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ORIGINAL ARTICLES

Primary Root Growth Regulation: The Role of Auxin and Ethylene Antagonists

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Abstract We investigated the growth and development of flax roots in the presence of auxin antagonists 4,4,4-trifluoro-3-(indole-3-)-butyric acid (TFIBA), *p*-chlorophenoxyisobutyric acid (PCIB), and the ethylene inhibitor silver thiosulfate. Of these compounds, silver thiosulfate was most effective in promoting root elongation. All compounds reduced root diameter and root hair development. The effects of TFIBA were reversed by exogenous indole-3-acetic acid (IAA) or 1-aminocyclopropane-1-carboxylic acid (ACC). Because the ethylene and ACC content of roots was reduced by TFIBA and PCIB but increased by silver thiosulfate, we measured the transcription level of five isoforms of ACC synthases (*Lu-ACS1-5*) and three isoforms of ACC oxidases (*Lu-ACO1-3*). *Lu-ACS1-3* were inhibited by TFIBA and PCIB but promoted by silver thiosulfate and by exogenous IAA. TFIBA inhibited all three ACC oxidase isoforms but PCIB inhibited only *Lu-ACO3*. Silver thiosulfate and IAA upregulated *Lu-ACO1* and *Lu-ACO2*. Exogenous IAA affected transcription of ACC synthases and ACC oxidases in a concentration-dependent manner. The root promotion by TFIBA and PCIB is related to ethylene, but may also involve auxin interactions.

Keywords Auxin · Antiauxin · Ethylene · ACC · ACC synthase · ACC oxidase

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Introduction

Auxins are known for their strong inhibitory effects on root elongation. However, some auxin derivatives such as *p*-chlorophenoxyisobutyric acid (PCIB) and 4,4,4-trifluoro-3-(indole-3-)-butyric acid (TFIBA) strongly promote root elongation. PCIB-induced promotion of root elongation was first noted in wheat (Burström 1950) and later in flax (Aberg 1950, 1951). McRae and Bonner (1953) proposed PCIB's function as an auxin antagonist because of its competitive inhibition of auxin-induced physiologic responses. However, PCIB-induced promotion of root growth is not universal because it inhibits root elongation in *Arabidopsis* (Oono and others 2003). Similarly, TFIBA's action is species-specific and its root-growth promotion ranges from 40% in lettuce (Zhang and Hasenstein 2000) to several fold in Chinese cabbage and rice (Katayama and others 1995). The TFIBA activity is higher for the [S+] than the [R−] enantiomer (Katayama and others 1995) and independent of pH and ethylene synthesis in Lactuca (Zhang and Hasenstein 2002). TFIBA can be viewed as an auxin antagonist because it inhibits avena coleoptile and hypocotyl elongation (Katayama and others 1995) and indole-3-butyric acid-induced formation of lateral roots (Kaldorf and Ludwig-Müller 2000).

The effectiveness of auxin antagonists such as TFIBA and PCIB could stem from antagonizing ethylene (Zhang and Hasenstein 2002), inhibiting supraoptimal endogenous auxin in roots (Thimann 1948), or blocking auxin transport. Comparing ethylene production after TFIBA and PCIB application with an established ethylene antagonist such as ionic silver, which stimulates root growth in lettuce (Zhang and Hasenstein 2002) and promotes ethylene production in tomato (Atta-Aly and others 1987) and *Arabidopsis*

(Guzman and Ecker 1990), could reveal that root-growth promotion is caused by a reduction in ethylene.

PCIB inhibits auxin activity by stabilizing the Aux/IAA protein in *Arabidopsis* roots (Oono and others 2003), which is in line with the observed PCIB-induced reduction of IAA-induced ethylene (Tsai and Arteca 1984) and reduced ACC oxidase activity (Trebitsh and Riov 1987). Assuming a similar mode of action, TFIBA like PCIB could affect auxin signaling and reduce ethylene biosynthesis by counteracting auxin-induced ethylene production or reducing ACC oxidase, which may be constitutive (Yang and Hoffman 1984) or auxin-modulated (Peck and Kende 1995).

To elucidate the interaction of TFIBA and PCIB with auxin and ethylene, we examined the effect of both compounds and silver thiosulfate on root growth, ethylene production, ACC content, and gene expression of ACC synthases and ACC oxidases. Despite a dramatic promotion of ethylene production, silver thiosulfate caused the strongest root-growth promotion in flax seedlings. TFIBA and PCIB reduced ethylene-related gene transcription, which at least partially explains their promotive effect on root elongation.

Material and Methods

Plant Material and Growth Measurements

Flax (*Linum usitatissimum*) seeds were surface-sterilized in 10% commercial bleach (v/v) for 5 min, washed in deionized H_2O for 5 × 5 min, and soaked in deionized H_2O for 1 h. Then the seeds were germinated on agar medium (1% agar, 5 mM Mes/Tris, pH 6.5) in vertically oriented 9-cm petri dishes. One-day-old seedlings were transferred to fresh agar medium containing the desired concentrations of the test compounds. Root length was measured from digital pictures (Nikon Coolpix 4500). Root thickness and root hairs were measured from images obtained with a dissection microscope connected to a Sony camera (DKC-ST5) using ImageJ software (v1.30, NIH, USA). All experiments were performed under continuous light (18 μmol s^{-1} m^{-2} PAR at 23°C).

Chemicals

TFIBA, PCIB, IAA, and ACC were prepared in 100-250 mM stock in DMSO and stored at 4°C. Silver thiosulfate was prepared by mixing equal volumes of 100 mM $AgNO_3$ and 400 mM $Na_2S_2O_3$ immediately before use. The stock solutions were diluted with growth medium and the concentration of DMSO was 0.2% or less for all treatments.

Ethylene Measurement

Fifty flax seeds were placed between two layers of filter paper in Corning 72-ml (25-cm^2) tissue culture flasks containing 3 ml of buffer (5 mM Mes/Tris, pH 6.0). The flasks were kept upright in a growth chamber (23°C, continuous light, 18 μmol m^{-2} s^{-1} PAR). After 1 day, the caps were removed for 20 min to vent any ethylene. Then TFIBA, PCIB, or silver thiosulfate was added, the flasks sealed, and the seedlings cultured for 24 h. One milliliter of headspace sample was injected into a gas chromatograph (SRI 8610) with a flame ionization detector and hydrogen as carrier gas on a Porapak Q column at 70°C and a flow rate of 13.4 ml min^{-1}. The system was calibrated with a standard of 12.4 ppm ethylene.

ACC Quantification

Catalytic conversion of ACC to ethylene with NaOCl and Hg^{2+} (Lizada and Yang 1979) was the basis for the determination of the ACC content. One-day-old agar-grown seedlings were exposed to TFIBA, PCIB, and silver thiosulfate for 1 day. Untreated seedlings served as controls. Sets of 75 roots were homogenized with a mortar and pestle in 2 ml of 80% ethanol. The extract was centrifuged (10 min × 20,000 *g* at 4°C) and the pellet was re-extracted twice with 2 ml of 80% ethanol each and centrifuged as before. The combined supernatant was dried in a rotary evaporator at 45°C. The residue was taken up in 3 × 300 μl H_2O and diluted to 900 μl in a 10-ml vial. After addition of 10 μl of 100 mM $HgCl_2$, the vials were sealed and kept on ice. Then, 100 μl of a mixture of cold 5% NaOCl and saturated NaOH (2:1 v/v) were added. The mixture was shaken (200 rpm) for 15 min before a 1-ml headspace gas sample was analyzed by gas chromatography. ACC was quantified based on the conversion of 0.25, 0.5, 0.75, 1, and 1.25 nmol ACC to ethylene.

