

Root growth regulation and gravitropism in maize roots does not require the epidermis

Thomas Björkman* and Robert E. Cleland

Department of Botany, KB-15, University of Washington, Seattle, WA 98195, USA

Received 11 November 1990; accepted 19 March 1991

Abstract. We have earlier published observations showing that endogenous alterations in growth rate during gravitropism in maize roots (*Zea mays* L.) are unaffected by the orientation of cuts which remove epidermal and cortical tissue in the growing zone (Björkman and Cleland, 1988, *Planta* **176**, 513–518). We concluded that the epidermis and cortex are not essential for transporting a growth-regulating signal in gravitropism or straight growth, nor for regulating the rate of tissue expansion. This conclusion has been challenged by Yang et al. (1990, *Planta* **180**, 530–536), who contend that a shallow girdle around the entire perimeter of the root blocks gravitropic curvature and that this inhibition is the result of a requirement for epidermal cells to transport the growth-regulating signal. In this paper we demonstrate that the entire epidermis can be removed without blocking gravitropic curvature and show that the position of narrow girdles does not affect the location of curvature. We therefore conclude that the epidermis is not required for transport of a growth-regulating substance from the root cap to the growing zone, nor does it regulate the growth rate of the elongating zone of roots.

Key words: Epidermis and root growth – Gravitropism (root) – Root (growth) – *Zea* (root growth and gravitropism)

Introduction

Current models for root growth regulation assume that the epidermal layer limits root growth (Tomos et al. 1989) and that the epidermal growth rate is regulated by the amount of growth regulator moving from the root cap (Evans et al. 1986). Using gravitropism as a means of endogenously altering the growth rate, we earlier re-

ported evidence that the epidermis was not necessary for growth regulation during gravitropic curvature (Björkman and Cleland 1988). To eliminate wounding as a variable, we removed tissue from two roots flanks and found that curvature was the same regardless of whether the remaining tissue was oriented to maximize or to minimize the gradients of growth regulator and mechanical restriction. A recent report (Yang et al. 1990) has caused us to reexamine how complete removal of the epidermis affects gravitropism, and to what extent wounding confounds the interpretation of such data.

In the present paper we test two hypotheses: 1) If the epidermis is the growth-limiting cell layer, then gravitropic curvature will be eliminated by removing the epidermis in the elongating zone; 2) if the epidermis is the sole path through which the growth regulator moves, then gravitropic curvature will be eliminated by removing the epidermis between its source, the root cap, and the elongating zone. If curvature occurs, these hypotheses may be rejected. Failure to curve does not allow them to be accepted however, because the present protocol does not allow us to distinguish between loss of response caused by the shock of wounding and that caused by removing essential cells.

Material and methods

Seeds of maize (*Zea mays* L. cv. 'Merit'; Asgrow Seeds, Kalamazoo, Mich., USA) were wrapped in germination paper (Anchor Paper, St. Paul, Minn., USA) wetted with 10 mM KCl, 0.5 mM CaCl₂, 1 mM 2-(N-morpholino)ethanesulfonic acid (Mes)-KOH, pH 6. Seedlings were grown under red lights at 23° C for approx. 40 h until the primary roots were 10–20 mm long. The roots were girdled by scoring the epidermis with a scalpel, then pulling off a thin layer of cells using fine forceps. The cells separated at a depth determined by the tissue structure, estimated as the epidermis and one or two cortical cell layers (Yang et al. 1990). For curvature assays, the roots were mounted in humidified plastic boxes (30 · 15 · 8 cm) and after 90 min in the vertical position (to eliminate effects of handling during girdling) the boxes were turned 90°.

The roots were photographed when turned and again 2 h later. The amount of curvature, the location of the girdle and of the

* Corresponding author; present address: Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456-0462, USA

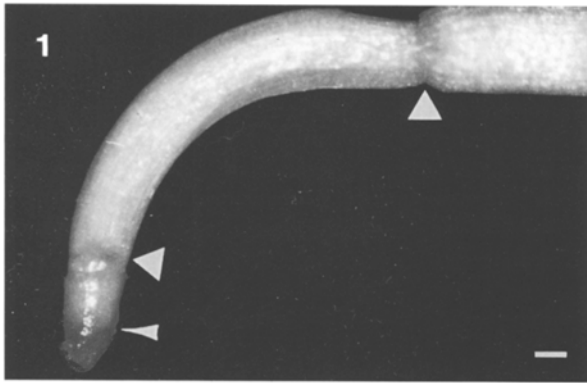


Fig. 1. Complete removal of the epidermis of a maize root from the cap through the elongating zone (0.3 to 3 mm from root pole) did not prevent gravitropic curvature. The root was gravistimulated for 2 h. *Triangles* indicate the extremes of the girdled region. *Arrowhead* indicates the root pole, used as the reference position throughout this paper. $\times 8.3$; bar = 0.5 mm

