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Hormones and Young Leaves Control Development of Cotyledonary Buds in Tomato Seedlings

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ABSTRACT

Hormonal and plant factors regulating the development of the inhibited cotyledonary buds of *Lycopersicon esculentum* Mill. cv. 'Fireball' seedlings were studied. Excision of the immature plumular leaves of 5- to 20-millimeter length significantly stimulated bud development after 2 to 4 days, but excision of leaves exceeding 20-millimeter length was without effect. Apical application of 20 microliters of 5 millimolar abscisic acid significantly promoted development of the cotyledonary buds after 6 days. A subapical ring of 0.1 millimolar concentration of 2,3,5-triiodobenzoic acid (TIBA) in lanolin significantly promoted cotyledonary bud development after 11 days. Twenty microliters of 0.1 millimolar 6-benzylaminopurine (BAP) applied directly to the cotyledonary bud loci significantly promoted bud development, but 1 micromolar gibberellin $A_{4/7}$ was ineffective. Application of 0.1 millimolar BAP in lanolin to the petiole or hypocotyl was ineffective. However, application of 0.1 millimolar TIBA as a ring around the petioles of the cotyledons or 1-centimeter on the hypocotyl below the cotyledons significantly promoted cotyledonary bud development.

The growth of lateral buds in plants and their correlative inhibition by the apical bud have been investigated by a number of investigators (1, 8, 15, 16, 19). In *Lycopersicon esculentum* Mill., understanding the nature and control of lateral bud development is of special interest because: (*a*) its morphology exhibits an initial phase of monopodial growth followed by a sympodial growth habit (3); and (*b*) manual or chemical removal of unwanted lateral buds or lateral shoots poses certain physiological problems such as disease and chemical injury in addition to being a labor-requiring and costly operation in tomato production (22, 25, 26).

In IAA-treated decapitated stumps of tomato plants, exogenous application of a combination of gibberellin A_3 and kinetin in lanolin paste stimulated lateral bud development, but the treatment effect was inconsistent on intact plants (7). Tucker (23, 24) found that the far red light suppression of the development of lateral buds of tomato was associated with an increase in endogenous levels of auxins and ABA, while stimulation of lateral bud development was associated with increased levels of endogenous gibberellins and cytokinins. The vigorous lateral bud situated immediately below the first main inflorescence was not inhibited by the far red light exposure following the 16-hr photoperiod. Aung (3) indicated that the factors controlling the development of the lateral bud located immediately below the first main inflorescence emanated from the inflorescence itself and from the roots.

Heretofore, studies on the development of the lateral buds of tomato have been concerned primarily with the buds subtended between the first inflorescence of the main stem and the cotyledons (3, 22-25). There is little information on the inhibition of the cotyledonary axillary buds during the ontogenetic development of the tomato plant. In an earlier study on the effects of gibberellins and cytokinin on the growth of the shoot apex, it was observed that 0.1 mM of BAP[1] stimulated the enlargement of the inhibited cotyledonary buds (4). Hussey (11) also found that removal of the young plumular leaves of the tomato seedling advanced the growth and enhanced the size of the apical bud. The present work reports further the influences of different phytohormones and young leaves on the development of the inhibited cotyledonary buds of tomato seedlings.

MATERIALS AND METHODS

Plant Materials. Seeds of *L. esculentum* Mill. cv. 'Fireball' were germinated in a medium of Vermiculite and fine white quartz sand (1:1, v/v). The seeded flats were kept moist and maintained at 26 C day and 20 C night in a growth chamber until transplanting 10 to 18 days later. The seedlings received a 12-hr photoperiod with an illuminance of 13 klux giving a photon flux density of photosynthetically active radiation of 160 $\mu E\ m^{-2}\ sec^{-1}$ from mixed cool-white fluorescent and incandescent lamps. Depending upon experiments, tomato seedlings 10 days or older from the growth chamber were transplanted into 15-cm clay pots and grown under greenhouse conditions at temperatures of 28 C day and 21 C night. The growing medium consisted of loam, coarse sand, and peat moss (1:1:1, v/v/v). The plants were fertilized weekly with a nutrient solution consisting of 3 g/l of a 10-30-20 (N-P-K) fertilizer plus complete micronutrients (9). A randomized complete block design of 8 to 10 replicates was used for each of the experiments reported.

