

## Correlations between proton-efflux patterns and growth patterns during geotropism and phototropism in maize and sunflower

Timothy J. Mulkey, Konrad M. Kuzmanoff, and Michael L. Evans

Department of Botany and Cellular, Molecular and Developmental Biology Program, Ohio State University, Columbus, OH 43210, USA

**Abstract.** By placing seedlings of sunflower (*Helianthus annuus* L.) or maize (*Zea mays* L.) on agar plates containing a pH indicator dye it is possible to observe surface pH patterns along the growing seedling by observing color changes of the indicator dye. Using this method we find that in geotropically stimulated sunflower hypocotyls or maize coleoptiles there is enhanced proton efflux on the lower surface of the organ prior to the initiation of curvature. As curvature develops the pattern of differential acid efflux becomes more intense. A similar phenomenon is observed when these organs are exposed to unilateral illumination, i.e. enhanced acid efflux occurs on the dark side of the organ prior to the initiation of phototropic curvature and the pattern of differential acid efflux intensifies as phototropic curvature develops. These observations indicate that differential acid efflux occurs in response to tropistic stimuli and that the acid efflux pattern may mediate the development of tropistic curvatures.

**Key words:** Acid growth – Geotropism – *Helianthus* – Phototropism – Proton secretion – *Zea*.

### Introduction

There is considerable evidence supporting the hypothesis that proton ( $H^+$ ) efflux mediates the action of auxin on growth (for a recent review, see Rayle and Cleland 1977). Growth-promotive concentrations of auxins promote  $H^+$  efflux in stems and stem-like tissues and this enhancement of  $H^+$  efflux precedes the stimulation of growth (Evans and Vesper 1980). These findings along with the well-established ability of exogenously applied acid to promote growth (Rayle and Cleland 1970) indicate that auxin-induced acid efflux plays a causal role in the enhancement of stem elongation.

If acid efflux is correlated with growth, then one would expect environmentally-induced modifications

of growth (e.g. tropistic responses) to be accompanied by modification of  $H^+$ -efflux patterns. We recently reported (Mulkey and Evans 1981) that, in geotropically responding primary roots of maize, there is more acid efflux on the more rapidly growing upper portion of the elongation zone of the root than on the more slowly growing lower portion. This pattern of differential acid efflux appears to be established before the initiation of geotropic curvature. These findings and the evidence that root growth is strongly dependent upon acid pH (Evans et al. 1980) indicate that differential acid efflux patterns may play a causal role in the curvature that develops in geotropically stimulated roots. If so, two questions come to mind: 1) What is the relationship between the gravitational stimulus and the establishment of differential acid efflux, i.e. is lateral redistribution of auxin required or does the differential acid efflux occur as a more direct response to gravity? 2) Does differential acid efflux occur during geotropism in other organs and does it occur during other tropistic responses such as phototropism? This report focuses on the latter question. We have examined  $H^+$  efflux patterns in geotropically and phototropically responding coleoptiles of maize and in hypocotyls of sunflower. In all cases we find greater  $H^+$  efflux on the rapidly growing side of the responding organ, indicating that differential  $H^+$  efflux may be a general feature of tropistically responding plant organs.

### Materials and Methods

The experiments were done with seedlings of maize (*Zea mays* L., hybrid WF9 × 38MS; Customaize, Momence, Ill., USA) and sunflower (*Helianthus annuus* L., cv. Russian; Boulevard Gardens, Columbus, OH., USA). Maize grains were soaked in tap water for 8 h and then placed between wet (distilled water) paper towels on plastic trays (24 × 32 × 1.5 cm, [long · wide · high]). The trays were held in a vertical position with the embryo of the grains aligned along the vertical axis. They were kept in the dark at about 26° C for 24 h and then exposed to fluorescent "ruby-red" lamps (F20T12-R; General Electric, Lexington, Ky., USA) for 8 h (fluence about 12.0 mW m<sup>-2</sup>). After this, they were returned to

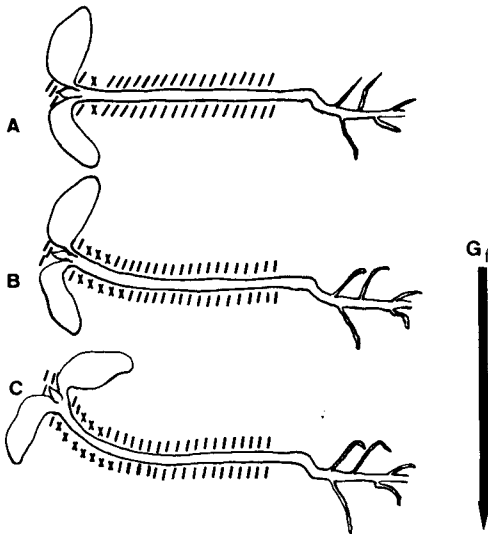


Fig. 1A–C. Proton efflux patterns in geotropically responding sunflower hypocotyls. In this and the subsequent figures, regions of  $H^+$  efflux are indicated by **XXXX**; regions of  $H^+$  uptake are indicated by **////**. Times elapsed after placing the seedling on the agar indicator-dye agar are: A 5 min; B 120 min; C 240 min.  $G_r$  indicates the direction of gravitational stimulation

