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Halogenated Auxins Affect Microtubules and Root Elongation in *Lactuca sativa*

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Abstract

We studied the effect of 4,4,4-trifluoro-3-(indole-3-) butyric acid (TFIBA), a recently described root growth stimulator, and 5,6-dichloro-indole-3-acetic acid (DCIAA) on growth and microtubule (MT) organization in roots of *Lactuca sativa* L. DCIAA and indole-3-butyric acid (IBA) inhibited root elongation and depolymerized MTs in the cortex of the elongation zone, inhibited the elongation of stele cells, and promoted xylem maturation. Both auxins caused the plane of cell division to shift from anticlinal to periclinal. In contrast, TFIBA (100 μM) promoted elongation of primary roots by 40% and stimulated the elongation of lateral roots, even in the presence of IBA, the microtubular inhibitors oryzalin and taxol, or the auxin transport inhibitor naphthylphthalamic acid. However, TFIBA inhibited the formation of lateral root primordia. Immunostaining showed that TFIBA stabilized MTs orientation perpendicular to the root axis, doubled the cortical cell length, but delayed xylem maturation. The data indicate that the auxin-induced inhibition of elongation and swelling of roots results from reoriented phragmoplasts, the destabilization of MTs in elongating cells, and promotion of vessel formation. In contrast, TFIBA induced promotion of root elongation by enhancing cell length, prolonging transverse MT orientation, delaying cell and xylem maturation (NASA grants NAG10-0190, NAG2-1423).

Key words: 5,6-dichloroindole-3-acetic acid; Indole-3-acetic acid; Indole-3-butyric acid; microtubules; *N*-1-naphthylphthalamic acid; quiescent center; 4,4,4-trifluoro-3-(indole-3-) butyric acid

Introduction

Auxin-induced plant cell elongation is partially based on proton excretion, which causes wall loosening and promotes growth by softening of the cell wall (Rayle and Cleland 1992). Typically, the elongation response can be observed within 15 min after auxin addition and lasts for several hours (Evans 1985). The growth mechanism of shoots and roots appears to be similar except for a shift in the dose-response toward lower concentrations in roots.

Root elongation can be promoted by auxin but only at low concentrations and when auxin-induced ethylene is inhibited (Mulkey and others 1982). The inhibition of root elongation by high auxin concentration is correlated with a rise of pH in the medium (Evans 1985). However, it is still controversial whether auxin changes the pH of the root cell wall (Buntemeyer and others 1998). Kutschera (1994) compared the effect of fusicoccin, IAA, and acid buffer on elongation of coleopitiles and found that the mechanism of IAA-induced growth is indepen-

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dent of cell-wall acidification. However, lateral root formation is stimulated by high auxin concentrations (Zhang and Hasenstein 1999). In addition, there is evidence that growth-inhibiting concentrations of IAA (10^{-7} M and higher) changes microtubule (MT) reorientation in apical root tissues (Blancaflor and Hasenstein 1995). These data imply that the effect of auxin on root elongation is not only controlled by acidification.

Because of differences in structure, function, and physiology of roots and shoots, plant growth regulators, which promote shoot elongation, such as auxins and gibberellins, fail to stimulate root growth. A notable exception from this rule is 4,4,4-trifluoro-3-(indole-3-) butyric acid (TFIBA, Katayama and others 1995). This compound significantly promotes root growth (Katayama and Gautam 1996, 1997) but inhibits *Avena* coleoptile elongation (Katayama and others 1995). Therefore, its physiologic properties are different from regular auxins. Furthermore, the mechanism of TFIBA promotion of root elongation is unknown.

Normal root elongation depends on the rate and direction of cell division and extent of subsequent elongation. These functions are tightly linked to the behavior of MTs. MTs determine the formation of the preprophase band before cell division, and cell elongation depends on the MT-controlled deposition of cellulose microfibrils (Giddings and Staehelin 1991; Wymer and Lloyd 1996). MTs in elongating cells are usually oriented perpendicular to the longitudinal axis before assuming an oblique orientation in mature cells. Any factors that accelerate or extend MT orientation normal to the root axis should promote cell elongation. In this article we test the hypotheses that MTs mediate promotion of root elongation by TFIBA and inhibition by high auxin concentrations.

Material and Methods

Lactuca sativa L. (cv. Baijianye) seeds were sterilized with 1% sodium hypochlorite for 5 min and batches of approximately 500 seeds were grown on two layers of wetted filter paper in 15-cm Petri dishes. After germination, seedlings with 1-mm long roots were transferred to other dishes. Seedlings with about 10-mm long roots were used for experimentation. All experiments were done in continuous light (12 μmol m^{-2} s^{-1}, 25°C). 2×10^{-3} M stock solutions of indole-3-acetic acid (IAA, Sigma, I-2886), indole-3-butyric acid (IBA, Sigma, I-5386), 5,6-dichloroindole-3-acetic acid (DCIAA), 4,4,4-trifluoro-3-(indole-3-) butyric acid (TFIBA), oryzalin (Chem Service, West Chester, PA), taxol (Sigma, T-1912), and 2 mg/mL naphthylphthalamic acid (NPA, Chem Service, West Chester, PA) were prepared in DMSO and stored at 4°C. All working solutions were obtained by diluting the stock solution with de-ionized H_2O.