Phytohormonal Treatments. Different molar concentrations of gibberellin $A_{4/7}$ ($GA_{4/7}$; Merck and Co., Rahway, N.J.), ABA (R. J. Reynolds Tobacco Co., Winston-Salem, N.C.), and TIBA (Sigma Chemical Co.) were prepared in 300 μl of ethanol and the solutions diluted with water containing 0.05% Tween 80 (polyoxyethylene sorbitan monooleate) for $GA_{4/7}$ and ABA, and with melted lanolin for TIBA. BAP (Nutritional Biochemicals Corp., Cleveland, OH) was prepared by first dissolving the chemical in 100 μl of 1 N KOH and then diluting to the desired concentration with water containing 0.05% Tween 80. The water with 0.05% Tween 80 and different concentrations of $GA_{4/7}$, ABA, and BAP were applied to tomato seedlings in 10-μl drops using a dispenser-attached Hamilton microsyringe, and TIBA was applied as a 50-μl volume paste.

Histology of the Cotyledonary Buds. In order to gain some information on the early response of the cotyledonary buds to

[1] Abbreviations: BAP: 6-benzylaminopurine; TIBA: 2,3,5-triiodobenzoic acid.

treatments, tomato seedlings were collected on the 3rd day (about 60 hr) after treatments (see Table IV for details). They were fixed in Craf III, dehydrated in a graded *tert*-butyl alcohol series, and embedded in Paraplast Plus with a melting point of 57 C. Serial longitudinal 10-μm sections were stained with periodic acid-Schiff reagent, a histochemical stain for carbohydrates. The height and width of the axillary cotyledonary bud shoot apices were measured from median longitudinal sections.

RESULTS

Excision of the first and second immature plumular leaves significantly stimulated the development of the cotyledonary buds 4 days after treatment. The stimulatory effect on bud development was similar to that of 0.1 mM BAP applied directly to the axils of the cotyledons. The apical application of 5 mM ABA significantly promoted the development of the cotyledonary buds after 6 days following treatment. Subapical treatment of 0.1 mM TIBA in lanolin, on the other hand, promoted cotyledonary bud development after 11 days. $GA_{4/7}$ at 1 μM concentration had no effect on the development of the cotyledonary buds (Table I).

Excision of the immature plumular leaves when they were between 5- and 10-mm lengths gave the greatest growth stimulation of cotyledonary buds. The effectiveness of the plumular leaves excision on cotyledonary bud development was diminished as the leaves grew older and increased in size (Table II).

Table I. Effect of exogenous hormones and excision of the young plumular leaves on the development of the cotyledonary bud of *Lycopersicon esculentum* Mill. cv. 'Fireball'[1]

Treatment[2]	Cotyledonary bud length (mm)				Response time[3] (days)
	July 16	July 20	July 23	July 27	
Untreated control	0.68	3.75	7.9	12.0	-
Lanolin control	1.13	7.28	17.5	27.8	-
1st & 2nd leaves excised	2.97	15.53	36.6	54.9	4
5 mM ABA (apical)	0.87	11.85	28.2	44.7	6
0.1 mM BAP + 1 μM $GA_{4/7}$	1.48	9.36	21.4	36.1	4
0.1 mM BAP + 0.1 mM BAP	1.67	14.92	31.4	47.7	4
1 μM $GA_{4/7}$ + 1 μM $GA_{4/7}$	0.73	4.93	10.0	16.2	-
0.1 mM TIBA (subapical)	0.69	9.23	31.0	65.1	11
HSD 5%	0.76	7.32	19.9	33.4	-

[1] 18-days old seedlings (seeded June 20 and transplanted July 8) were treated on July 12, 1976.

[2] Combined treatments were applied 24-hr apart in the sequence indicated; HSD refers to the Tukey's test (21) at 5% level of probability.

[3] Days after treatment showing significant bud growth.