Cloning of ACC Synthases and ACC Oxidases

Total RNA was exacted from control and 24-h TFIBA-, PCIB-, and silver thiosulfate-treated roots using Trizol (Invitrogen). Aliquots of RNA (2 μg) were reverse-transcribed using Superscript III (Invitrogen) according to the manufacturer's instructions. Degenerate primers modified after Woltering and others (2005) (Table 1) were used for PCR amplification. After separation on a 1% agarose gel, the product was excised and purified using QIAquick Gel Extraction Kits (Qiagen). The purified product was cloned into pDrive vector (Qiagen). After transformation with DH5α *E. coli*-competent cells (Invitrogen), transformants were selected on LB medium containing kanamycin (50 μg/ml). Colonies were checked by PCR with T7 and

Table 1 Primers used for cloning and real-time PCR

Gene	Primer sequence
Lu-ACS	F: ATTCARATGGGTCTHGCNGARAAYCAG
	R: AARCARACACGRAACCAVCCMGGYTC
Lu-ACO	F: TGYGARAAYTGGGGHTTCTTTGAG
	R: CATKGCYTCRAAYCTBGGCTCYTTDGC
Lu-ACS1	F: AGCATTGCTGAAGTACTCAACGAT
	R: GTAGTTCTCAGTAAACTCCGCGT
Lu-ACS2	F: GCTAGCGTGCATGCTTTCCAG
	R: CGGACCCAAGTTCATCCAGCAG
Lu-ACS3	F: TGACTACCGCAAGAAGGATGTCG
	R: CGGACCCATGTTCATCCAGCAA
Lu-ACS4	F: TAGAGGCGACAGAGTGAAATTCG
	R: GATCGGCACCAGTTGGATTC
Lu-ACS5	F: GACTACCATGGCTTACCAGAGTTC
	R: CCCGGTCGAATCCAGGGTAG
Lu-ACO1	F: GTTTCAGGATGATAAAGTGAGCGGT
	R: GAACGACGCAATGGACATCCTC
Lu-ACO2	F: GGAGAGCACTTTCTTCCTCCG
	R: ATTCTCGCACAACAGATCGAGC
Lu-ACO3	F: GGAGAGCACCTTCTACCTCAAG
	R: CCTTCTTCAAGTAGCCCTTCTCG
Lu-Actin1	F: CCAATCTACGAAGGGTATGCTCTC
	R: CGTTGTGAACATGTACCCTCTCTC

SP6 primers following the manufacturer's protocol (GenHunter Co.) and cultured in liquid LB overnight at 37°C. Plasmids were extracted from liquid culture with GenElute Plasmid Miniprep Kit (Sigma). Sequencing was performed with T7 and SP6 primers on an ABI 3100 system (Applied Biosystems).

Quantitative RT-PCR

RNA samples were quantified with a Nanodrop ND-1000, and the integrity confirmed using agarose gel electrophoresis (Sambrook and others 1989). Reverse transcription was based on 2 µg RNA and performed in 20 µl with Superscript III. Genomic DNA contamination was assessed by parallel reactions in the absence of RT. The RT product was diluted in 200 µl nanopure H_2O. PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems) in a volume of 12 µl on a StepOne Real-time PCR System (Applied Biosystems). The PCR mixture consisted of 3 µl of cDNA, 3 µl of 0.8 µM primers, and 6 µl master mix (ABI No. 4367659). Actin1 served as an internal control with primers listed in Table 1. Denaturation and Taq activation at 95°C for 10 min preceded 40 amplification cycles (95°C for 15 s and 60°C for 1 min). Melt-curve analysis of the amplified products confirmed uniformity. The results were reported as averages of three biological repeats, each replicated three times per plate. mRNA abundance was calculated as fold change = 2^[ΔCt(actin1) − ΔCt(target)]. ΔCt represents the difference in cycle numbers at which amplification first exceeds the threshold fluorescence level.

Data Analysis

Growth measurements (control, 100 µM TFIBA, 50 µM PCIB, and 1 mM silver thiosulfate) were repeated three times with 12 seedlings each. Ethylene measurements were repeated five times with TFIBA and PCIB and three times with silver thiosulfate-treated seedlings; ACC measurements were repeated four times. Ethylene and ACC data were analyzed by ANOVA (Proc GLM v9.1; SAS Institute, Cary, NC) with Tukey's adjusted test for multiple comparisons. Real-time PCR data were analyzed in Excel (Microsoft, Redmond, WA) and subjected to Student's *t* test.

Results

TFIBA, PCIB, and Silver Thiosulfate had Comparable Effects on Root Growth and Morphology

TFIBA, PCIB, and silver thiosulfate promoted root elongation but reduced root diameter and hair growth. The strongest growth promotion was observed in the presence of 50-100 µM TFIBA (Fig. 1a), 50 µM PCIB (Fig. 1b), and 1 mM silver thiosulfate (Fig. 1c). Two days after application of optimal concentrations of TFIBA, PCIB, and silver thiosulfate, roots were 2, 1.9, and 3.1 times as long as control roots, respectively (Fig. 1a–c).

The diameter of control roots increased steadily from the root tip to about 12 mm, but the enhancement of root elongation by the tested compounds resulted in a reduction of the root diameter (Fig. 1d–f). The most effective concentration for growth promotion (100 µM TFIBA and 50 µM PCIB) generated the thinnest roots and higher concentrations did not reduce the diameter further. Silver thiosulfate produced the thinnest roots at 2 mM (Fig. 1f), which was higher than the optimal concentration for root elongation (Fig. 1c).

In addition to thinning, the tested compounds also inhibited root hair development (Fig. 2). Increasing the TFIBA concentration reduced the number and length of root hairs, and at the optimal concentration for elongation (100 µM), root hairs were completely absent (Fig. 2b–d). PCIB affected root hair growth less than TFIBA; hair density was greatly reduced, but even at supraoptimal concentration (300 µM), PCIB did not completely eliminate root hairs (Fig. 2e–g). Silver thiosulfate was most

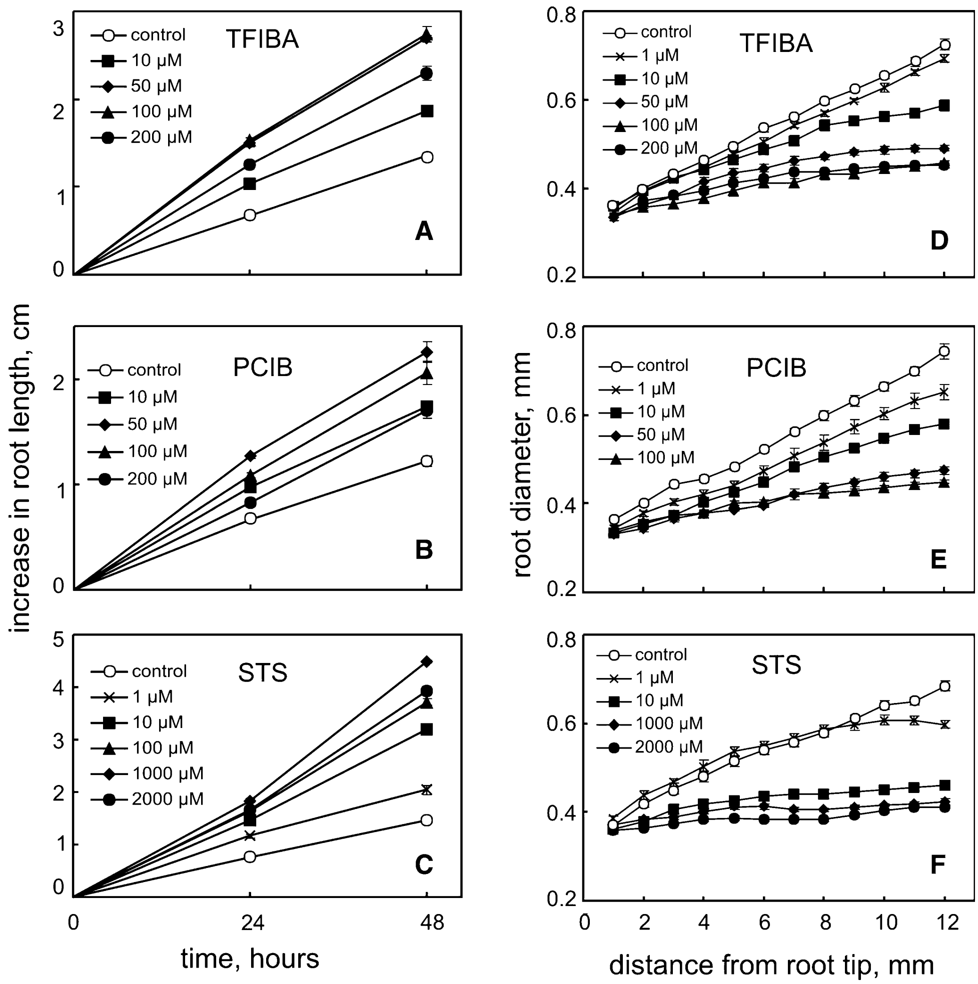

Fig. 1 Effect of TFIBA (**a**, **d**), PCIB (**b**, **e**), and silver thiosulfate (STS) (**c**, **f**) on root elongation (**a**, **b**, **c**) and root diameter (**d**, **e**, **f**) of 1-day-old flax seedlings grown vertically in petri dishes on 1% agar medium containing different concentrations of each compound. Root length was measured after 24 and 48 h and root diameter was measured after 48 h. Averages of three replicates ± SE with 12 seedlings each

effective in suppressing root hairs; application of 1 μM severely inhibited (Fig. 2h) and 10 μM silver thiosulfate completely eliminated root hair formation (Fig. 2i).

