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Effect of 2, 3, 5-Triiodobenzoic Acid on the Growth of Lateral Bud and on Tropism of Petiole[1)]

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2, 3, 5-Triiodobenzoic acid (TIBA) is known to be an antagonist against growth promoting substances (3, 7, 9, 10), but the mechanism has not yet been clear. In the course of author's investigation on correlative growth, it was found that TIBA not only interfered with the bud-inhibiting action of leaf blade and indole-3-acetic acid (IAA), but also blocked the conduction of influence from the leaf blade or IAA causing the phototropic and geotropic bending of petiole. The results reported here may be of some value in considering the effects of leaf blade and auxin on the bud inhibition and on the tropistic movement of petiole, as well as on the interference with them by TIBA.

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Material and methods

Single-node stem cuttings of sweet potato (*Ipomoea Batatas*, var. Norin No. 1) were used in the experiments. Age of a node was represented as follows: the lowermost (oldest) node bearing a leaf of which the blade had not yet been unfolded was named A 1, the younger nodes being respectively called A 2, A 3, ..., acropetally. The node that bore youngest expanded leaf was named B 1, and the older nodes were respectively numbered B 2, B 3, ..., basipetally. Leaves and lateral buds were named according to the nodes which bore them. In the experiments reported below the node A 1, B 1 or B 3 was used. Both the internode and the midrib of A 1 or B 1 were rapidly elongating, but the growth in length of internode was almost going to cease and the midrib had reached about 70% of its final length towards B 3.

Materials were selected so as to be as uniform as possible. Stems

1) This work was aided by the Scientific Research Expenditure of the Department of Education.

were cut on both sides of the nodes to be used, so as to put the nodes at the middle of the isolated stem pieces. The pieces were made 5 or 6 cm. long, according to cases. The lateral buds were 1–2 mm. long at the beginning of experiments. Treated and control pieces of stem were supported erect by putting their basal parts through holes bored through pieces of thick paper which covered beakers of 100 ml. capacity. The beakers contained 100 ml. of the nutrient solution,[2] which soaked the bases of the pieces of stem. They were placed about 1.5 m. apart from the south window of a room, except the experiments in the dark. The nutrient solution was renewed every 2 days. For lanolin paste, sodium salt of TIBA or acid form of IAA (Eastman Kodak Co.) was well mixed with dehydrated plain lanolin.

Length of lateral buds was measured every 2 days. In case the lateral bud grew well one or more leaves became expanded on it during the course of experiment. So the number of the newly developed leaves was recorded at the close of experiment. And then the buds, which might better be called new shoots in case of good growth, were excised and oven-dried, to measure the dry weight. Though the initial dry weight of the bud actually used in the experiment is not known, the dry weight data roughly indicates the growth in mass, for the initial weight is 0.3–0.5 mg. The growth in length is represented in Tables by the increment.

Results

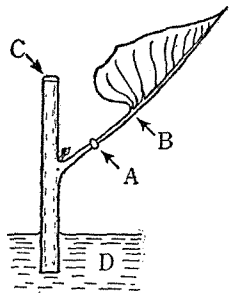
Experiment 1. Treatment with TIBA of petioles with intact and cut off blades

Stem pieces, 5 cm. long, of A 1 nodes were used. The apical cut surface of each stem was smeared with plain lanolin. The stem pieces were divided into two groups. In the one group the leaf blades were cut off at their bases, while in the other the blades were left intact. For a half number of each group the middle parts of the petioles were smeared with 2% TIBA-paste, while for the rest the same parts were smeared with plain lanolin. In either case the smear formed a ring, about 2 mm. broad, around the petiole. Increments in length, dry weight and number of expanded leaf of each axillary bud were recorded after 7 days in the condition already described.

The result represented in Table 1 shows that the very young leaf strongly inhibited the growth of its axillary bud, if no TIBA was applied. When the petiole was treated with TIBA, however, the inhibiting action of blade was very much weakened. Buds of the TIBA-treated bladeless samples grew a little better than those of the control bladeless. The explanation may

2) The composition of nutrient solution was KH_2PO_4 0.55g., $MgSO_4 \cdot 7H_2O$ 0.55g., $Ca(NO_3)_2 \cdot 4H_2O$ 0.73g., KNO_3 0.15g., $FeSO_4 \cdot 7H_2O$ 2.5mg., $MnSO_4$ 1.4mg., H_3BO_3 0.5mg., $ZnSO_4$ 1.0mg. in 1 l. distilled water.

Table 1. Effect of TIBA and leaf blade on the growth of lateral bud, tested with the pieces of stem as shown by the figure (A: Ring of lanolin paste, B: Point of excision of blade, C: Plain lanolin, D: Culture solution). Measured 7 days after the treatment on Aug. 29, 1952. Mean of 5 samples.