Table II. Effect of excision of the 2 young plumular leaves at different stages of development on cotyledonary axillary bud growth[1]

Treatment[2]	Cotyledonary bud length (mm)		
	Sept. 3	Sept. 10	Sept. 24
1. Intact control	1.40	5.6	9.8
2. 10 off & 5 off	10.12	41.5	69.9
3. 10 off & 5 intact	2.78	14.9	28.2
4. 10 intact & 5 off	3.59	13.6	22.1
5. 20 off & 10 off	6.03	28.5	46.5
6. 20 off & 10 intact	1.96	13.7	25.8
7. 20 intact & 10 off	3.80	18.8	29.7
8. 35 off & 20 off	2.24	8.3	17.0
9. 55 off & 35 off	2.29	9.8	27.0
HSD 5%	2.8	13.8	23.0
1%	3.3	16.1	26.9

[1] 17-days old seedlings were used. They were seeded on Aug. 9 and transplanted Aug. 24.

[2] Treatments 2, 3 and 4 were excised on Aug. 26 when the plumular leaves were less than 10 mm length; Treatments 5, 6 and 7 were excised on Aug. 28 when the young leaves were less than 20 mm length; Treatments 8 and 9 were excised on Sept. 1 when the leaves were greater than 20 mm length. For example, 10 off & 5 off indicates the excision of the 1st and 2nd plumular leaves when they were 10 mm and 5 mm lengths respectively.

Table III. Effect of N^6-benzylaminopurine (BAP) and young leaves excision on cotyledonary bud development of *Lycopersicon esculentum* Mill. seedlings[1]

Treatment[2]	Cotyledonary bud length (mm)
1. Intact control	0.45
2. 1st and 2nd leaves excised	1.09
3. Excision 1 & 2 + lanolin on petiole	0.89
4. Excision 1 & 2 + 0.1 mM BAP on petiole	1.03
5. Excision 1 & 2 + 0.5 mM BAP on petiole	1.04
6. Intact + 0.1 mM BAP on hypocotyl & petiole	0.37
7. Intact + 0.1 mM BAP on petiole	0.38
8. Intact + 0.1 mM BAP on hypocotyl	0.39
9. Intact + lanolin on hypocotyl & petiole	0.32
HSD 5%	0.66

[1] 16-days old seedlings (seeded Oct. 25 and transplanted Nov. 5) were used. The seedlings were treated on Nov. 10 and measured on Nov. 18, 1976.

[2] Excision indicates the removal of the 1st and 2nd immature plumular leaves.

Table IV. Effect of N^6-benzylaminopurine (BAP) and abscisic acid (ABA) on cotyledonary bud development of tomato seedlings[1]

Treatment[2]	Cotyledonary bud length (mm)	
	Jan. 25	Feb. 18
Untreated control	0.07	7.8
1 mM BAP (twice)	1.13	122.0
5 mM BAP (twice)	0.07	14.8
1 mM BAP + 5 mM ABA	0.91	91.0
5 mM ABA + 1 mM BAP	0.85	69.4
HSD 5%	0.77	64.0

[1] 18-days old seedlings (seeded Jan. 4 and transplanted Jan. 14) were used.

[2] 10 μl of BAP or ABA solution in 0.05% Tween 80 was applied; BAP (twice) denotes that BAP was applied 2 times at 24-hr interval, and BAP + ABA indicates that BAP was applied 24-hr before the application of ABA.

Table V. Effect of hormones and young leaves excision on cotyledonary bud development of tomato seedlings[1]

Treatment	Meristematic Dome (μm)[2]	
	Height	Width
Intact control	215 ± 45	142 ± 24
Excision of 1st & and leaves	201 ± 46	209 ± 50
1 mM BAP (twice)	264 ± 60	225 ± 40
5 mM ABA (twice)	120 ± 44	116 ± 35
1 mM BAP + 5 mM ABA	279 ± 102	180 ± 50
5 mM ABA + 1 mM BAP	192 ± 33	170 ± 50

[1] Seedling age and treatments same as those reported in Table IV.

[2] Means of 8 specimens except intact control where values are for only 2 specimens since the remaining specimens showed no initiated cotyledonary bud development at the time of examination; plant materials collected 60-hrs after treatments.

