscisic acid cause explants to produce increasing amounts of ethylene and that there was a close correlation between the loss of break strength and ethylene evolution.

Table II presents the results of experiments in which explants treated with abscisic acid were exposed to 10 % CO_2. The data indicate that abscisic acid increased ethylene evolution, but that the decrease in break strength of cotton caused by abscisic acid was only partially overcome by CO_2.

The data in table III show that ethylene was unable to mask the effect of abscisic acid on the break strength of cotton and bean explants. When explants were treated with saturating levels of ethylene and abscisic acid, an abscisic acid effect on break strength was still apparent.

Table I. *Effect of Abscisic Acid on Break Strength and Ethylene Production in Cotton and Bean Explants*

Explants were placed in 43-ml gas collection bottles fitted with rubber vaccine caps and kept at 25° with continuous 400 ft-c fluorescent light. Wound ethylene was flushed out of bottles 8 hr after excision. Break strength and ethylene production were measured at 28 hr.

Abscisic acid	Cotton		Bean	
	Break strength	C_2H_4	Break strength	C_2H_4
m	*g*	*ppm*	*g*	*ppm*
0	50[a]	0.121[a]	174[a]	0.061[a]
5×10^{-6}	47[a,b]	0.136[a]	163[a]	0.084[a,b]
5×10^{-5}	38[b]	0.136[a]	140[a,b]	0.072[a,b]
5×10^{-4}	0[c]	0.316[b]	108[b]	0.096[b]

[a, b, c] Means having the same letter within a column are not significantly different at the 5 % level.

Table II. *Effect of 10 % CO_2 on Abscission of Cotton and Bean Explants Treated With 5×10^{-4} M Abscisic Acid*

Explants were placed in 43-ml gas collection bottles fitted with rubber vaccine caps and kept at 25° with continuous 400 ft-c fluorescent light. Wound ethylene was flushed out of bottles 8 hr after excision. Break strength and ethylene production were measured at 28 hr.

Treatment	Cotton		Bean	
	Break strength	C_2H_4	Break strength	C_2H_4
	g	*ppm*	*g*	*ppm*
Control	63[a]	0.192[a]	160[a]	0.076[a]
Abscisic acid	16[c]	0.332[b]	92[b]	0.110[a, b]
CO_2	60[a]	0.170[a]	180[a]	0.106[a, b]
Abscisic acid + CO_2	39[b]	0.241[a]	189[a]	0.146[b]

[a, b, c] Means having the same letter within a column are not significantly different at the 5 % level.

The data in Fig. 1 show the effect of abscisic acid on changes in the break strength of A) cotton and B) bean explants stored in an atmosphere of 10 ppm ethylene. The break strength of cotton and bean explants in an ethylene atmosphere remains constant for about 16 hr and then decreases. The effect of abscisic acid in cotton explants appears to shorten the time required for a loss of break strength by about 2 hr. The data obtained with bean explants indicated no effect on induction period but rather an accelerated loss of break strength once abscission was started.

IAA was able to prevent a loss of break strength of explants when applied shortly after excision (Fig. 2A and 2B). When the time of IAA application was delayed by 5 hr for cotton (Fig. 2A) and 8 hr for bean (Fig. 2B) then its effect was reduced and delaying the time of application even further caused a corresponding loss of effectiveness. As shown in Fig. 2A and 2B, abscisic acid did not appear to have an effect on the period of time IAA was able to prevent a loss of break strength. However, abscisic acid caused a lower break strength once the aging requirements were met.

The ability of actinomycin D to inhibit abscission in a 10 ppm ethylene gas phase lasts 4 hr after excision in cotton explants (Fig. 3A) and 6 hr in bean explants (Fig. 3B). Abscisic acid had no influence on the length of time explants retained full sensitivity to actinomycin D but it again caused a lower break strength once the inhibitory effect of actinomycin D was lost.