Root elongation was tested by transferring seedlings to various concentrations of TFIBA, IAA, IBA, and DCIAA. After 72 h, the seedlings were fixed in ethanol and acetic acid (3:1) overnight and kept in 70% ethanol before the length of roots was measured. The effect of TFIBA on the formation and elongation of lateral roots induced by IBA was tested by treating seedlings with 2×10^{-5} M TFIBA + 0 – 10^{-4} M IBA. The plants were fixed as previously described and the length of primary roots and lateral roots was measured (Zhang and Hasenstein 1999).

The effect of 0–10 mg/L NPA, 0–10M^{-5} M oryzalin, and taxol on TFIBA-promoted root elongation was tested with or without 2×10^{-5} M TFIBA. In these experiments the root length was measured after 3 days.

Microtubules were visualized by immunocytochemistry (Blancaflor and Hasenstein 1993). After fixation in 4% formaldehyde in PHEMD buffer (pH 7.0) for 2 h, the roots were washed with buffer three times for 5 min. The terminal 6 mm of the roots were used for immunolocalization. Seventy micrometer sections were cut on a vibratome-1000 (Technical Products International, St. Louis, MO), and transferred to slides. The samples were treated with 1% cellulase and 0.5% pectolyase in PHEMD for 12 min and then incubated in 0.1% Triton X-100 in PHEMD for 20 min. After three washes (PHEMD), the slides were incubated for 2 h with antitubulin MAb (clone YOL 1/34; Accurate Chemical and Scientific Corp., Westerbury, NY) in PBS (4 mM NaH_2PO_4, 6 mM Na_2HPO_4, 150 mM NaCl, pH7.0) and then rinsed three times with PHEMD buffer. The bound anti-MT Ab was stained for 2.5 h with DTAF-labeled goat anti-rat IgG (Accurate Chemicals). After five rinses, the samples were mounted in 20% Mowiol 4-88 (Calbiochem) in PBS (8 mM Na_2HPO4, 25 mM KCl, 135 mM NaCl, pH 8.5) containing 0.1% *p*-phenylenediamine (Sigma, P-1519) and examined with a confocal microscope (MRC 1024ES, Bio-Rad).

Results

Root Elongation by TFIBA and Auxins

TFIBA promoted root elongation in a concentration-dependent manner with an optimal concentration of about 10^{-4} M (Figure 1). Concentrations of less

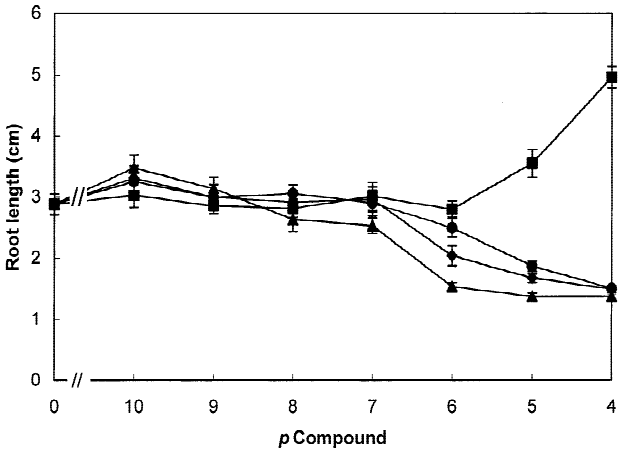

Figure 1. Length of primary roots of *Lactuca sativa* after application of 0 to 10^{-4} M (expressed as negative log) IAA (♦), DCIAA (▲), IBA (●), and TFIBA (■) for 72 h to 3-day-old seedlings. $n = 20 \pm$ SE.

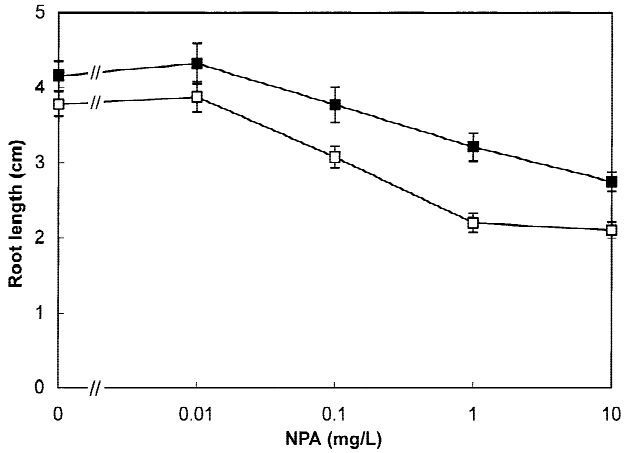

Figure 2. Length of primary roots of *Lactuca sativa* after application of NPA (□) or NPA + 2×10^{-5} M TFIBA (■) for 72 h to 3-day-old seedlings. $n = 20 \pm$ SE.

than 10^{-6} M TFIBA had no effect. Roots became necrotic when TFIBA was administered at 3×10^{-4} M. The promoting effect was obvious after 48 h. Although low concentrations (10^{-10} M) of IAA, DCIAA, and IBA slightly promoted root elongation, concentrations of DCIAA greater than 10^{-8} M, and IAA and IBA greater than 10^{-7} M inhibited primary root elongation (Figure 1).