Application of 1 mM BAP to the axillary bud sites of the cotyledons significantly promoted the development of the cotyledonary buds. The BAP-induced development of the buds was not inhibited by 5 mM ABA applied 24 hr after BAP treatment (Tables III and IV). The size of the cotyledonary bud shoot apex was increased by BAP and by excision of immature leaves (Table V and Fig. 1).

TIBA at 1 mM concentration applied in lanolin to the hypocotyl one cm below the cotyledons or on the cotyledonary petioles significantly promoted the development of the cotyledonary buds. It should be noted, however, that the stimulation of bud development was obtained only after 2 weeks of treatment (Table VI), in contrast to the shorter response time obtained with BAP or excision of immature leaves.

DISCUSSION

The suppression of cotyledonary bud development of the to-

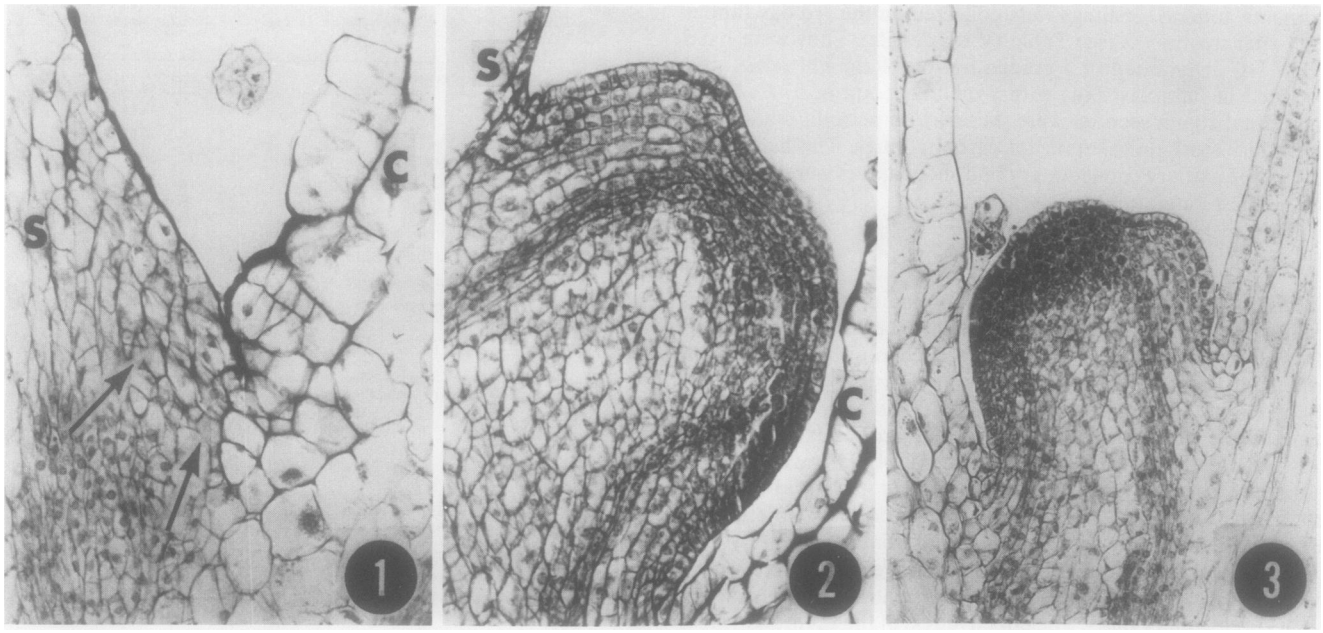

FIG. 1. Development of tomato cotyledonary axillary buds as viewed in median longitudinal section (all × 260). 1: Untreated control specimen; note the initiation of an axillary bud (arrows); 2: specimen treated with 20 µl of 1 mM BAP showing the greatly enlarged shoot apex with an incipient leaf primordium; 3: specimen treated with the excision of the first and second immature plumular leaves. C: cotyledons; S: shoot (main axis).

