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ORIGINAL ARTICLE

Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*

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Abstract Certain plant growth-promoting rhizobacteria (PGPR), in the absence of physical contact with a plant stimulate growth via volatile organic compound (VOC) emissions, through largely unknown mechanisms. To probe how PGPR VOCs trigger growth in plants, RNA transcript levels of *Arabidopsis* seedlings exposed to *Bacillus subtilus* (strain GB03) were examined using oligonucleotide microarrays. In screening over 26,000 protein-coded transcripts, a group of approximately 600 differentially expressed genes related to cell wall modifications, primary and secondary metabolism, stress responses, hormone regulation and other expressed proteins were identified. Transcriptional and histochemical data indicate that VOCs from the PGPR strain GB03 trigger growth promotion in *Arabidopsis* by regulating auxin homeostasis. Specifically, gene expression for auxin synthesis was up regulated in aerial regions of GB03-exposed plants; auxin accumulation decreased in leaves and increased in roots with GB03 exposure as revealed in a transgenic DR5::GUS *Arabidopsis* line, suggesting activation of basipetal auxin transport. Application of the auxin transport inhibitor 1-naphthylphthalamic acid (NPA) restricted auxin accumulation to sites of synthesis thereby preventing GB03-mediated decreases in shoot auxin levels as well as thwarting GB03-mediated growth promotion. In addition, microarray data revealed coordinated regulation of cell wall loosening enzymes that implicated cell expansion with GB03 exposure, which was confirmed by comparative cytological measurements. The discovery that bacterial VOCs, devoid of auxin or other known plant hormones regulate auxin homeostasis and cell expansion provides a new paradigm as to how rhizobacteria promote plant growth.

Keywords Auxin transport · *Bacillus subtilus* GB03 · Cell expansion · Plant growth promotion · Transcriptional profiling · Rhizobacterial signaling

Abbreviations

IAA	Indole acetic acid
NPA	1-Naphthylphthalamic acid
PGPR	Plant growth promotion rhizobacteria
rt-PCR	Real time PCR
RT-PCR	Reverse transcriptase PCR
VOCs	Volatile organic compounds

Introduction

Plants and certain rhizobacteria form mutually beneficial associations mediated through an exchange of chemical metabolites. Root exudates provide energy-rich organic

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acids that are metabolized within hours by soil microorganisms (Jones et al. 2003), while specialized microbes, referred to as plant growth-promoting rhizobacteria (PGPR), generate an array of biologically active compounds that elicit growth promotion and induced systemic resistance (ISR) in plants (Kloepper et al. 1999; Ryu et al. 2004; Paré et al. 2005). Widely accepted mechanisms for plant-growth promotion by PGPR include bacterial synthesis of plant hormones (Loper and Schroth 1986; Timmusk et al. 1999; MacDonald et al. 1986), breakdown of plant-produced ethylene (Glick et al. 1999), and increased mineral and nitrogen availability in the soil (Lin et al. 1983).

Blends of volatile chemicals emitted from specific strains of PGPR, in the absence of physical contact with plant roots, also trigger growth promotion in *Arabidopsis* (Ryu et al. 2003). Of the several PGPR assayed, two strains *Bacillus subtilis* GB03 and *B. amyloliquefaciens* IN937a elicited plant growth promotion via volatile emissions. To elucidate signaling networks involved in growth promotion via PGPR VOCs, a series of mutant lines were tested (Ryu et al. 2003). Enhanced total leaf surface area was observed from exposure to GB03 VOCs for mutant lines ethylene-insensitive (*etr*1), auxin-transporter-deficient and ethylene-insensitive (*eir*1), gibberellic acid-insensitive (*gai*2), and brassinosteroid-insensitive (*cbb*1) mutants, thereby negating the essential involvement of brassinosteroid-, gibberellic acid-, or ethylene-signaling in the activation of growth promotion by VOCs. Considering that the auxin efflux carrier EIR1 (also known as AGR and AtPIN2) is a root-specific protein (Luschnig et al. 1998; Sieberer et al. 2000), a mutation in this auxin transporter (*eir1*) does not necessarily affect basipetal auxin transport or auxin action in leaves. Therefore, participation of auxin in VOC-induced growth promotion cannot be excluded. The cytokinin receptor-deficient (*cre*1) and cytokinin- and ethylene-insensitive (*ein*2) mutants were also tested and exhibited no growth promotion with exposure to GB03 VOCs. Although the function of EIN2 has not been clearly resolved, *ein2* mutants have been recovered in screens for *Arabidopsis* mutants resistant to auxin transport inhibitors, cytokinins, or abscisic acid and in screens for delayed senescence (Alonso et al. 1999), implicating auxin, cytokinin, and/or abscisic acid as possible mediators of growth promotion by GB03 VOCs.