NPA and IBA Reduced TFIBA-Induced Root Elongation

The inhibition of root elongation by the auxin transport inhibitor NPA was concentration dependent, and 2×10^{-5} M TFIBA only partially compensated inhibition if NPA was applied at levels greater than 0.1 mg/L (Figure 2). Although the inhibitory effect of IBA levels less than or equal to 10^{-6} M could be reversed by 2×10^{-5} M TFIBA, concentrations greater than or equal to 10^{-5} M IBA could not be compensated (Figure 3). Similarly, TFIBA reduced the number of lateral root primordia induced by IBA at low concentration (less than 10^{-6} M), but had no effect at 10^{-4} M IBA (Figure 3). TFBIA (2×10^{-5} M) increased the length of the primary root by about 1 cm independent of the duration of IBA application and enhanced the length of lateral roots (Figure 4).

Effect of Oryzalin and Taxol on TFIBA-Induced Root Elongation

The MT depolymer oryzalin reduced root elongation in a concentration-dependent manner and 2×10^{-5} M TFIBA compensated the inhibitory effect of less

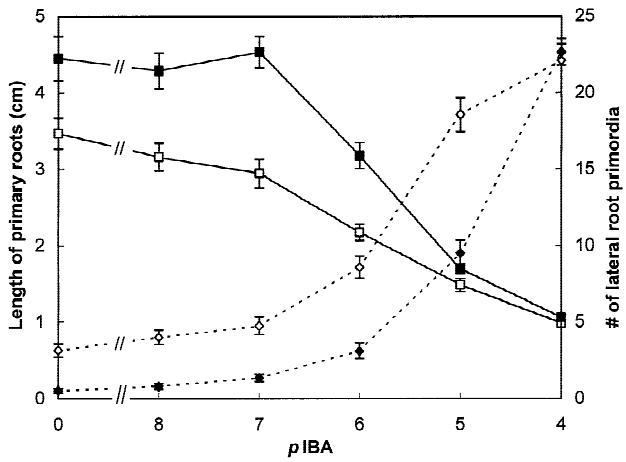

Figure 3. Length of primary root (solid lines) and number of lateral root primordia (dashed lines) per root of 3-day-old *Lactuca sativa* seedlings that were treated either with IBA (□, ◇) or IBA + 2×10^{-5} M TFIBA (■, ♦) for 72 h. Concentrations are expressed as negative log. $n = 20 \pm$ SE.

than 10^{-7} M oryzalin. However, 10^{-6} M oryzalin completely voided the elongation-promoting effect of TFIBA (Figure 5). Low concentrations of taxol (less than 10^{-6} M) did not affect root growth, but 10^{-5} M taxol reduced root growth by about 20%. When 2×10^{-5} M TFIBA was applied together with taxol, the inhibition by taxol was overcome and elongation was promoted (Figure 5).

Arrangements of MTs

In the quiescent center (QC) of control roots, MTs appeared randomly organized, but they were well

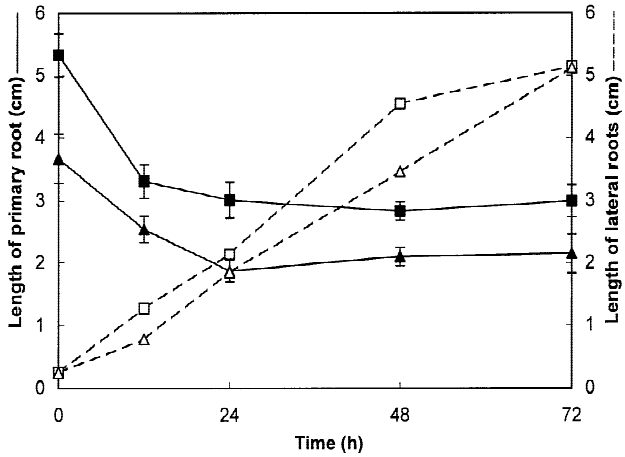

Figure 4. Length of primary (■, ▲) and collective length of lateral roots (□, △; dashed lines) of 3-day-old *Lactuca sativa* after application of 10^{-5} M IBA for 0, 12, 24, 48, or 72 h and subsequent treatment with 2×10^{-5} M TFIBA (■, □) or de-ionized H_2O (▲, △) for 3 additional days. $n = 20 \pm$ SE.

