Effects of Absciscic Acid on the Sprouting of Aerial Tubers of *Begonia evansiana* and *Dioscorea batatas*

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Abstract

d-Absciscic acid (ABA) inhibited the sprouting of aerial tubers of *B. evansiana*, which had been vernalized, at concentrations from 3×10^{-6} to 10^{-5} M. In some experiments it caused promotion of sprouting at 10^{-6} M. Though weaker, similar effects were observed with cut aerial tubers of *D. batatas*. Intact tubers of this species did not respond to ABA.

The low responsiveness of *D. batatas* to added ABA may not originate from its inactivation or decomposition, since the ABA added was recovered without any change.

ABA was ineffective in inducing the formation of aerial tubers on *B. evansiana* plants under long-day conditions.

Begonia evansiana has been shown to form aerial tubers at axils on short-day induction¹⁾. At maturity the tubers are in dormancy, which is broken by a cold treatment at 2-8° for about a month²⁾. *Dioscorea batatas* also forms aerial tubers in early autumn, which are in dormancy and released from it by cold treatment. In contrast to buds and seeds of other species which experience dormancy, the tubers of the above-stated plants show a unique mode of dormancy as intensified by gibberellin treatment³⁾.

Recently we have isolated absciscic acid (ABA) from dormant aerial tubers of *Dioscorea* as a substance probably responsible for the dormancy⁴⁾, but have not succeeded yet in showing the occurrence of the acid in *Begonia* tubers. In this paper we report effects of ABA on the formation of aerial tubers in *Begonia* as well as on the sprouting of vernalized aerial tubers of *Begonia* and *Dioscorea*.

Physiological and biochemical functions of ABA are being investigated extensively⁵⁾, and particularly it has been shown that ABA inhibits germinations of lettuce⁶⁾, ash⁷⁾, peach⁸⁾, and hazel⁹⁾ seeds, and sprouting of potato tubers¹⁰⁾. The acid has also been reported to suppress the cytokinin-induced swelling of *Begonia* aerial tubers¹¹⁾.

Materials and Methods

Begonia tuber formation. From plants of *Begonia evansiana* Andr. grown in a shade in a garden, about 20 cm long shoots with the apex were excised, inserted in 180-ml milk bottles (two cuttings each) filled with half-strength Hoagland's nutrient solution, and kept in the original habitat of the plants. Throughout this experiment the natural day-length was extended by illuminating from the dusk to 10 p.m. with a 100-watt incandescent filament lamp hanged 50 cm above the plants. The culture solution was

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renewed once a week. Ten days later when the cuttings formed roots, treatment of ABA started. Each axil and apex of the cuttings were treated with $10\mu\text{l}$ of $100\mu\text{g/ml}$ d, l-ABA solution dissolved in 50% aqueous acetone containing 0.025% Tween 20. Control lot was similarly treated with plain Tween-aqueous acetone.

Sprouting of Begonia tubers. Aerial tubers of *B. evansiana* were harvested in October, 1967, and stored in polyethylene bags at about 5° until use. During the storage the tubers were vernalized. At the time of use tubers were partially sterilized in 500-times diluted "Osban" (10% benzalkonium chloride solution, Takeda Chemical Ltd., Osaka) for 30 min, thoroughly rinsed with tap water and cut into half. The upper halves were placed on thin cotton layer in 9-cm petri dishes moistened with 8 ml of distilled water or d-ABA water solution.

In another experiment, tubers treated with "Osban" were further sterilized with 0.5% sodium hypochlorite solution for 10 min, rinsed with sterile water and cut into half. A single upper half was placed on a cotton piece in an aseptic test tube ($1.5\text{ cm}\times 10\text{ cm}$) moistened with 0.5 ml of ABA solution or water. On the 9th day of culture 0.4 ml of ABA solution or water was supplemented aseptically.

