Short Communication

Effects of Cytokininson Growth and Auxin in Coleoptiles of Derooted Avena Seedlings

William R. Jordan and Folke Skoog
Institute of Plant Development, Birge Hall, University of Wisconsin, Madison, Wisconsin 53706

It is known that removal of the endosperm (deseeding) reduces subsequent auxin production in the oat coleoptile tip and the "regeneration of the physiological tip" (auxin production) in the decapitated coleoptile as determined by the quantities of auxin which are secreted into agar blocks and assayed by the Avena curvature test (10). This decrease in auxin production is associated with parallel decreases in growth rate and geotropic responses of the coleoptile. However, the capacity of the coleoptile to respond to unilaterally applied auxin (the curvature response) is increased in deseeded plants as compared with intact controls, mainly owing to an increase in the time of active bending. Van Overbeek (11) found that removal of the roots of the Avena seedling brought about a similar decrease in auxin production and an increase in the curvature response of the coleoptile to unilaterally applied auxin. Subsequently Went (13, 14) showed that, in tomatoes, shoot growth is dependent upon hormone-like substances supplied by the root. Mothes and co-workers (3, 8) have demonstrated that substances from the roots are required for the maintenance of chlorophyll, protein, and RNA levels in the leaves, and that in detached leaves these factors can be replaced by cytokinins. The above observations, together with the evidence for cytokinins in the transpiration stream of many plants (5-7) have led Mothes and others to conclude that the roots supply cytokinins needed for growth and normal metabolism in the shoot.

Earlier work in this laboratory has shown that tobacco tissue cultures will grow without exogenous auxin if supplied with high levels of cytokinins, and we have now found that the tobacco cultures synthesize the auxin they require for growth. In the light of this effect of cytokinin on auxin biosynthesis, it appeared possible that the application of cytokinin would restore normal auxin production and growth rate in the coleoptiles of derooted seedlings. Results of experiments to test this premise are presented below.

MATERIALS AND METHODS

Hulled oats (Avena sativa L. var. Victory I) were soaked in tap water for 2 to 3 hr, arranged on wet cloth in Pyrex dishes, and placed in a Avena darkroom maintained at 24 C and high relative humidity. They were immediately exposed to red light for 3 to 4 hr to reduce first internode growth and then left in darkness. All subsequent manipulations were performed under red light. Plants prepared in this way were used for Avena assays and for both series of experiments described below.

For experiments on the effect of derooting on growth and the effect of cytokinins on coleoptile growth of derooted and intact plants, seedlings between 56 and 60 hr old were selected for uniform size and marked with India ink 3 or 5 mm below the coleoptile apex. The roots of some of these plants were snipped off at the seed with sharp scissors. Sets of 20 intact and derooted seedlings were immediately treated with various adenine derivatives by placing a 0.5-ml drop of 50% ethanol containing 5% carbowax 1500 and 15 μg of the substance to be tested on the tip of each plant. Synthetic preparations of the tested cytokinins, 2iP and BAP, and of the inactive analogues, 3-A2-isopentenyladenine (triacanthine) and 3-allyladenine, were kindly furnished by Professor N. J. Leonard. A microsyringe was used for the application. Plain ethanol-carbowax solution was applied to sets of intact and derooted plants used as controls. In a few cases test substances were applied to the base of the plants by placing the plants on a layer of cotton saturated with a water solution of the substance. The length of the marked apical portion of each coleoptile was measured with a millimeter ruler, after growth periods varying from 16 to 20 hr in different experiments.

For experiments on the effect of cytokinins on auxin secretion from coleoptile tips, the ethanol-carbowax solutions were applied to the tips either after 56 to 60 hr of growth or just 5 min before excision of the tips. For each treatment 36 2-mm tips were excised with a scalpel after 72 to 74 hr of growth and arranged basal end down on 0.15-ml agar platelets. Every hour for 5 hr each set of tips was transferred to a fresh platelet. Each platelet was then divided into 12 blocks for auxin assays by the Avena curvature test.

The assay was performed in the standard way (15) with uniform plants selected from the same batch of seedlings from which the experimental plants had been selected. During the assay the relative humidity in the Avena room was maintained near 88%. For quantitation of the response each assay included a standard series of IAA concentrations.

RESULTS

As illustrated by a typical experiment (Fig. 1), increase in length of the measured apical portion of the coleoptiles was about 50% less in derooted plants in the 20 hr following derooting than in intact controls. In coleoptiles of derooted plants supplied with 2iP immediately upon derooting the increase was about the same as in intact controls. The increase in intact plants treated with 2iP was only slightly greater than in intact controls. Thus treatment with cytokinin overcomes the effect of

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1 This work is dedicated to Professor Kurt Mothes on the occasion of his 70th birthday.