For field applications, GB03 is thought to survive on seeds until planted and then uses seed exudates during seed germination, directionally multiplying to reach young roots, and maintaining a robust population in the presence of field crops via plant–microbial interactions (Kloepper et al. 2004). The minimum bacterial density in the soil for triggering observable plant responses is ca. 10^4 colony forming units (cfu)/root. GB03 can maintain soil populations of 10^5 cfu/root for over 60 days after planting; negative plant development effects are not observed with bacterial populations as high as 10^{10} cfu/root (Kokalis-Burelle et al. 2006). The inoculum in Petri dish bioassays for VOC-mediated growth promotion contains ca. 10^7 cfu.

To probe plant-signaling pathways activated by bacterial VOCs that mediate growth, we have begun to characterize global changes in the *Arabidopsis* transcriptome using available microarray technology. Microarray results identified a series of physiological changes associated with growth, photosynthesis, and stress tolerance. In this study, we report that bacterial VOCs induce endogenous auxin synthesis and regulate auxin transport *in planta*; the regulation of transcripts associated with cell wall loosening provides insight into the mechanism of leaf cell expansion that is observed in GB03 exposed plants.

Material and methods

Plant materials and treatments

Arabidopsis thaliana (*Col-0*) seeds were surface sterilized then planted onto one side of specialized plastic Petri dishes (100 × 15 mm) that contained a center partition (I plates, Fisher Scientific, Pittsburgh); both sides contained one-half-strength MS solid media with 0.8% (w/v) agar and 1.5% (w/v) sucrose. Before being placed in the growth room, seeds were vernalized for 2 days at 4°C in the absence of light. The growth room is set to a 16-h-light/8-h-dark cycle under metal halide and high pressure sodium lamps with a total light intensity of 200 µmol m^{-2} s^{-1}, a temperature of 25 ± 4°C and a relative humidity of 40±10%.

One day before plant experiments, PGPR strain *Bacillus subtilis* GB03 were streaked onto TSA (tryptic soy agar) plates and incubated at 28°C in the absence of light for 24 h. PGPR cells were harvested from TSA plates in double distilled water (DDW) to yield 10^9 CFU ml^{-1} as determined by optical density and serial dilutions with plate counts. To the non-plant side of a given Petri dish containing 2-day-old *Arabidopsis* seedlings, 20 µl of GB03 suspension culture or DDW was applied drop wise. By positioning plants and bacteria on separate sides of the partitioned Petri dish, plants are exposed to bacterial VOCs without physical contact.

For auxin inhibition assays, plant growth and inoculations were the same as described above except that on the side of the I-plate in which *Arabidopsis* seedlings were to be grown, the MS media contained 2-((1 naphthalenylamino)-carbonyl) benzoic acid (NPA) with concentrations of 1 or 10 µM; solvent controls contained only DMSO. For total leaf surface area quantification, seedlings were photographed fourteen days after the treatments using an Olympus C-4000 camera (Olympus American Inc., USA). Images were imported into Adobe Photoshop 5.5 and leaf

surface area measurements were determined by using an available histogram function; pixel areas were calibrated based the total Petri dish area.

Gene expression profiling

Two-day-old seedlings were treated with GB03 VOCs or water as control, then harvested 48 or 72 h after treatment. For each time set, microarray hybridizations consisted of three biological replicates with dye swapping for one replicate. Each replicate consisted of approximately 300 seedlings (ca. 4 g); total RNA was extracted using TRI Reagent (Molecular Research Center Inc, Cincinnati, OH, USA).

