Lateral Movement of Radioactivity from $[^{14}\text{C}]$Gibberellic Acid (GA₃) in Roots and Coleoptiles of Zea mays L. Seedlings during Geotropic Stimulation

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Summary. An upward lateral movement of radioactivity from $[^{14}\text{C}]$gibberellic acid (GA₃) has been found to occur in geotropically stimulated coleoptiles and primary roots of intact Zea mays (L.) seedlings.

Introduction

The development of an asymmetric distribution of gibberellin-like activity between agar blocks in contact with the upper and lower halves of the basal end of detached horizontal shoots of Helianthus annuus and coleoptiles of Zea mays has recently been reported (Phillips, 1972; Railton and Phillips, 1973). Horizontal organs yielded a greater total of gibberellin-like activity than did vertical ones, and the lowermost receiver block of horizontal shoots contained up to 10 times more gibberellin-like activity than the upper block. More recently El-Antably and Larsen (1974) extracted the upper and lower halves of horizontal primary roots of Vicia faba and found the upper half to contain more gibberellin-like activity than the lower half.

In an attempt to establish the mechanism whereby the gradient of gibberellin-like activity was established in shoots, Wilkins and Nash (1974) investigated the movement of $[^{3}\text{H}]$GA₃ in sub-apical segments of Zea mays coleoptiles. No evidence of radioactivity emerging into a basal receiver block could be found even after a transport period of 24 h, and no downward lateral transport of radioactivity in the tissue was detected following the application of asymmetric donor blocks to sub-apical coleoptile segments.

In this paper we report a more detailed study of the possible lateral movement of radioactive gibberellic acid in geotropically stimulated coleoptiles and primary roots of intact, undamaged seedlings of Zea mays.

Materials and Methods

Fruits of Zea mays L. w. Giant White Horsetooth (Martin's Seeds Ltd., Norwich, England) were soaked for 8 h in flowing tap water and then planted out, embryo uppermost, on moist paper in covered plastic boxes which were placed in darkness at 25°C. Under these conditions the primary roots grew horizontally along the surface of the paper.

* Abbreviation: GA₃ = gibberellic acid.
For the experiments with roots, seedlings were used approximately 68 h after soaking when the primary roots had attained a length of about 25 mm. Immediately before an experiment, the seedlings were brought into white fluorescent light under which they were maintained for the duration of the experiment.

For the experiments with coleoptiles, seedlings were transferred individually, 56 h after soaking, to Pyrex vials which had previously been filled with 1% (w/v) agar (“Purified”, Oxoid Ltd., London). The seedlings were maintained in darkness at 25°C except for the transfer and experimental procedures which were carried out under dim green light. The seedlings were used when they had attained an age of 6 days.

The specific activity of the [17-14C]gibberellic acid (GA3) was 6.1 Ci M⁻¹ (Radiochemical Centre, Amersham, Berkshire, U.K.). Thin layer chromatography of the stock solution revealed one major peak of activity at Rf 0.11 using di-isopropyl ether:acetic acid, 95:5 v/v as the ascending solvent. The labelled GA3 cochromatographed with unlabelled GA3 which was located by spraying with 5% sulphuric acid in ethanol, and heating at 80°C.

The GA3 was applied to the seedlings as an aqueous solution at 2 × 10⁻³ M taken up in glass micropipettes similar to those used previously by Shaw and Wilkins (1974), and Shaw et al. (1973). The pipette containing the radioactive GA3 was placed on the surface of the coleoptile or root so that the sharp tip just penetrated the plant tissue for several seconds before being withdrawn. No change in the volume of solution in the pipette was detected as the result of this procedure and the pipette method thus provides effectively a point source for diffusion of GA3 into a highly localised region of the organ. The GA3 was applied to one side of the coleoptile or root at a point approximately 1 mm behind the apex. During the GA3 application, the seedlings were supported vertically. They were subsequently orientated vertically, or horizontally with the point of application on either the upper side or the lower side. At the end of the transport period, each coleoptile or root was bisected longitudinally and then divided transversely into several portions, as indicated in Figs. 1 and 3 for radioactive assay. Because of the low specific activity of the GA3, it was necessary to combine equivalent pieces of tissue from 12 seedlings to obtain workable levels of radioactivity.

The tissue was placed in scintillation vials and left to extract in 2 ml of ethanol for at least 24 h in darkness at 4°C. The ethanol was subsequently removed by evaporation under reduced pressure and 10 ml of scintillation fluid (PPO 4g/l in toluene) were added to each vial before counting. Radioactivity is expressed in disintegrations per minute (dpm). In an individual experiment there were three samples, each of 12 seedlings, for each particular treatment. Since the exact amount of GA3 applied to each seedling varied, the results have been calculated as the percentage distribution of radioactivity within each sample of twelve seedlings. Every experiment was carried out on at least 2 separate occasions; the data presented here are the mean of the separate experiments. Significant differences have been determined by students 't' test.

**Results**

**Coleoptiles.** The distribution of radioactivity in both horizontal and vertical shoots of *Zea mays* seedlings after asymmetric application of [14C] GA3 was followed as a function of time (Fig. 1). In each orientation most of the radioactivity remained in the apical 5 mm of the coleoptile even after 6 h; very little radioactivity moved into the more basal regions of the coleoptile. The distribution of radioactivity in the sub-apical region of the coleoptile was similar, regardless of the orientation of the seedlings with respect to gravity. The lateral distribution of radioactivity in the apical 5 mm of the coleoptile was, however, dependent upon orientation (Fig. 1). The proportion of radioactivity moving into the non-donated half of coleoptiles that were vertical, or orientated horizontally with the point of application on the upper side, was closely similar and increased with time from 7.4% at 0.5 h to 37% at 6 h (Fig. 2). In contrast, the proportion of radioactivity in the non-donated half of the horizontal coleoptile was much