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Short Communication

Root-Growth Inhibitors from Root Tips of *Zea mays* L.

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Summary. At least two root-growth-inhibiting substances have been detected in extracts of *Zea mays* root tips. One has chromatographic properties similar to those of abscisic acid (ABA) and is largely confined to the cap. Another, which has not been identified, is present in the root apex but not in the cap. Indole-3yl-acetic acid (IAA) is never detected in the root cap. Xanthoxin may be present in the root tip.

For years doubt has been expressed that indol-3yl-acetic acid (IAA) has a role to play in geotropic responses of roots and a separate inhibitor arising in the tip has been postulated as the specific regulator involved (Audus and Brownbridge, 1957a, b). The elusiveness of IAA in roots, and its predominantly acropetal polarity of movement when applied to isolated segments (Scott and Wilkins, 1968; Wilkins and Scott, 1968), strongly supported these contentions. Recently the demonstrations that the root cap is the site of geotropic perception (Juniper, Groves, Landau-Schachar and Audus, 1966) and also the source of a polar-moving growth inhibitor (Gibbons and Wilkins, 1970; Shaw and Wilkins, 1973; Pilet, 1973) prompted an attempt to isolate and characterise this specific inhibitor. A preliminary account of this work follows.

Roots of 6-day-old seedlings of *Zea mays* L. var Golden Bantam were used. Three types of material were extracted, (1) caps dissected under the binocular microscope making sure to exclude all other tissue, (2) 5-mm root tips minus the cap and (3) whole 5-mm root tips including the cap. Samples, usually of 100 tips or caps, were plunged directly on harvesting into methanol at $-10°$C in a freezing mixture and then allowed to extract for 24 hours in the deep freeze. Extracts were fractionated by conventional methods (Kefford, 1955) and the acid fractions run on Whatman No. 1 paper by ascending chromatography in the isopropanol: ammonia:water (10:1:1) solvent system. The chromatograms were assayed for root-growth inhibitors by a root-segment micro-assay (*Zea mays* roots) sensitive to about 50 pg of IAA (Kundu and Audus, 1974) and by the *Commelina* stomata-closing assay (Tucker and Mansfield, 1971) for abscisic acid (ABA).

Cap extracts show predominantly one root-growth inhibitor running at Rf 0.5 to 0.7 (Fig. 1A). This has been called the "cap inhibitor". On the other hand, in tips without caps (Fig. 1B) a rather greater inhibition

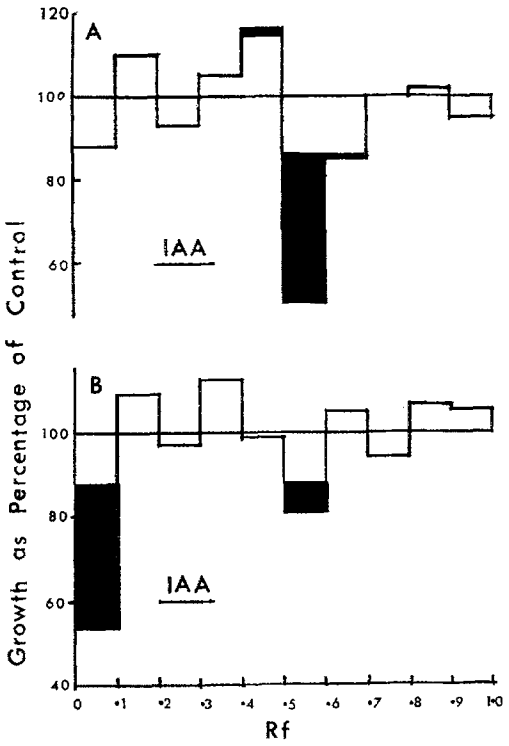

Fig. 1. A. Chromatogram of root cap extract assayed by the root-growth inhibition micro-assay. B. Chromatogram of extract of tips minus their caps assayed by the root-growth-inhibition micro-assay. The shaded areas of the chromatograms represent the responses which are outside the 5% fiducial limits of the assay

always occurs at Rf 0 to 0.1 and, like the cap inhibitor, is also completely consistent in its appearance. This has been called the "meristem inhibitor". Traces of the cap inhibitor are also occasionally observed in meristem extracts. A third inhibitor sometimes appears at Rf 0.9 to 1.0 in extracts of whole tips (Fig. 2A). Very rarely a slight stimulation or inhibition of growth has been found at the Rf of IAA but never in extracts of root caps. This could well be due to IAA, as indicated by the recent results of Bridges, Hillman and Wilkins (1973) who have shown it to be present predominantly in the stele. From our results it is clear that the "cap inhibitor" is *not* IAA.

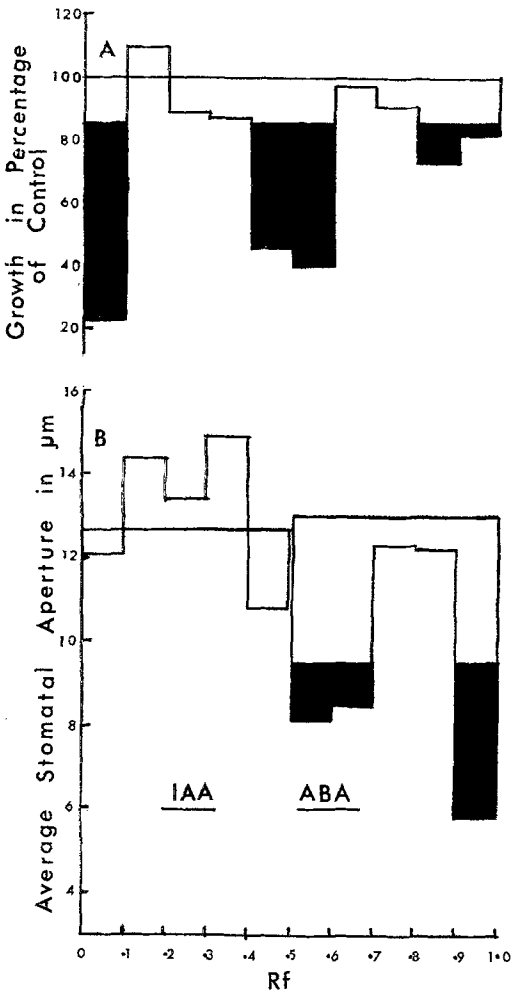

Fig. 2A and B. Chromatograms of extracts of whole tips (meristem plus cap). (A) Assayed by the root-growth-inhibition micro-assay. (B) Assayed by the *Commelina* stomata-closing assay. This chromatogram was assayed in two halves because of the restrictions of the experimental facilities and this accounts for the slightly different control levels in the two halves. The shaded areas of the chromatograms represent the responses which are outside the 5% fiducial limits of the assay

The *Commelina* assay of whole tip extracts (Fig. 2B) demonstrated the clear presence of abscisic acid-like activity at Rf values corresponding precisely with those of two of the inhibitors, i.e. at Rf 0.5 to 0.7 and 0.9 to 1.0 respectively. Furthermore authentic samples of abscisic acid (ABA)

ran at an Rf value in this solvent system corresponding with that of the cap inhibitor. Experiments are now under way to confirm this tentative identification by unambiguous methods.

The nature of the second ABA-like root inhibitor is more speculative. It has chromatographic properties resembling those of xanthoxin, which runs at Rf 0.85 in this solvent system (Taylor, 1969). Unpublished gas-chromatographic evidence from bulk extracts of *Zea* roots, obtained by Mrs. Lesley Stanbury in our laboratory, shows xanthoxin to be present.

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