Seventy-mer oligonucleotide microarray slides containing the complete *Arabidopsis* genome (26,751 genes) were purchased from Galbraith's laboratory (University of Arizona, Tucson, AZ, USA). Slides contain 29,000 spots; multiple spots with same gene ID were averaged when results were reported. A complete listing of probes can be accessed at http://www.ag.arizona.edu/microarray. Target amplification, labeling and hybridization were performed following the protocol provided by the array producer (http://ag.arizona.edu/microarray/methods.html). Target cDNAs were labeled using Amino-Allyl aRNA Amplification Kit (Ambion, Austin, TX, USA) and NHS dyes Cy3 or Cy5 (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) according to the manufacturer's protocol. The arrays were scanned using a GenePix 4100 array scanner (Axon Instruments, Sunnyvale, CA, USA). Spot statistical analysis was performed according to the manufacturer's guidelines (GeneSpring 7.0; Silicon Genetics, Redwood, CA, USA). A 40% change, either up- or down-regulation, in the expression level compared with the control was selected as the threshold for a gene to be classified as altered in response to PGPR VOCs. As a reference, gene expression fold-change ratios $\geq$1.25–2.0 (or $\leq$0.75–0.5) have been found significant with plant exposure to other environmental changes (Halitschke and Baldwin 2003; Salzman et al. 2005). Only genes that pass the flag filtering, identified as present (GeneSpring7.0), and pass the *T*-test *P*-values $\leq$0.05 for at least one of the two time points tested were considered differentially regulated with PGPR treatment.

Reverse-transcriptase PCR (RT-PCR)

First strand cDNA was synthesized from 5 µg of total RNA using MuMLV-RT (Fisher Scientific, Houston, TX, USA). The following primers were used (5′ to 3′): [*UBQ10*], CGATTACTCTTGAGGTGGAG and AGACCAAGTGA AGTGTGGAC; [ARR5], GAGCGGTTACTCAGAGTC TC and GCTTCAAGCTCTCTCTTGTG; [*IPSI*], TGG AGAACATTGTGCCACTC and TCCATGGCTCGCTT ACTATC; [*GT20*], TCCTTCTTCCGTCACACAAG and TCTACCACCAAGATCAGGAG; [*UGE4*], GGAGCTTG TTGGTCCTGATC and CAGACGTTCCTTGACCAGTC; [*EXP5*], TAGTAATCTCGCTTCTCGTG and CGTTGAT CGTGAACCTTATC; [*IRT1*], GCAATCTCTCCAGCAA CTTC and TCTTGCTGGTGTATAGGCTC. Agarose gel electrophoresis images were taken by Kodak Gel Logic 100 Imaging System (Fisher Scientific, Houston, TX, USA) and quantified by using Image J 1.33u (http://rsb.info.nih.gov/ij/, National Institute of Health, USA).

Quantitative real-time PCR

Reverse transcription of all the RNA samples was carried out using random hexamers. ABI TaqMan Reverse Transcription Reagent Kit (Applied Biosystems) was used for first strand synthesis in 50 µl reactions (containing 1 µg of total RNA) at 37°C for 60 min. Quantitative real-time PCR was used to assay the gene expression levels. The expression levels of the *AEC*, *EXP5*, *NIT1*, and *NIT2* genes in different *Arabidopsis* tissues were detected by ABI PRISM 7000 Sequence Detection System. The primers were designed by using PrimerQuest program (Integrated DNA Technology). The following primers were used (5′ to 3′): [AEC], TCTGATCGTTGCAAGCTCTTCCCT and AAC CACCCAAGAAGTCCTCCGATT; [EXP5], AAAGGCC GATCCATTGTGGTCACT and ACTTCTCTTGCACCG AACCCTTCT; [NIT1], AAGCAAGGGAGCAGAGCTA GTGTT and TTTCCTAGCCACGTCAGCCAATCT; [NIT2], TCCGCAAGTACCATGCTTCTGCTA and ACC CAAGAACTGACCTTGTGGACT. 18S rRNA primers were used for RNA normalization. SYBR Green PCR Master Mix (ABI) was used for 50 µl PCR reactions as follow: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 60s. Each sample was assayed three times. The relative expression levels of all the samples were calculated and analyzed (User Bulletin #2, ABI PRISM 7700 Sequence Detection System). The threshold cycle (C*t* value) of the target genes and 18S RNA in different samples were obtained after quantitative real-time PCR reaction. In brief, the normalizer 18S RNA C*t* value is subtracted from the gene of interest C*t* (target gene) to produce the dC*t* value of the sample. The dC*t* value of the calibrator (the sample with the highest dC*t* value) was subtracted from every other sample to produce the ddC*t* value. Two to the $-$ddC*t* power ($2^{-\mathrm{ddCt}}$) was taken for every sample as the relative expression levels.

