

Abscission

MOVEMENT AND CONJUGATION OF AUXIN

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LYLE E. CRAKER,¹ ARTHUR V. CHADWICK,² AND GERALD R. LEATHER
Plant Sciences Laboratories, Fort Detrick, Frederick, Maryland 21701

ABSTRACT

A 1-hour application of indole-3-acetic acid to bean (*Phaseolus vulgaris* L. cv. Red Kidney) explants inhibited abscission for an 8-hour aging period. Use of indole-3-acetic acid-¹⁴C showed that the applied indole-3-acetic acid was conjugated within explant tissue and that this conjugation mechanism accounts for loss of effectiveness of indole-3-acetic acid in inhibiting abscission after 8 hours. Reapplication of indole-3-acetic acid to an explant at a later time, before the induced aging requirement was completed re-inhibited abscission. 2,4-Dichlorophenoxyacetic acid, which is not destroyed or conjugated by this system, did not lose its ability to inhibit abscission. It was concluded that indole-3-acetic acid destruction is one of the processes involved in the aging stage of abscission in explants.

Indoleacetic acid has been demonstrated to effectively inhibit abscission in many plant systems if it is applied to the distal cut surface of petioles (10, 14, 15, 17). The applied auxin has been supposed to replace the continuous auxin supply from the leaf (14, 20). However, the mechanism by which IAA exerts its inhibition properties on abscission is unknown. Research has been primarily concerned with auxin transport systems, auxin balances, and auxin concentrations. Such work is well summarized in recent review articles (2, 12).

There has been no correlation between time of application, location, or form of auxin at the time it functions to inhibit abscission processes. This paper presents a series of experiments designed to determine movement and conjugation of IAA in explants as correlated with initiation of abscission.

MATERIALS AND METHODS

Plant Material. Bean plants (*Phaseolus vulgaris* L. cv. Red Kidney), were grown at 24 ± 2 C, 64% relative humidity, and a 12-hr photoperiod from fluorescent and incandescent lamps with 1200 ft-c at plant level. Petiole abscission zone explants from the primary leaf were excised from 14-day-old plants and inserted into a 3-mm layer of 1.5% agar contained in 43 ± 2-ml gas collection bottles (5, 6). During the experiments, the explants were exposed to 25 C and 400 ft-c of continuous fluorescent light. Unless otherwise noted, each experimental datum represents experi-

ments repeated on three different occasions with three sets of 10 explants.

Application of Chemicals. Indole-3-acetic acid, the conjugates and derivatives of IAA (indoleacetyl aspartate, indoleacetamide, and ethyl indoleacetate), and 2,4-D were applied by placing a 50- μ l drop of 1.5% agar containing the compound on the distal cut surface of the explant. In instances where the agar was subsequently removed, the explant was wiped with a moist tissue (10). The concentration of growth substance supplied in the agar was 50 μ M for IAA and its derivatives and 500 μ M for 2,4-D. These concentrations of IAA and 2,4-D were selected as being effective for inhibition of abscission (1, 8, 10).

Radioactive Assays. In studies on the movement of conjugation mechanisms of IAA in explants, IAA-1-¹⁴C (specific radioactivity 25.6 mc/mmmole) obtained from Amersham/Searle was used. The IAA-¹⁴C was applied at 50 μ M concentration in the same way as the nonradioactive IAA at 7 hr after excision of explants. After a 1-hr treatment period, the agar containing IAA-¹⁴C was removed and the explant was wiped clean with a moist tissue and rinsed with distilled H₂O from a squeeze bottle. Incubation after IAA-¹⁴C treatment was done in fresh gas collection bottles. At the end of the incubation period, the explants were cut into three sections: pulvinus, top 3 mm; separation layer, middle 2 mm; and petiole, bottom 5 mm (3). These sections were sealed in scintillation vials and extracted for 24 hr in the dark three times with 5-ml portions of ethanol. The ethanol samples were pooled, taken to dryness on a Buchler Instruments rotary Evapo-Mix, and then resuspended in 150 μ l of ethanol. The 150 μ l of extract plus a 50 μ l wash were spotted in 3.8-cm wide strips under a nitrogen atmosphere on Eastman silica gel Chromogram sheets with fluorescent indicator. Standards of cold IAA, indoleacetyl aspartate, indoleacetamide, and ethyl indoleacetate were spotted on top of the extract for cochromatography. The silica gel sheets were run for 15 cm in chloroform:ethyl acetate:formic acid (50:40:10 v/v [21]). The chromatographs were air-dried and stored in desiccators under a nitrogen atmosphere until used. A ¹⁴C trace of the chromatograph was prepared by counting in a strip scanner. The sheets were then viewed under ultraviolet light for location of applied standards of IAA and conjugates of IAA. The IAA spot was subsequently cut from the chromatograph sheet, placed in scintillation fluid, and quantitatively assayed with a scintillation counter.