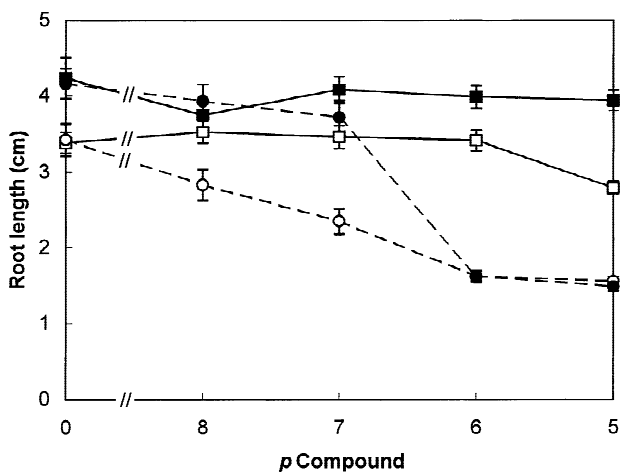

Figure 5. Root length of *Lactuca sativa* seedlings treated with oryzalin (○), oryzalin + 2×10^{-5} M TFIBA (●), taxol (□), or taxol + 2×10^{-5} M TFIBA (■) for 3 days. Concentrations are expressed as negative log. n 20 ± SE.

ordered in the mitotic and elongation zone (Figure 6). Cell divisions were mainly observed between 200 and 400 µm from the QC and the direction of the cell division was primarily transverse to the root axis (Figures 6A–B). In the cortex, active cell division was observed as far as 1.2 mm from the QC. Cells began to elongate at 400 µm from the QC, and the orientation of MTs in elongating cells was uniformly perpendicular to the root axis (Figure 6C).

IBA promoted the rearrangement of MTs from perpendicular to root axis to oblique in the cortex of

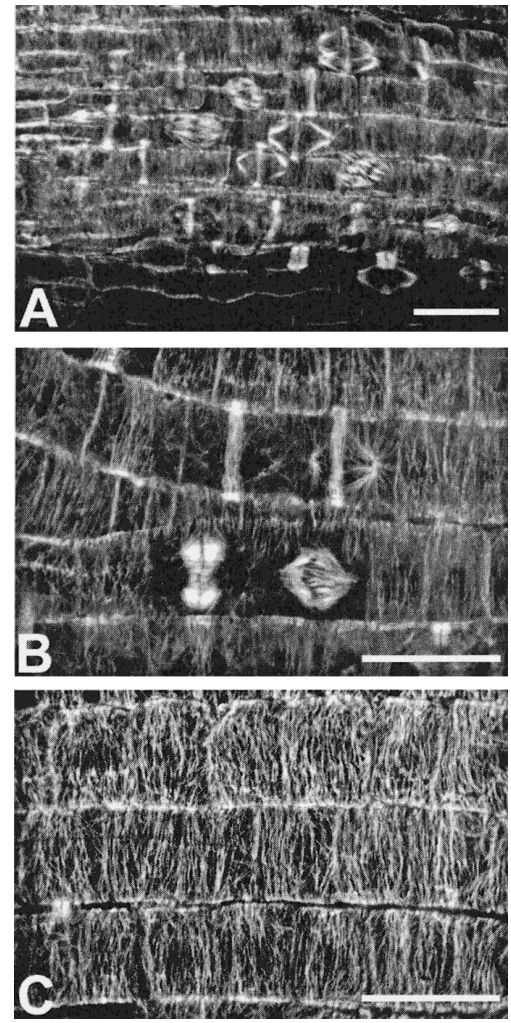

Figure 6. MT cytoskeleton in roots of *Lactuca sativa*. Cell division was confined within the mitotic zone and cell plates were oriented transverse to the root axis (**A** and **B**, 200 and 400 µm from QC, respectively). MTs in cortical cells were highly ordered (**C**, 400 µm from QC). Bars = 25 µm.

the elongation zone within 3 h (Figure 7A). IBA reduced the frequency of cell division and the density of MTs in the mitotic region (Figure 7B). After 9 h, the elongation zone started to swell (Figures 7C, D). At the site of swelling the cortical cells no longer showed MTs, and cells expanded isodiametrically (Figure 7C). Cell division in the pericycle (Figure 7D) and mature cortex (Figure 7E) was observed in regions that typically show no mitotic activity. Moreover, the elongation of stele cells was inhibited, and xylem matured earlier than in controls (Figure 7F).

Similar to IBA treatment, dichloro-IAA caused MTs fragmentation within 12 h. The density of MTs in the QC (Figure 8A) and mitotic region (Figure 8B)

