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Suppression of Asymmetric Acid Efflux and Gravitropism in Maize Roots Treated with Auxin Transport Inhibitors or Sodium Orthovanadate

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ABSTRACT

In gravitropically stimulated roots of maize (Zea mays L., hybrid WF9 x 38MS) there is more acid efflux on the rapidly growing upper side than on the slowly growing lower side. Since the Cholodny/Went hypothesis of gravitropism states that gravitropic curvature results from lateral redistribution of auxin, we examined the effects of auxin transport inhibitors on the development of acid efflux asymmetry and curvature in gravistimulated roots. All the transport inhibitors tested prevented both gravitropism and the development of asymmetric acid efflux in gravistimulated roots. The results indicate that auxin redistribution may cause the asymmetry of acid efflux, a finding consistent with the Cholodny/Went hypothesis of gravitropism. As further evidence that auxin-induced acid efflux asymmetry may mediate gravitropic curvature, we found that sodium orthovanadate, an inhibitor of auxin-induced H^+ efflux, prevented both gravitropism and the development of asymmetric acid efflux in gravistimulated roots.

INTRODUCTION

Earlier work from this laboratory showed that asymmetric acid efflux occurs in gravitropically responding roots and shoots (Mulkey and Evans, 1981; Mulkey et al., 1981). In both cases there is more H^+ efflux on the most rapidly growing side of the gravi-responding organ, i.e. in roots more H^+ efflux occurs on the top while in shoots more H^+ efflux occurs on the bottom. Since acid pH stimulates cell elongation in both roots and shoots (Edwards and Scott, 1974; Rayle and Cleland, 1977), it is possible that this asymmetric acid efflux mediates the asymmetric growth which causes gravitropism. Further evidence that this may be the case was recently reported by Wright and Rayle (1982) who showed that neutral buffers can prevent gravitropism in a variety of hypocotyls and coleoptiles.

According to the Cholodny/Went hypothesis, gravitropism is mediated by lateral redistribution of auxin toward the lower side of horizontal shoots and roots (Went and Thimann, 1937) with the elevated level of auxin promoting growth on the lower side of shoots but inhibiting growth on the lower side of roots. This model of gravitropism is supported by reports that inhibitors of auxin transport such as naphthylphthalamic acid (NPA), 2,3,5-triiodobenzoic acid (TIBA), DPX-1840 and morphactins inhibit gravitropism (Gaither, 1975; Gaither and Abeles, 1975).

These observations raise the question of the relationship between the asymmetric H^+ efflux associated with gravitropism and the apparent requirement for lateral redistribution of auxin in gravitropism. Does asymmetric acid efflux occur independently of auxin redistribution? If so, asymmetric acid efflux should occur even in gravistimulated roots treated with auxin transport inhibitors. Is asymmetric acid efflux linked to asymmetric auxin distribution? If so, auxin transport inhibitors should prevent the development of asymmetric acid efflux as well as gravitropism. The possibility that gravity-induced auxin redistribution might cause the asymmetry in H^+ efflux is suggested by studies showing that auxin stimulates acid efflux from shoots (Rayle and Cleland, 1977) and inhibits acid efflux from roots (Evans et al., 1980). In order to test the idea that lateral redistribution of auxin causes the asymmetry of H^+ efflux, we examined the effects of a variety of auxin transport inhibitors as well as vanadate on the development of asymmetric acid efflux in gravistimulated roots of maize. A preliminary report of these findings has been published (Mulkey and Evans, 1982). We have recently learned that Wright and Rayle (in press) have tested the effects of vanadate plus a variety of antiauxins and inhibitors of auxin action on asymmetric acid efflux and growth patterns during gravitropism in shoots. Their results are consistent with ours in showing an apparent dependence of the development of asymmetric acid efflux on auxin redistribution.

