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Sources of Free IAA in the Mesocotyl of Etiolated Maize Seedlings

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ABSTRACT

Sources of free indole-3-acetic acid (IAA) for the mesocotyl of intact etiolized maize (*(Zea mays* L.) seedlings are evaluated. The coleoptile unit, which includes the primary leaves and the coleoptilar node, is the main source of free IAA for the mesocotyl. The seed and the roots are not immediate sources of IAA supply. Dependence of the apical growing region of the mesocotyl on the coleoptile unit as a source of free IAA is almost total. One-half or more of the supply of IAA comes from the coleoptile tip, the rest mainly from the primary leaves. Removal of the coleoptile tip results in inhibition of mesocotyl elongation. The hypothesis that growth of the mesocotyl is regulated by auxin supplied by the coleoptile is supported. Conjugated forms of IAA appear to play little part in regulating the levels of free IAA in the shoot.

In 1928, Went (27) expressed the opinion that the growth of the mesocotyl is determined by a growth substance (later called auxin) which reached it from the tip of the coleoptile. This hypothesis was experimentally supported by van Overbeek, (23–25) who showed that inhibition of mesocotyl elongation due to a genetic factor (dwarfism) or external treatments, such as heat and light, is accompanied by a lower yield of diffusible auxin from the tip or the base of the coleoptile. He was also able to reverse the heat-induced inhibition by applying hetero-auxin (IAA) to the tip of the coleoptile (24). Went (27) and van Overbeek (24) cited Beyer's (3) finding, that decapitation of the oat coleoptile tip inhibits the mesocotyl elongation, in support of the hypothesis. With maize, a similar effect of decapitation was found by Inge and Loomis (12). Light-induced inhibition has been shown recently to be reversed by applied IAA (22).

On the other hand, the auxin hypothesis has not always received support. Schneider (18), working on inhibition by light, concluded that 'if there is an indirect mechanism involving the mediation of another part of the plant, the mediation can hardly be by way of auxin.' Mer (13) also marshalled evidence from his many experiments to contradict the auxin hypothesis. The most striking evidence was the claim that decapitation of the coleoptile tip exerted no appreciable effect on the elongation of the oat mesocotyl, even though successive decapitations of the coleoptile were carried out to reduce the possibility of auxin production at the coleoptile stump, known (28) as 'regeneration of the physiological tip.' Dattary and Mer (5) further claimed to show that the effect of light or heat treatment on mesocotyl elongation is not accompanied by a fall in the auxin content of the mesocotyl. Thus, the experimental evidence that mesocotyl growth is controlled by auxin originating in the coleoptile is contradictory, and further research is needed.

The results of Iino and Carr (10) provide a reaffirmation of the classical concept of IAA production at the coleoptile tip. The mesocotyl, however, is unlikely to depend totally on the coleoptile tip for its IAA. The primary leaves, for instance, appear able to supply relatively large amounts of IAA (10). There is also a possibility that the mesocotyl receives some IAA in free (8, 17, 19) or conjugated (6, 15) form from the seed. If regulation of mesocotyl growth by IAA is to continue to be regarded as an example of hormone-mediated growth correlations, it is necessary, at least, to show that the IAA reaches it from elsewhere in the seedling. The present study investigates the extent of the dependence of the maize mesocotyl on other parts of the seedling as sources of IAA.

MATERIALS AND METHODS

Etiolated seedlings of maize (*Zea mays* L.) were raised in seedling boxes, as previously reported (9), and used when 3 d old (the variety used was GH 390, a field corn produced at the Agricultural Plant Breeding Station, Grafton, N. S. W.). The box was opened only when seedlings were treated. Surgical treatments to seedlings were carried out in the dark using an IR-scope and physiological safe IR radiation (9). Dim green light (9) was used when plant materials were harvested and frozen in liquid N_2 for estimation of their free IAA content. All these procedures were carried out in a dark room at 25°C and 80% RH. The RH was raised to about 90% when the seedling box was opened for surgical treatments. Free, conjugated, and diffusible IAA were estimated essentially as previously reported (10, 11).