TFIBA Effects Are Reversed by IAA or ACC

Antiauxin effects should be reduced by elevated auxin or relevant downstream compounds such as ethylene. Therefore, we tested the coapplication of IAA and ACC in the presence of TFIBA. IAA reduced and ultimately reversed TFIBA-induced growth promotion (Fig. 3a), enlarged the root diameter (Fig. 3b), and restored root hair growth (Fig. 3c, d). A combination of 0.1 μM IAA and 100 μM TFIBA generated roots similar to controls but with different thickness patterns. Despite the reduction of root length, the root diameter was smaller than in controls in the apical 4 mm but larger beyond 4 mm (Fig. 3b). Coapplication of the ethylene precursor ACC partially reversed the effect of TFIBA. Similar to auxin application, ACC shortened the root (Fig. 3a), increased the root diameter (Fig. 3b), and restored root hair growth (Fig. 3c, e). Roots treated with 5 μM ACC and 100 μM TFIBA approached the morphology of controls but were shorter with still reduced diameter. ACC caused root curling when 5 μM or higher concentrations were applied.

TFIBA and PCIB Inhibit but Ag^+ Increases Ethylene Production

Because ethylene production in vegetative tissues is under the control of IAA (Abeles 1973; Hansen and Grossmann 2000), we studied the effect of TFIBA, PCIB, and silver thiosulfate on ethylene production in intact seedlings. The ethylene production by 1-day old seedlings during the next 24-h period was reduced by 100 μM TFIBA and PCIB to 58% and 72% of control values, respectively. Ethylene production decreased further with higher concentrations of TFIBA or PCIB (Fig. 4a). TFIBA consistently inhibited ethylene production more strongly than PCIB. In contrast, silver thiosulfate increased ethylene production (Fig. 4b); the concentration that eliminated root hair growth (10 μM) resulted in 1.7-fold higher ethylene levels, and 1 mM silver

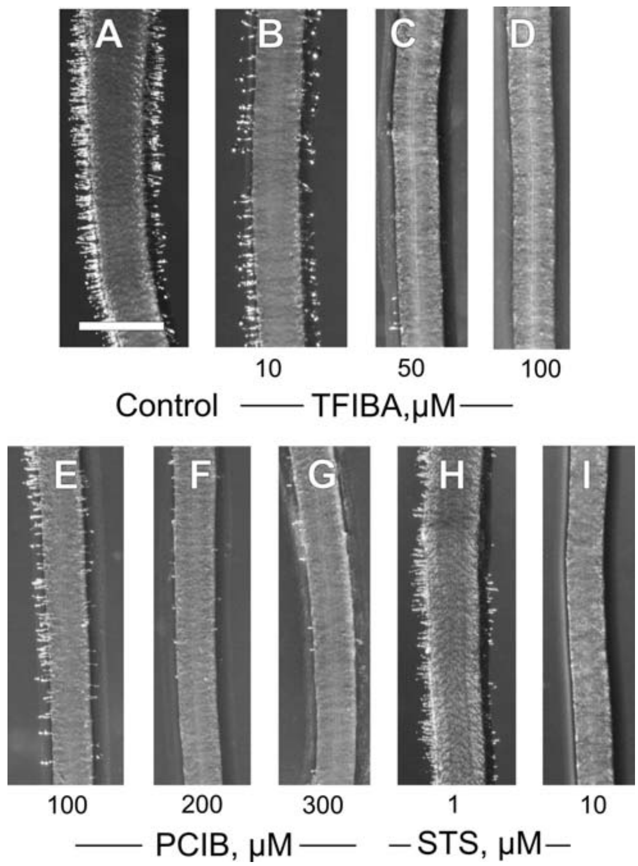

Fig. 2 Root hair growth on primary roots of 3-day-old flax seedlings after 2 days of application of TFIBA, PCIB, and silver thiosulfate. Controls show vigorous growth of root hairs (**a**) and increasing concentrations of TFIBA gradually eliminated hair development (**b–d**). Despite higher concentrations, PCIB (**e–g**) did not completely suppress hair formation. Silver thiosulfate at 1 μM (**h**) strongly reduced and at 10 μM (**i**) completely prevented hair formation. Images show root sections between 6 and 10 mm from the root tip. Scale bar = 1 mm

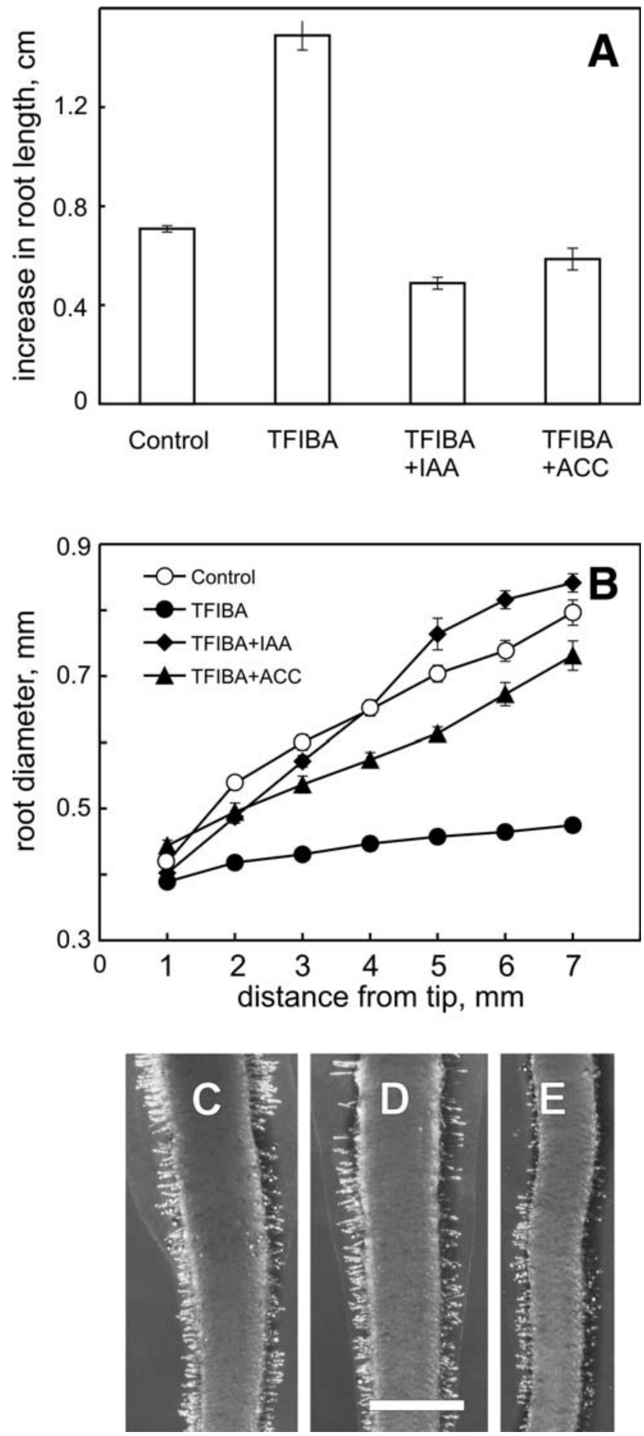

Fig. 3 Effect of coapplication of TFIBA and IAA or ACC on root elongation (**a**), root thickness (**b**), and root hair growth (**c**, control; **d**, TFIBA + IAA; **e**, TFIBA + ACC) in flax. TFIBA concentration was either 0 (controls) or 100 μM. One-day-old seedlings were treated for 1 day. 0.1 μM IAA or 5 μM ACC restored root morphology to control levels, but ACC treatment induced wavy growth. Root length was measured in three replicates with 12 seedlings each. Root diameter was measured from nine representative roots. Root hair images were taken from 3 to 7 mm from the root tip. Scale bar = 1 mm

thiosulfate, which induced the strongest growth promotion, resulted in a more than 4-fold increase of ethylene production.