The cellulase content of explants in an atmosphere of 10 ppm ethylene increases after 6 hr for

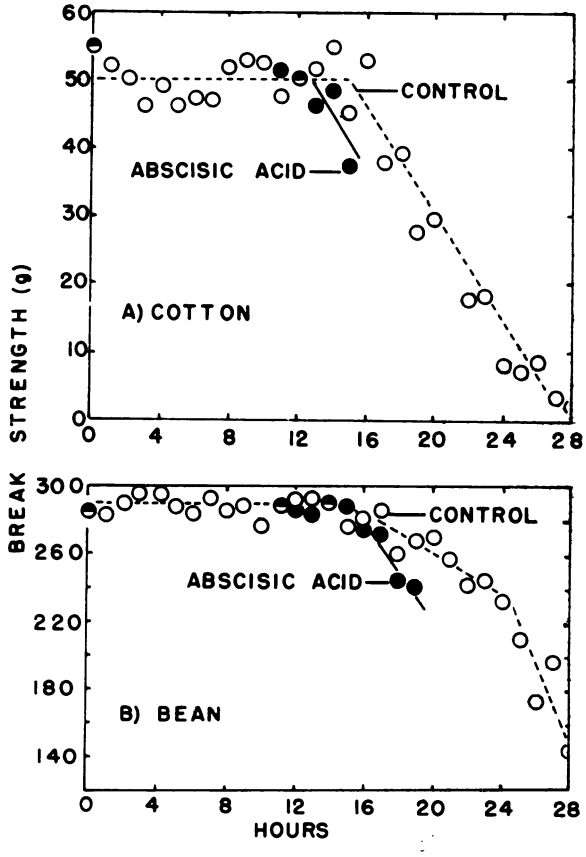

FIG. 1. Effect of abscisic acid on break strength of A) cotton and B) bean explants. Gas phase was 10 ppm ethylene. Abscisic acid was applied at 0 hr. Break strength determinations made at times indicated.

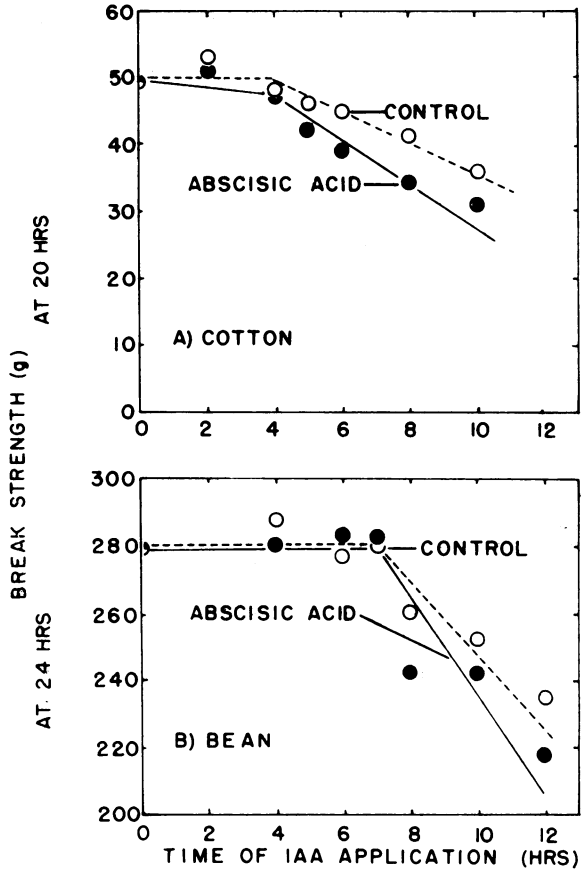

FIG. 2. Effect of abscisic acid on the ability of IAA to retard abscission of A) cotton and B) bean explants. Gas phase was 10 ppm ethylene. Abscisic acid or plain agar controls added at 0 hr. Abscisic acid and control agar were carefully wiped off with moist tissue and replaced with IAA at times indicated.