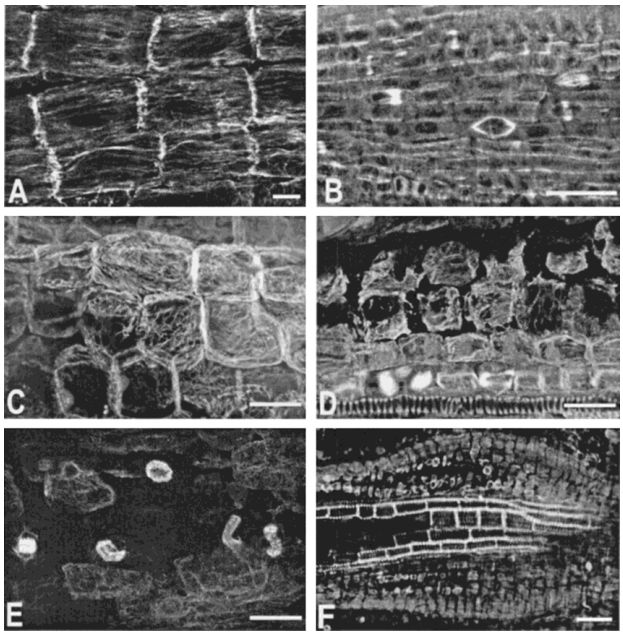

Figure 7. The effect of 5 x 10^{-5} -µM IBA on MTs in *Lactuca sativa* roots. The orientation of MTs shifted from perpendicular to parallel to the root axis in the cortex of the elongation zone 3 h after IBA application **(A).** The activity of cell division and the density of MTs in the mitotic region were reduced in roots treated with IBA for 9 h **(B).** After 9 h of IBA treatment, the elongation zone became swollen and MTs in cortical cells depolymerized. The proximal end of the swelling developed about 1.1 mm from QC **(C)** and showed a gradual depolymerization of MTs. Cells without MTs expanded primarily in the transverse direction (**C** to **D**). IBA-stimulated cell division in pericycle cells **(D)** and mature, partially separated cortical cells **(E)** after 9 h of treatment. The elongation of stele cells was inhibited, and the maturation of xylem was noticeable after 24 h of IBA treatment **(F).** Bars = 25 µm in (**A** to **E**) and 50 µm in **(F).**

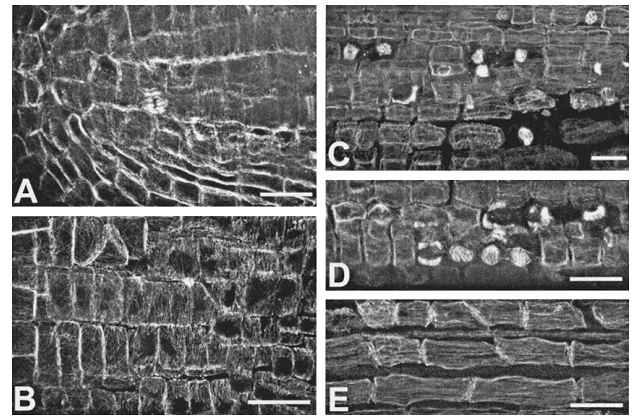

Figure 8. MTs in 3-day-old *Lactuca sativa* roots treated with 10^{-5} M DCIAA for 12 h. The density of MTs declined near the QC **(A),** and cell division was dramatically reduced (**B,** 100 µm from QC). However, cells were dividing at 1.2 mm from the QC **(C)** and beyond, often with the cell plate in periclinal direction. At 0.6–1 mm from the QC, stele and pericycle cells were actively dividing in the periclinal direction **(D).** The MTs in the stele became oriented longitudinally and elongation was inhibited (**E,** 2 mm from QC). Bars = 25 µm.

decreased, the MTs became obliquely or randomly oriented and cell division was reduced (Figure 8B). In contrast, cell division was promoted and extended into the elongation zone in the cortex (Figure 8C) and pericycle (Figure 8D). Cell division in the cortex was observed into the maturation zone. In addition to extending the zone of mitotic activity, DCIAA altered the orientation of phragmoplasts such that cells extended less aligned to the root axis (Figures 8C, D). MTs in the stele were oriented longitudinally, and the elongation of cells was inhibited (Figure 8E).

After 24 h of DCIAA treatment, MTs showed increased fragmentation in the area of the QC (Figure 9A). In cortical cells the shift from transverse to oblique or parallel to the long axis occurred at 100 µm from the QC (Figure 9B) rather than the 2–3 mm observed in controls. Cell division in stele and cortical cells in the mitotic region resumed, but shifted to the periclinal plane (Figures 9C–D). Between 300–600 µm from the QC roots expanded laterally (Figure 9E). In the elongation zone MTs in outer cortical cells were fragmented, and the cortical cells expanded isodiametrically. In the center of the swelling, cells separated, MTs were randomly oriented and partially depolymerized (Figure 9E). DCIAA also induced early secondary thickening of xylem before cell elongation (Figure 9F).

When roots were treated with 10^{-5} M TFIBA, transverse orientation of MTs was observed in the immediate vicinity of the QC, considerably closer than in controls. The MT bundles appeared thinner than in controls (Figures 10A–B). Unlike IBA and DCIAA, which promoted cell division in all tissues except mature stele, cell divisions of TFIBA-treated roots were concentrated in the cortex (Figure 10B) and stele (Figure 10C) of the mitotic region, and cell plates were oriented normal to the root axis. Stele cells began to elongate about 400 µm from QC (Figure 10D).