Because IAA stimulates ethylene production via ACC (Jones and Kende 1979; Yu and Yang 1979), auxin inhibitors could inhibit ACC formation. This concept is supported by the consistent ratio between measured ACC (Fig. 4c) and released ethylene (Fig. 4a, b). One day after application of 100 μM TFIBA or PCIB, the quantity of ACC was half of that found in controls. The ACC content of 1 mM silver thiosulfate-treated roots increased about 1.4-fold over controls (Fig. 4c).

TFIBA, PCIB, Silver Thiosulfate, and IAA Affect Transcription of ACC Synthases and ACC Oxidases

The antagonistic effect between auxin and TFIBA or PCIB could be related to altered transcription of

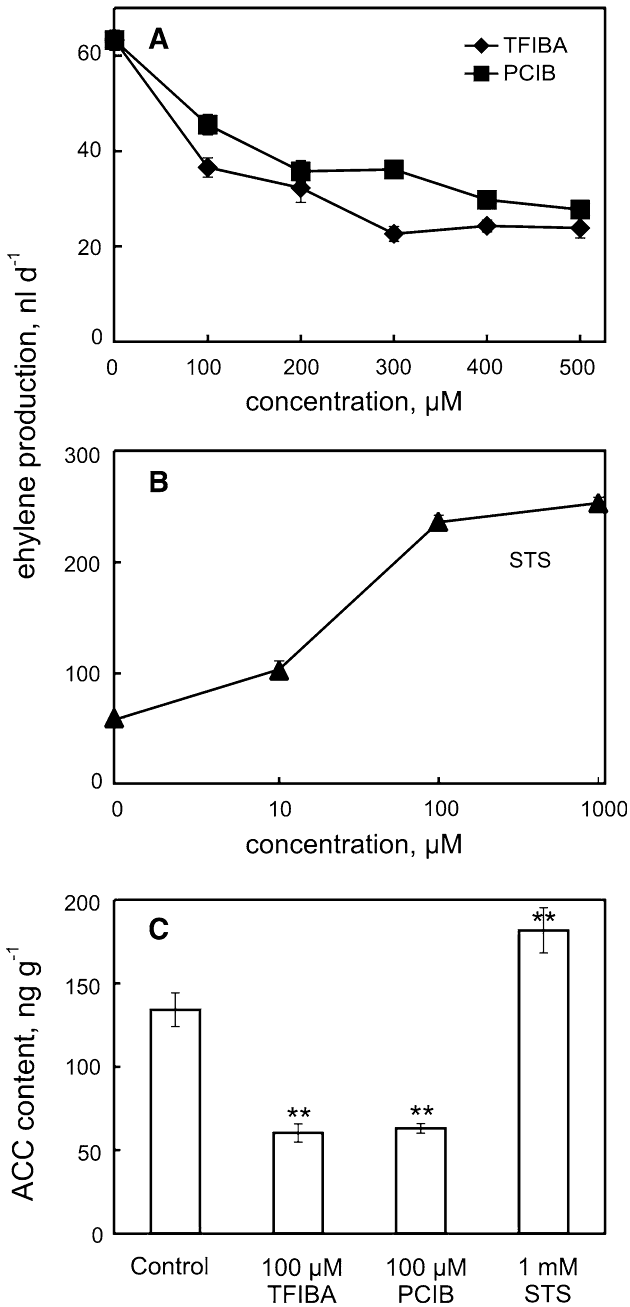

Fig. 4 Effect of TFIBA and PCIB (**a**) and silver thiosulfate (**b**) on ethylene production in flax seedlings. Fifty flax seeds were sealed between two layers of filter paper wetted with 3 ml buffer (5 mM Mes/Tris, pH 6.0) in 75-ml tissue culture flasks for 1 day. Seedlings were treated for 1 day. Average ± SE of five repeats with 50 roots each for TFIBA and PCIB; three repeats for silver thiosulfate. **c** ACC content in the flax root after treatment with TFIBA, PCIB, and silver thiosulfate. One-day-old seedlings were transferred to agar medium in vertical petri dishes and allowed to grow for 24 h. TFIBA and PCIB significantly inhibited and silver thiosulfate increased the ACC content of the roots. Average ± SE of four repeats with about 75 roots each; $^{**}P < 0.01$

ethylene-producing enzymes such as ACC synthase and/or ACC oxidase. Therefore, we identified five ACC synthase genes (GenBank accession No. EF661820–EF661824) and three ACC oxidase genes (GenBank accession No. EF661825–EF661827) in flax. The cloned and sequenced ACC synthase fragments (about 1100 bp) were more diverse than the ACC oxidase fragments (about 840 bp). The ACC synthase isoforms were 48% and the ACC oxidase isoforms 88% identical at the amino acid level. However, Lu-ACS1 and Lu-ACS2 differed by only one amino acid. Lu-ACS3 was 96% identical to the previous two isoforms, but the remaining two isoforms (Lu-ACS4-5) were different from each other (81% identity) and from the other three isoforms (Fig. 5a). Based on the phylogenetic tree of ACC synthase isoforms from *Arabidopsis*, Lu-ACS1, 2, and 3 grouped together and are closely related to AtACS 4, 5, 7, 8, 9, 11. On the other hand, Lu-ACS4 and Lu-ACS5 are more similar to AtACS2 and AtACS6 (Fig. 5b). As for ACC oxidases, Lu-ACO1 is more closely related to Lu-ACO3 (98% identity), whereas Lu-ACO2 is 89% identical to both Lu-ACO1 and Lu-ACO3 (Fig. 6).

Based on the Ct values relative to actin1, the transcription levels of *ACS1* and *ACS2* were similar and about two times more abundant than those of *ACS4* and *ACS5* and five times more abundant than *ACS3* in control roots at all examined time points. The time courses of *ACS1*, *2*, and *3* transcriptions were similar (Fig. 7a–c). These isoforms were downregulated by TFIBA and PCIB but upregulated by silver thiosulfate. Downregulation of *ACS1* by TFIBA and PCIB began 6 h after application (80 and 60%, respectively) and lasted for the next 18 h, when *ACS1* was reduced to about 40% of controls (Fig. 7a). Similarly, TFIBA and PCIB reduced *ACS2* transcription to about 40% of control levels (Fig. 7b). PCIB inhibited *ACS3* within 3 h (50% of control); TFIBA reduced *ACS3* transcription significantly 6 h after application (Fig. 7c). By 24 h, *ACS3* transcription decreased to less than 20% of controls. Conversely, silver thiosulfate caused upregulation of *ACS1*, *ACS2*, and *ACS3* within 6 h to 1.5-2 times the control level (Fig. 7a–c), but the stimulation was short-lived and after 12 h was similar to controls.

ACS4 transcription declined after about 12 h of treatment with TFIBA and PCIB to about 40 and 60% of controls, respectively (Fig. 7d). The inhibition lasted for the next 12 h. Silver thiosulfate inhibited *ACS4* more than TFIBA or PCIB within 6 h to about half of controls and at all time points (Fig. 7d). None of the three chemicals affected *ACS5* (Fig. 7e).