Table VI. Effect of 2,3,5-triiodobenzoic acid (TIBA) on cotyledonary axillary bud development of tomato seedlings[1]

Treatment	Cotyledonary bud length (mm)		
	Feb. 6	Feb. 11	Feb. 21
Control, lanolin petiole	1.0	1.9	2.6
Control, lanolin hypocotyl	2.1	3.3	4.5
1 mM TIBA on hypocotyl	8.6	16.6	21.1
1 mM TIBA on petiole	11.6	23.5	30.2
HSD 5%	5.5	10.6	16.5
1%	6.8	13.5	20.5

[1] 17-days old seedlings (seeded Jan. 4 and transplanted Jan. 14) were treated on Jan. 21, 1977.

mato plant during ontogeny can be successfully overcome by: (*a*) the excision of immature leaves; (*b*) inhibiting the growth of the main apical bud with ABA; (*c*) direct application of BAP to the axillary bud sites; and (*d*) TIBA application as a ring below the apical bud, on the cotyledonary petioles, or 1 cm below the cotyledons on the hypocotyl. It should be remembered that the response time of the different treatments differs.

The inhibitory influence of the young developing leaves on axillary bud development of seedlings has long been recognized (20). In the tomato, excision of the immature leaves has been shown to enhance flowering and fruit ripening (5). Hussey (11) found that removal of the young leaves of tomato seedlings accelerated the development of the apical bud. The excision of the two immature plumular leaves (5- to 10-mm length) with the main apex intact effectively promoted the outgrowth of the inhibited cotyledonary axillary buds (Table II and Fig. 1). It may be of interest to note that the intensity of the young leaves inhibition of the tomato cotyledonary buds observed in this study resembled similar effects of the young leaves on the inhibition of pea axillary buds reported by Snow (20). It is not known whether the influence exerted by the young leaves of the two plant species is mediated by similar or different mechanisms.

In an excised *in vitro* tomato apical shoot system, Hussey (12) found that a combination of GA_3 and coconut milk resulted in the fastest growth of the main shoot apex. Kinetin, in addition to sucrose and inorganic salts, also promoted apical shoot growth. Application of $GA_{4/7}$ directly to the cotyledonary axillary bud sites of intact tomato seedlings in our study, however, did not give any growth promotion. Earlier, Aung and Byrne (4) showed that 0.1 mM BAP and 1 µM $GA_{4/7}$ applied apically caused significant enlargement of the shoot apex of intact tomato seedlings. In the present study, BAP when applied directly to the cotyledonary bud sites promoted cotyledonary bud development rapidly, but failed to give growth stimulation when applied indirectly to adjacent regions (Table III). The stimulatory effects of BAP applied locally on bud growth agreed with similar responses observed on other plant species (10, 18). The lack of response to BAP when the hormone was applied at some distance from the cotyledonary bud sites, however, may be due to the limited mobility of the hormone to the cotyledonary buds in order to evoke a response (14).

In contrast to the direct influence of BAP in stimulating the development of the tomato cotyledonary buds, ABA applied indirectly to inhibit the main apical shoot growth of the tomato significantly promoted cotyledonary bud outgrowth (Table I). Bellandi and Dorffling (6) also found that ABA application to the shoot apex of pea seedling inhibited apical growth but promoted the development of the lateral buds. It is conceivable that the ABA-inhibited apical growth stimulation of tomato cotyledonary bud development may be due to the lessening of nutrients competition by the young developing leaves (11). Analogous to the excision of young leaves, ABA inhibition of apical shoot growth could make available a greater supply of nutrients for the growth of the cotyledonary buds.

The TIBA-induced growth stimulation of tomato cotyledonary buds is probably mediated through the interference with auxin transport (13). Auxins have been shown to be present in the different organs of the tomato (24, 25) and particularly in the seedling cotyledons (2). Thus, TIBA treatment could lower the level of auxins in the cotyledonary buds so as to favor their growth by altering the cytokinin to auxin ratio (17). A high cytokinin to low auxin content favors shoot growth (25). It is possible, therefore, that the observed cotyledonary bud development elicited by TIBA and BAP may be due to the alteration in the ratio or proportion of the two hormones.

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