The average length of cortical cells in the elongation zone (3–6 mm from the QC) of control roots was about 73 µm, compared with 143 µm in TFIBA-treated roots. Thus, TFIBA doubled cell length. The cell length of meta-xylem in control roots was about 302 µm (Table 1). It was not possible to reliably determine the cell boundaries of protoxylem; there-

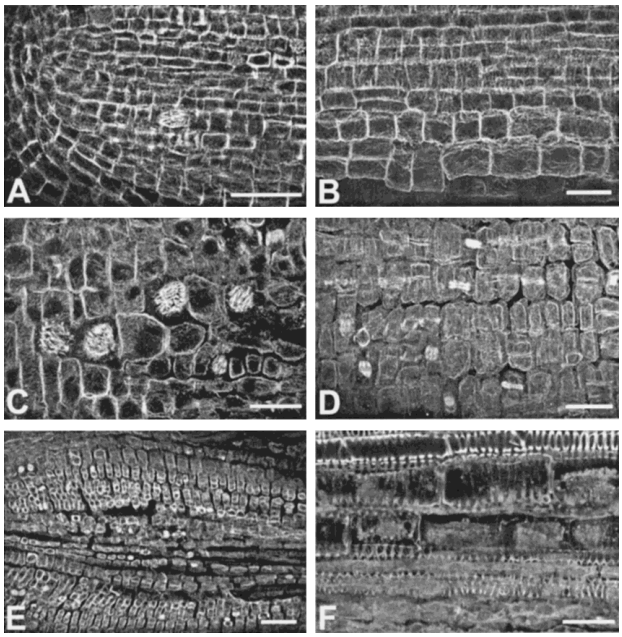

Figure 9. MTs in roots with 10^{-5} M DCIAA treatment for 24 h were further fragmented in the QC **(A)** and oriented parallel to the axis in cortex (**B,** 100 µm from QC). Cell division in stele and cortical cells in the mitotic region recovered but shifted to the periclinal direction **(C, D).** The elongation zone became swollen **(E),** MTs in outer cortical cells depolymerized and enlarged laterally. In the center of the swelling, cells separated and MTs partially depolymerized. DCIAA inhibited elongation of roots by enhanced secondary thickening of xylem (**F,** 700 µm from QC). Bars = 25 µm in (**A** to **D, F**) and 50 µm in **(E).**

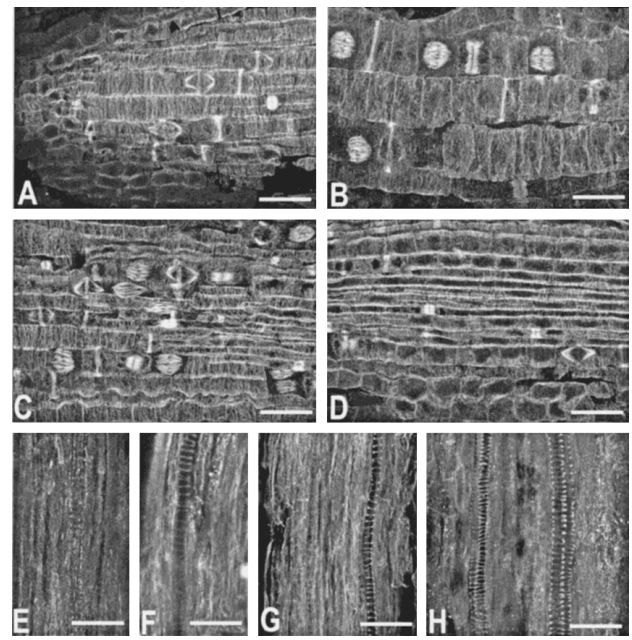

Figure 10. The effect of 10^{-5} M TFIBA on MTs in *Lactuca sativa* roots after 12 h (**A** to **F**) or 24 h (**G** and **H**) treatment. The transversely oriented MTs in the QC **(A)** and cortical cells of the mitotic region (**B,** 300 µm from QC) were enhanced. Compared with Figure 9, cell division was normal to root axis (**C,** 300 µm from QC), and stele cells were elongating at 400 µm from the QC **(D).** Xylem formation was delayed, and secondary thickening was noticeable at 2.8 mm from the QC **(E).** No metaxylem was observed as far as 8 mm from the QC **(F).** The MTs in stele were parallel to root axis (**G,** 5.6 mm from QC). The second vessel was found at 4 mm from the QC in some roots, but the secondary thickening pattern was spiral, not reticular **(H).** Bars = 25 µm.

fore, the lengths of vessel elements of protoxylem were not measured. The cell length in DCIAA-treated roots was expected to be shorter than in controls. However, the length of cortical cells at the sections 3–6 mm from the QC of 10^{-5} M DCIAA-treated roots was similar to that of the controls, but the length of metaxylem elements in DCIAA-treated roots was about two thirds of the controls (Table 1). Cell dimensions in the mitotic region were comparable between DCIAA-treated roots (length 18.8 ± 1.0 µm, width 18.0 ± 0.9 µm, volume 4.8 pL) and controls (length 19.5 ± 1.4 µm, width 17.6 ± 0.6 µm, volume 4.7 pL).