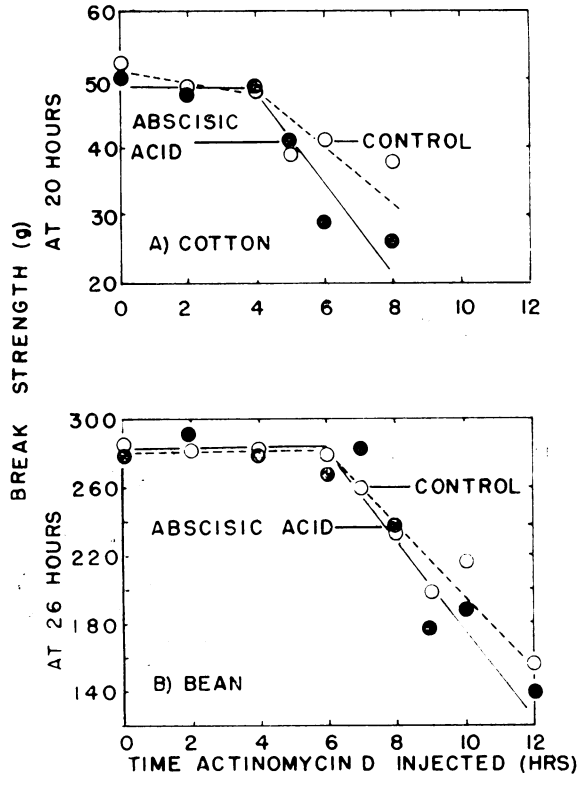

FIG. 3. Effect of abscisic acid on the ability of actinomycin D to inhibit abscission in A) cotton and B) bean explants. Gas phase was 10 ppm ethylene. Abscisic acid and control agar droplets applied at 0 hr and left on throughout the course of the experiment. Actinomycin D injected into explants at the times indicated.

cotton (Fig. 4A) and 12 hr (Fig. 4B) for bean. Abscisic acid did not decrease the lag period before cellulase appeared in cotton explants and it increased cellulase activity over controls only after 14 hr. However, in bean explants abscisic acid did appear to decrease the lag period as well as increasing cellulase activity.

Discussion

The data in table I present evidence in favor of the view that enhanced ethylene production plays a role in the ability of abscisic acid to accelerate abscission of isolated abscission zone explants. However, the data in tables II and III show that enhanced ethylene production is only a part of the explanation of why abscisic acid stimulated abscission.

Table II shows that only a part of the abscisic acid effect on cotton could be overcome by CO_2, a competitive inhibitor of ethylene action (5, 11). All previous work on abscission in this laboratory with cotton and beans has shown inhibition of abscission with CO_2 (5). However, abscisic acid's ability to accelerate abscission could still be due to ethylene stimulation, since other cases of the inability of CO_2 to reverse ethylene effects have been reported (2, 8). It did, however, encourage us to measure the effect of abscisic acid in 10 ppm ethylene, which we have found earlier to represent a saturating concentration of the gas for abscission (5).

The observation (table III) that abscisic acid still had a promotive effect on abscission in the presence of saturating levels of ethylene substantiates the idea that enhanced ethylene production is only a part of the explanation of the abscisic acid mechanism of action.

Fig. 5 outlines some of the processes thought to occur during abscission and delineates the abscission process into a number of discrete stages or steps. A review discussing this scheme in greater detail has been published earlier (3). The experiments presented in Figs. 1 thru 4 were designed to distinguish whether abscisic acid accelerated the aging process (Stage 1) or the induction of cell wall degrading

enzymes (Stage 2). All of the experiments reported in Figs. 1 thru 4 were done in the presence of 10 ppm ethylene. This was done to insure that the effects of abscisic acid and other treatments performed were

Table III. *Stimulation of Abscission by 5×10^{-4} M Abscisic Acid and 10 ppm Ethylene*

Explants were placed in desiccators containing air or ethylene and kept at 25° with continuous 400 ft-c fluorescent light for the times indicated.

Treatment	Break strength Cotton (16 hr)	Beans (26 hr)
	g	*g*
Control	122	158
Abscisic acid	79	115
C_2H_4	37	52[a]
Abscisic acid + C_2H_4	60	44[a]

[a] Means significantly different at 10 % level. All other means within a column significantly different at 5 % level.