Ethylene Treatment. Earlier papers from this laboratory have described the treatment of explants with ethylene in gas collection bottles and the measurement of ethylene by gas chromatography (3, 6). Since the addition of auxin to explants will promote ethylene production (6), explants were kept in a 10- μ l/liter ethylene atmosphere to eliminate any influence auxin-induced ethylene would have on abscission.

Break strength of the separation layer was measured with a recording abscissor (9). Initial break strength refers to the break strength of a freshly excised explant.

¹ Present address: Department of Environmental Sciences, University of Massachusetts, Waltham, Massachusetts 02154.

² Present address: Department of Biology, Loma Linda University, Loma Linda, California 92701.

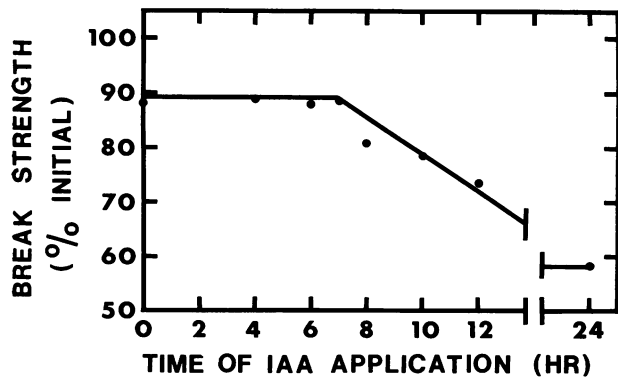


FIG. 1. Time sequence for IAA inhibited abscission of bean explants. IAA was applied to explants at times indicated. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 . Break strength was measured at 24 hr.

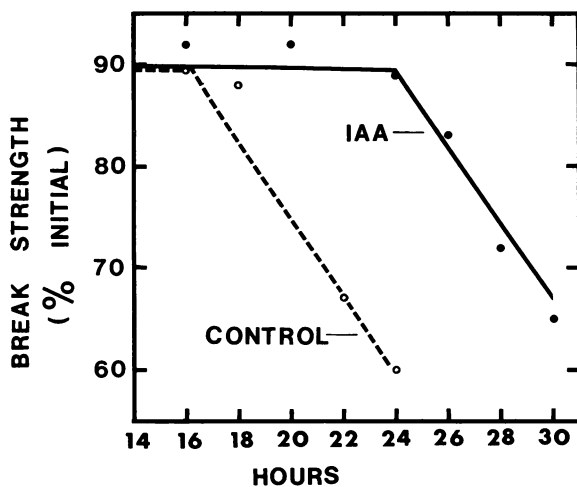


FIG. 2. Time of abscission after 1-hr IAA application. IAA was applied at 7 hr after excision of explants from leaf. Control explants received plain agar. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 . Break strength was measured at times indicated. Time of explant excision equals 0 hr.

RESULTS

The effect of exogenously applied IAA on ethylene-treated bean explants is shown in Figure 1. Application of auxin to a bean explant up to 7 hr after excision from the leaf inhibited the normal loss of break strength. Delaying application of IAA more than 7 hr reduced its ability to prevent abscission.

Figure 2 demonstrates that a 1-hr application of IAA from 7 to 8 hr after excision delayed the loss of break strength by approximately 8 hr. The loss of break strength then paralleled the loss of break strength in agar-treated control explants. Abscission at this time appeared to be normal in all respects.

The loss in effectiveness of a 1-hr application of IAA in preventing abscission after 8 hr suggests that there might have been some changes in the transport, conjugation, or destruction of auxin so that it was no longer able to inhibit abscission. The use of IAA- ^{14}C allowed us to follow movement and destruction of free IAA in bean explants.

Preliminary experiments showed no difference in total ^{14}C activity of explant fractions with progressing times after the 1-hr IAA- ^{14}C application. Extraction and chromatographic separation of the ^{14}C activity from explants indicated that the free IAA was rapidly lost in all three fractions of the explant (Fig. 3).

The conjugates of IAA form within 1 hr and remain after the

IAA has disappeared. Quantitative assay for free IAA- ^{14}C indicated that by 3 hr after application almost no free IAA remained (Table I).

The conjugates of IAA were not effective as abscission inhibitors (Table II). Ethyl indoleacetate was most effective, but it only inhibited loss of break strength 76% compared with IAA, which inhibited loss of break strength 92%.

This information suggested that the conjugation mechanism could be a primary factor in the loss of effectiveness of IAA in preventing abscission. To test this hypothesis, we applied 2,4-D. Since 2,4-D is not conjugated in the tissue (7), there should be no loss of break strength at 8 hr after the 1-hr treatment. 2,4-D is similar to IAA in its ability to prevent abscission, and application of 2,4-D to an explant 7 hr after excision will prevent loss of break strength (Fig. 4). However, the data in Figure 5 indicate that 2,4-D does not lose its effectiveness in inhibiting loss of break strength at 8 hr after a 1-hr application.