TFIBA inhibited development of vessel elements, especially metaxylem (Figure 10E–H). In TFIBA-treated roots, the first visible protoxylem with spiral MT patterns was observed at 2.5–3 mm from the QC (Figure 10E), whereas untreated controls show such differentiation between 1–1.5 mm. In the stele the orientation of MTs parallel to the root axis was extended (Figures 10F,–G). The second vessel appeared at about 4 mm from the QC (Figure 10H).

Oryzalin totally depolymerized MTs in roots within 12 h (Figure 11A). After 12 h, the elongation zone became swollen (Figure 11B), and the cortical cells enlarged isodiametrically, but oryzalin did not affect the shape or development of pericycle and endodermis cells (Figure 11C). When 10^{-5} M oryzalin and 5×10^{-5} M IBA were applied together, no depolymerization of the spindle MTs in dividing cells was observed. In contrast, the application of 10^{-5} M taxol together with 5×10^{-5} M IBA did not prevent the inhibition of root elongation, depolymerization of MTs in cortical cells, or swelling of the elongation zone (Figure 11D).

Discussion

Our data suggest that inhibition and promotion of root growth is controlled by common parameters. Previous data have shown that auxin affects the

Table 1. Cell Length, Width, and Volume of Cortical Cells and Metaxylem Elements of 3-day-old *Lactuca sativa* Roots (µm). After Treatment with 10^{-5} M TFIBA or DCIAA for 24 h.

	Control			TFIBA			DCIAA		
	Length	Width	Volume*	Length	Width	Volume*	Length	Width	Volume*
Cortex	72.9 ± 2.2	22.7 ± 1.0	29.5	143.3 ± 5.4	18.0 ± 0.9	36.4	73.4 ± 1.9	21.5 ± 1.2	26.6
Xylem	302 ± 27	17.9 ± 0.5	76	n/a	n/a	n/a	211 ± 11	19.7 ± 1.3	64.2

**The cell volume (in pL) is calculated for cylinders on the basis of the measured length and width.*
The data pertain to cells from 3–6 mm from the quiescent center.

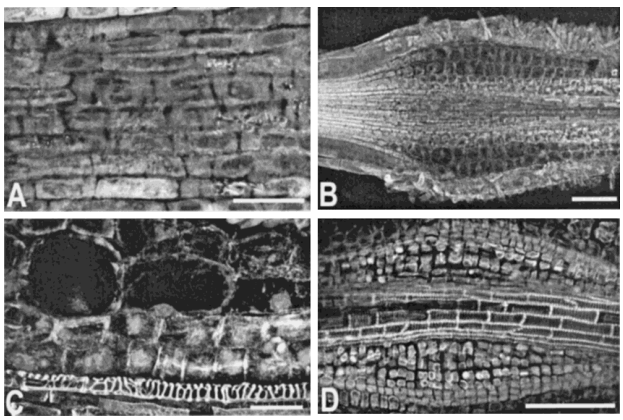

Figure 11. The effect of oryzalin and taxol on the MTs in roots of *Lactuca sativa*. After treatment with 10^{-5} M oryzalin for 12 h, MTs in the mitotic zone were completely depolymerized (**A,** 200 µm from QC), and the elongation zone expanded laterally **(B).** Treatment with 10^{-5} M IBA + 10^{-5} M oryzalin caused cortical cells to expand isodiametrically to a spherical shape, but the MTs in IBA-induced division of pericylce (**C,** 800 µm from QC) and cortical cells resisted depolymerization by oryzalin. Simultaneous application of 10^{-5} M taxol and 5×10^{-5} M IBA for 24 h did not prevent MT depolymerization in cortical cells or swelling of the elongation zone **(D).** Bars = 25 µm in **(A, C),** 100 µm in **(B),** and 50 µm in **(D).**

MT cytoskeleton in a concentration-dependent and site-specific manner (Blancaflor and Hasenstein 1995). Our present data show that the longer term effect of auxins includes a modification of the cell division and xylem and cortex-specific responses. Under the effect of high-auxin concentration, the plane of the cell division shifted, and xylem matured earlier. In the presence of TFIBA, the cells grew parallel to the root axis, and maturation of xylem was delayed, supporting the notion that growth regulation in roots depends on cell division, elongation, and maturation of the xylem.

Although the molecular structure of TFIBA is similar to that of auxins, its mode of action is different. In contrast to IAA, IBA, and DCIAA, TFIBA had no promoting effect on root elongation at low concentrations (Figure 1) but stimulated root elongation at high concentrations (Figure 1). Aside from elongation growth, TFIBA also inhibited the formation of lateral root primordia (Figure 3) but promoted lateral root elongation (Figure 4). In contrast, IBA and other auxins promoted the formation of lateral root primordia (Figure 3, Zhang and Hasenstein 1999) but not their elongation. One of the best documented functions of auxins is the promotion of elongation in the *Avena* coleoptile test, but TFIBA inhibited coleoptile elongation (Katayama and others 1995). This effect classifies TFIBA as a special, possibly root-specific growth promoter.