	Control		2% TIBA	
	Leaf blade cut off	Leaf blade intact	Leaf blade cut off	Leaf blade intact
Elongation of bud (mm)	12.0	3.8	14.2	12.2
Ratio	**100**	**32**	**118**	**102**
Dry weight of bud (mg)	10.1	1.2	11.1	5.6
Ratio	**100**	**12**	**110**	**56**
No. of new leaves on lateral bud	1.0	0	1.0	0.6

be either 1) TIBA promotes the bud growth, or 2) TIBA reverses the inhibition exerted by the tip part of petiole, which is much weaker than the inhibition by the blade. The first explanation may seem to be applicable also to the case of bearing intact blades. But Experiments 3 and 4 will show that TIBA rather inhibits the growth of lateral bud, and next experiment will indicate that TIBA interrupts the inhibiting influence of auxin coming from the tip part of petiole.

Experiment 2. Treatments of debladed petiole with TIBA and/or IAA at the middle and/or the tip

Cuttings used were 5 cm-long pieces bearing A 1 nodes. The apical cut end of each stem was smeared with plain lanolin. The leaf blades were cut off at their bases, and the cut ends were smeared with 0.1% IAA or 2% TIBA lanolin paste or plain lanolin. The middle parts of petioles were treated also with the three kinds of smear. The result is summarized in

Table 2. Effect of IAA and TIBA on the growth of the axillary bud of defoliated petiole, when lanolin pastes were applied around the middle part or on the cut end of it. Measured 7 days after the treatment on Aug. 25, 1952. Mean of 5 samples.

Treatment at :	(I)	(II)	(III)	(IV)	(V)
Cut tip of petiole	Plain lanolin	0.1% IAA	0.1% IAA	Plain lanolin	2% TIBA
Middle of petiole	Plain lanolin	Plain lanolin	2% TIBA	0.1% IAA	0.1% IAA
Elongation of bud (mm)	12.0	5.8	9.2	5.6	5.6
Ratio	**100**	**48**	**77**	**47**	**47**
Dry weight of bud (mg)	8.9	3.8	8.1	2.7	3.1
Ratio	**100**	**43**	**91**	**30**	**35**
No. of new leaves on lateral bud	1.2	0.4	0.8	0.2	0.4

Table 2. IAA applied in place of blade, which was of the same age as the foregoing experiment, inhibited the bud growth nearly to the extent as the intact blade (compare Table 2, II with Table 1, II). And the inhibition by IAA was reversed to some extent by TIBA applied at the middle part of petiole (Table 2, III). When the middle part of petiole was treated with IAA (IV), bud inhibition was found to be of the same degree as in II. But in this case, the inhibition was not reversed by TIBA applied to the cut end of petiole. Hence TIBA can cancel the IAA-inhibition when it is applied on the midway between the place of IAA application and the bud, but not when the points of application of the two substances are reversed.

Experiment 3. Treatment of apical cut surface of stem with IAA or TIBA

Stem pieces bearing B 3 nodes were used. They were 6 cm. long, and the blades were cut off at their bases. The apical cut surfaces of stems were smeared with 0.1% IAA or 2% TIBA lanolin paste or plain lanolin.

Table 3. Effect of IAA and TIBA on the growth of lateral bud when the cut surface of stem was treated. Measured 10 days after the treatment on Aug. 5, 1952. Mean of 5 samples.

Treated with	Plain lanolin	0.1% IAA	2% TIBA
Elongation of bud (mm)	17.7	0.4	9.7
Ratio	**100**	**2**	**55**
Dry weight of bud (mg)	10.6	0.5	4.2
Ratio	**100**	**5**	**40**
No. of new leaves on lateral bud	1.0	0	0.3

As shown in Table 3, elongation of lateral bud was inhibited by IAA and TIBA by 98% and 45%, respectively. Large callus was formed on the IAA-treated surface of stem, but no apparent callus was observed on the surface treated by plain lanolin or TIBA. Hence the bud inhibition by TIBA does not seem to be explained by compensated growth.

Experiment 4. Treatment with TIBA at the base and IAA at the top of stem

B 3 cuttings, 5 cm. long, were divided into two groups: in the one the nutrient solution which soaked the bases of cuttings was as in the usual case, and in the other the solution was enriched with 1 mg./l. of TIBA. And each group was subdivided into three, as seen in Table 4, according to the presence or the absence of the blade and IAA treatment at the apical

end of stem.

Table 4. Effect of TIBA and IAA on the growth of lateral bud when TIBA was given as a solution (1 mg/l) to the base and IAA-lanolin to the apical cut end of stem. Measured 8 days after the treatment on Sept. 20, 1952. Mean of 5 samples.

