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TECHNICAL MANUSCRIPT 530

ABSCISSION: ROLE OF ABSCISIC ACID

Lyle E. Craker
Frederick B. Abeles

MAY 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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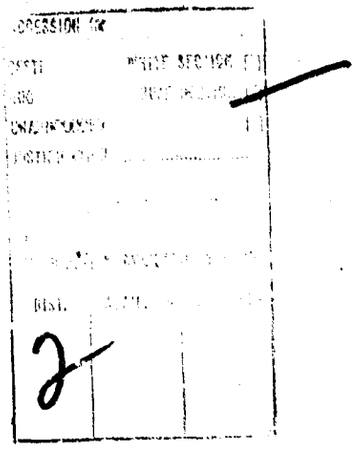

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ABSCISSION: ROLE OF ABSCISIC ACID

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PLANT SCIENCES LABORATORIES

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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 twofold. Abscisic acid increased ethylene production from the explants, which accounted for some of its ability to accelerate abscission. Abscisic acid also increased the activity of cellulase. Increased synthesis of cellulase was not caused by 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.

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I. INTRODUCTION*

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.[1, 2] The role of abscisic acid in abscission, however, remains uncertain. The substance promotes the abscission of explants, but so do a large number of other compounds, many of which are not normal constituents of leaves.[3] Evidence both for[1] and against[4] a role for this hormone in abscission has been presented.

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[5] and the production of cell wall - degrading enzymes.[6]

II. MATERIALS AND METHODS

A. 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.[3,7,8] Each experimental datum with bean explants represents experiments repeated on three different occasions with three sets of 10 explants. Cotton experiments were repeated twice, but because there are two separation layers per explant, each experimental datum represents a total of 120 observations.

B. APPLICATION OF CHEMICALS

Indole-3-acetic acid (IAA) and abscisic acid were applied by placing a 50-µliter drop of 1.5% agar containing the IAA or abscisic acid on the distal cut surface of the explant. IAA concentration was 5×10^{-5} M, and abscisic acid concentration, 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 Co. 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 Co., and a number of preliminary experiments with this material indicated that there was no essential difference in the properties of the two preparations.

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Earlier papers from this laboratory have described the treatment of explants with ethylene and CO_2 in gas-collection bottles,[6] the measurement of ethylene by gas chromatography,[9] the injection of actinomycin D (1 µg/µliter water) into the separation layers of explants,[7] the determination of cellulase activity by the loss of viscosity of sodium carboxymethyl cellulose (CMC),[8] and break strength measurements by a recording abscissor.[5]

III. RESULTS

The effect of abscisic acid on break strength and ethylene production from explants is summarized in Table 1. The data are in agreement with earlier observations[3] that increasing amounts of abscisic acid cause explants to produce increasing amounts of ethylene and that loss of break strength and ethylene evolution were closely correlated.

TABLE 1. EFFECT OF ABSCISIC ACID ON BREAK STRENGTH AND ETHYLENE PRODUCTION IN COTTON AND BEAN EXPLANTS[a/]

	Cotton		Bean	
Abscisic Acid, M	Break Strength, g	C_2H_4, ppm	Break Strength, g	C_2H_4, 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. Explants were placed in 43-ml gas-collection bottles fitted with rubber vaccine caps and kept at 25 C with continuous 400 ft-c fluorescent light. Wound ethylene was flushed out of bottles 8 hours after excision. Break strength and ethylene production were measured at 28 hours. Means having the same superscript letter within a column are not significantly different at the 5% level.

Table 2 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 partially overcome by CO_2.

TABLE 2. EFFECT OF 10% CO_2 ON ABSCISSION OF COTTON AND BEAN EXPLANTS TREATED WITH 5×10^{-4} M ABSCISIC ACID[a]

Treatment	Cotton		Bean	
	Break Strength, g	C_2H_4, ppm	Break Strength, g	C_2H_4, 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. Explants were placed in 43-ml gas-collection bottles fitted with rubber vaccine caps and kept at 25 C with continuous 400 ft-c fluorescent light. Wound ethylene was flushed out of bottles 8 hours after excision. Break strength and ethylene production were measured at 28 hours. Means having the same superscript letter within a column are not significantly different at the 5% level.