Sprouting of Dioscorea tubers. Aerial tubers of *Dioscorea batatas* Decne. harvested in October, 1967, were stored in the same manner as *Begonia* tubers. At the time of use tubers were sterilized likewise and the apical part of tubers, 10-14 mm in diameter and 3-5 mm in height, was excised and placed in test tubes (the same size as above). Since the cut surface of *Dioscorea* tubers was large, 1 ml of 1% agar was used as a substitute for cotton piece so as to secure the cut surface to contact the culture bed. ABA was added to the agar.

For intact tubers 20 g of air-dried garden soil was placed in each 9-cm petri dish, to which 9.1 ml of water was added to give a moisture of 60% water holding capacity of the soil. The dishes thus prepared were autoclaved. Ten tubers treated with "Osban" were placed on the soil of dishes so that the lower part (approximately 1/4) of each tuber was buried. This culture method was shown to be the best for intact aerial tubers. d-ABA was dissolved at 400, 40, and $4\mu\text{g/ml}$ in water containing 0.05% Tween 20, and $2.5\mu\text{l}$ of the solutions was applied with a microsyringe to each tuber apex on the first and second days of incubation.

Incubation of ABA with Dioscorea tuber tissues. Aerial tubers stored at 5° for 7 months (until June of 1968) were cut into slices, 0.5-1 mm thick, and 2 g in fresh weight of slices were placed in a 30-ml Erlenmeyer flask containing 5 ml of 0.1 M tris-HCl buffer (pH 7.2) with or without the addition of $2\mu\text{g/ml}$ d-ABA, and incubated by shaking at 120 cycles/min at 26.5° for 17.5 hrs. At the end of the incubation the tissues were killed by adding concentrated phosphoric acid until the pH of the media was 2.0. The media and slices were then extracted altogether with three 10 ml portions of ether to determine the recovery of ABA added.

Thin-layer chromatography. Kieselgel G plates were used throughout. Among three solvent systems tested, I was the best to separate ABA from gibberellins, and III was preferable for discrimination of ABA from indole-3-acetic acid as shown in Table 1. Based on the result of Thomas et al.¹²⁾ that gibberellins interfere with determination of ABA by the *Avena* coleoptile section test rather than IAA, solvent system I was used for the separation of the ABA extract mentioned above. Authentic ABA on a chromatogram was detected as yellow spot with iodine vapor.

Optical rotatory dispersion. Sample was dissolved in 5 ml of ethanol containing 0.01 N hydrochloric acid, and its ORD was recorded with a spectropolarimeter (Japan Spectroscopic Ltd., ORD/UV-5).

Table 1. Rf values in thin-layer chromatography of growth regulating compounds.

Compounds	Solvent system		
	I	II	III
Absciscic acid	.27	.83	.57
Deoxygibberellin C	.26	.86	.69
Gibberellin A ₇	.21	.78	.56
Gibberellin A ₉	.42	—	—
Gibberellin A ₂₀	—	.80	.58
Indole-3-acetic acid	.31	.67	.43

Solvent system I, benzene/ethyl acetate/acetic acid (75:20:5 v/v); II, n-propanol/ammonium hydroxide (15 N)/water (6:1:2 v/v); III, n-propanol/n-butanol/ammonium hydroxide (15 N)/water (6:2:1:2 v/v). Developed 12.5 cm at about 20°.

Avena coleoptile section test. Seedlings were grown under a week red light at 25° for about 72 hrs., and a single 5-mm section was cut 4 mm below the tip from a 25 mm long coleoptile without the first decapitation. Sections were cultured on 2.5 ml of medium containing 1 µg/ml IAA, 2% sucrose, and various concentrations of test sample for 18 hrs. at 25°.

Absciscic acid (ABA). d-ABA was isolated from *D. batatas* aerial tubers and examined to be pure by IR spectrum and thin-layer chromatography⁴⁹. d,1-ABA was generously supplied by Dr. J. W. Cornforth, Shell Research Ltd.

Results and Conclusion

Effect of ABA on the aerial tuber formation of Begonia plants. Ten plants were treated with 1 µg ABA per bud once a day from July 18 to August 18 and subsequently every other day until August 31, 1967. Another set of 10 plants served as control. Around August 20 some flowers began to open, but no difference in number and flowering date was observed between the treated and control lots. Aerial tubers were formed neither in the treated nor control plants by the end of the experiment.