Culture solution	No TIBA added			1 mg/l TIBA added		
Leaf blade :	(I) Cut off	(II) Intact	(III) Intact	(IV) Cut off	(V) Intact	(VI) Intact
Stem-end treatment :	Plain lanolin	Plain lanolin	0.1% I A A	Plain lanolin	Plain lanolin	0.1% I A A
Elongation of bud (mm)	14.0	23.2	0.8	7.8	5.6	0
Ratio	**100**	**166**	**6**	**56**	**40**	**0**
Dry weight of bud (mg)	8.2	15.4	0.5	2.4	2.3	0.4
Ratio	**100**	**188**	**6**	**29**	**28**	**5**
No. of new leaves on lateral bud	1.0	1.0	0	0	0	0

In contrast to the case of very young leaf (Experiment 1), the blade promoted, instead of inhibiting, the bud growth in the present case of nearly mature leaf (Table 4, I and II). TIBA given to the base of stem inhibited the bud growth, and the blade did not foster the bud in this case (Table 4, IV and V). Hence TIBA showed no indication of bud-promoting effect. IAA applied to the apical cut surface of stem piece inhibited the bud growth very strongly, and TIBA did not reverse the inhibition in this case (Table 4, III and VI). This corresponds to the case of Experiment 2 in which TIBA applied, but not between IAA lanolin and bud, did not undo the IAA inhibition.

Experiment 5. Effect of TIBA on phototropic curvature of petiole

In the course of experiments as described above, it was observed that the phototropic and geotropic movement of petiole was disturbed in a characteristic way by the treatment of its middle part by the TIBA paste. So some simple experiments were performed. The nodes B 1 whose petioles growing unbent were selected. Cut stem pieces were made to stand erect on beakers as described before. They were illuminated laterally from the side of their blades by a 250-watt incandescent lamp, about 50 cm. apart. In 24 hours, the midrib, the petiole and the stem of each piece lay in a plain, and the angle between the latter two became about 45°, as shown in Fig. 1, A. Then the following treatments were made :

Leaf blade	Middle of petiole ringed with	Cut surface of petiole treated with	
Intact	Plain lanolin	———	(I)
	2% TIBA	———	(II)
Cut off	Plain lanolin	Plain lanolin	(III)
		0.1% IAA	(IV)
	2% TIBA	Plain lanolin	(V)
		0.1% IAA	(VI)

Immediately after the treatment, all the cuttings were put to be illuminated oppositely to the preillumination (Fig. 1, B). And the petioles were

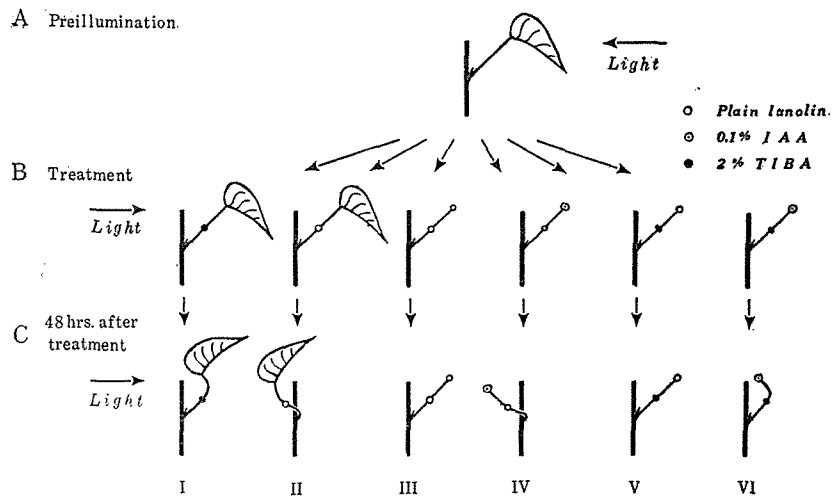


Fig. 1. Effect of TIBA and IAA on phototropic curvature of petiole.

as shown in Fig. 1, C, after 2 days. The petioles with leaf blades intact and those without blades but treated with IAA showed the typical phototropic reaction (Fig. 1, II and IV). But if the middle parts of petioles were treated with TIBA, the phototropic movement occurred only on the distal halves, the halves below the sites of TIBA treatments remaining nearly straight (Fig. 1, I and VI). The petioles treated with plain lanolin or plain and TIBA-lanolin did not show any phototropic curvature (Fig. 1, III and V).