RESULTS AND DISCUSSION

Origin of Free IAA in the Mesocotyl. In 3-d-old seedlings, the yield of diffusable IAA from the base of the whole coleoptile (coleoptile plus primary leaves and nodal region, obtained by cutting at about 1 mm below the node) was 0.6 ng/h (10). Inasmuch as we know that a mesocotyl (about 3-cm long at this age) contains only 1.5 ng of free IAA (10), the yield of diffusable IAA suggests that IAA in the mesocotyl originates largely in the coleoptile unit. To evaluate its dependency on the coleoptile unit, the free IAA content of the mesocotyl was monitored after removal of the whole coleoptile (Fig. 1). The result, however, did not permit a clear-cut evaluation, which can be made only when the IAA content reaches a steady state following removal of the source. A decrease was followed by an increase. At the lowest level, 3 h after decapitation, the content was more than one-half that of the undecapitated control. However, when the free IAA content of the apical 5 mm of the mesocotyl stump was monitored following removal of the whole coleoptile, the content fell almost to zero at 1 h after cutting, despite the following increase (Fig. 2).

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The result clearly indicates that the free IAA in the apical part of the mesocotyl is almost entirely derived from the coleoptile unit.

It has been suggested that the seed of cereals supplies IAA to the shoot (8, 17, 19). The results indicate that there is no substantial acropetal supply of IAA, at least for the apical region of the mesocotyl, but there may be some supply to the basal region. To investigate the supply of IAA to the mesocotyl from the parts below it, *i.e.* the seed and the roots, the whole shoot was excised from the seedling, and changes in the free IAA content of the mesocotyl were then monitored. The excised shoots were incubated in vials, their basis in contact with water. The free IAA content of the mesocotyl was unaffected (Fig. 3); therefore, the seed and the roots are unlikely to supply free IAA to the mesocotyl in appreciable amounts.

The rise in IAA content of the mesocotyl following the initial fall is probably due to production of IAA in the mesocotyl itself, induced by removal of the coleoptile unit, as has been shown in the coleoptile after its decapitation (see below). In the apical 5 mm of the mesocotyl, the increase is apparent from 1 h after cutting (Fig. 2). The induced IAA production appears to start very soon after cutting.

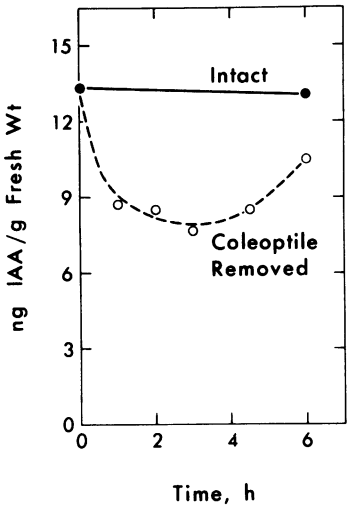

FIG. 1. Effect of removal of the whole coleoptile on the free IAA content of the mesocotyl. The coleoptiles were removed from seedlings, at time zero, by cutting at about 1 mm below the node. Following incubation of the seedlings for a given time, mesocotyls were excised, and their free IAA content was estimated. For each estimate, 30 mesocotyls were used. Controls were mesocotyls freshly excised from intact seedlings.