GUS histochemical analysis

Wild type *Arabidopsis Col-0* and transgenic line *DR5::GUS* were grown as described in Plant materials and treatments. Seedlings were collected for analysis as indicated in Results. The GUS staining buffer was composed

of: 100 mM $NaPO_4$ (pH 7.0); 1 mM EDTA; 5 mM potassium ferrocyanide; 5 mM potassium ferricyanide; 0.1 Triton X-100; and 1 mM X-Gluc. Samples were put into Eppendorff tubes containing the staining buffer and incubated at 37°C overnight, followed by ethanol (70% in H_2O) rinsing (3×) at 60°C for 30 min each rinse.

Cell size measurements

Twelve-day-old seedlings exposed to GB03 VOCs or water for 10 days, were used for cell size measurements by microscopic analysis. For leaf study, the first true leaves of 12-day-old seedlings exposed to GB03 VOCs or water for 10 days, were used for cell size measurements by microscopic analysis. Leaves were sectioned and prepared for light microscopic observations as described by Inan et al. (2004). Fixed leaves were cut at three different positions, with the cutting edge being perpendicular to the central vein (Fig. 6a). Positions 1, 2, and 3 refer to ¼, ½, and ¾ of the whole leaf starting from the leaf tip. Cells area was measured using SimplePCI software (Olympus America Inc., USA). To obtain representative cell types (e.g.epidermal, mesophyll and cell vein regions) cells in a ring area, with the central vein as the center, were measured. Radii of each ring were set individually to include both upper epidermal and lower epidermal cells in each sample, since a fixed radius would not include the same cell types for both the larger and smaller GB03- and water-exposed leaves, respectively. For root study, primary roots and secondary roots (lateral roots) were sectioned randomly and perpendicularly. Epidermal and endodermal cells were used to obtain cell size population in roots. Tissues from different plants were measured for each of the replicates for each treatment ($n = 3$ for leaves; $n = 4$ for roots).

Statistical analysis

Statistical analysis of total leaf surface area was performed by SAS software (SAS Institute, Cary, NC, USA). Significant difference between treatments was based on P-values ≤ 0.05.

Results

Regulation of gene expression by PGPR VOCs

To provide insight into underlying mechanisms responsible for the induction of plant growth promotion, genome-wide analysis of gene expression was performed using oligonucleotide microarray slides (described at http://www.ag.arizona.edu/microarray and http://omad.operon.com/download/index.php). Transcriptional gene regulation essential for early growth enhancement was assumed to occur at or before phenotypic changes in leaf area and biomass. The time interval required for activating observable growth promotion with PGPR VOC exposure was 5 days as determined by measuring seedling fresh weight (data not shown). To identify the complement of transcripts that participate in plant growth promotion triggered by GB03 VOCs and not exclude non-specific bacterial-VOC responses that may overlap with PGPR growth promotion, water was selected for all non-GB03 controls. Analysis of the *Arabidopsis* transcriptome at 48 and 72 h post GB03 exposure revealed differential expression of 631 (379 up regulated) and 666 (350 up regulated) genes, respectively (supplementary data).