Sprouting of Begonia aerial tubers. Vernalized aerial tubers were incubated from December 13 to 31, 1967 and from March 1 to 16, 1968 in experiments 1 and 2, respectively (Table 2). ABA suppressed sprouting at concentrations of 3×10^{-6} and 10^{-5} M (Exp. 1 and 2), and in experiment 2 sprouting was promoted at 10^{-6} M. Although in the dark the sprouting percentages were generally lower than those in the light, ABA exhibited similar effects.

Sprouting of Dioscorea aerial tubers. Cut aerial tubers were incubated from March 4 to 22, 1968. In some tubers, eyes began to grow and then entered dormancy again forming processes of tubers. Such tubers were counted as non-sprouted ones (Table 3). Although effects of ABA were weaker, a similar trend of inhibition at 10^{-5} M and promotion at 10^{-6} M was observed as in the case of *Begonia* tubers.

In intact tubers no appreciable effect of ABA was observed either in the light or in the dark. ABA did not affect rooting of either cut or intact tubers (Table 4). *Stability of ABA in Dioscorea tuber tissues.* To examine a possible cause for the low responsiveness of *Dioscorea* tubers to ABA, the recovery of added ABA was determined by incubating 10 µg of ABA (5 ml of 2 µg/ml solution) with sliced tubers for

17.5 hrs. The following combinations of ABA and tuber tissues were designed, i.e. ABA only, ABA plus tissues, tissues only and the basal medium only (tris-HCl buffer), and one fourth of the acidic ether extracts from these incubation mixtures were bioassayed by the *Avena* coleoptile section test. As shown in Fig. 1 no difference in amount was observed between ABA plus tissues and ABA only.

Another one-fourth aliquot of the ether extracts was purified by thin-layer chromatography using solvent system I (Table 1), ABA zone was eluted with ethyl acetate, and the O.R.D.'s of the eluates were recorded. The result in Table 5 confirms that ABA was not decomposed by the tuber tissues.

Table 2. Effect of ABA on the sprouting of cut tubers of *Begonia*.

		0	ABA concentrations (M)			
			10^{-7}	10^{-6}	3×10^{-6}	10^{-5}
Light incubation						
Exp. 1	% sprouting	69	70	50	38	42
	No. of viable tubers	98	50	50	45	45
Exp. 2	% sprouting	54	54	84		35
	No. of viable tubers	57	28	32		25
Dark incubation						
Exp. 1	% sprouting	17	18	15	10	7
	No. of viable tubers	93	50	48	42	48
Exp. 2	% sprouting	38	47	65		17
	No. of viable tubers	61	19	23		23

Exp. 1, from Dec. 13 to 31, 1967; Exp. 2, March 1 to 16, 1968. In Exp. 1, 50 upper halves of tubers were cultured on a cotton piece in a 9-cm petri dish containing 8 ml of d-ABA solution or water. In Exp. 2 a single cut half of a tuber was placed on a cotton piece in each test tube, supplied with 0.5 ml of d-ABA solution or water. In both experiments 50 tubers for each of the ABA treatments and 100 tubers for the water control were used. Light intensity, 3000 lux, temperature, 25°.

Table 3. Effect of ABA on the sprouting of cut tubers of *Dioscorea*.

		0	ABA concentrations (M)		
			10^{-7}	10^{-6}	10^{-5}
Light incubation					
	% sprouting	64	69	73	50
	No. of viable tubers	50	48	49	48
Dark incubation					
	% sprouting	72	73	77	67
	No. of viable tubers	47	48	47	48

Incubation, from March 4 to 22, 1968 at 25°. Light intensity, 3000 lux. Cut tuber pieces were cultured on agar in test tubes. Fifty tubers for each treatment were used.

Table 4. Effect of ABA on the sprouting of intact *Dioscorea* aerial tubers.