Fig. 5 **a** Multiple alignment of deduced amino acid sequences of flax ACC synthases using Clustal W software (http://www.ebi.ac.uk/Tools/clustalw/index.html). * represents identical amino acid residues; - represents gaps. **b** Phylogenetic tree built with ACC synthases in flax and in *Arabidopsis* using Mega3 (http://www.megasoftware.net/mega.html). Neighbor-joining method was used with 1000 bootstrap replicates

A

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Lu-ACS1    IQMGLAENQVSFDLLEDYLEKHPEASSTTHN----FKENALYQDYHGLKSFRQAMASFMS
Lu-ACS2    IQMGLAENQVSFDLLEDYLEKHPEASSTTHN----FKENALYQDYHGLKSFRQAMASFMS
Lu-ACS3    IQMGLAENQVSFDLLEDYLAKHPEASSTTHN----FKENALYQDYHGLKSFRQAMASFMS
Lu-ACS4    IQMGLAENQLCFDLVENWVKNNPASSICTPEGSSGFKDIAIFQDYHGLPQFRNAVANFMA
Lu-ACS5    IQMGLAENQLCFDMVEQWMNNNPSASICTSQGARGFKDIAIFQDYHGLPEFRNAVAKFMG
           *********  **  *      *  *  *      **  *  ******  ** * * **

Lu-ACS1    QIRGGRATFDPDRIVLTAGATAANELLTFILANPGDALLVPTPYYPGFDRDLRWRTGVKI
Lu-ACS2    QIRGGRATFDPDRIVLTAGATAANELLTFILANPGDALLVPTPYYPGFDRDLRWRTGVKI
Lu-ACS3    QIRGGRATFDPDRIVLTAGATAANELLTFILANPGDALLVPTPYYPGFDRDLRWRTGVKI
Lu-ACS4    KVRGDRVKFDPDRIVMAGGATGAHETLAFCVANPGEALLVPTPYYPGFDRDLRWRTGIQL
Lu-ACS5    KVRGERVKFNPDRIVMAGGATGAHETVAFCLADPGDAFLIPTPYYPGFDRDLRWRTGVQL
             ** *  * *****   *** * *   *  * ** * * *****************

Lu-ACS1    VPIHCDSSTNFQVTKEALQSAYNEAVSMGLKVRAVLITNPSNPLGATVQRVVLEDILDFS
Lu-ACS2    VPIHCDSSTNFQVTKEALQSAYNEAVSMGLKVRAVLITNPSNPLGATVQRVVLEDILDFS
Lu-ACS3    VPIHCNSSTNFQVTKEALEAAYDDAVSMGLKVRAVLITNPSNPLGATVQRVVLEEILDFS
Lu-ACS4    VPIECKSSNKFKLTIEDLEAAYETAKLNNIKVKGLLLTNPSNPLGTILDGETLRSVVAFT
Lu-ACS5    VPIECQSSNNFKLRIEDLESAYDMAKLNNVRVKGLLLTNPSNPLGTILDGNTLRSIVSFT
           *** * **  *    * *  **  *      *   * ********       *     *

Lu-ACS1    VEKNIHLVSDEIYSGSTFS--PDEFVSIAEVLND-------------RGSIQSES----E
Lu-ACS2    VEKNIHLVSDEIYSGSTFS--PDEFVSIAEVLND-------------RGSIQSES----E
Lu-ACS3    VEKNIHLVSDEIYSGSTFS--PDEFVSIAEVVND-------------RGSIRSES----E
Lu-ACS4    NSKSIHLICDEIYSATVFDGDAAEYVSIAEIVDE------------DRNSNVAPNRLNLD
Lu-ACS5    NDNNIHLICDEIYSATVFD--KPDYVSVAEVVDEYLNNANDDGEGDDGNSNGPRPTLNLD
               ***  *****   *       ** **                   *

Lu-ACS1    RVHIVYSLSKDLGLPGFRVGTIYSYNDAVVTTARRMSSFTLISSQTQHLLACMLSNAEFT
Lu-ACS2    RVHIVYSLSKDLGLPGFRVGTIYSYNDAVVTTARRMSSFTLISSQTQHLLACMLSSAEFT
Lu-ACS3    RVHIVYSLSKDLGLPGFRVGTIYSYNDAVVTTARRMSSFTLISSQTQHLLACMLSNAEFT
Lu-ACS4    LIHLVYSLSKDMGFPGFRVGIIYSYNDVVVGCARKMSSFGLVSAQTQHLLGSMLADDDFV
Lu-ACS5    LIHIVYSLSKDMGFPGFRVGIIYSYNDVVVSCARKMSSFGLVSTQTQHLIGSMLSDDDFV
             * ******* * ****** ****** **  ** **** * * *****   **    *

Lu-ACS1    ENYISKNRERLRRRYEMIIAGLRRAGIECL-EGN-AGLFCWMNLGPVLVDPTREAELSLW
Lu-ACS2    ENYISKNRERLRRRYEMIIAGLRRAGIECL-EGN-AGLFCWMNLGPVLVDPTREAELSLW
Lu-ACS3    ENYISKNRERLRKRYEMIISGLRRAGIECL-EGN-AGLFCWMNMGPVLVEPTREAELSLW
Lu-ACS4    GEFIAANRRRLSERHRRFTAGLERVGIRCLRSGNAAGLFVWMDLRRMMKEQSVEAETALW
Lu-ACS5    DEFIAANRRRLAERHRAFTGGLAEVGIRCLKTSN-AGLFVWMDLRRLLKEQSVEAETALW
              *  ** **  *      **   ** **   * **** **           ***  **

Lu-ACS1    KLILKDVRLNISPGSSCHCSEPGWFRVC
Lu-ACS2    KLILKDVRLNISPGSSCHCSEPGWFRVC
Lu-ACS3    KLILKDVRLNISPGSSCHCSEPGWFRVC
Lu-ACS4    KVIIDEVKLNVSPGTSFHCSEPGWFRVC
Lu-ACS5    RVIINEVKLNVSPGSSFHCSEPGWFRVC
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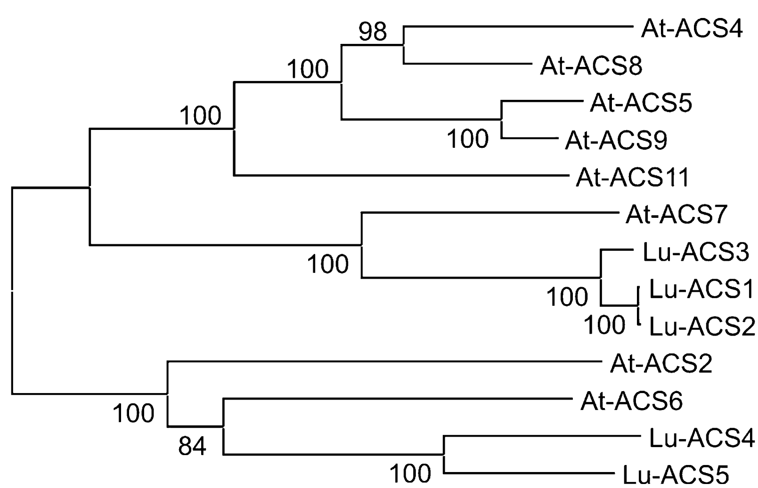
B



ACO3 was the most prominent among the ACC oxidase isoforms and two and five times more abundant than *ACO2* or *ACO1*. *ACO1* was reduced by TFIBA to 60% of controls but transiently stimulated by silver thiosulfate; PCIB had no effect (Fig. 7f). *ACO2* transcription changed relatively little during the 24-h observation time. As for *ACO1,* silver thiosulfate transiently upregulated *ACO2* levels and PCIB had no effect (Fig. 7g). TFIBA and PCIB inhibited *ACO3* after 12 and 24 h (about 60% of controls) but silver thiosulfate had no effect (Fig. 7h).

IAA treatment of flax roots for 6 h did not affect ACC synthase transcription at less than 10^{-8} M (Fig. 8a).

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Lu-ACO1      CENWGFFEILNHPIPVELLDMVEAMTKEHYRKCLEQRFKELVKSKGLDEVDSEIKDMDWE
Lu-ACO3      CENWGFFEILNHPIPVELLDTVEAMTKEHYRKCLEQRFKELVKSKGLEELDSEIKDMDWE
Lu-ACO2      CENWGFFEVLNHPIQVELLDTVEKMTKEHYRKCMEQRFKELVKSKGLEEVDSEIKDMDWE
             ******** ***** ***** ** ********* ************* * **********

Lu-ACO1      STFYLKHLPESNINEVPDLEERYREVMKDFAGRLEKLAEELLDLLCENLGLEKGYLKKAF
Lu-ACO3      STFYLKHLPESNINEVPDLEDRYREVMKDFAGRLEKLAEELLDLLCENLGLEKGYLKKAF
Lu-ACO2      STFFLRHLPDSNINDIPDLEEDYRKVMKEFAVKLEKLAEELLDLLCENLGLEKGYLKKAF
             *** * *** ****  ****  ** *** **  ***************************

Lu-ACO1      YGSKGLPTFGTKVSNYPPCPKPDLIKGLRAHTDAGGIILLFQDDKVSGLQLLKDGEWVDV
Lu-ACO3      YGSKGLPTFGTKVSNYPPCPKPDLIKGLRAHTDAGGIILLFQDDKVSGLQLLKDGEWVDV
Lu-ACO2      YGSKGAPTFGTKVSNYPPCPKPDLIKGLRAHTDAGGIILLFQDDKVSGLQLLKDGKWVDV
             ***** ************************************************* ****