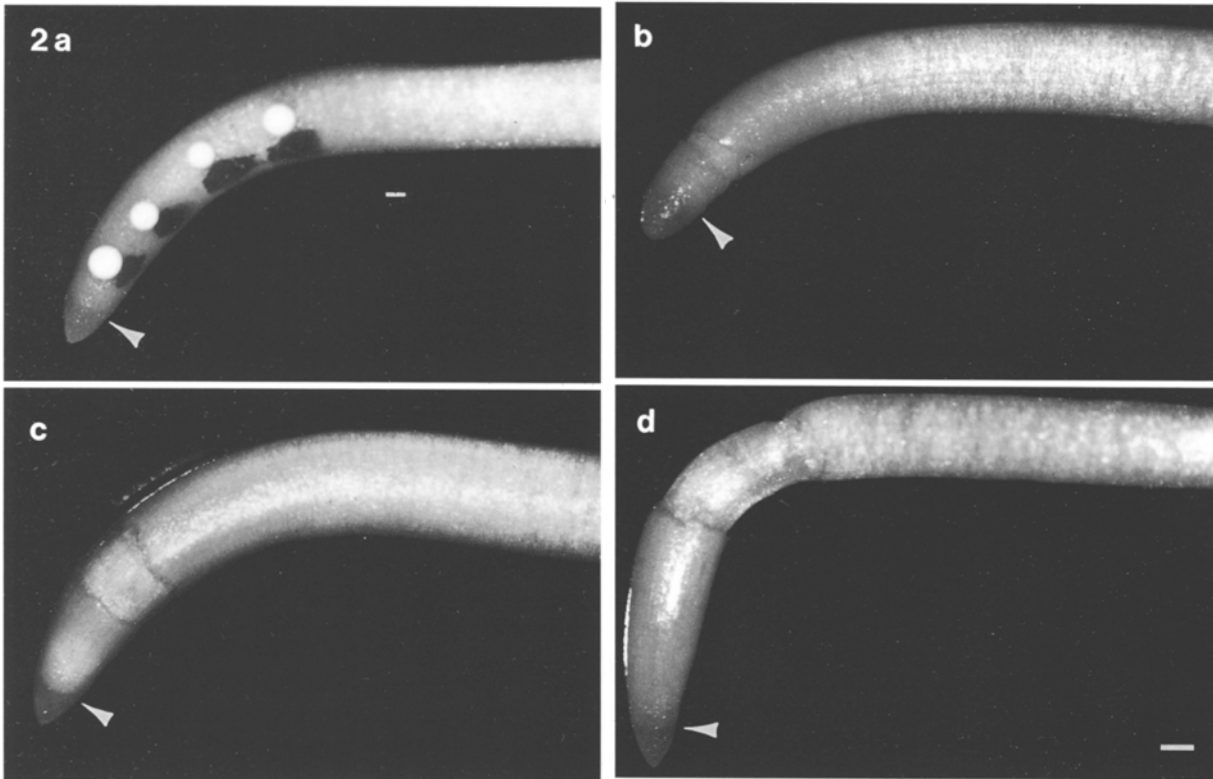


Fig. 2a–d. Gravitropic curvature in maize roots girdled at different distances from the tip. After girdling, roots were vertical for 1 h then horizontal for 3 h. These are the treatments referred to in Figs. 3 and 4. $\times 8.3$; bar = 0.5 mm. **a** Intact root marked with Sephadex beads at 0.5, 1, 1.5 and 2 mm from the pole, but not girdled, then treated the same as girdled roots. These marks indicate where the cells which would have been removed by girdling end up after 4 h. **b** Root girdled 0.5 to 0.7 mm from pole. **c** Root girdled

1.0 to 1.5 mm from pole. **d** Root girdled 1.5 to 2.0 mm from pole. *Arrowheads* indicate root pole. In **b** and **c**, the girdle has not prevented a differential growth signal from reaching the growing region. In **d**, note that 4 h after girdling, growth has displaced the girdle relative to the tip so that it is in the curved region (it extends from 2.2 to 3.8 mm from pole); growth was well-constrained in the absence of the epidermis

curvature were determined from enlarged images of the film negatives. The micrographs shown in this article were made at the end of the 2-h curvature period on a Zeiss DRC stereomicroscope with an MC63 camera (Carl Zeiss, Oberkochen, FRG).

Results

When the outer cell layers were removed from both the region where regulator transport occurs and from the elongating zone, gravitropic curvature was strong and rapid (Fig. 1). Therefore, removing the outer cell layers in this manner did not produce a severe wound response,

the gradient of gravitropic effector was preserved during transport, and differential growth was possible.