The data in Table 3 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 3. STIMULATION OF ABSCISSION BY 5×10^{-4} M ABSCISIC ACID AND 10 PPM ETHYLENE[a]

Treatment	Break Strength, g	
	Cotton (16 hr)	Bean (26 hr)
Control	122	158
Abscisic acid	79	115
C_2H_4	37	52[a]
Abscisic acid + C_2H_4	20	44[a]

a. Explants were placed in desiccators containing air or ethylene and kept at 25 C with continuous 400 ft-c fluorescent light for the times indicated. Superscript a indicates means significantly different at 10% level. All other means within a column significantly different at 5% level.

The data in Figure 1 show the effect of abscisic acid on changes in the break strength of cotton and 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 hours and then decreases. Abscisic acid in cotton explants appears to shorten the time required for a loss of break strength by about 2 hours. The data obtained with bean explants indicated no effect on induction period but rather an accelerated loss of break strength once induction was started.

IAA was able to prevent a loss of break strength of explants when applied shortly after excision (Fig. 2). When the time of IAA application was delayed by 5 hours for cotton (Fig. 2A) and 8 hours for bean (Fig. 2B) its effect was reduced; delaying the time of application even further caused a corresponding loss of effectiveness. As shown in Figure 2, 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 hours after excision in cotton explants (Fig. 3A) and 6 hours 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 hours for cotton (Fig. 4A) and 12 hours (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 hours. However, in bean explants, abscisic acid does appear to both decrease the lag period and increase the cellulase activity.

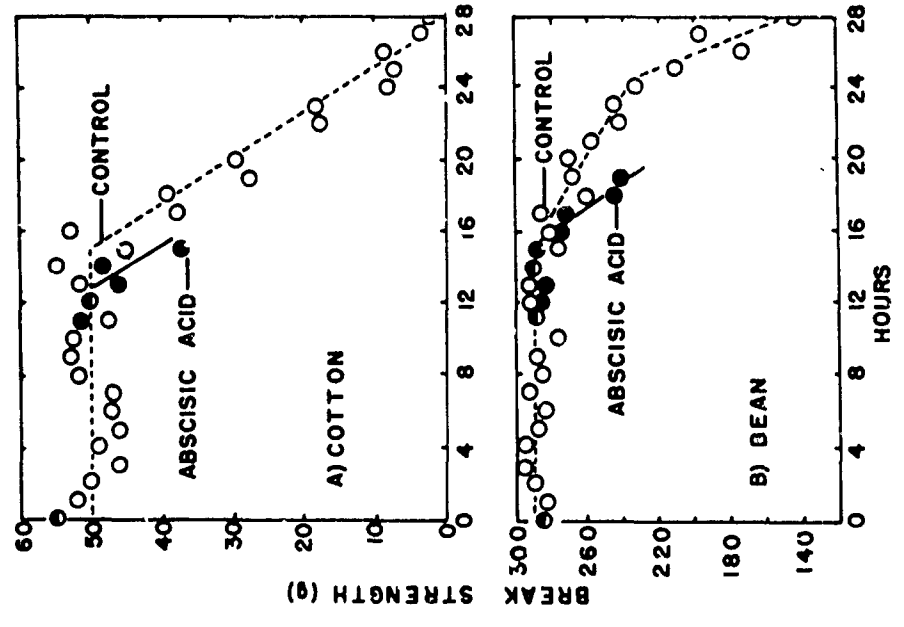

FIGURE 1. Effect of Abscisic Acid on Break Strength of (A) Cotton and (B) Bean Explants. Gas phase was 10 ppm ethylene. Abscisic acid was applied at 0 hour. Break strength determinations made at times indicated.