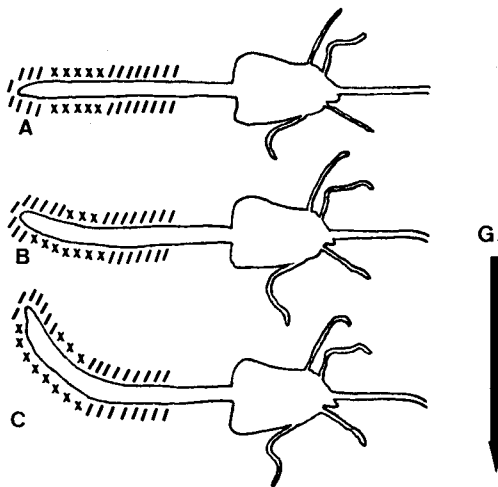


Fig. 2A–C. Proton efflux patterns in geotropically responding maize coleoptiles. A 5 min; B 90 min; C 180 min.  $G_r$  indicates the direction of gravitational stimulation

the dark and used for experiments 3.5 d after planting. Sunflower seeds were soaked in tap water for 8 h with the water changed each hour. They were then planted between wet paper towels on plastic trays as with the maize grains except that they were allowed to grow for 6 d in a growth chamber with 16 h light daily and a temperature of 25° C during the light and 20° C during the dark periods. Light was from cool-white fluorescent lamps (F96T12-CW-1500; General Electric) supplemented with incandescent lamps. Fluence was about 2.5  $W\ m^{-2}$  at the plant surface.

The  $H^+$  efflux patterns were visualized using the method of Weisenseel et al. (1979) with modifications as described by Mulkey and Evans (1981). Briefly, the seedling to be studied is placed on a 4-mm plate of agar (0.4%) containing the pH indicator dye, bromocresol purple (0.71 mM), plus inorganic nutrients (Mulkey

and Evans 1981). The organ to be observed (coleoptile or hypocotyl) is pressed into the agar so that about half of its circumference is in contact with the agar. The agar is prepared at pH 5.0. At this pH the indicator dye is orange. In regions along the seedling where sufficient  $H^+$  efflux occurs to reduce the pH of the medium to 4.8 or less, the agar turns yellow. Over the pH range 5.5–6.4 the indicator color is red; above pH 6.4 it turns purple. Thus regions of  $H^+$  efflux or uptake along the surface can be visualized by observing color changes.

For geotropism experiments the plate is mounted in a vertical position with the coleoptile or hypocotyl oriented perpendicular to the direction of gravity and observations of color changes are made under normal laboratory lighting. For phototropism experiments, the plate is mounted in a vertical position with the coleoptile or hypocotyl oriented parallel to the direction of gravity. The plate is placed in a black box with a slit on one side through which cool-white fluorescent light (F15T8-CW; General Electric) is shone on the seedlings (fluence about 0.9  $W\ m^{-2}$  at the plant surface).

When using this method to study  $H^+$  efflux patterns in roots (Mulkey and Evans 1981) no alteration of the root surface was necessary. In order to obtain color changes on plates containing coleoptiles or hypocotyls we found it necessary to abrade (Evans and Vesper 1980) the organ prior to placing the seedling on the agar.

## Results

The acid efflux/influx patterns which develop in geotropically stimulated shoots are illustrated in Fig. 1 for sunflower hypocotyls and Fig. 2 for maize coleoptiles. In these figures and in Fig. 3 and 4, the yellow (acidification) zones are indicated by **XXXX** while the reddish zones where pH has increased above 5.5 are represented by **////**. In horizontally mounted sunflower hypocotyls a uniform zone of acidification develops just below the cotyledonary node. This becomes apparent within 3–5 min after placing the seedling on the agar (Fig. 1A). After 120 min negative geotropic curvature is underway and it is clear that the region of acidification is more intense and more widespread on the lower portion of the hypocotyl than on the upper portion (Fig. 1B). After 240 min, geotropic curvature is stronger and the differential  $H^+$  efflux pattern is even more striking (Fig. 1C). Using maize coleoptiles the same results are obtained except that both the curvature and the differential  $H^+$  efflux patterns develop more rapidly (Fig. 2).

The acid efflux/influx patterns which develop in unilaterally illuminated shoots are illustrated in Fig. 3 for sunflower hypocotyls and Fig. 4 for maize coleoptiles. In both cases a uniform region of acidification develops within 3–5 min after placing the seedlings on the agar. In sunflower seedlings the acid region is just below the cotyledonary node (Fig. 3A). In maize coleoptiles the acid region begins 1 or 2 mm behind the tip (Fig. 4A). After 90 min phototropism is underway and  $H^+$  efflux is clearly stronger on the dark side in both cases (Figs. 3B, 4B). As phototropic