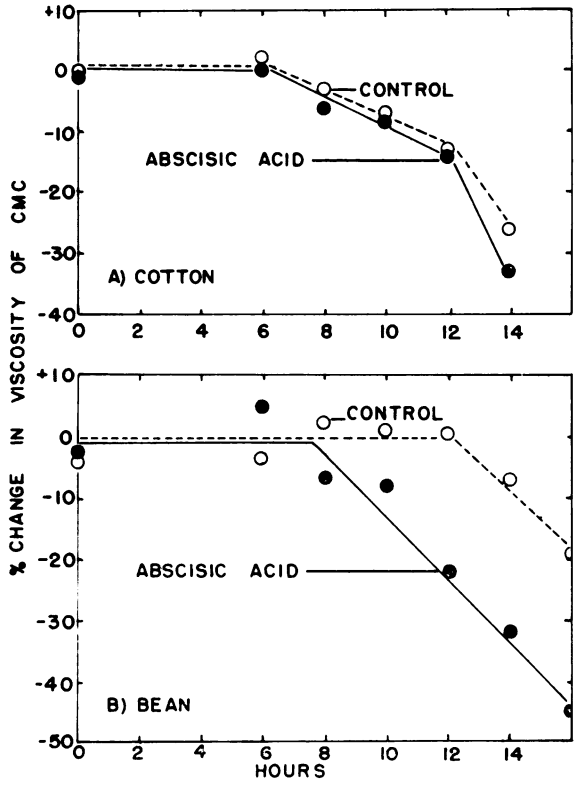

FIG. 4. Effect of abscisic acid on the production of cellulase from separation layers of A) cotton and B) bean explants. Gas phase was 10 ppm ethylene. Abscisic acid and control agar drops applied to explants at 0 hr and left on throughout the course of the experiment. Explants were harvested and frozen at times indicated until analyzed for cellulase. The cellulase was incubated with 1.5 % sodium carboxymethyl cellulose in 0.05 M potassium phosphate buffer, pH 7, for 16 hr at 40°.

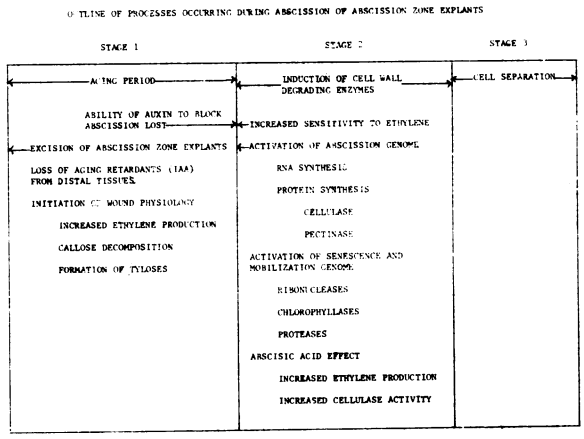

FIG. 5. Outline of processes occurring during abscission of abscission zone explants.

not due to an increase in ethylene production. It was assumed that 10 ppm ethylene saturated any requirement the explants had for the gas (5).

One way of measuring the effect of abscisic acid on aging is to determine the effect of abscisic acid on the time required for a loss of break strength. Since an aging period is required before cell wall degrading enzymes start acting, an increased rate of aging should appear as an earlier loss in break strength in explants treated with abscisic acid. The data obtained with cotton (Fig. 1A) indicate that a loss of break strength may have occurred 2 hr sooner in abscisic-acid-treated explants. Abscisic acid had no effect on the time required for a loss of break strength in bean explants (Fig. 1B) but, rather, caused a more rapid loss of break strength once the process was initiated.

We believe that the available data suggest that the major function of IAA in abscission is to prevent aging of explants. As shown in Figs. 2A and 2B, IAA can be added to explants for a fixed period of time after excision and still prevent a loss of break strength measured some hours later. If abscisic acid did accelerate aging, it should be able to shorten the time that IAA is still able to keep the explants in their original unaged state. If the role of abscisic acid is to accelerate aging, we would have expected that IAA would have lost the ability to block abscission sooner in treated explants. However, the data in Figs. 2A and 2B indicate that the length of Stage 1, measured as the ability of IAA to retard abscission, was the same in control and abscisic-acid-treated explants.