MATERIALS AND METHODS

The experiments were performed using 3-day-old seedlings of maize (*Zea mays* L., hybrid WF9 x 38MS; Customaize, Momence, Ill., USA) germinated and grown as described by Mulkey et al. (1981). Patterns of acid efflux during gravitropism were observed using a modification of the method of Weisenseel et al. (1979) described by Mulkey and Evans (1981). Briefly, the seedling was placed on a 4-mm-thick plate of 0.6 percent agar containing the pH indicator dye, bromocresol purple (0.71 mM). The pH of the agar-dye mixture was adjusted to 5.0 before pouring the plates. The primary root was pressed gently into the agar so that about half of the cylindrical root was imbedded. The plate was then mounted vertically with the root perpendicular to the direction of gravity. At the initial pH of 5.0, the agar-dye is a dull orange. In regions where H^+ efflux from the root occurs, the pH of the medium increases causing the indicator dye adjacent to the root to become yellow. In regions where H^+ uptake by the root occurs, the pH of the medium increases causing the indicator dye to turn red. The figures presented are black and white photographs of seedlings on the agar-dye plates. In these figures, the yellow (acid) regions appear as light regions adjacent to the root. The red (higher pH) regions appear as dark zones (e.g. the dark halos surrounding the root tips).

Agar plates containing auxin transport inhibitors or p-chlorophenoxyisobutyric acid (PCIB) were prepared by incorporating the chemicals into the agar-dye mixture prior to pouring the plates. The concentrations of inhibitors used are indicated in the figure legends. In order to prepare agar-dye plates containing sodium orthovanadate (vanadate), the vanadate was first dissolved in distilled water and titrated to pH 7.0 using HCl. The solution was then boiled for 3 min to depolymerize the vanadate, and the solution was cooled in an ice bath. Since boiling releases H^+ from polymerized vanadate, the pH of the vanadate solution fell below 6 during the depolymerization. The pH of the cooled vanadate solution was therefore adjusted to 6.0 using NaOH. Agar (0.6 g/l) and bromocresol purple (0.71 mM) were added to the vanadate solution and the mixture was boiled for 5 min. Agar/dye/vanadate plates were then poured for immediate use.

The bromocresol purple and the PCIB were purchased from Sigma Chemical Co., St. Louis, MO, USA. The TIBA was purchased from Polysciences Inc., Warrington, PA, USA. NPA was obtained from Pfaltz and Bauer, Inc., Stamford, CT, USA. The two morphactins were purchased from Aldrich Chemical Co., Milwaukee, WI, USA. DPX-1840 was a gift from Dr. E. Beyer, Jr., Dupont Experimental Station, Wilmington, DW, USA. Sodium orthovanadate was a gift from Dr. R. Saftner, USDA, Beltsville, MD, USA.

RESULTS AND DISCUSSION

Effect of Auxin Transport Inhibitors.

Figure 1 shows the effect of a variety of inhibitors of auxin transport on the development of gravitropism and H^+ efflux asymmetry in gravistimulated roots. In untreated roots (Fig. 1A) strong gravitropism occurred within 2 h and there was asymmetry in the H^+ efflux pattern, with more H^+ efflux (light areas) on the top than on the bottom. In the presence of the morphactin, 9-HFCA (Fig. 1B), no asymmetry in the H^+ efflux pattern occurred and there was no gravitropic curvature, even though the growth rate of the root was unaffected. Similar results were obtained with the morphactin, 9-chloro-9-hydroxyfluorenecarboxylic acid (data not shown). The auxin transport inhibitors, DPX-1840, TIBA and NPA also prevented the development of asymmetric acid efflux and gravitropism in horizontal roots (Fig. 1 C through E). However, each of these three auxin transport inhibitors also inhibited straight growth of the roots, DPX-1840 by 60%, TIBA by 40% and NPA by 70%. Notice that in Figure 1, roots C,D, and E were photographed after 18 h on the agar-dye plates while roots A and B were photographed after only 2 h. Although DPX-1840, TIBA and NPA partially inhibited root growth, we found that gravitropism did not occur in roots treated with these inhibitors, even when they were left on the agar plates in a horizontal position long enough to allow growth equivalent to that occurring in strongly responding control roots, i.e. the failure to develop gravitropic curvature was not due to insufficient elongation to allow expression of curvature.

The fact that these auxin transport inhibitors prevented both gravitropism and the development of asymmetry in the acid efflux pattern suggests that auxin redistribution is necessary to the development of acid efflux asymmetry.