As a first approximation, differentially expressed genes at both 48 and 72 h were categorized into 24 groups based on function (Table 1). Each functional gene category includes at least five common genes for one of the time points. An overlap in gene induction for both collection times was observed for flavonoid and auxin synthesis, as well as genes encoding wall-loosening enzymes suggesting elevated flavonoid accumulation, auxin production and cell-wall flexibility. Ribosomal proteins were almost four times more represented 72 h post treatment (31 genes) than 48 h (8 genes), indicating GB03-elevated protein synthesis. Most other categories showed no discernable pattern of gene regulation (Table 1 and Supplementary data). Microarray results were selectively validated by RT-PCR (Fig. 1). In measuring differential gene expression (GB03-treated versus water-control), microarray and RT-PCR results differed by less than 30% based on an arithmetic scale.

GB03 VOCs regulate plant auxin homeostasis

All differentially regulated genes associated with auxin synthesis were up regulated for at least one time-point measurement (Table 2). Three nitrilases genes (At3g44300, At3g44310, and At3g44320) encode for catalysis of the terminal activation step in indole acetic acid (IAA) biosynthesis (Hillebrand et al. 1998). In contrast, a gene (At2g17500) annotated as a putative auxin efflux carrier involved in auxin polar transport was down regulated.

Auxin response in the presence or absence of GB03 VOCs was further examined by assaying a transgenic auxin-responsive reporter, *DR5::GUS*. The *DR5::GUS* construct with seven highly active tandem repeats of the auxin-responsive TGTCTC elements (Ulmasov et al. 1997) exhibited opposing responses in leaves and roots with exposure to GB03 VOCs. GB03-exposed leaves exhibiting lower auxin accumulation than water controls while increased auxin accumulation was observed in GB03 exposed roots compared with water alone (Fig. 2). Only in

Table 1 Functional gene classification differentially expressed by GB03 VOCs

Gene class	Subclass (example provided)	Observable pattern	Identified genes		Common genes
			48 h	72 h	
Metabolism	Amino acid (asparagine synthetase, At3g47340)	*	5	5	2
	Flavonoid (chalcone synthase, At5g13930)	+	11	11	8
	Lipid (phospholipase, At3g15650)	*	14	14	4
	Sugar (starch synthase, At1g32900)	*	16	16	6
Growth	Auxin (nitrilase 1, At3g44310)	+	12	9	4
	Cell wall (β-expansin, At2g20750)	+	28	32	14
	Chloroplast (chloroplast lumen protein, At2g37400)	*	14	19	7
	Nodulin (nodulin MtN21 protein, At1g11450)	*	6	6	3
	No apical meristem (NAM protein RD26, At4g27410)	−	0	5	0
Stress	Heat shock (HSP101, At1g74310)	*	14	20	9
	Stress (universal stress protein, At3g11930)	*	46	41	24
Signaling	Kinase/phosphatase (protein kinase, At5g11020)	*	24	23	6
	Nucleic acid binding (transcription factor, At1g56170)	*	38	42	6
	Other hormone related (GA4, At1g15550)	*	8	9	3
Other	Cytochrome (cytochrome P450, At1g16410)	*	23	11	7
	Expressed protein (expressed protein, At4g04745)	*	124	120	39
	Germin-like protein (GER2, At5g39190)	*	8	5	5
	Hydrolase (epoxide hydrolase, At3g05600)	*	12	13	4
	Methyltransferase (HMT-3, At3g22740)	*	6	2	2
	Oxidase/reductase (sulfite reductase, At5g04590)	*	35	35	14
	Protease (cysteine proteinase, At4g11320)	*	32	33	17
	Ribosomal protein (RPL31B, At4g26230)	+	8	31	3
	Transporter (ammonium transporter, At3g24300)	*	27	33	10
	Miscellaneous (glycine-rich protein, At2g05540)	*	120	133	42
Total			631	666	239

+ Predominantly up-regulated; − predominantly down-regulated; * a mixed pattern of up and down regulation