In order to reconcile this observation with the data of Yang et al. (1990), we made narrow girdles at different positions along the root tip. Examples of the curvature obtained are shown in Fig. 2. In Fig. 3, the location of the girdle after the 2-h horizontal exposure is plotted against the position of the middle of the curved region. If the girdle was blocking the transmission of the gravitropic effector, bending should always have been on the tip side of the girdle (to the right of the diagonal line in Fig. 3). In fact, when girdles were placed close to the tip,

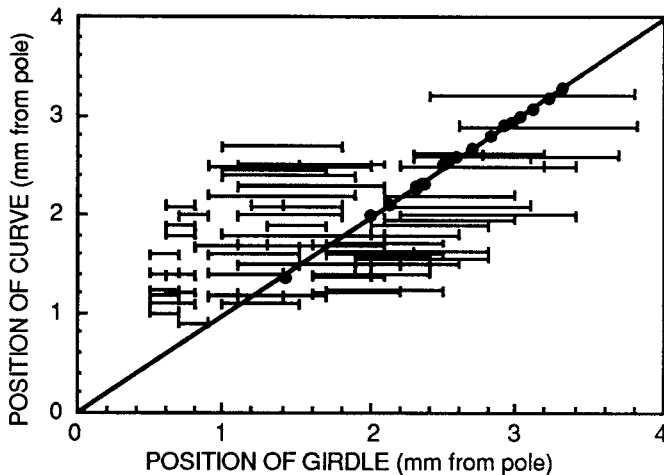


Fig. 3. Relationship between the position of the girdle and the resulting curvature. Maize roots were girdled from 0.5 to 0.7, 1.0 to 1.5, or 1.5 to 2.0 mm from the root pole; the horizontal lines indicate the position of the girdled zone at the end of the experiment. Solid circles on the diagonal line indicate where curvature was on intact roots. Data left of the diagonal line are roots which curved basipetal to the girdle. If the epidermis were the pathway for growth-regulator transport, no such curvature would be observed. If the epidermis had an essential role in growth regulation, all the observations would have been below the diagonal

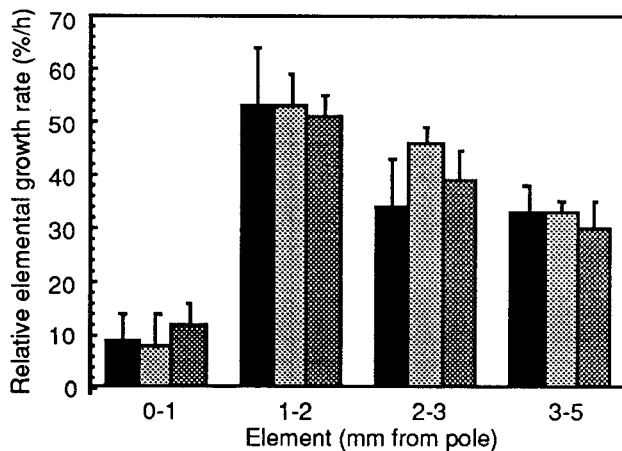


Fig. 4. Relative elemental growth rates of maize root tips during the first 2 h after gravistimulation, measured at the central axis of the root. Sections are in mm from the root pole. Roots were intact (black), or girdled at 1.0–1.5 mm (light gray) or 1.5–2.0 mm (dark gray) from the root pole

the girdle was always between the tip of the root and the apex of curvature. After 2 h, the middle of the curved region was 1.8 ± 0.5 mm ($n=20$) from the root pole (the junction of the root body with the root cap) in girdled roots. The curvature in all the girdled treatments was nearer the tip of the root than in intact roots (2.6 ± 0.5 mm), $n=66$; $P<0.0001$), but it made no difference whether the girdle was made acropetal or basipetal to the zone of curvature (Fig. 3). Although girdles caused roots to bend closer to the tip, there was no

evidence that they hindered bending basipetal to the girdle.

In those roots which were girdled between 1.5 and 2.0 mm from the pole growth of the root during the experiment resulted in displacement of the girdle beyond the zone where curvature developed (Fig. 2d). The displacement of the girdled section away from the tip as the root grew while bending, combined with wounding causing curvature closer to the tip, sometimes caused the girdle to be basipetal to the curved region. This shifting of reference points could give the impression that the girdle inhibited transport from the cap even if that was not happening.

The growth rate of marked segments of roots was measured for different girdling treatments. If the growth regulator moved exclusively in the epidermis, the growth rate behind the girdle should have increased because of relief from growth inhibition, and the region in front of the girdle should have grown more slowly. In fact, the elemental growth rate was unaffected by girdling (Fig. 4).

