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Short Communication

Inhibition of Shoot Geotropism by Neutral Buffers

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ABSTRACT

Submerged hypocotyl sections from *Helianthus* have been used to test the effect of neutral buffers on shoot geotropism. When hypocotyls have been abraded, it is found that increasing the molarity (0.25 to 20 mM) of pH 6.8 K-phosphate buffer, as well as other buffering systems, results in a strong inhibition of geotropic curvature. Buffer strength has no such effect on the curvature of nonabraded segments. One possible explanation for these data is that asymmetric shoot growth following geostimulation may require the establishment of a proton gradient across the cell walls of the shoot. When neutral buffers have access to the wall space (*i.e.* in abraded segments), they may prevent the establishment of such a gradient.

There is substantial evidence from straight-growth systems that auxin-induced cell elongation is mediated, at least in part, by the excretion of protons from the protoplast into the cell wall region (3, 4, 10). In spite of the strength of these observations, there is little direct evidence that the acid-growth theory can be applied satisfactorily to the growth of intact plants or the differential growth response of plant organs to environmental stimuli. Exceptions to this paucity of information are two recent reports by Mulkey *et al.* (8, 9). These authors have qualitative data indicating that under geostimulation a proton gradient develops across *Zea* roots as well as across shoots of *Zea* and sunflower. This proton asymmetry may lead to differential rates of acid-induced growth on the upper and lower sides of the organ which, in turn, may cause its georesponse.

We have begun a line of investigation to determine if proton gradients may play a role in the negative geotropic behavior of shoots. To facilitate application of some of the methodology used in straight-growth systems, we have devised a system in which geostimulation and the subsequent curvature of hypocotyl segments occur while the sections are submerged in aqueous solutions. In this publication we report on the effects of neutral (pH 6.8) buffers on the geotropic behavior of *Helianthus*.

MATERIALS AND METHODS

Seeds of sunflower (*Helianthus annuus* L. cv. Sungold) were germinated and grown in growth chambers programmed for cycles of 16 h light (25°C) and 8 h dark (20°C). After 4 to 5 days, seedlings were harvested, and 2.5-cm segments were cut from the region beginning just below the cotyledonary node. After cutting, the cuticle of some segments was abraded (scrubbed) by stroking individual segments (5 strokes/segment) with a paste prepared by mixing water with rottenstone (finely decomposed siliceous limestone; Dowman Products, Inc., Long Beach, CA). After preparation, the hypocotyl segments were rinsed with distilled H_2O, then placed in 0.25 mM K-phosphate buffer (pH 6.8) and randomized on a rotary shaker for 30 to 45 min. After preincubation, segments were mounted in custom Plexiglas holders (see Fig. 1). To prevent rotation or movement of the sections after insertion into the holder, their basal portions were embedded in liquid 5% (w/v) agar which quickly solidified. The final length of the hypocotyl segments which extended beyond the side of the holder was approximately 1.5 cm.

After mounting (10 segments/holder), each Plexiglas platform was placed in a 1-liter beaker containing 1,000 ml buffer maintained at 32°C, the optimum temperature (unpublished data). No aeration of the solution surrounding the segments was employed, inasmuch as our experiments indicated that aeration retards geocurvature of scrubbed shoots. All manipulations prior to submerging the segments in the test solution were carried out under normal laboratory lighting. However, the exposure of the segments to gravitational stimulation and their subsequent curvature occurred in the dark.

After 4 h geostimulation, the Plexiglas holders were removed from the test solution and the segments photographed with a 35mm camera. The response of each segment at 4 h could then be compared with photographs of the segments at 0 time and the amount of tip displacement calculated.

RESULTS AND DISCUSSION

Hypocotyl segments of light-grown *Helianthus* were exposed to gravitational stimulation while submerged in 0.25 to 20 mM concentrations of K-phosphate buffer (pH 6.8, 32°C). Those segments in 0.25 mM buffer exhibited noticeable upward curvature by at least 30 min (data not shown), and by 4 h complete reorientation of the tip and a portion of the hypocotyl had occurred (Fig. 1). Scrubbed and nonscrubbed segments behaved similarly. However, as the buffer strength was progressively increased, the curvature of scrubbed segments was less pronounced; at 20 mM virtually no curvature occurred. In contrast, the curvature of nonscrubbed segments was not affected by increasing the buffer strength (Figs. 1 and 2). Similar results have been obtained using other species including cucumber and soybean hypocotyls as well as *Avena* and *Zea* coleoptiles.

The observed inhibition of curvature in scrubbed sections with increasing buffer strength does not appear to result from a reduction of wall pressure due to the osmolarity of the solution, as equal or greater concentrations of nonbuffering substances (*e.g.* mannitol) do not result in the inhibition of curvature.