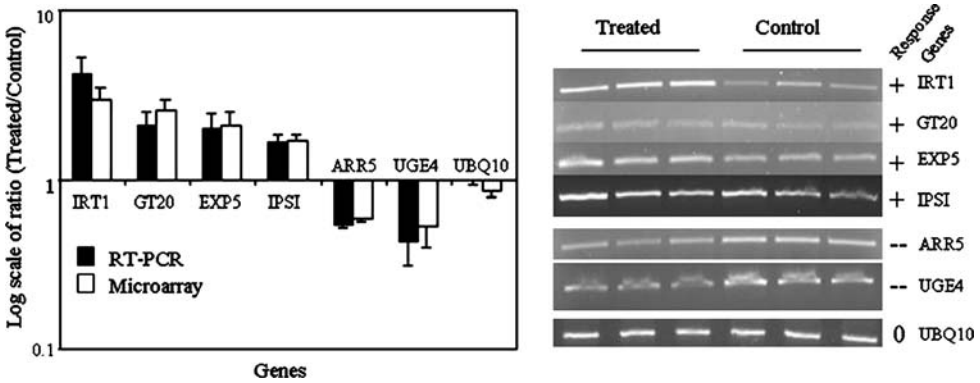

Fig. 1 *Arabidopsis* transcript induction comparison by microarray and RT-PCR analysis; seedlings exposed for 72 h to GB03 VOCs (treated) or water alone (control). Quantified expression with $\log_{10}$ scale (mean ± SD, $n = 3$) (*left*) and RT-PCR gel bands (*right*) shown. Selected genes include: iron-responsive transporter (IRT1); glycosyl transferase family 20 protein (GT20); expansin A 5 (EXP5); inositol-3-phosphate synthase isozyme (IPSI); *Arabidopsis* response regulator 5 (ARR5); UDP-glucose-4-epimerase (UGE4); and a loading control, polyubiquitin (UBQ10)

petiole and shoot meristem regions was *DR5::GUS* activity observed three days after GB03 exposure, while control plants accumulated auxin throughout the aerial portion (Fig. 2a, b). Roots of VOC-treated plants showed a greater abundance of *DR5::GUS* expression after three days of exposure (DOE) (Fig. 2a, b, e, f). At 8 DOE, auxin distribu-

Table 2 Auxin-related genes differentially regulated by GB03 VOCs

Expressed gene (GB03/Control)	48 h	72 h
Auxin synthesis		
At4g27070, tryptophan synthase	1.4 *	1.8
At5g05730, anthranilate synthase	0.8	1.6 *
At3g44310, nitrilase 1	1.4 *	2.2 *
At3g44300, nitrilase 2	1.4	1.5 *
At3g44320, nitrilase 3	1.5 *	2.0
At1g51760, IAA-amino acid hydrolase 3	1.1	1.9 *
Auxin-responsive genes		
At1g16510, auxin induced protein	1.6*	1.7
At1g56150, auxin-induced protein	2.9 *	0.9
At3g07390, auxin-induced protein (AIR12)	1.5*	1.1
At4g34750, small auxin up RNA	1.4*	1.3
At5g18030, small auxin up RNA	1.3*	1.4 *
At5g18060, small auxin up RNA	1.2*	1.6 *
At1g10230, E3 ubiquitin ligase	1.4*	1.5
At3g04730, auxin-responsive protein IAA16	0.6 *	0.6*
Auxin transport		
At2g17500, putative auxin efflux carrier	0.4 *	0.5 *
Other		
At1g28330, dormancy/auxin associated	0.3 *	0.2 *
At1g56220, dormancy/auxin associated	0.7 *	0.6 *
At2g33830, dormancy/auxin associated	0.4 *	0.03

Asterisk (*) indicates P values ≤ 0.05 between GB03 and control treatments. Values are means of ratios of three biological replications. For the arithmetic scale used, values greater than 1 are up regulated while values less than 1 are down regulated. At least one of the two time points meets all of the threshold requirements (see Materials and methods

tion in GB03-exposed seedlings was highly skewed, with leaves devoid of blue staining while roots were highly specked with blue; a reverse tissue color pattern was observed in water controls (Fig. 2c, d). VOC-treated seedlings had greater lateral root development than controls, along with higher auxin accumulation (Fig. 2g, h). In both GB03 and water exposed roots, auxin accumulation associated with sites of lateral root primordia.