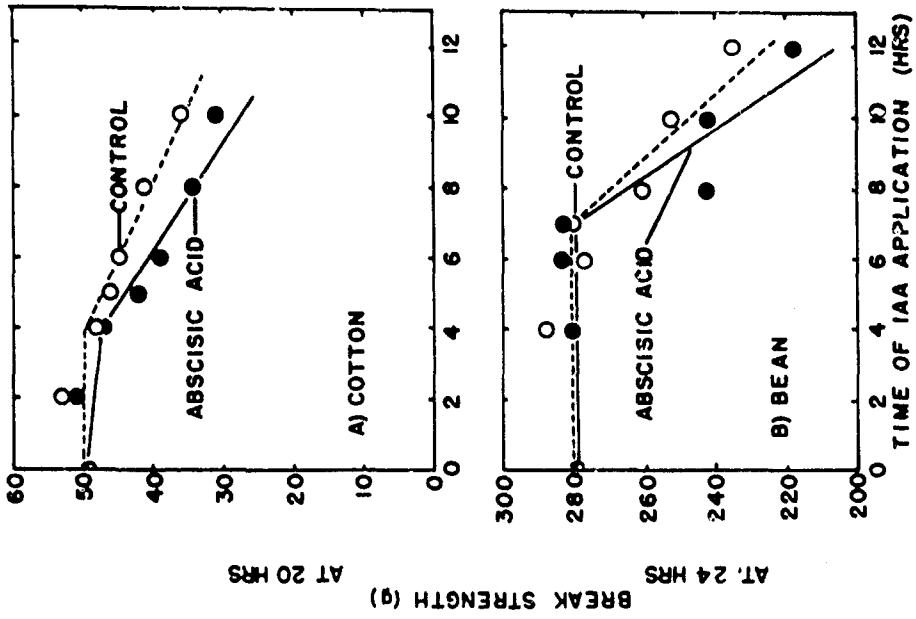

FIGURE 2. Effect of Abscisic Acid on Ability of IAA to Retard Abscission of (A) Cotton and (B) Bean Explants. Gas phase was 10 ppm ethylene. Abscisic acid or plain agar controls added at 0 hour. Abscisic acid and control agar were carefully wiped off with moist tissue and replaced with IAA at times indicated.

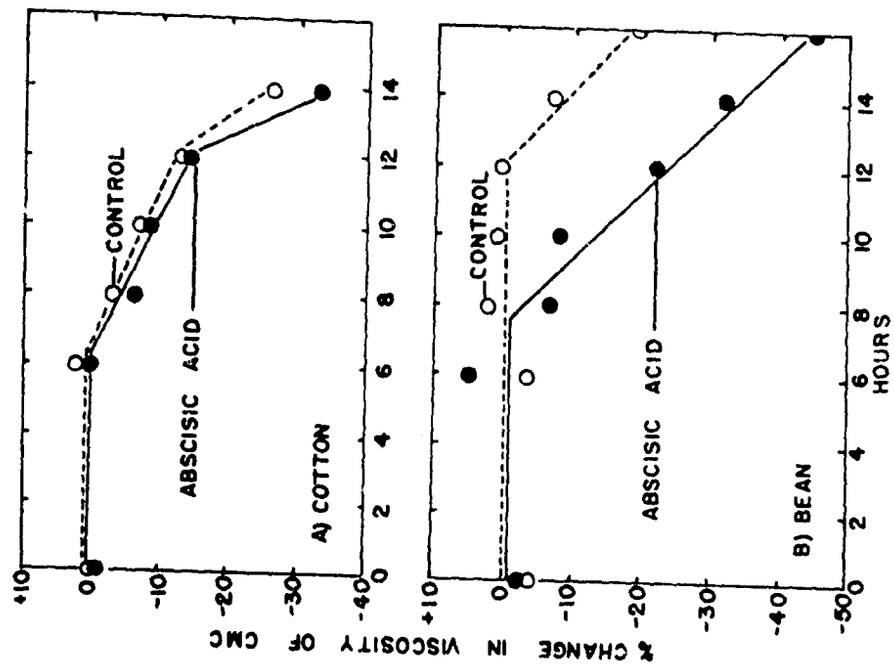

FIGURE 4. Effect of Abscisic Acid on Production of Cellulase from Separation Layers of (A) Cotton and (B) Bean Explants. Gas phase was 10 ppm ethylene. Abscisic acid and control agar drops applied to explants at 0 hour and left on throughout the experiment. Explants were harvested and frozen at times indicated until analyzed for cellulase. The cellulase was incubated with 1.5% sodium CMC in 0.05 M potassium phosphate buffer, pH 7, for 16 hours at 40 C.