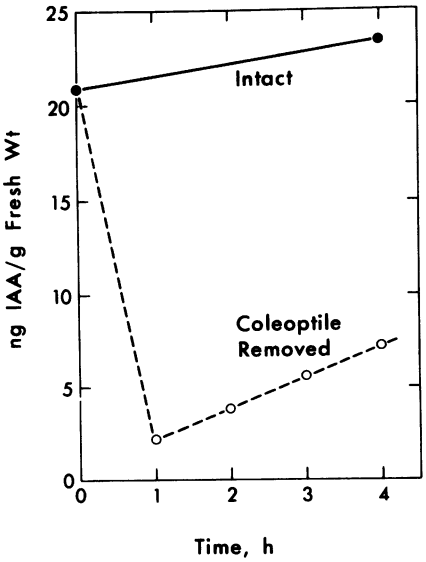

FIG. 2. Effect of removal of the whole coleoptile on the free IAA content of the apical 5 mm of the mesocotyl. Experimental treatments were as given in the legend to Figure 1, but only the apical 5 mm of the mesocotyl were subjected to estimation of free IAA. For each estimate, 30 mesocotyls were used

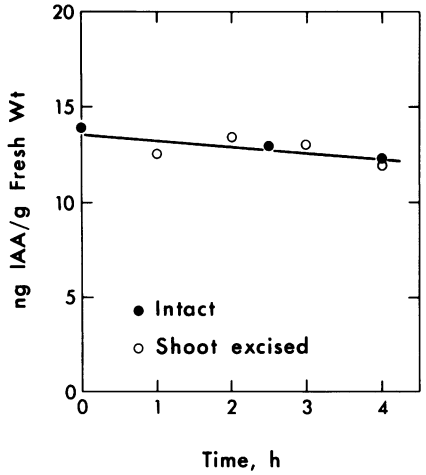

FIG. 3. Effect of removal of the seed and the roots on the free IAA content of the mesocotyl. Ten whole shoots, excised from seedlings, were stood in a vial, cut surfaces in contact with water (3 ml). Following incubation for a given period, the mesocotyls were excised, and their free IAA content was estimated. For each estimate, mesocotyls from three vials were used.

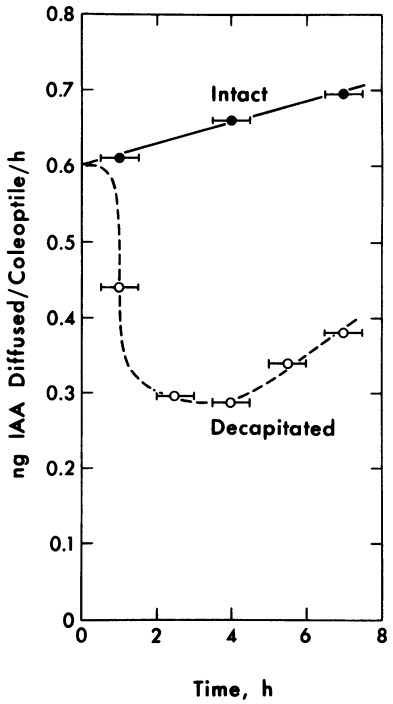

FIG. 4. Effect of removal of the coleoptile tip on diffusible IAA yield from the base of the whole coleoptile. Coleoptile tips (3 mm) were removed from seedlings at time zero. Following incubation of the seedlings for a given time, the coleoptiles were excised from the seedlings by cutting at about 1 mm below the node; the yield of diffusible IAA, collected from the basal cut surfaces for 1 h, was estimated. For each estimate, 24 coleoptiles were used. Controls were prepared from nondecapitated coleoptiles. Each estimate was plotted at the center of the diffusion time of 1 h, indicated by a horizontal bar.

We conclude that the external source of free IAA in the mesocotyl of the intact seedling is the coleoptile unit, and the dependence of the apical region of the mesocotyl on the coleoptile unit as the source is almost total. Considering that direct measurement (M. Iino, unpublished data) shows that the growth of the mesocotyl is located in its apical region, the important source of free IAA for mesocotyl growth must be the coleoptile unit.

Supply of IAA from the Coleoptile Unit. The most active site of IAA production in the coleoptile unit is demonstrably the coleoptile tip (10). However, the primary leaves may also be such sites. Skoog (20) could detect some auxin diffusing out of oat primary leaves using his sensitive 'deseeded *Avena* test'. Iino and Carr (10) estimated the yield of diffusible IAA from the base of a single set of primary leaves as about 0.2 ng/h, a substantial part of the yield (0.6 ng/h) from the base of the whole coleoptile.