Observed auxin-induced GUS expression patterns suggested tissue-specific gene expression of auxin-regulated genes. Transcriptional levels of the putative auxin efflux carrier (AEC), At2g17500, two nitrilases, At3g44300 and At3g44320, and a potential auxin-related expansin gene EXP5 (At3g29030) were probed in a tissue-specific fashion by quantitative real-time PCR (Fig. 3). AEC was found to be down-regulated by VOC exposure only in roots, which may contribute to belowground auxin accumulation in the plant. Gene expression of EXP5 was significantly up-regulated by GB03 exposure only in the aerial portion of the plant, consistent with cell size increases observed in the leaves alone with GB03 exposure. Both NIT1 and NIT2 were up-regulated in the aerial portion by GB03 VOCs, while NIT1 transcript level exhibited a reduction in roots with VOC exposure. The disparity between DR5::GUS localization and NIT gene expression suggests enhanced basipetal auxin transport, so that although auxin synthesis in leaves is up regulated, auxin movement out of leaves results in a net accumulation of auxin in the roots.

To test the possibility that GB03 VOCs augment growth by increasing auxin transport from leaves to roots, the auxin transport inhibitor NPA was added to the plant growth

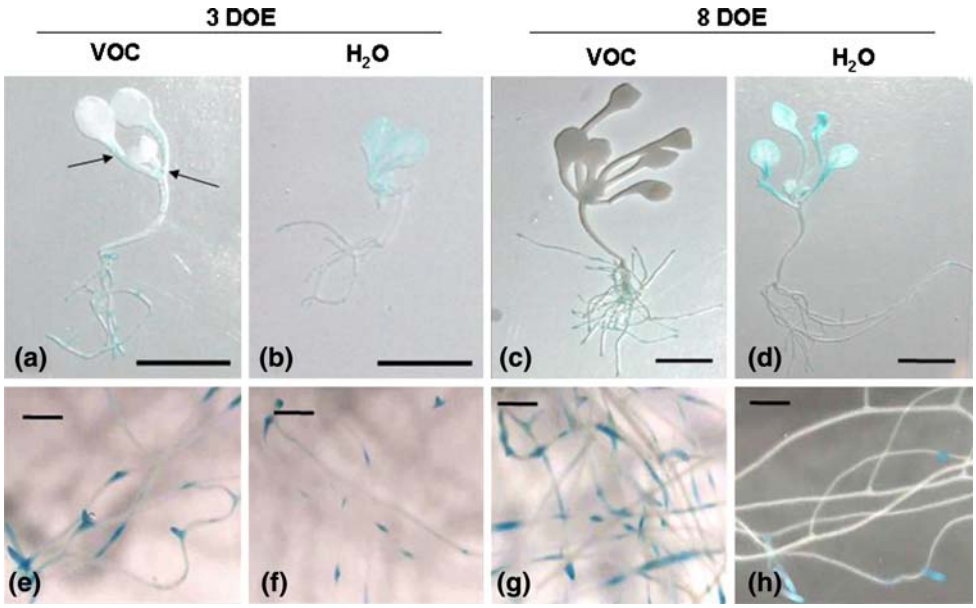

Fig. 2 Differential GUS expression with GB03 VOCs or water control in *DR5::GUS* transgenic plants 3 and 8 days of exposure (DOE). Row 1, images of whole seedling (bar represents 5 mm; *arrows* indicate elevated GUS expression in the petiole and apical meristem); row 2, root mat (bar represents 0.5 mm). Three replicates for each treatment were performed; total seedling population 20. Representative images are shown