Lu-ACO1      PPMRHSIVINLGDQIEVITNGRYKSVEHRVVAQTDGTRMSIASFYNPGNDAVIYPAPQLL
Lu-ACO3      PPMRHSIVINLGDQIEVITNGRYKSVEHRVVAQTDGTRMSIASFYNPGNDAVIYPAPQLL
Lu-ACO2      PPMHHSIVVNLGDQIEVITNGKYKSVEHRVVAQTDGTRMSIASFYNPGSDAVIYPAPELI
             *** **** ************ ************************** ******** *

Lu-ACO1      EGESETEKK-SITYPKFVFDDYMKLYAGLKFEAKEPRFEAM 280
Lu-ACO3      EGESETEKK-SITYPKFVFDDYMKLYAGLKFEAKEPRFEAM 280
Lu-ACO2      EKEEEEEKKVATTYPKFVFEDYMKLYAGLKFEAKEPRFEAM 281
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Fig. 6 Comparison of deduced amino acid sequences of flax ACC oxidases. Clustal W software (http://www.ebi.ac.uk/Tools/clustalw/index.html) was used for multiple alignments. * represents identical amino acid residues

Increasing IAA concentrations transiently upregulated *ACS1* and *ACS2* (1.2-fold of controls at 10^{-7} M). Higher IAA concentrations (10^{-6} M) downregulated these isoforms (Fig. 8a). *ACS3* expression was upregulated almost 2-fold by 10^{-7} M IAA and remained elevated at higher IAA concentrations. Increasing IAA concentration progressively inhibited *ACS4* and *ACS5* transcription.

ACC oxidases showed upregulation with increasing IAA concentrations; the strongest effect was observed for *ACO1*, which was upregulated at all auxin concentrations with a maximum at 10^{-6} M IAA (Fig. 8b). *ACO2* was upregulated by 10^{-7} M IAA (1.6-fold) but showed no response to higher auxin concentrations. Similarly, *ACO3* transcription decreased with increasing auxin concentrations.

Discussion

TFIBA, PCIB, and Silver Promote Root Growth

Although the effectiveness of the compounds differed at optimal concentrations, the three compounds similarly affected root elongation, diameter, and root hair development (Figs. 1 and 2). Previous work on TFIBA mentioned only promotion of root elongation (Katayama and others 1995; Zhang and Hasenstein 2000, 2002) but did not consider root diameter or root hair development. A combination of root elongation, diameter, and hair formation better assesses physiologic effects than does elongation alone. The variability of these three parameters suggests that TFIBA interferes with ethylene because ethylene inhibits root elongation, causes swelling of the tip (Bertell and others 1990), and enhances formation of root hairs (Masucci and Schiefelbein 1994, 1996). During early stages of root growth, auxin and ethylene occur at their highest concentrations and inhibit root elongation (Bialek and others 1992; Epstein and others 1980; Lalonde and Saini 1992; Tillberg 1977). TFIBA was most effective in promoting root elongation during the early stages of root growth, and the effect of TFIBA was reversed by both IAA and ACC (Fig. 3). Thus, TFIBA is likely to promote root growth when auxin concentrations are inhibitory for root elongation. If auxin concentrations are limited, antiauxins are more likely to inhibit growth, as has been shown for PCIB in *Arabidopsis* (Oono and others 2003). Furthermore, the inhibition of ethylene and ACC production by TFIBA and PCIB but promotion by silver thiosulfate (Fig. 4) suggests a different mode of action for Ag^{+}, despite the common effect on root growth and morphology. The reduction of ethylene and ACC by TFIBA and PCIB (Fig. 4a, c) may originate from the inhibition of auxin, which is thought to control ethylene biosynthesis (Abeles 1973; Kang and others 1971).

The partial but incomplete compensation of TFIBA effects by ACC on root growth (Fig. 3) may also stem from overlapping functions of auxin and ethylene on root growth (Alonso and others 2003; Pitts and others 1998; Rahman and others 2002; Stepanova and others 2007; Swarup and others 2002). Li and others (2006) reported that auxin response factors ARF19 and ARF7 not only participate in auxin signaling, but also play a critical role for the ethylene pathway. The enhancement of primary root elongation by TFIBA, PCIB, and silver thiosulfate (Fig. 1) suggests that ethylene inhibits early root elongation, as was also demonstrated by the promotional effect of the ethylene biosynthesis inhibitor L-α-(2-aminoethoxyvinyl) glycine (Zhang and Hasenstein 2002).

Therefore, inhibiting auxin or ethylene can produce similar effects on root growth and may explain the

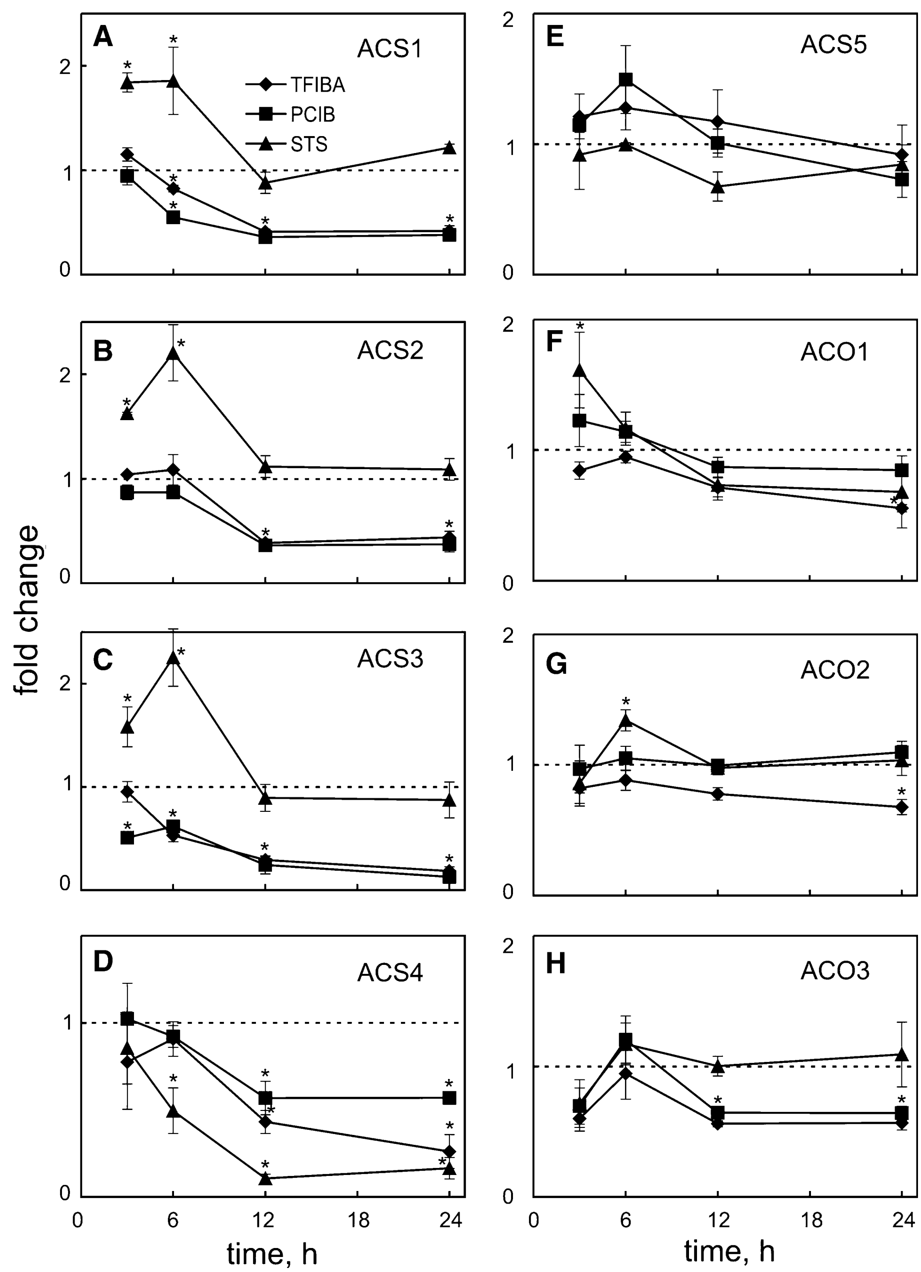

Fig. 7 Time course of ACC synthase and ACC oxidase transcription in flax roots treated with 100 µM TFIBA, 100 µM PCIB, and 1 mM silver thiosulfate. ACS1 (**a**); ACS2 (**b**); ACS3 (**c**); ACS4 (**d**); ACS5 (**e**); ACO1 (**f**); ACO2 (**g**); ACO3 (**h**). One-day-old seedlings were transferred to fresh medium and treated for the indicated time. *Actin1* was used as reference gene and showed less than 10% variability. The onefold line indicates expression of genes in untreated controls. Each treatment was repeated three times, with three replicates per PCR plate; $*P < 0.05$

interaction between TFIBA and ACC. Although it is possible that TFIBA interferes with the auxin signaling system, similar to PCIB (Oono and others 2003), this mechanism remains to be discovered.