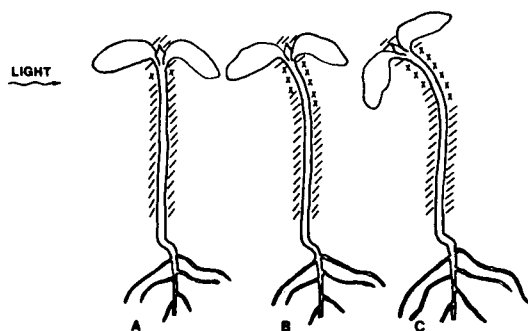


Fig. 3A-C. Proton efflux patterns in phototropically responding sunflower hypocotyls. Times elapsed after placing seedlings on the indicator-dye agar with unilateral illumination are: A 5 min; B 90 min; C 240 min

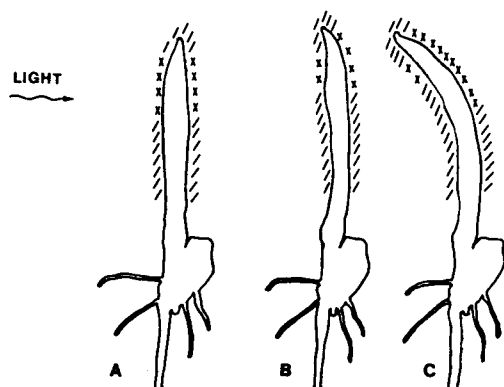


Fig. 4A-C. Proton efflux patterns in phototropically responding maize coleoptiles. Times elapsed after placing seedlings on the indicator-dye agar with unilateral illumination are: A 5 min; B 90 min; C 240 min

curvature develops the differential H<sup>+</sup>-efflux pattern becomes more intense in both cases (Figs. 3C, 4C).

### Discussion

The fact that differential H<sup>+</sup> efflux occurs during both phototropism and geotropism in both the monocotyledonous and dicotyledonous seedlings studied here indicates that directional H<sup>+</sup> efflux may be a common feature of tropistic responses. This suggestion is supported by our earlier finding that geotropism in maize roots is accompanied by enhanced acid efflux in the elongation zone on the rapidly growing upper half of the root. In geotropism of maize roots (Mulkey and Evans 1981) as well as in both geotropism and phototropism in shoots of the seedlings studied here, one can observe the development of differential acid efflux patterns before the initiation of curvature. This leaves open the possibility that differential acid efflux plays a causal role in the development of tropistic curvatures.

Ganot and Reinhold (1970) found that acid but not auxin was capable of inducing curvature in sunflower hypocotyl segments which had been depleted

of nutrients and exposed to geostimulation. They pointed out that their findings indicate that geotropic stimulation may induce asymmetry in the tissue with respect to acid sensitivity, with the lower half of the hypocotyl developing enhanced sensitivity to low pH. Since they were using segments which were neither peeled nor abraded, a second interpretation of their results may be that the curvature observed upon application of acid was the consequence of a combination of slow penetration of exogenously applied acid on all sides plus enhanced acid efflux from cells on the lower side. Since the tissue was depleted of nutrients it may have been that the preferential H<sup>+</sup> efflux on the bottom half was weak enough to require the combined effect of slow uniform penetration of exogenously applied acidic buffer to trigger an acid-growth response in the lower half of the segments.

In a related study Bridges and Wilkins (1973) reported that growth can be initiated in segments of the geotropically sensitive leaf sheath base of wheat (*Triticum aestivum*) either by placing the segment in a horizontal position or by treating the segment with acid. They speculated that "a localized increase in proton concentration in the lower half of the horizontal organ could give rise to growth and hence upward curvature". Similarly, Jaffe and Nuccitelli (1977) suggested that the electrical potentials which develop across geotropically and phototropically responding shoots might result from auxin-mediated asymmetric proton secretion. Our results provide experimental support for these suggestions.

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### References

- Bridges, I.G., Wilkins, M.B. (1973) Acid-induced growth and the geotropic response of the wheat node. *Planta* **114**, 331-339
- Evans, M.L., Mulkey T.J., Vesper M.J. (1980) Auxin action on proton influx in corn roots and its correlation with growth. *Planta* **148**, 510-512
- Evans, M.L., Vesper, M.J. (1980) An improved method for detecting auxin-induced hydrogen ion efflux from corn coleoptile segments. *Plant Physiol.* **66**, 561-565
- Ganot, D., Reinhold, L. (1970) The "acid-growth effect" and geotropism. *Planta* **95**, 62-71
- Jaffe, L.F., Nuccitelli, R. (1977) Electrical controls of development. *Ann. Rev. Biophys. Bioeng.* **6**, 445-476
- Mulkey, T.J., Evans, M.L. (1981) Geotropism in corn roots: Evidence for its mediation by differential acid efflux. *Science*, **212**, 70-71
- Rayle, D.L., Cleland R.E. (1970) Enhancement of wall loosening and elongation by acid solutions. *Plant Physiol.* **46**, 250-253
- Rayle, D.L., Cleland, R.E. (1977) Control of plant cell enlargement by hydrogen ions. *Curr. Topics Dev. Biol.* **11**, 187-214
- Weisenseel, M.H., Dorn A., Jaffe, L.F. (1979) Natural H<sup>+</sup> currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). *Plant Physiol.* **64**, 512-518

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