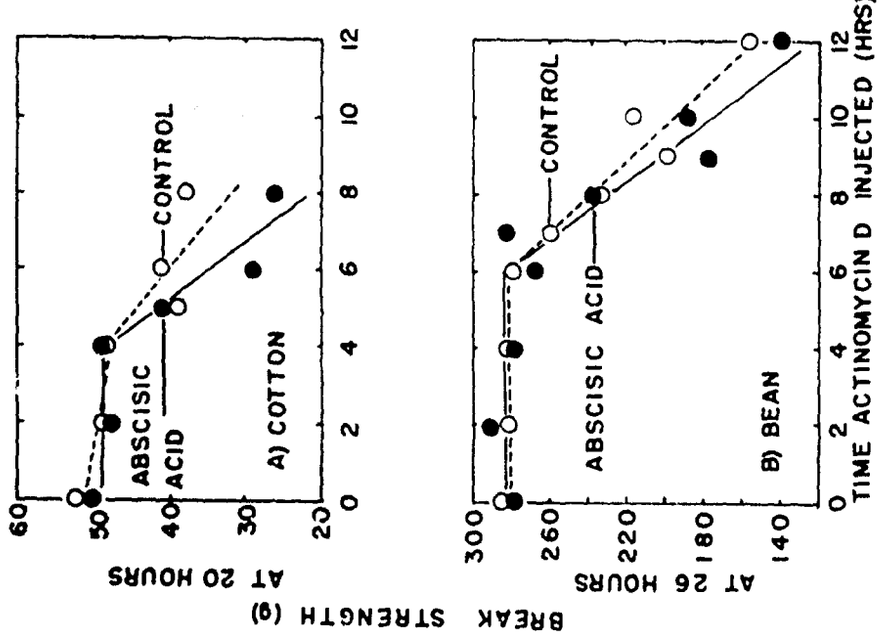

FIGURE 3. Effect of Abscisic Acid on Ability of Actinomycin D to Inhibit Abscission In (A) Cotton and (B) Bean Explants. Gas phase was 10 ppm ethylene. Abscisic acid and control agar droplets applied at 0 hour and left on throughout the experiment. Actinomycin D injected into explants at times indicated.

IV. DISCUSSION

The data in Table 1 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 2 and 3 show that enhanced ethylene production is only a part of the explanation of why abscisic acid stimulated abscission.

Table 2 shows that only a part of the abscisic acid effect on cotton could be overcome by CO_2, a competitive inhibitor of ethylene action.[10,11] All previous work on abscission in this laboratory with cotton and beans has shown inhibition of abscission with CO_2.[10] However, the ability of abscisic acid to accelerate abscission could still be due to ethylene stimulation, inasmuch as other cases of the inability of CO_2 to reverse ethylene effects have been reported.[9,12] We were, however, encouraged to measure the effect of abscisic acid in 10 ppm ethylene, which we found earlier to represent a saturating concentration of the gas for abscission.[10]

The observation (Table 3) 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.

Figure 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.[13] The experiments presented in Figures 1 through 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 Figures 1 through 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 not due to an increase in ethylene production. It was assumed that 10 ppm ethylene saturated any requirement the explants had for the gas.[10]

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. Because an aging period is required before cell wall - degrading enzymes start, 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 hours 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.