TFIBA Is not a Universal Root-Growth Promoter

The TFIBA-promoted root elongation caused speculations of improved rooting of seedlings (Katayama and Gautam 1997) and the mimicking as of yet unidentified root-specific growth promoters (Zhang and Hasenstein 2000). However, root elongation coincided with reduced root thickness, reduced root hair growth (Figs. 1 and 2b–d), and reduced lateral root formation (Zhang and Hasenstein 2000). Because root thickness is important for mechanical strength, hairs are important for water and mineral absorption, and lateral roots are essential for anchorage and soil exploitation, TFIBA may not be unequivocally beneficial for seedling development despite its promotion of root elongation. The promotional effect of TFIBA on root elongation also depends on the nutrient status and age of seedlings (data not shown). The TFIBA effect on root growth decreased as seedlings developed or when nutrients were added to the growth medium. Therefore, TFIBA is not likely to improve the overall performance of a root system, soil utilization, or plant productivity.

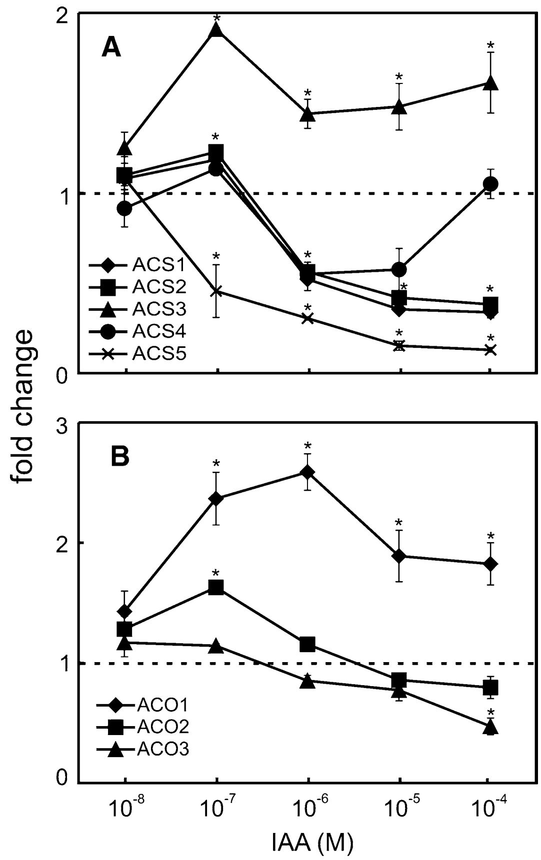

Fig. 8 Expression levels of ACC synthases (**a**) and ACC oxidases (**b**) in flax roots after treatment with different concentrations of IAA. One-day-old seedlings were transferred to fresh medium and treated with IAA for 6 h. *Actin1* was used as reference gene and showed less than 10% variability. The onefold line indicates expression of genes in untreated controls. Averages ± SE of three experiments; $*P < 0.05$

TFIBA and PCIB Affect Ethylene Synthesis

ACC synthase and ACC oxidase represent gene families. *Arabidopsis* has eight active ACC synthases (Tsuchisaka and Theologis 2004) and at least two ACC oxidase genes (Raz and Ecker 1999) similar to tomato, where eight ACC synthases (Shiu and others 1998) and four ACC oxidases (Nakatsuka and others 1998) have been identified. We identified five isoforms of ACC synthase and three isoforms of ACC oxidase (Figs. 6 and 7). Although the complete number of copies for these genes in flax is unknown, the identified isoforms should be representative for these two gene families in this species. Despite the high similarity, real-time PCR was able to distinguish all ACC synthase and ACC oxidase isoforms.

TFIBA and PCIB reduced the transcription of *ACS1*, *ACS2*, and *ACS3* similarly (Fig. 7a–c). The upregulation of these three isoforms by silver thiosulfate underscores their importance for ethylene production. The similarity among these isoforms is also supported by their phylogenetic relationship with *AtACS4* in *Arabidopsis* (Fig. 6b), which is induced by IAA (Abel and others 1995; Wang and others 2005). IAA enhanced the transcription of *ACS3*, *ACO1*, and, at low concentrations, *ACO2* (Fig. 8) but did not induce *ACS4* or *ACS5* transcription despite their similarity with the auxin-susceptible isoforms *AtACS2* and *AtACS6* (Tsuchisaka and Theologis 2004; Yamagami and others 2003). The auxin susceptibility of ACC synthase isoforms may be species specific because *AtACS9* has been demonstrated as not IAA inducible (Tsuchisaka and Theologis 2004; Yamagami and others 2003). In contrast, TFIBA and PCIB decreased the expression of all ACS isoforms except *ACS5* (Fig. 7). These observations suggest that TFIBA and PCIB at least partially counteract IAA-induced ethylene biosynthesis.

The ACC oxidase isoforms *ACO1* and *ACO2* were inhibited by TFIBA but promoted by silver thiosulfate and IAA (Fig. 7f, g, 8b). *ACO1* was promoted by the entire range of tested IAA concentrations (Fig. 8b). *ACO3*, the most abundant isoform, was not induced by IAA but inhibited by TFIBA and PCIB (Fig. 7h). This result suggests that *ACO3* transcription may be saturated by endogenous auxin but suppressible by antiauxins such as TFIBA and PCIB.

The sensitivity of ACC synthase and ACC oxidase isoforms' transcription to auxin concentration (Fig. 8) indicates that these enzymes may be under auxin control. Transcription of abundant isoforms may be saturated by endogenous IAA and not be inducible (*ACS4*, *ACS5*, *ACO3*) or stimulated only by low IAA concentrations (*ACS1*, *ACS2*, *ACO2*). The well-documented sensitivity of roots to exogenous auxin is in line with saturation of transcription of most ACC synthase and ACC oxidase isoforms by 0.1 μM IAA.

Possible Mode of Action of TFIBA

Comparing TFIBA and PCIB with other auxin antagonists shows important differences. Yokonolide B stimulates lateral root formation and does not inhibit root hair growth (Hayashi and others 2003). Terfestatin A does not compete with auxin for the receptor because it does not inhibit the interaction between SCF^{TIR1} and Aux/IAA (Yamazoe and others 2005). However, these compounds inhibit auxin-induced gene expression by blocking the degradation of Aux/IAA repressor proteins in *Arabidopsis* (Hayashi and others 2003; Yamazoe and others 2005).

The structural similarity between TFIBA and PCIB and the auxins IAA and 2,4-D, respectively, suggests some competition at a recognition site. This concept is supported by different activities for IAA and 2,4-D (Rahman and others 2006, 2007) and by observations that the

Arabidopsis mutant *aar1* is resistant to PCIB and 2,4-D but responds normally to IAA (Rahman and others 2006). The low effectiveness of PCIB on some auxin-controlled processes could be the result of some structural specificity. Compared with PCIB, TFIBA is the stronger promoter of root elongation (Fig. 1), more powerful inhibitor for root hair growth (Fig. 2), and a more potent ethylene inhibitor (Fig. 4a). Although the experiments described here focus on ethylene, our data do not rule out that TFIBA might be an auxin antagonist similar to PCIB.

Although the antiauxin mode of action for PCIB results from its stabilization of the Aux/IAA protein (Oono and others 2003), similar modes of action for TFIBA are possible but remain to be detected.