Because none of the common plant growth regulators effectively stimulate root elongation, TFIBA is an exception and provides important clues for the mechanism of root elongation. Although roots do not elongate without auxin (Figure 2), it is still unclear whether auxin is the only agent that controls root elongation. Auxin promotion of growth is limited to low concentrations (less than 10^{-10} M, Figure 1, Evans 1985), and auxin effects are masked by ethylene (Mulkey and others 1982). The mode of action of TFIBA on root elongation is different, because it acts at high concentrations, without cell wall acidification and independent of the pH of the growth medium (data not shown). Although TFIBA is a man-made plant growth regulator (Katayama and Gautam 1996, 1997; Katayama and Tanaka 1999), its chemical structure and/or charge distribution may alter or bind to auxin or ethylene receptors, reducing the (inhibitory) auxin activity, or it may mimic a still elusive natural root growth promoter. The halogenation of indole-3-acetic acid results in both a stronger inhibitory activity for root growth (DCIAA) and a stronger promotive activity (TFIBA) compared with indole-3-acetic acid.

Although a natural root growth promoter has not yet been identified, our studies of MTs suggest its

likely mode of action. Because the increase of cell number and volume is affected by the spatial organization of MTs, growth promotion is likely to result from earlier and/or spatially and temporally extended MT organization around the long cell axis that favors elongation growth (Sakoda and others 1992). Similarly, newly formed cell plates must be aligned normal to the root axis. Last, structures of great tensile strength such as lignified xylem will reduce or inhibit elongation. Therefore, a root growth promoter is likely to delay xylem maturation.

IBA and other auxins affect MT orientation and elongation of roots as a function of cell number and the orientation of the phragmoplast. If cell division occurs with the phragmoplast normal to the root axis, the two daughter cells will develop parallel to the root axis, and subsequent elongation will promote root elongation. This spatial arrangement of cell division was found in control and TFIBA-treated roots (Figures 6 and 10). In contrast, IBA and DCIAA shifted the direction of cell division from normal to the root axis to parallel (Figures 7–9). The subsequent cell expansion caused swelling and reduced elongation (Figures 7F, 9E). Treatment with TFIBA improved the perpendicular alignment of MTs to the root axis (Figures 10A–C). This MT pattern is likely to lead to a more orderly alignment of cellulose microfibrils, facilitating cell elongation parallel to the root axis. In contrast, IBA and DCIAA disorganized the MT arrays in the mitotic zone and depolymerized MTs in cortical cells of the elongation zone, which led to isodiametric enlargement (Figures 7–9).

Because TFIBA counteracted low concentrations of oryzalin (Figure 5), it overcame oryzalin-induced MT depolymerization. However, high concentrations of oryzalin completely depolymerized MTs and inhibited root elongation (Figure 5), similar to results reported earlier (Baskin and others 1994; Hasenstein and others 1999). The MT stabilizer taxol reduced root growth by about 20% but had no effect on root elongation when applied together with 2×10^{-5} M TFIBA (Figure 5). These results suggest that TFIBA improves the functionality of the MTs.

The distinct MT organization caused by TFIBA and auxins also affected the development of xylem. TFIBA maintains MTs in longitudinal orientation in stele cells (Figures 10E–H) and prevents MTs arrangement to the spiral or reticular pattern, which precedes secondary thickening. Therefore, TFIBA delayed the development of xylem and increased the length of stele cells. In contrast, typical auxins stimulated the reorganization of MTs necessary for secondary thickening (Figures 7F and 9F) and thus promoted xylem maturation. The earlier lignification and thus improved xylem development is likely to inhibit root elongation.

DCIAA was the most potent inhibitor of root elongation of all compounds tested (Figure 1). This effect is consistent with the observation that DCIAA shows great resistance to peroxidase degradation and has the strongest activity of all known natural and synthetic auxins (Hatano and others 1987; Katayama and others 1998). Compared with controls, DCIAA did not affect the length of cortical cells at 3–6 mm from the QC, in which cortical elongation had been completed. However, the metaxylem that continued to elongate in control roots, was inhibited by DCIAA (Table 1).

Cells at different developmental stages are likely to have different sensitivities to auxin (Trewavas 1982). The depolymerization by DCIAA and IBA partially in the mitotic zone and completely in the elongation zone is in line with previously reported concentration and site-specific effects of auxins (Blancaflor and Hasenstein 1995). In addition, cell divisions were promoted in the pericycle and reached the cortex of what is typically referred to as the maturation zone. Similarly, xylem development was promoted (Figures 7–9). Because of the inability of oryzalin to depolymerize IBA-induced spindle MTs, auxin may either act on (a subset of) MTs or affect (specific) organization centers (MTOCs).

In conclusion, our investigation of the growth pattern of roots after auxin and TFIBA application indicates that promotion of root growth is at least in part controlled by MTs. Three factors characterize promotion of root elongation, increase in cell division, prolonged MT organization perpendicular to the cell axis, and delayed xylem maturation. In contrast, destabilized MTs, reoriented cell divisions, and early xylem lignification are associated with (auxin-induced) inhibition of root elongation.

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