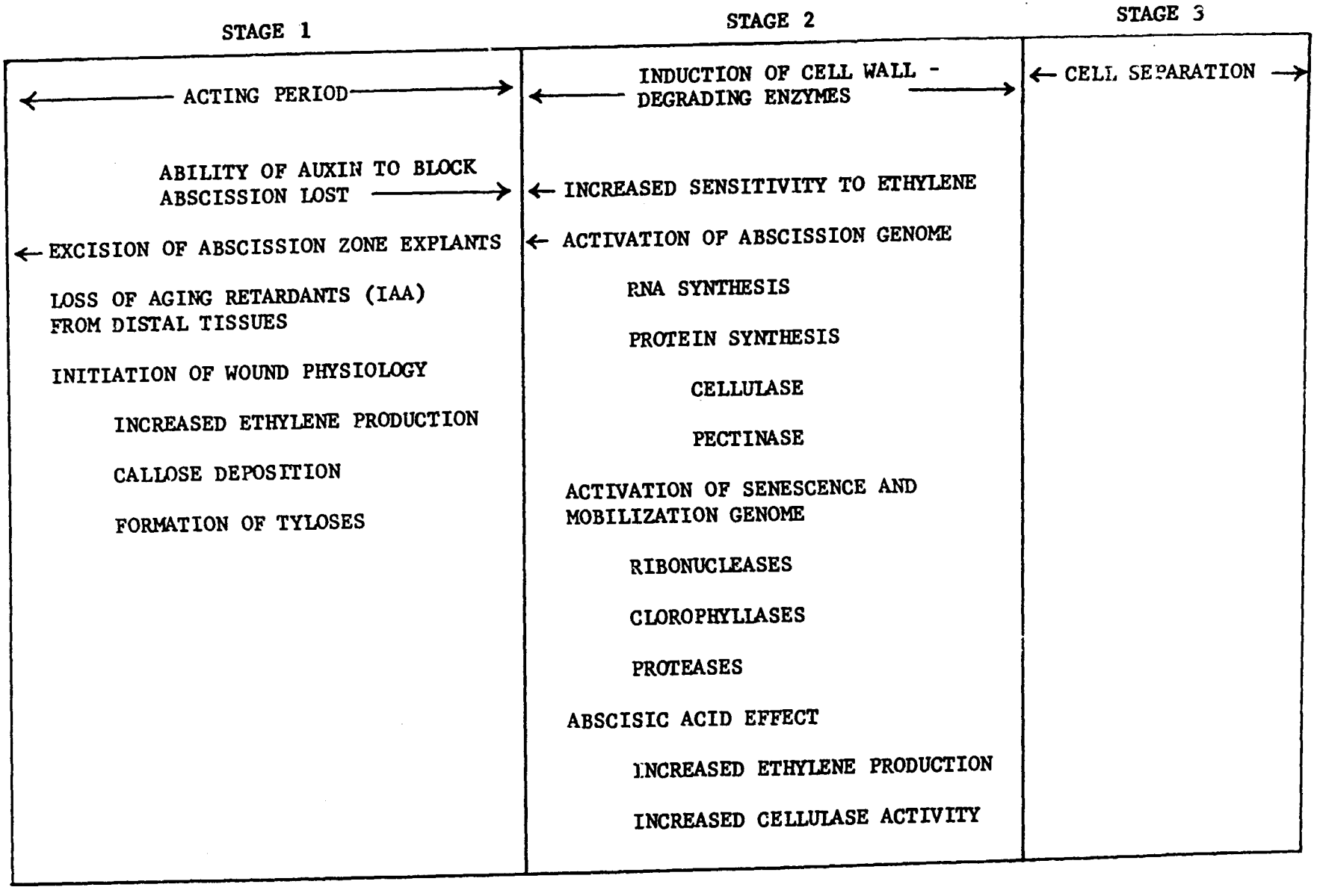

FIGURE 5. Outline of Processes Occurring During Abscission of Abscission Zone Explants.

We believe that the available data suggest that the major function of IAA in abscission is to prevent aging of explants. As shown in Figures 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 Figures 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 and other cellular constituents.[8,14] We found that abscisic acid had no effect on chlorophyll degradation in bean and cotton explants using methods described earlier.[8] However, other workers[2,15] have observed that abscisic acid increased the loss of chlorophyll in other experimental systems.

We have shown earlier that actinomycin D blocked RNA synthesis[16] and abscission,[7] 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 (Fig. 3). 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, it should shorten the time for actinomycin D to lose its effectiveness. The data in Figure 3 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-hour 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 one sample of explants, 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 Figure 4. We have shown that abscisic acid accelerated the loss of break strength of cotton and bean explants (Fig. 1) and that an increase in cellulase activity preceded the loss of explant break strength.[5] If, in addition to the ability of abscisic acid 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 hours. 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.

V. 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.[2,17,18] 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, Heslop, and Whisler[18] found that concentrations of abscisic acid that defoliated olive trees had no effect on the ethylene production of the leaves. Cooper et al.,[17] 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,[2] inhibition of seed germination,[19] inhibition of α-amylase,[20,21] promotion of phenylalanine ammonia-lyase,[22] floral morphogenesis,[2,23,24] or inhibition of growth.[2,25] In fact, we reported earlier that increased ethylene production did not account for the inhibition of growth of excised soybean hypocotyls by abscisic acid.[25]

We conclude from the data presented here that the action of abscisic acid on abscission of isolated explants was twofold. 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.