	Doses of ABA ($\mu\text{g}/\text{tuber}/\text{day}$)			
	0	0.01	0.1	1
Light incubation				
% sprouting	94	93	86	98
No. of viable tubers	69	57	59	59
Dark incubation				
% sprouting	91	88	97	90
No. of viable tubers	70	60	59	59

Incubation, from May 15 to 22, 1968 at 25°. Light intensity, 3000 lux. Application of ABA was done on the first and second days of incubation. Sixty and 70 tubers were planted, respectively, for the ABA treatment and water control.

Table 5. Determination of recovery of ABA by optical rotatory dispersion.

	Rotations ($^{\circ}$) at		Calculated amount of ABA*
	245 $m\mu$	297 $m\mu$	(μg)
without tissues	0.018	not measurable	1.8
with tissues	0.022	not measurable	2.2
standard (10 μg ABA)	0.100	0.036	

* Calculated from the O. R. D.s' at 245 $m\mu$. The original amount of ABA added in the incubation experiment, 2.5 μg each.

Fig. 1. Recovery of d-ABA incubated with slices of vernalized *Dioscorea* aerial tubers. Solid circles, basal medium (HCl-tris buffer) only; solid triangles, tissues only; open circles, ABA only; crosses, ABA plus tissues. Assay of ABA, *Avena* coleoptile section test. Abscissa, relative concentrations of test samples in media for *Avena* coleoptiles, representing as one the concentration obtained when one sixth of the whole extract (see the text) was dissolved in 2.5 ml of medium.

In conclusion, the present experiments have shown that ABA suppresses the sprouting of vernalized aerial tubers of *B. evansiana* at high concentrations and, in some experiments, promotes it at low concentrations. Cut aerial tubers of *D. batatas* also responded to ABA in a similar manner, though slightly. The sprouting of intact tubers of the same species was, however, not affected by the application of ABA, although much larger dose was applied than that used for potato eyes¹⁰⁾. Several possible causes for the low responsiveness of *Dioscorea* tubers may be conceivable, but the experimental results on the ABA recovery (Fig. 1, Table 5) would exclude the possibility that ABA may be decomposed by the tubers.

The present experiments have also shown that ABA is completely ineffective in inducing the formation of aerial tubers in *B. evansiana* plants growing under long-day conditions. El-Antably and Wareing¹⁴⁾ reported that the acid accelerated the floral initiation in *Pharbitis nil* and *Chenopodium rubrum*, but not in *Xanthium pensylvanicum* and *Nicotiana tabacum*. The same workers and Hillman¹⁵⁾ succeeded in inducing the formation of apical resting buds in *Betula pubescens*, *Acer pseudoplatanus*, and *Ribes nigrum* by ABA. In spite of all our effort we were unable to obtain even small aerial tubers.

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摘 要

橋本 徹*・田村三郎*： シュウカイドウおよびナガイモの
地上塊茎の発芽におよぼすアブサイシン酸の影響

低温処理によって休眠の打破されたシュウカイドウ (*Begonia evansiana*) およびナガイモ (*Dioscorea batatas*) の地上塊茎の発芽に対する d-アブサイシン酸 (d-ABA) の影響を調べた。塊茎の上半切片を ABA を含む培地で培養した場合、ABA は $3 \times 10^{-6} \sim 10^{-5}$ M の濃度で発芽をおさえ、 10^{-6} M では、場合によっては、発芽を促進した。ナガイモの塊茎切片も、やや微弱ながら同様の反応を示したが、インタクトのナガイモ塊茎は ABA の影響を全然受けなかった。ナガイモの反応性が低いことの原因を知るために、

ABA が塊茎組織によって不活性化されるかどうかを調べたが、17時間の塊茎スライスとのインキュベーションによっては、ABAの不活性化は全然認められなかった。

シュウカイドウの腋芽と頂芽を ABA ($1\mu\text{g}/\text{bud}/\text{day}$) で44日間にわたって処理したが、長日条件下で地上塊茎を形成させることはできなかった。また花芽形成の促進も認められなかった。(*東京大学農学部農芸化学科)