It may be concluded from the above results that 1) even the debladed petiole can perceive phototropic stimulus, 2) the blade serves as the source of auxin, and can be replaced by IAA so far as the observed phototropic response concerns, and 3) TIBA applied at the middle part of petiole inhibits the basal half of petiole from being affected by auxin which is transported from the tip of it.

Experiment 6. Effect of TIBA on geotropic curvature of petiole

Selected were B 1 nodes whose petioles were straight. The stems of cuttings were kept horizontal for 24 hours, to make their petioles erect (Fig. 2, A). Then leaf blades were cut off from a group of cuttings. The middle part of every petiole was smeared with plain or TIBA-lanolin. And all were left standing in the dark, as shown in Fig. 2, B. The result after 2 days is represented by Fig. 2, C.

Even the petiole without blade reacted geotropically if IAA is applied to its tip (Fig. 2, I and III). And when the middle part of petiole was treated with TIBA, the response was restricted only in the distal half, whether the blade was present or was replaced by IAA (Fig. 2, II and IV). The circumstance is the same as in the case of phototropism.

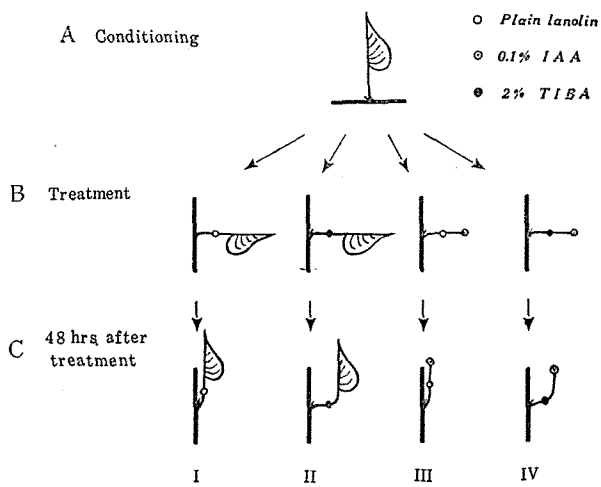


Fig. 2. Effect of TIBA and IAA on geotropic curvature of petiole.

Discussion

Concerning various plants it has been reported that a young developing leaf inhibits the elongation of lateral bud at its axil (6, 8 etc.), and now the same is proved true with sweet potato plant. Some authors showed that such young leaves produced much diffusible auxin (2, 4, 5, 11). It has not been measured how much auxin is actually produced by very young leaves of sweet potato. But IAA applied in place of leaf blade inhibited growth of the bud at its axil (Table 2) and caused tropistic movements of petiole (Figs. 1 and 2) as well. When, on the other hand, the leaf was drawing to a close of its growth phase, it rather promoted the bud growth (Table 4). The difference

due to the age of leaf may be accounted for in either one of the two ways: 1) auxin produced by maturing leaf is insufficient to inhibit bud, and 2) maturing leaf supplies substances needed for bud growth, whereas rapidly growing one rather competes with bud for them. Although the experiments described above are insufficient for definite conclusion, it is highly probable that the bud inhibition by the very young leaf is due to auxin, the growth competition playing a role, too.

BRAUNER and VARDER (1) found that the leaf blade served, not as the perception organ for phototropic and geotropic stimuli, but as the source of auxin for the responsive movement of the petiole. It was confirmed also to be the case with sweet potato by Experiments 5 and 6. And it is interesting that TIBA applied to the middle part of petiole blocks the effect of blade and IAA coming from the tip of petiole downwards, in the case of tropistic movement as well as in the case of bud inhibition. THIMANN and BONNER (9) think that TIBA competes with IAA for *a* common substrate. If it should be true, however, TIBA applied distal to IAA (Table 2, V) and at the base of stem (Table 4, VI) would have reversed the IAA-inhibition, to some extent at least. Hence it might be concluded that the auxin coming down through a petiole is blocked or inactivated at the point of TIBA treatment.

Summary

The following facts were observed in regard to excised pieces of sweet potato stems, carrying single nodes of different ages:

1. A very young leaf inhibited growth of the bud at its axil, while a leaf which had grown to some extent rather promoted it.
2. IAA applied at the apical end of stem or at the tip or the middle part of petiole strongly inhibited the bud growth.
3. TIBA applied at the apical or the basal end of stem weakly inhibited the bud growth.
4. TIBA applied at the middle of petiole which had leaf blade intact, or IAA-paste in place of it, reversed the bud-inhibiting effect of them.
5. TIBA applied, but not between the bud and the blade or IAA, did not reverse the inhibiting effect.
6. The effect of blade, or IAA applied in place of it, causing photo- and geotropic movement of its petiole was blocked just at the point of application of TIBA at the middle part of petiole.

It is concluded that auxin is blocked or inactivated at the part of petiole treated by TIBA.

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