If an initial 1-hr application of IAA from 7 to 8 hr after excision is followed by a second 1-hr application of fresh IAA at a later time, the subsequent loss of break strength is again effectively inhibited (Table III).

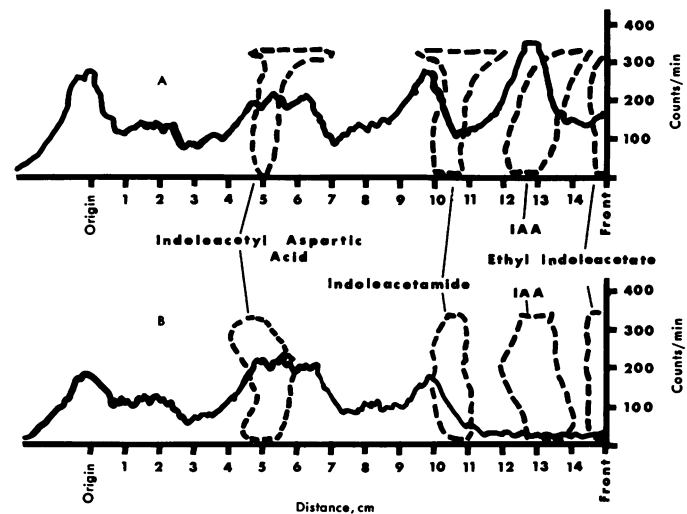


FIG. 3. Conjugation of IAA in bean explants. Explants were aged 7 hr before 1 hr application of IAA- ^{14}C . Solid line is ^{14}C scan with strip scanner. Dotted lines are applied standards for cochromatography as viewed by ultraviolet light. A: Top section 1 hr after application; B: top section 3 hr after application. Similar results were obtained with the separation layer and bottom sections.

Table I. *Decrease of Free IAA in Tissues of Bean Explants*

Explants were aged for 7 hr before treatment with IAA- ^{14}C . After 1 hr the IAA- ^{14}C was removed with moist tissue and the explants were rinsed with distilled H_2O . The explants were placed in fresh gas collection bottles for the remaining time before harvest. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 .

IAA- ^{14}C Application	IAA- ^{14}C		
	Pulvinus	Separation layer	Petiole
hr	dpm		
1	5820	958	2700
3	575	50	770
5	355	47	233
7	395	36	138

Table II. Influence of Conjugates and Derivatives of IAA on Abscission

Explants were aged 7 hr before treatment. After 1 hr the compounds were removed with moist tissue. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 for the experiment. Break strength was measured at 24 hr. Dispersion is average deviation from the mean.

Treatment	Break Strength	
	% initial	
IAA	92	± 8
Indoleacetyl aspartate	66	± 5
Indoleacetamide	68	± 4
Ethyl indoleacetate	76	± 8
Agar control	46	± 2

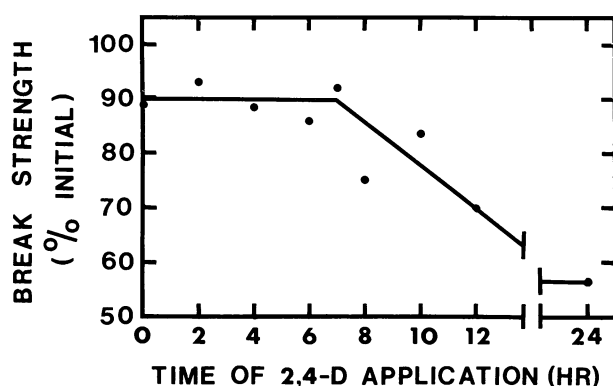


FIG. 4. Time sequence for 2,4-D inhibited abscission of bean explants. 2,4-D was applied at times indicated. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 . Break strength was measured at 24 hr.

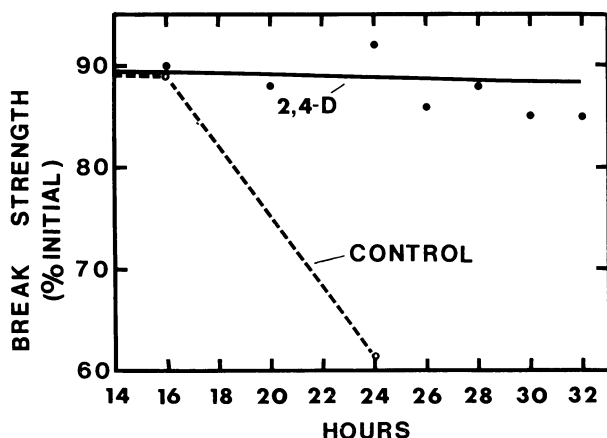


FIG. 5. Time of abscission after 1 hr 2,4-D application. 2,4-D was applied at 7 hr after excision of explants from leaf. Control explants received plain agar. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 . Break strength was measured at times indicated. Time of explant excision equals 0 hr.