To evaluate what fraction of the IAA supplied by the coleoptile unit to the mesocotyl originates in the coleoptile tip, the yield of diffusible IAA from the base of the coleoptile unit was monitored after removal of the tip (Fig. 4). The yield fell rapidly after decapitation. The fall was followed by a gradual rise, due probably to development of IAA production in the coleoptile stump induced by decapitation (26, 28) (see also below). The minimum yield obtained, at around 3 h after decapitation, was about one-half that of the undecapitated control. From this, we concluded that at least one-half of the diffusible IAA at the base originates in the coleoptile tip. The amount, however, could be greater, since IAA production induced by decapitation probably begins earlier than does the measured rise. A large part of the diffusible IAA not derived from the coleoptile tip is probably supplied by the primary leaves (see above). However, the data do not exclude the possibility that parts of the coleoptile unit other than the coleoptile tip and the primary leaves contribute to some small extent.

Production of IAA in the coleoptile induced by removal of its tip was demonstrated by monitoring diffusible IAA from the apical region of the decapitated coleoptile. Each h, apical 5-mm stumps of coleoptiles, without included leaves, were excised from seedlings decapitated at time zero (3 mm of tip removed). The yield of diffusible IAA in an h from these excised coleoptile cylinders was estimated. The yield fell to a minimum during the first 2 h, then increased until at least 8 h from the time of decapitation (data not shown). A similar increase, following an initial decrease, was induced even in coleoptile cylinders (5-mm long) excised from intact seedlings and incubated in H_2O, and these isolated segments were found to give off diffusible IAA in amounts far exceeding their initial free IAA content. These results are consistent with those obtained by van Overbeek (26), using a bioassay. It is not, however, clear whether this increased IAA production is due to newly generated or merely enhanced production, since the results do not exclude the possibility that the non-tip tissues of the intact coleoptile produce IAA.

The yield of diffusible IAA from the base of the whole coleoptile showed a gradual increase with seedling age (see undecapitated control, Fig. 4). This probably reflects a normal increase of IAA production in the intact coleoptile, since the yield of diffusible IAA from the coleoptile tip also showed an increase with age (data not shown). An age-dependent increase in diffusible auxin yield from the maize coleoptile tip has already been reported by van Overbeek (25) and Briggs (4).

Roles of Conjugated IAA. Conjugated IAA is known to occur in seeds (2, 16, 21) and seedling shoots (1, 10) of cereals. Bandurski and coworkers (6, 15) have developed a hypothesis, based on the transport and metabolism of radioactive indole-3-acetyl-*myo*-inositol applied to the cut surface of the maize endosperm. They have hypothesized that this substance, a major component of IAA conjugates in the maize seed, is transported into the shoot and serves as the source of free IAA. Nevertheless, the most active IAA production at the coleoptile tip has been shown to be able to proceed without a supply of conjugated IAA (10). Conjugated IAA might be converted to free IAA in the mesocotyl, since the free IAA content of the whole mesocotyl did not fall greatly after removal of the coleoptile unit (Fig. 1). However, such supplies of IAA are probably not important for growth in the intact seedling, because, as shown above, the apical growing portion of the mesocotyl depends largely on the coleoptile unit for its supplies of free IAA.