Discussion

A growth-regulating substance, presumed to be auxin (Jackson and Barlow 1981), moves from the root cap and inhibits the growth of the elongating zone of roots. Variation in the amount of this growth regulator coming from the cap would vary the growth rate of the root. We are trying to locate the pathway through which the growth regulator moves, and to determine on which cells it acts. Green (1980), in his seminal review of morphogenic regulation, ascribed a special role to the epidermis in constraining growth. This model has been extended to root gravitropism to give a model in which a growth regulator moves from the root cap through the outer layers of the root, and in which the outer wall of the epidermis is the mechanically limiting structure (Tomos et al. 1989). Our data lead us to reject both of these ideas.

Bilateral removal of tissue (Björkman and Cleland 1988) allowed us to distinguish the decreased responsiveness caused by wounding from the specific requirement for a particular piece of tissue. Such a comparison is not possible in the present experiments, nor in those of Yang et al. (1990) because the intensity of the wounding is different in each treatment. The position of the girdle affects whether meristematic or mature cells are removed, the depth of the girdle also affects the number and type of cells removed. We cannot determine by this circumferential girdling which cell layer below the epidermis is essential for regulating growth because progressively deeper wounding will also inhibit curvature through a progressively greater wound response. However, the present girdling experiments do allow us to eliminate the possibility that the asymmetric gradient of growth substances was being carried in the epidermis of the lateral strips.

If the epidermis plays a special role in transport or response, placing a girdle where growth-regulator transport occurs or in the part of the elongating zone where

the root bends, should prevent curvature in that region and instead cause it to occur acropetal to the girdle. The position of the girdle had little effect on the location of curvature (Fig. 3). We obtained all possible relative positions of curvature and the girdle, the proportion of each depending on where the girdle was placed relative to where the root normally bends. In fact, the root could be stripped from the root cap through the entire growing region without blocking curvature (Fig. 1). Therefore, the epidermis does not appear to have a special role in transport of, or response to, the gravitropic effector.

There is also evidence from wheat roots that the epidermis is not essential for regulating root growth. Burström (1949) found that application of 10 μ M diamylacetic acid caused root epidermal cells to stop elongating but root growth was unhindered, the root simply shed the curled-up epidermal cells. The outer epidermal wall clearly limited extension of the epidermal cells, but not of the entire root. The roots lacking an epidermis grew normally and responded to auxin (α -naphthylacetic acid), as we found with mechanically stripped roots (Björkman and Cleland 1988). Thus, the epidermis does not constrain root elongation, nor is it required for auxin to regulate root growth.

We do not believe that a gradient in growth regulator at the apical end of a cut would be maintained after diffusion around the girdle, as suggested by Yang et al. (1990), because diffusion is uniform, not axial. The distinct curvature on the basipetal side of the girdle (Fig. 2) requires that the differential in growth regulator be well maintained, and that this requires a substantially intact transport pathway.

For roots in their natural growing conditions, it would be inefficient for a growth regulator to move through the apoplast at the root surface. The auxin gradient is estimated at about 1.5:1 between the two sides

of the tissue (Young et al. 1990). It seems unlikely that such a small gradient could be precisely maintained in the epidermal apoplast in the presence of soil solution since the root has no cuticular barrier. It seems more likely that such a precise transport would occur in a more internal part of the root.

We observed normal curvature even when all of the epidermis that might play a role in gravitropism was removed. Therefore, we conclude that the effector regulating root growth need not move through the outer cell layers in primary roots of maize. We further conclude that the growth-regulating tissue, which is the target of the regulating signal from the cap, is not the epidermis.

References

- Björkman, T., Cleland, R.E. (1988) The role of the epidermis and cortex in gravitropic curvature of maize roots. *Planta* **176**, 513–518
- Burström, H. (1949) Studies on growth and metabolism of roots. I. The action of *n*-diamyl acetic acid on root elongation. *Physiol. Plant.* **2**, 197–209
- Evans, M.L., Moore, R., Hasenstein, K.-H. (1986) How roots respond to gravity. *Sci. Am.* **254**, Dec., 112–118
- Green, P.B. (1980) Organogenesis – a biophysical view. *Annu. Rev. Plant Physiol.* **31**, 51–82
- Jackson M.B., Barlow, P.W. (1981) Root geotropism and the role of growth regulators from the cap: a re-examination. *Plant Cell Environ.* **4**, 107–123
- Tomos, A.D., Malone, M., Pritchard, J. (1989) The biophysics of differential growth. *Environ. Exp. Bot.* **29**, 7–23
- Yang, R.L., Evans, M.L., Moore, R. (1990) Microsurgical removal of epidermal and cortical cells: Evidence that the gravitropic signal moves through the outer cell layers in primary roots of maize. *Planta* **180**, 530–536
- Young, L.M., Evans, M.L., Hertel, R. (1990) Correlations between gravitropic curvature and auxin movement across gravistimulated roots of *Zea mays*. *Plant Physiol.* **92**, 792–796