DISCUSSION

The available data suggest that auxin acts as an aging retardant and thus inhibits abscission by preventing the metabolic changes necessary for the formation of enzymes that degrade cell tissue in the separation layer. The data in Figure 1 agree with previous

work from this (10) and other laboratories (15, 17), that there is a fixed period of time in which IAA can be added to an explant and inhibit abscission.

However, data in Figure 2 show that explants treated with IAA for 1 hr begin to abscise 8 hr after control tissue. This suggests that stage 1 of the abscission process, aging (5, 10), is delayed for approximately 8 hr in bean explants by a 1-hr treatment with IAA, and that it takes the 8 hr by which IAA delays aging to effectively remove auxin from the explant tissue containing its active site for inhibiting abscission. The loss in effectiveness could be due to either IAA transport or IAA destruction mechanisms within the tissue.

Other workers (13) have reported on transport systems that indicate a basipetal movement of auxin in freshly excised bean explants. Our data do not disagree with these results, but indicate that movement of auxin probably plays a minor role in the loss of effectiveness of IAA as an abscission inhibitor. Total ^{14}C activity of the explant tissue sections changed very little during the short experimental time following IAA- ^{14}C removal. However, data in Figure 3 and Table I demonstrated that the IAA was unavailable to inhibit abscission 3 hr after application.

Indications were that IAA was rapidly converted to the conjugates of IAA. Figure 3 shows that indoleacetyl aspartate is formed with another unidentified fraction with an R_F similar to that reported for indoleacetyl- β -glucoside (21). The indoleacetamide is probably due to ammonolysis of indoleacetyl- β -glucoside (22). Other workers have reported conjugation of naphthaleneacetic acid in *Coleus* explants (18, 19) and conjugation of IAA in cotton explants (16). In these studies, the aspartic acid derivative was reported as the conjugate formed.

Previous work involving auxin and abscission have used a continuous auxin supply and thus could not be used to indicate what happens to application of auxin where the auxin source is removed. The latter would more nearly represent the natural situation in explant tissue where the primary leaf is removed or under natural conditions where the leaf could no longer supply auxin to basipetal tissue because of death or other modification of leaf cells.

It is assumed in these studies that auxin stimulation of ethylene production within the explant would have no effect, since any ethylene requirement for abscission would be satisfied by the 10 μ l/liter ethylene present in the gas collection bottles (4, 14).

The conjugates of IAA did not appear to be an important controller of abscission. The ethyl indoleacetate inhibited loss of break strength to a greater extent than the other conjugates, but it too does not appear in tissue at 3 hr after application of IAA (Fig. 3).

If the conjugation mechanism is a primary method by which

Table III. The Effect of Reapplication of IAA to Bean Explants

Explants were treated for the 1-hr time periods indicated and then the IAA or agar was removed with moist tissue. Explants were kept at 400 ft-c and 25 C with 10 μ l/liter C_2H_4 for the experiment. This experiment was repeated two times with three sets of 10 explants per treatment.

Time of IAA Treatment	Break Strength	
	24 hr	30 hr
<i>hr after excision</i>	% initial	
7-8	90	53
7-8, 10-11		84
7-8, 12-13		84
7-8, 14-15		84
Agar control	63	45

IAA is removed from tissue to prevent it from inhibiting abscission, then replacement with an auxin that is not conjugated should prevent the loss of break strength that occurs 8 hr after a 1-hr application. The herbicide 2,4-D has been used as an abscission inhibitor (8) and is similar to IAA in preventing abscission if applied by 7 hr after excision (Fig. 4). Data in Figure 5 show that 2,4-D, which is not conjugated (7), did not lose its effectiveness 8 hr after a 1-hr application, but instead continued to inhibit abscission for the entire time this experiment was conducted.

To establish whether unconjugated IAA could still inhibit abscission after the first 8-hr IAA induced aging delay, we re-applied auxin at a later time. Data in Table III demonstrate that IAA will inhibit loss of break strength if available to the explant tissue before delayed aging is completed.

Our data support the idea of Gorter and Veen (11) that auxin controlled abscission is not dependent upon translocation of auxin, but is probably mediated by immobilization of auxin through the conjugation mechanism. However, this does not fully explain the time period of aging (10, 17), since the applied IAA is effectively removed by 3 hr after application, yet the extended aging stage is 8 hr long. It appears that IAA destruction is only one of the steps in aging of explant tissue, but that all the steps can be inhibited by the presence of IAA at any time during aging.

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