The outer epidermis of *Avena* and maize coleoptiles is not a unique target for auxin in elongation growth

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Received 30 January; accepted 25 August 1991

**Abstract.** A controversy exists as to whether or not the outer epidermis in coleoptiles is a unique target for auxin in elongation growth. The following evidence indicates that the outer epidermis is not the only auxin-responsive cell layer in either *Avena sativa* L. or *Zea mays* L. coleoptiles. Coleoptile sections from which the epidermis has been removed by peeling elongate in response to auxin. The magnitude of the response is similar to that of intact sections provided the incubation solution contains both auxin and sucrose. The amount of elongation is independent of the amount of epidermis removed. Sections of oat coleoptiles from which the epidermis has been removed from one side are nearly straight after 22 h in auxin and sucrose, despite extensive growth of the sections. These data indicate that the outer epidermis is not a unique target for auxin in elongation growth, at least in *Avena* and maize coleoptiles.

**Key words:** Auxin-induced growth — *Avena* — (auxin-induced growth) — Coleoptile (peeled, growth) — Epidermis — *Zea* (auxin-induced growth)

**Introduction**

Coleoptiles have often been treated as if all cells were equally auxin-responsive in cell elongation. However, in 1937 Van Overbeek and Went reported that removal of the outer epidermis of *Avena* coleoptiles rendered them insensitive to auxin. More recently, Pope (1982) and Kutschera et al. (1987) concluded that auxin-induced wall loosening only occurs in the outer epidermal cell layers of *Avena* and maize coleoptiles. On the other hand, Thimann and Schneider (1938), Rayle (1973) and Cleland (1975) all reported extensive auxin-induced elongation in *Avena* coleoptiles from which the epidermis had been removed, and concluded that other cell layers were auxin-responsive as well. Since the peeling process often fails to remove the epidermis completely (Rubinstein and Stein 1980), Brummell and Hall (1980) and Kutschera et al. (1987) have suggested that the remaining longitudinal strips of epidermis are responsible for the auxin-growth response.

Resolution of this controversy is important. For example, the idea that auxin sensitivity is restricted to the outer epidermal cells has been used by Löbler and Klämbt (1985) as evidence that the auxin-binding protein, which was localized to the outer epidermis of maize coleoptiles by immunofluorescence, is the receptor responsible for auxin-induced growth. If coleoptile sections are capable of auxin-induced growth in the absence of the outer epidermis this argument would not be valid. Likewise, Bergfeld et al. (1988) found that auxin causes a reorientation of the cellulose microfibrils in the outer epidermal wall of maize coleoptiles but not in any of the inner walls, and suggested that this reorientation is responsible, at least in part, for the auxin-induced wall loosening (however, Edelmann et al. (1989) rejected this idea for other reasons). Kutschera et al. (1987) reported that auxin caused the deposition of electron-dense deposits in the outer epidermal wall of maize coleoptiles, and linked these deposits to wall loosening. Such phenomena which are restricted to outer epidermis cannot constitute a general mechanism of auxin-induced wall loosening, however, if cells other than the outer epidermis are capable of auxin-induced elongation.

The present studies were undertaken to determine the extent to which auxin could cause elongation in peeled *Avena* and maize coleoptiles, and whether such an auxin-induced growth could simply be due to remaining strips of epidermal cells.

**Materials and methods**

**Plant material.** Plant material consisted of 5- or 14-mm sections cut 3 mm from the tip of 25-32 mm coleoptiles of *Avena sativa* L., cv. Victory (Swedish General Seed Co., Svalöf) or *Zea mays* L., cv.
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B73 × Mo17 (Brayton Seeds, Ames, la., USA). Seeds (caryopses) were planted in wet vermiculite and plants were grown for 90 h (Avena) or 120 h (maize) under dim red light (> 5 μmol · m⁻² · s⁻¹). The enclosed leaf was removed.

To obtain peeled sections, the epidermis was removed from the apical 20 mm of the coleoptile with fine jewelers forceps in two (Avena) or four (maize) strips. Sections were then cut to length, their initial length was measured, and they were placed immediately into the incubation solutions. In some experiments the epidermis was removed from only one side of the section.

Incubations. The incubation solutions contained, where indicated, 0.1 mM K-phosphate buffer, pH 6.0, 10 μM indole-3-acetic acid (IAA), 10–50 mM sucrose, 5 or 10 mM KCl, 0.1 mM CaCl₂. In some experiments sections were incubated in 3 ml of solution in test tubes, 100 mm long, 25 mm wide, which were rotated at 1 rpm on a Rollardrum (New Brunswick Scientific, New Brunswick, N.J., USA) and the solutions were changed after each measurement. In other experiments the sections were incubated in 10 ml of solution in plastic Petri dishes, 60 mm diameter, 15 mm high, rotated at 50 rpm on a rotary shaker. In this case the solutions were not changed.

Assays. Section lengths were measured with a microscope fitted with an eyepiece micrometer. The extent to which the epidermis had been removed in peeled sections was determined at the end of each experiment by incubating the sections in 0.1% Neutral red in distilled water for 1 min. After rinsing in water, the sections were examined under a dissecting microscope. Wherever the epidermis had been removed the tissue was stained pink; wherever the epidermis was intact it was yellow. The percentage of the circumference from which the epidermis was removed was then estimated by examining the section.

Replication. All experiments reported here were carried out a minimum of 3 times, and most were repeated over 10 times. Standard errors were determined for all measurements. They are not shown in Fig. 1 for clarity and because they were only slightly larger than the symbols.

Results

*Avena* coleoptile sections with an intact epidermis elongated rapidly in response to auxin. Sucrose and KCl had little effect on this initial elongation, but when they were present in optimal concentrations, the auxin-induced growth persisted at a nearly constant rate for at least 18 h (Fig. 1A); in the absence of an absorbable solute the growth rate began to decline after 3–6 h and fell to the minus-auxin control level after 12–15 h (Fig. 1B).

Sections from which the epidermis had been stripped also elongated in response to auxin. In the presence of 20 mM sucrose and 10 mM KCl, auxin-induced growth occurred with nearly identical kinetics in peeled and intact sections (Fig. 1A). Under these conditions the growth of both intact and peeled sections often exceeded 100% extension. In the absence of auxin the initial growth of peeled sections exceeded that of intact sections, but by 24 h there was no difference in length.

In the absence of an absorbable solute the growth curves for auxin-treated peeled and intact sections were similar. After an initial period of rapid elongation the growth rate declined until growth was almost stopped by 12 h (Fig. 1B). In the absence of both solutes and auxin, peeled sections elongated more rapidly than intact sections during the first hour, but thereafter the growth rate was low in both cases.

Removal of the epidermis with its associated cuticle did not change the kinetics of auxin-induced growth, but it did alter the dosage-response curve to auxin. Figure 2