Abbreviations: 2iP: 6-(3-methyl-2-butenylamino)purine; BAP: 6-benzylaminopurine.
derooting on the growth of the coleoptile but has relatively little effect on the growth of intact plants. When derooted plants were placed on a layer of cotton saturated with a solution of 2iP, growth of the coleoptiles of derooted plants was restored to the level of intact controls when the concentration of 2iP was 100 \( \mu M \). A concentration of 5 \( \mu M \) only partially restored the growth rate. Thus 2iP restores the growth rate in the coleoptiles of derooted plants whether it is applied to the coleoptile tip or to the seed.

In order to determine whether the observed restoration of growth in coleoptiles of derooted plants is a true cytokinin effect, we tested four other adenine derivatives for this effect. The results of one of two experiments with essentially identical results (Fig. 2) show that derooted plants treated at the apex with 15 m\( \mu \)moles of two cytokinin-inactive substances (tri-

Fig. 1. Effects of derooting and 2iP on coleoptile growth in *Avena* seedlings. The seedlings were derooted 56 hr after germination. 2iP, 15 m\( \mu \)moles, was applied to each coleoptile tip. The increase in length of the upper 3-mm portion of the coleoptile in 16 hr after treatment are recorded. The vertical lines on bars represent standard deviations of the means.

Fig. 2. Effects of four adenine derivatives on coleoptile growth in derooted *Avena* seedlings. The seedlings were derooted 56 hr after germination. Fifteen millimicromoles of BAP, kinetin, triacanthine, or 3-allyladenine were applied to each coleoptile tip. The increases in length of the upper 5-mm portion of the coleoptiles in 16 hr are recorded.

acanthine or 3-allyladenine) dissolved in ethanol-carbowax solution elongate no more during the 20 hr following derooting than controls treated with plain ethanol-carbowax solution, while coleoptiles of derooted plants treated with cytokinin active substances (BAP or kinetin) elongate about 50% more rapidly than coleoptiles of derooted controls and nearly as rapidly as those of intact plants. Thus 2iP, kinetin, and BAP, which are highly active cytokinins as determined by the tobacco callus assay, were effective while both triacanthine and 3-allyladenine, which are inactive in the bioassay, were ineffective. Thus the capacity of the five adenine derivatives tested to overcome the effect of derooting on coleoptile elongation parallels the effectiveness of these compounds as cytokinins in the tobacco bioassay.

Similarly, when applied as 100 \( \mu M \) aqueous solution to the base of the plants, cytokinins (2iP and kinetin) restored elongation in the coleoptiles of derooted plants to the level of the intact controls, while 3-allyladenine had no effect.

In further experiments we tested the effect of cytokinins on
COLEOPTILE GROWTH AND AUXIN CYTOKININ

The observed restoration of growth rate in coleoptiles of derooted plants brought about by applied cytokinins suggests that coleoptile growth normally depends on a supply of cytokinins from the root. As the compounds tested, which, though chemically closely related to cytokinins, are not active as cytokinins in the cellus assay (11) did not restore root-dependent coleoptile growth, this is probably a specific cytokinin effect. The fact that cytokinin is as effective when applied in aqueous solution to the base of seedlings as when applied to the coleoptile tips probably means that the cytokinins are transported to and act in the tips. This is in accordance with Schrank's (9) earlier observations.

Secrecion of auxin by excised coleoptile tips normally declines steadily with time. The observed increase in the amount of auxin secreted and prolongation of the period of secretion resulting from cytokinin application suggest that secretion normally may depend on a cytokinin supply which rapidly becomes limiting in the feared tips. In that case the effect of roots on auxin secretion by coleoptile tips which Van Overbeek (12) described and correlated with coleoptile growth appears to be due to cytokinins.

The observed increase in auxin secretion brought about by cytokinin application is probably due to increased auxin production in the tip. This would be in accord with our finding that tobacco callus tissue provided with high concentrations of cytokinin can synthesize auxin as well as thiamine (1, 2). However, alternative or additional effects on rates of degradation or transport are not excluded. It is recognized that the origin of auxin in the coleoptile tip may differ from that in callus. The possibility that the increase is due to a cytokinin-directed accumulation of auxin precursors from the seed or other parts of the seedling (a Mothes eect) would seems practically precluded by experiments (Fig. 3A) in which the tips were excised within 5 min after the cytokinin was applied.

Application of cytokinin to coleoptiles of intact plants resulted in a repeatable though slight increase in elongation rate. Application of auxin (naphthaleneacetic acid, 1.5 or 15 μmoles per plant) to either derooted or intact plants may have increased growth slightly but by no means restored coleoptile growth of derooted plants to the level of the intact controls. Thus, the growth-promoting effect of cytokinins in derooted plants is not accounted for merely in terms of an influence on auxin secretion. Nevertheless, it appears likely that cytokinins are involved in the regulatory function exerted by roots on the growth of coleoptiles in intact seedlings.

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LITERATURE CITED