LITERATURE CITED

1. Addicott, F.T.; Carns, H.R.: Lyon, J.L.; Smith, O.E.; McMeans, J.L. 1964. On the physiology of abscisins. In Regulateurs Naturels de la Croissance Vegetale. Colloq. Int. Centre Nat. Rech. Sci. (Paris) 123:687-703.

2. El-Antably, H.M.M.; Wareing, P.F.; Hillman, J. 1966. Some physiological responses to d,l abscisin (dormin). Planta 73:74-90.

3. Abeles, F.B. 1967. Mechanism of action of abscission accelerators. Physiol. Plant. 20:442-454.

4. Dale, J.E.; Milford, G.F. 1964. The role of endogenous growth substances in the fruiting of upland cotton. New Phytologist 64:28-37.

5. Craker, L.E.; Abeles, F.B. May 1969. Abscission: Quantitative measurement with a recording abscissor, (Technical Manuscript 528). Plant Physiology Division, Fort Detrick, Frederick, Maryland.

6. Abeles, F.B. 1969. Abscission: Role of cellulase. Plant Physiol. 44:447-452.

7. Abeles, F.B.; Holm, R.E. 1967. Abscission: Role of protein synthesis. Ann. N.Y. Acad. Sci. 144:367-373.

8. Abeles, F.B.; Holm, R.E.; Gahagan, H.E. 1967. Abscission: The role of aging. Plant Physiol. 42:1351-1356.

9. Abeles, F.B.; Lonski, J. 1969. Stimulation of lettuce seed germination by ethylene. Plant Physiol. 44:277-280.

10. Abeles, F.B.; Gahagan, H.E. 1968. Abscission: The role of ethylene, ethylene analogues, carbon dioxide and oxygen. Plant Physiol. 43:1255-1258.

11. Burg, S.P.; Burg, E.A. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42:144-152.

12. Abeles, F.B. 1968. Herbicide-induced ethylene production: Role of the gas in sublethal doses of 2,4-D. Weed Sci. 16:498-500.

13. Abeles, F.B. 1968. Role of RNA and protein synthesis in abscission. Plant Physiol. 43:1577-1586.

14. Scott, P.C.; Leopold, A.C. 1966. Abscission as a mobilization phenomenon. Plant Physiol. 41:826-830.

15. Aspinall, D.; Paleg, L.G.; Addicott, F.T. 1967. Abscisin II and some hormone-regulated plant responses. Australian J. Biol. Sci. 20:869-882.

16. Holm, R.E.; Abeles, F.B. 1967. Abscission: The role of RNA synthesis. Plant Physiol. 42:1094-1102.

17. Cooper, W.C.; Rasmussen, G.K.; Rogers, B.J.; Reece, P.C.; Henry, W.H. 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43:1560-1576.

18. Hartmann, H.T.; Heslop, A.J.; Whisler, J. 1968. Chemical induction of fruit abscission in olives. Calif. Agr. 22:14-16.

19. Khan, A.A. 1968. Inhibition of gibberellic acid - induced germination by abscisic acid and reversal by cytokinins. Plant Physiol. 43:1463-1465.

20. Chrispeels, M.J.; Varner, J.E. 1967. Hormonal control of enzymic synthesis: On the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiol. 42:1008-1016.

21. Hemberg, T. 1967. Abscisin II as an inhibitor of α-amylase. Acta Chem. Scand. 21:1665-1666.

22. Walton, D.C.; Sondheimer, E. 1968. Effect of abscisin II on phenylalanine ammonia-lyase activity in excised bean axes. Plant Physiol. 43:462-469.

23. Evans, L.T. 1966. Abscisin II: Inhibitory effect on flower induction in a long-day plant. Science 151:107-108.

24. Nitsch, C.; Nitsch, J.P. 1967. The induction of flowering in vitro in stem segments of Plumbago indica L: II. The production of reproductive buds. Planta 72:371-384.

25. Holm, R.E.; Abeles, F.B. 1968. The role of ethylene in 2,4-D induced growth inhibition. Planta 78:293-304.

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13. 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 twofold. Abscisic acid increased ethylene production from the explants, which accounted for some of its ability to accelerate abscission. Abscisic acid also increased the activity of cellulase. Increased synthesis of cellulase was not caused by 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.

14. Key Words

*Abscission
*Growth regulators
Cotton plants
Beans
Ethylene
Cellulase
*Plant regulators

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