Effect of Vanadate

Sodium orthovanadate has been shown to inhibit auxin-induced H^+ secretion and growth in pea epicotyls and oat coleoptiles (Jacobs and Taiz, 1980). In order to test further the dependence of root gravitropism on H^+ efflux, we examined the effects of vanadate on H^+ secretion and growth in gravistimulated roots of maize (Fig. 2). In roots placed on agar-dye plates containing 1 mM vanadate, the pattern of H^+ efflux from the root was altered with the region of H^+ efflux extending over the entire root tip. In the region of the elongation zone behind the tip, H^+ efflux continued, but it was somewhat reduced compared with control roots. It is difficult to illustrate the partly reduced H^+ efflux exhibited by vanadate-treated roots using this photographic method. There is some variation among control roots in the magnitude of H^+ efflux, and the black and white photographs show the H^+ efflux pattern less distinctly than the original colored plates. An indication of the partial reduction of H^+ efflux by vanadate can be gained by comparing the light zone which develops around the control in Figure 3B in 2 h with that which develops around the vanadate-treated root in Figure 2C after 18 h. Also, notice that in Figure 2, the acid (light) zone shown in part A developed in 15 min while the pattern shown by the vanadate-treated root in Figure 2C is shown after 18 h.

In gravistimulated roots on vanadate-containing plates, no gravitropism occurred even after 18 h (Fig. 2C), and the H^+ efflux which occured toward the tip remained symmetrical. Although vanadate-treated roots grew more slowly than control roots, the failure of vanadate-treated roots to exhibit gravitropism appears not to be simply a result of their reduced growth rate. Vanadate-treated roots failed to respond to gravity even after 18 h, during which time the roots grew as much or more than control roots responding normally to gravity over a 2 h period. The data indicate that rapid H^+ efflux from the elongation zone may be necessary to rapid root elongation and that suppression of the development of asymmetric acid efflux along the elongation zone can prevent root gravitropism.

Effect of PCIB

The "antiauxin" PCIB inhibited gravitropism less effectively than the auxin transport inhibitors tested. At low concentrations ($<= 10^{-6}$ M) PCIB had either no effect or a slightly promotive effect on growth and the development of gravitropic curvature. The slight enhancement of gravitropism may be related to the ability of PCIB to stimulate maize root elongation (Moloney et al., 1981). At higher concentrations (e.g. 10^{-5} M) PCIB reduced the rate of gravitropic curvature with little or no effect on growth (Fig. 3). The retardation of curvature was paralleled by a retardation of the development of asymmetry in the acid efflux pattern across the root. Although the rate of development of gravitropic curvature and H^+ efflux asymmetry were slowed by 10^{-5} M PCIB, the final curvature and degree of acid efflux asymmetry were as great as that of control roots.

Since PCIB is thought to act as an antiauxin, its ability to retard both gravitropism and the development of asymmetric acid efflux suggests that both may be controlled by endogenous auxin. These data are consistent with the ability of both auxin transport inhibitors and vanadate to prevent acid efflux asymmetry and gravitropism. Taken together, the data provide strong, though indirect, evidence that auxin redistribution is necessary for the development of acid efflux asymmetry in gravistimulated roots and that this acid efflux asymmetry may cause the differential growth leading to gravitropic curvature. This is essentially a restatement of the Cholodny/Went hypothesis of gravitropism extended to include induction of asymmetric acid efflux as the means of auxin-mediated differential growth and hence, curvature.

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LEGENDS FOR FIGURES

Fig. 1: Effect of auxin transport inhibitors on gravitropism and acid efflux patterns in intact gravistimulated roots of maize. This figure and those that follow show black and white photographs of maize seedlings on agar-dye plates prepared as described in Materials and Methods. On the original plates the acid zones are bright yellow and the regions of higher pH are red. On these black and white photographs, the yellow (acid) zones appear as light regions and the red (higher pH) zones appear as dark regions. A. Control (2 h). B. Morphactin (10^{-5} M 9-HFCA, 2 h). C. DPX-1840 (10^{-5} M, 18 h). D. TIBA (1mM, 18 h). E. NPA (4.86 mM, 18 h). The times indicated represent the time elapsed between placing the seedling on the agar-dye and photographing the plate.

Fig. 2: Effect of 1 mM vanadate on gravitropism and acid efflux in intact gravistimulated roots of maize. A. Control (15 min). Pattern was the same in vanadate-treated roots at this time. B. Control (2 h). C. Vanadate (1mM, 18 h).