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References

Abel S, Nguyen MD, Chow W, Theologis A (1995) ASC4, a primary indole acetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana*. J Biol Chem 270:19093–19099

Abeles F (1973) Ethylene in plant biology. Academic Press, New York

Aberg B (1950) On auxin antagonists and synergists in root growth. Physiol Plant 3:447–461

Aberg B (1951) The interaction of some auxin antagonists and 2,4-D in root growth. Physiol Plant 4:627–640

Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, Ausubel FM, Ecker JR (2003) Five components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in *Arabidopsis*. Proc Natl Acad Sci USA 100:2992–2997

Atta-Aly MA, Saltveit ME, Hobson GE (1987) Effect of silver ions on ethylene biosynthesis by tomato fruit tissue. Plant Physiol 83:44–48

Bertell G, Bolander E, Eliasson L (1990) Factors increasing ethylene production enhance the sensitivity of root-growth to auxins. Physiol Plant 79:255–258

Bialek K, Michalczuk L, Cohen JD (1992) Auxin biosynthesis during seed germination in *Phaseolus vulgaris*. Plant Physiol 100:509–517

Burström H (1950) Studies on growth and metabolism of roots. IV. Positive and negative auxin effects on cell elongation. Physiol Plant 3:277–292

Epstein E, Cohen JD, Bandurski RS (1980) Concentration and metabolic turnover of indoles in germinating kernels of *Zea mays* L. Plant Physiol 65:415–421

Guzman P, Ecker JR (1990) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell 2:513–523

Hansen H, Grossmann K (2000) Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiol 124:1437–1448

Hayashi K, Jones AM, Ogino K, Yamazoe A, Oono Y, Inoguchi M, Kondo H, Nozaki H (2003) Yokonolide B, a novel inhibitor of auxin action, blocks degradation of AUX/IAA factors. J Biol Chem 278:23797–23806

Jones JF, Kende H (1979) Auxin-induced ethylene biosynthesis in subapical stem sections of etiolated seedlings of *Pisum sativum* L. Planta 146:649–656

Kaldorf M, Ludwig-Müller J (2000) AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. Physiol Plant 109:58–67

Kang BG, Newcomb W, Burg SP (1971) Mechanism of auxin-induced ethylene production. Plant Physiol 47:504–509

Katayama M, Gautam RK (1997) Synthesis and biological activities of fluorinated plant growth regulators, 4,4,4-trifluoro-3-(3-indolyl)butyric acids and 4,4,4-trifluoro-3-(2-indolyl)butyric acid bearing a methyl group on the indole nucleus. J Pestic Sci 22:331–337

Katayama M, Kato K, Kimoto H, Fujii S (1995) (S)-(+)-4, 4, 4-Trifluoro-3-(indole-3-) butyric acid, a novel fluorinated plant-growth regulator. Experientia 51:721–724

Lalonde S, Saini HS (1992) Comparative requirement for endogenous ethylene during seed germination. Ann Bot 69:423–428

Li JS, Dai XH, Zhao YD (2006) A role for auxin response factor 19 in auxin and ethylene signaling in *Arabidopsis*. Plant Physiol 140:899–908

Lizada MCC, Yang SF (1979) Simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100:140–145

Masucci JD, Schiefelbein JW (1994) The RHD6 mutation of *Arabidopsis thaliana* alters root-hair initiation through an auxin-associated and ethylene-associated process. Plant Physiol 106:1335–1346

Masucci JD, Schiefelbein JW (1996) Hormones act downstream of TTG and GL2 to promote root hair outgrowth during epidermis development in the *Arabidopsis* root. Plant Cell 8:1505–1517

McRae DH, Bonner J (1953) Chemical structure and antiauxin activity. Physiol Plant 6:485–509

Nakatsuka A, Murachi S, Okunishi H, Shiomi S, Nakano R, Kubo Y, Inaba A (1998) Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. Plant Physiol 118:1295–1305

Oono Y, Ooura C, Rahman A, Aspuria ET, Hayashi K, Tanaka A, Uchimiya H (2003) p-Chlorophenoxyisobutyric acid impairs auxin response in *Arabidopsis* root. Plant Physiol 133:1135–1147

Peck SC, Kende H (1995) Sequential induction of the ethylene biosynthetic enzymes by indole-3-acetic-acid in etiolated peas. Plant Mol Biol 28:293–301

Pitts RJ, Cernac A, Estelle M (1998) Auxin and ethylene promote root hair elongation in *Arabidopsis*. Plant J 16:553–560

Rahman A, Hosokawa S, Oono Y, Amakawa T, Goto N, Tsurumi S (2002) Auxin and ethylene response interactions during *Arabidopsis* root hair development dissected by auxin influx modulators. Plant Physiol 130:1908–1917

Rahman A, Nakasone A, Chhun T, Ooura C, Biswas KK, Uchimiya H, Tsurumi S, Baskin TI, Tanaka A, Oono Y (2006) A small acidic protein 1 (SMAP1) mediates responses of the Arabidopsis root to the synthetic auxin 2,4-dichlorophenoxyacetic acid. Plant J 47:788–801

Rahman A, Bannigan A, Sulaman W, Pechter P, Blancaflor EB, Baskin TI (2007) Auxin, actin and growth of the *Arabidopsis thaliana* primary root. Plant J 50:514–528

Raz V, Ecker JR (1999) Regulation of differential growth in the apical hook of *Arabidopsis*. Development 126:3661–3668

Sambrook J, Fritsch EF, Maniatis T (1989) In: Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Shiu OY, Oetiker JH, Yip WK, Yang SF (1998) The promoter of LE-ACS7, an early flooding-induced 1-aminocyclopropane-1-carboxylate synthase gene of the tomato, is tagged by a Sol3 transposon. Proc Natl Acad Sci USA 95:10334–10339

Stepanova AN, Yun J, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in *Arabidopsis* roots. Plant Cell 19:2169–2185

Swarup R, Parry G, Graham N, Allen T, Bennett M (2002) Auxin cross-talk: integration of signalling pathways to control plant development. Plant Mol Biol 49:411–426

Thimann KV (1948) Plant growth hormones. In: Thimann KV (ed) The hormones. Academic Press, New York, pp 5–119

Tillberg E (1977) Indoleacetic-acid levels in *Phaseolus*, *Zea*, and *Pincus* during seed germination. Plant Physiol 60:317–319

Trebitsh T, Riov J (1987) Inhibition of activity of the ethylene-forming enzyme by alpha(para-chlorophenoxy)isobutyric acid. J Plant Growth Regul 5:207–215

Tsai DS, Arteca RN (1984) Inhibition of IAA-induced ethylene production in etiolated mung bean hypocotyl segments by 2,3,5-triiodobenzoic acid and 2-(para-chlorophenoxy)-2-methyl propionic-acid. Physiol Plant 62:448–452

Tsuchisaka A, Theologis A (2004) Unique and overlapping expression patterns among the *Arabidopsis* 1-amino-cyclopropane-1-carboxylate synthase gene family members. Plant Physiol 136:2982–3000

Wang NN, Shih MC, Li N (2005) The GUS reporter-aided analysis of the promoter activities of *Arabidopsis* ACC synthase genes *AtACS4*, *AtACS5*, and *AtACS7* induced by hormones and stresses. J Exp Bot 56:909–920

Woltering EJ, Balk PA, Nijenhuis-De Vries MA, Faivre M, Ruys G, Somhorst D, Philosoph-Hadas S, Friedman H (2005) An auxin-responsive 1-aminocyclopropane-1-carboxylate synthase is responsible for differential ethylene production in gravistimulated *Antirrhinum majus* L. flower stems. Planta 220:403–413

Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, Theologis A (2003) Biochemical diversity among the 1-amino-cyclopropane-1-carboxylate synthase isozymes encoded by the *Arabidopsis* gene family. J Biol Chem 278:49102–49112

Yamazoe A, Hayashi K, Kepinski S, Leyser O, Nozaki H (2005) Characterization of terfestatin A, a new specific inhibitor for auxin signaling. Plant Physiol 139:779–789

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol Plant Mol Biol 35:155–189

Yu YB, Yang SF (1979) Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. Plant Physiol 64:1074–1077

Zhang NG, Hasenstein KH (2000) Halogenated auxins affect microtubules and root elongation in *Lactuca sativa*. J Plant Growth Regul 19:397–405

Zhang NG, Hasenstein KH (2002) 4,4,4-Trifluoro-3-(indole-3-)butyric acid promotes root elongation in Lactuca sativa independent of ethylene synthesis and pH. Physiol Plant 116:383–388