Interconversion of free IAA and conjugated IAA shown by Bandurski and coworkers (6, 15) also may have little importance in the control of free IAA levels in the etiolated seedling. To investigate this problem, coleoptile segments (1-cm long) excised 3 mm below the tip were used. In such a segment, incubated in H_2O, the free IAA content deceases after excision, owing to removal of the supply of IAA from the coleoptile tip and diffusion of IAA from the segment into water. This decrease is then followed

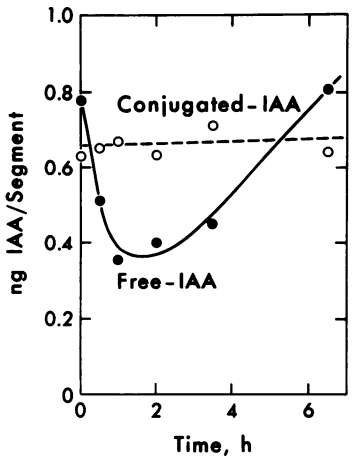

FIG. 5. Changes in free and conjugated IAA content of coleoptile segments following excision. A coleoptile segment, 1-cm long, was excised at 3 mm below the tip, and the leaves were removed. Twenty such segments were incubated for a given time floating on 10 ml H_2O in a shaken vial. The segments were then subjected to estimation of free and conjugated IAA content.

Table I. *Effect of Removal of the Coleoptile Tip on Elongation of the Mesocotyl*

Seedlings in two boxes were uncovered at the same time under safe IR radiation. From the seedlings in one box, coleoptile tips (3 mm) were removed; the seedlings in the other box were kept as undecapitated controls. Immediately afterward, a third box was transferred to a cold room (4°C) for an estimate of the initial mesocotyl length. These procedures, which took about 10 min to perform, were repeated three times. After another 8-h incubation, the decapitated and control seedlings were transferred to the cold room. Mesocotyl length was measured to the nearest 1 mm. Mean length (±SE) of 30 to 33 seedlings in each box is presented.

	Mesocotyl Length		
	Initial	After 8 h	
		Control	Decapitated
	mm		
	36.7 ± 0.9	50.5 ± 1.8[a]	45.3 ± 1.2[a]
	36.8 ± 1.2	51.3 ± 1.6[b]	46.5 ± 1.2[b]
	37.4 ± 1.0	52.9 ± 1.9[c]	47.3 ± 1.4[c]
Mean	37.0	51.6	46.4
Increment		14.6	9.4

[a, b, c] Means (control, decapitated) are significantly different (a and c, $0.01 < p < 0.02$; b, $0.02 < p < 0.05$).

by an increase due probably to IAA production induced by removal of the coleoptile tip. In spite of these changes, however, the conjugated IAA content remained constant (Fig. 5). Thus, the changes in the content of free IAA were not reflected in that of conjugated IAA. This suggests that free IAA and conjugated IAA are not freely interconvertible, at least in the coleoptile tissue used.

The IAA supplied from the coleoptile unit to the mesocotyl is probably produced from precursors, such as tryptophan or tryptamine, and not by release of IAA from its conjugates. Some of the IAA produced in the coleoptile unit appeared to be lost during its downward transport (10). This loss is more likely to be due to decomposition than to conjugation (10).

IAA and Mesocotyl Growth. Contrary to the finding of Beyer (3), Mer (13) has reported that decapitation of the coleoptile tip of oats does not inhibit elongation of the mesocotyl. If mesocotyl elongation is regulated by IAA, the result shown in Figure 4 leads to the expectation that coleoptile decapitation should inhibit mesocotyl growth. During 8-h incubation following decapitation, the increment of elongation was reduced to 64% of the undecapitated control (Table I). Mer's (13) claim cannot be sustained, at least for maize.

The hypothesis that mesocotyl elongation is regulated by auxin produced at the coleoptile tip is supported, with the provision that parts other than the coleoptile tip, particularly primary leaves, are also sources of auxin. The concept of auxin as a correlation carrier, which extends back to the time of its discovery (28), found support in parallel effects of environmental factors, *e.g.* light, on the growth and on the auxin status of plants. However, the supporting experimental evidence was later contradicted or questioned (7, 13, 14, 18). The dependence of the apical growing region of the mesocotyl on supplies of IAA from the coleoptile unit is shown in this study. Mesocotyl elongation will be an excellent subject for the further study of growth correlations mediated by auxin.

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