Fig. 3: Effect of PCIB on gravitropism and acid efflux in intact, gravistimulated roots of maize. A. Control (15 min). Pattern was the same in PCIB-treated roots at this time. B. Control (2 h). C. PCIB (10^{-5} M, 2 h). D. PCIB (10^{-5} M 18 h).

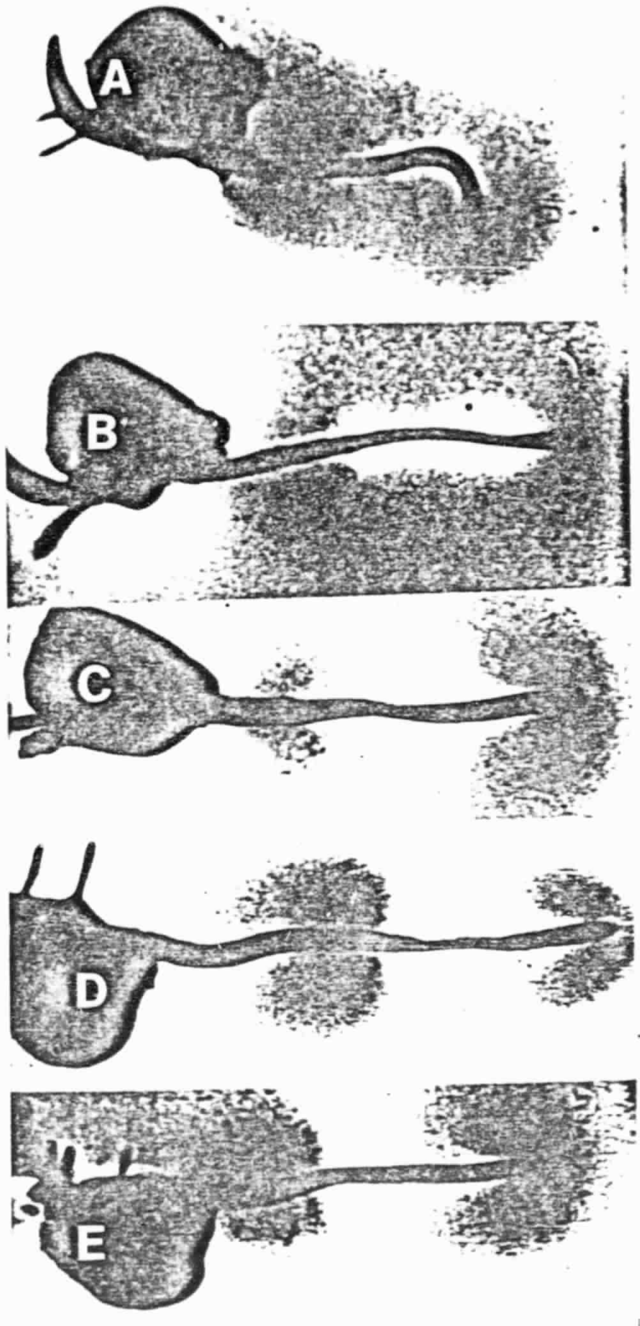

FIGURE 1

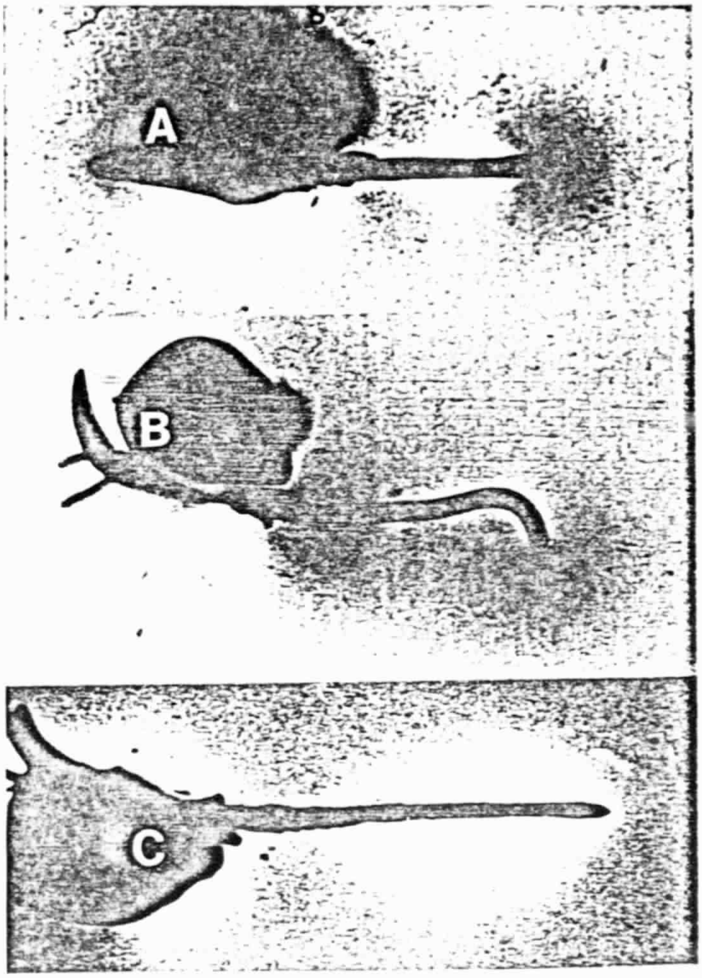

FIGURE 2

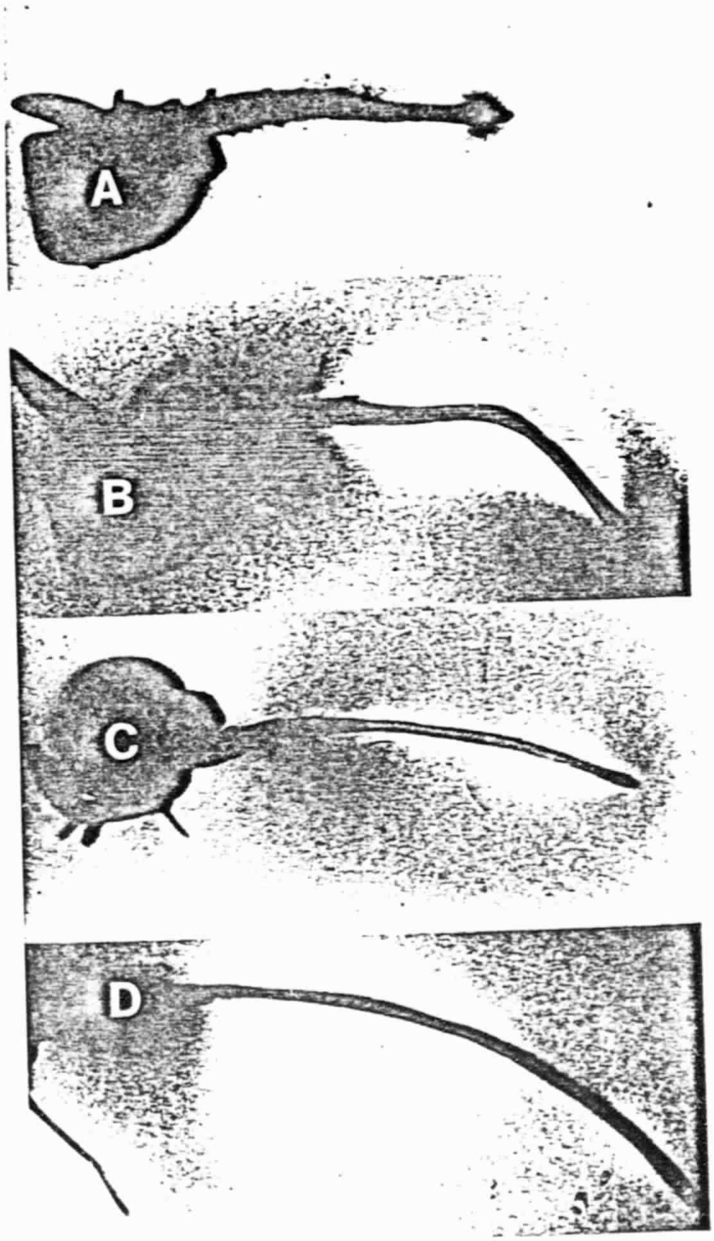

FIGURE 3