The possibility that inhibition of geocurvature by high-strength buffer systems is due to toxicity rather than their buffering action cannot be eliminated completely. Regarding this possibility, however, three points should be made. First, our experiments show that Mes-Tris, Pipes-KOH (Fig. 2), and Hepes-KOH buffering systems produce results similar to those of K-phosphate. Second, Hepes Mes, and Pipes are zwitterionic buffers thought to be incapable of biological membrane penetration. Third, as Figure 2

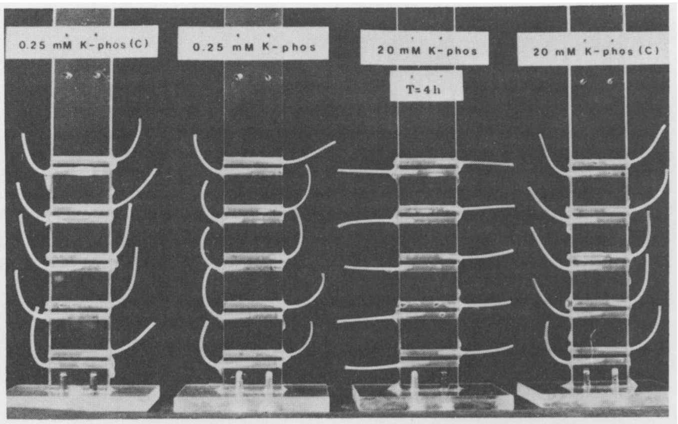

FIG. 1. Effect of 0.25 and 20 mM K-phosphate buffer on the geocurvature of sunflower hypocotyl segments after 4 h. Nonscrubbed sections (labeled C) are at the far left and far right of the photograph. Scrubbed sections are shown in the middle two Plexiglas holders.

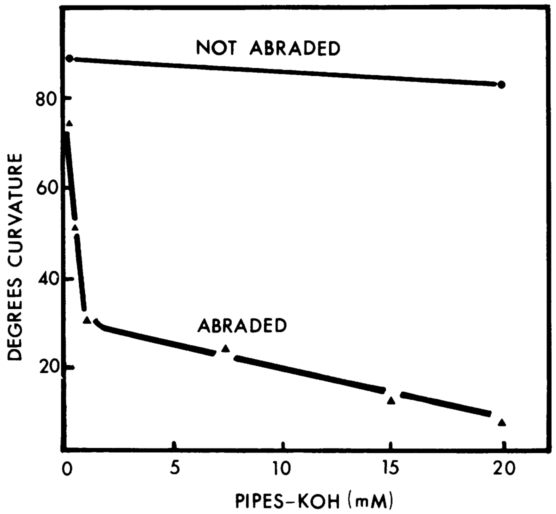

FIG. 2. Effect of various concentrations of Pipes-KOH on the geocurvature of sunflower hypocotyl segments after 4 h. Photographic negatives taken at 0 time and after 4 h geostimulation were superimposed and the resulting print was used to measure the degrees of arc through which the upper 2 mm of the tip passed in 4 h.

indicates, even relatively weak buffers (*e.g.* 0.5 mM) produce a marked decrease in geocurvature.

If one eliminates turgor reduction and/or toxicity effects as viable possibilities, there remain at least two possible explanations for the inhibition of curvature in submerged scrubbed segments by neutral buffers. One possibility is that asymmetric growth may require the development of a proton gradient across the cell wall region of the segment. The asymmetry would be such that the cells near the lower surface are exposed to a more acidic environment than those on the upper surface, and thus differential rates of acid growth would occur. One could further rationalize that this asymmetry is possible only when the medium surrounding the segments is of low buffering capacity or when a cuticular barrier (*i.e.* nonscrubbed segments) prevents buffer penetration.

Interestingly, the possibility that a gravitational stimulus could bring about a lateral pH gradient in geostimulated shoots has been previously suggested from indirect evidence (2, 5–7). Unfortunately, this possibility has not been pursued extensively in the literature. Direct quantitative means to test for a pH gradient have not been employed for shoot systems, nor has the inhibition of geocurvature by neutral buffers been previously noted.

Alternatively, geostimulation could result in a differential sensitivity of the hypocotyl cells to acid. In this case, one might imagine the normal wall pH to be slightly acidic but no asymmetry to be present. Enhanced sensitivity of cells on the lower surface to existing wall protons might then lead to growth. Neutral buffers in this case would act then to "stat" the wall pH at a value higher than the threshold level required to trigger the extension of the sensitized cells.

The possibility that geostimulation may bring about a physiological asymmetry in the tissue apart from an asymmetric distribution of auxin and/or protons has also been previously suggested by others (1, 5). Of these reports, the finding of Ganot and Reinhold (5) that the curvature developed by sunflower hypocotyl segments exposed to gravitational stimulus was enhanced in acidic solutions (*e.g.* pH 4.0) may be of particular importance.

Work is underway in our laboratory to test the above two alternative explanations for the inhibition of geotropic curvature by neutral buffers. If an asymmetric distribution of protons across the walls of a geostimulated shoot can indeed be quantitatively measured, it will be interesting to investigate whether an asymmetric redistribution of auxin must necessarily precede the development of such a gradient or if it occurs in response to a lateral proton gradient.

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