The completion of Stage 1 also sets into motion the degradation of chlorophyll as well as other cellular constituents (7, 24). We found that abscisic acid had no effect on chlorophyll degradation in bean and cotton explants using methods described earlier (7). However, other workers (10, 16) have observed that abscisic acid increased the loss of chlorophyll in other experimental systems.

We have shown earlier that actinomycin D

blocked RNA synthesis (20) and abscission (6), suggesting that RNA synthesis was essential to the cell separation process. When actinomycin D was injected into explants soon after excision it prevented a subsequent loss of break strength (Figs. 3A and 3B). However, actinomycin D loses its effectiveness after RNA molecules essential for abscission have been synthesized. This loss of effectiveness can be thought to represent the onset of Stage 2. If abscisic acid accelerates the completion of Stage 1 then it should shorten the time for actinomycin D to lose its effectiveness. The data in Figs. 3A and 3B indicate that abscisic acid did not shorten the time before actinomycin D lost its ability to block abscission completely. However, as in the earlier figures, abscisic acid did cause lower break strengths once Stage 1 was completed. It should be pointed out that there was a 1-hr difference in the duration of Stage 1 as measured by the auxin-induced lag and actinomycin D-induced lag in bean explants. This difference was primarily due to the fact that these experiments were done at different times. If experiments on auxin-induced and actinomycin D-induced retardation of abscission were done simultaneously with 1 sample of explants then the durations of Stage 1 were found to be similar.

An experiment designed to measure the effect of abscisic acid on Stage 2 is shown in Fig. 4. We have shown that abscisic acid accelerated the loss of break strength of cotton and bean explants (Fig. 1) and that increase in cellulase activity preceded the loss of explant break strength (14). If in addition to abscisic acid's ability to increase ethylene production, it has a second effect of increasing cellulase activity, then this should be observable with the sensitive viscometric techniques used to measure cellulase activity. Cotton explants (Fig. 4A) treated with abscisic acid did have greater cellulase activity, but the difference was small until 14 hr. Bean explants (Fig. 4B) treated with abscisic acid also had greater cellulase activity but, unlike cotton explants, the change in cellulase activity occurred sooner than in the controls. The cellulase data obtained from bean explants suggest that abscisic acid shortened the induction period for cellulase because it decreased the time required for the initial appearance of cellulase activity.

Conclusions

The action of abscisic acid in accelerating explant abscission may or may not be applicable to its effects on the abscission of intact leaves (13, 16, 18). Spray applications result in contact of abscisic acid with the blade as well as the separation layer, and ultimate control of abscission may rest on modification of blade physiology. (The same would also hold true for intact fruits.) Hartmann *et al.* (18) found that concentrations of abscisic acid that defoliated olive trees had no effect on the ethylene production of the leaves. Cooper *et al.* (13), however, found that defoliating concentrations of abscisic acid did promote ethylene production from citrus leaves.

There is no reason to believe that the role of abscisic acid in explant abscission accounts for its action on other physiological systems such as dormancy (16), inhibition of seed germination (22), inhibition of α-amylase (12, 19), promotion of phenylalanine ammonia-lyase (25), floral morphogenesis (16, 17, 23), or inhibition of growth (16, 21). In fact, we reported earlier that increased ethylene production did not account for the inhibition of growth of excised soybean hypocotyls by abscisic acid (21).

We conclude from the data presented here that the action of abscisic acid on abscission of isolated explants was 2-fold. First, it accelerated ethylene production and, second, it increased cellulase activity. We believe that abscisic acid has no effect on the aging of explants but rather it increases the activity of cellulase. Whether such control over cellulase is at the level of RNA-protein synthesis or of enzyme release-activation-degradation remains to be determined.

Acknowledgment

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