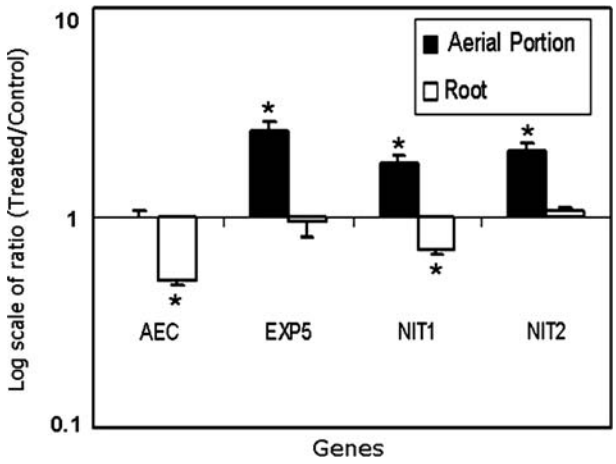

Fig. 3 Tissue-specific gene expression with 72 h plant exposure to GB03 VOCs (treated) or water alone (control); quantitative rt-PCR results in a $\log_{10}$ scale (mean ± SD, $n = 3$) includes the putative auxin efflux carrier (AEC), expansin A5 (EXP5), nitrilase 1 (NIT1), and nitrilase 2 (NIT2)

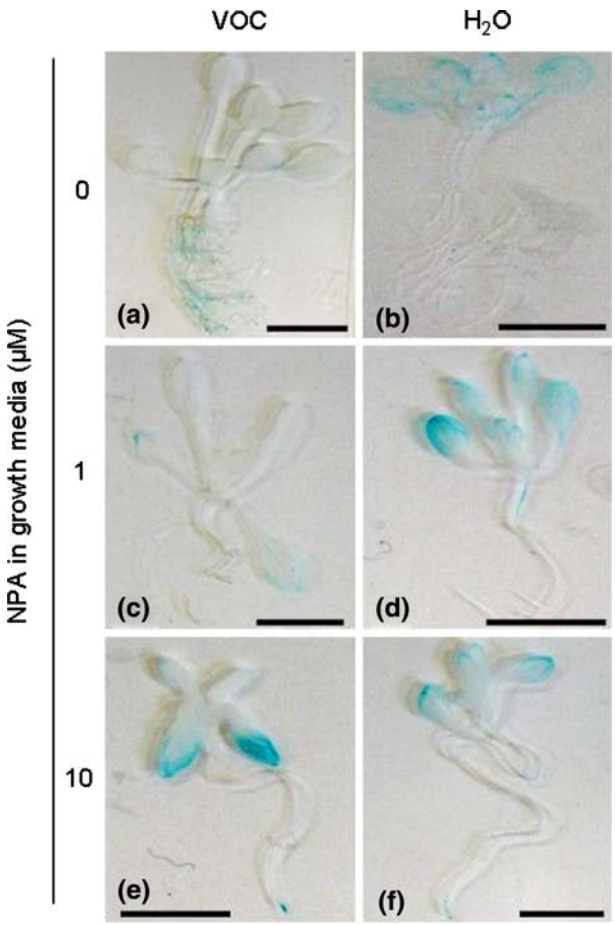

Fig. 4 Differential GUS expression with seedling exposure to GB03 VOCs (**a**, **c**, **e**) or water alone (**b**, **d**, **f**) in the *DR5::GUS* transgenic line 8-days post treatment and containing 0-, 1-, or 10-μM of the auxin transport inhibitor 2-((1 naphthalenylamino)-carbonyl) benzoic acid (NPA). Images of whole seedling (bar represents 5 mm). Three replicates were performed with a total seedling population of 20; representative images are shown

media. For *Arabidopsis* as well as bean, NPA restricts leaf auxin translocation and concurrently reduces leaf size (Keller et al. 2004). With eight days of GB03 exposure and in the presence of a 1 μM NPA, a slight increase in auxin in the aerial portion of GB03-exposed plants was observed compared with exposed plants in the absence of NPA (Fig. 4a, c). An introduction of 10 μM NPA resulted in greater blue staining in leaves of GB03-exposed plants than the lower NPA dose (Fig. 4a, c, e). Indeed blue staining in leaves treated with 10 μM NPA were comparable whether plants were GB03 or water exposed (Fig. 4e, f) indicating that changes in leaf-auxin distribution that occur with GB03 exposure can be reversed by blocking auxin transport. The localization of auxin when translocation is blocked corresponds to identified sites of auxin synthesis including leaf parameters (previously reported by Mattsson et al.2003). In the roots, GB03-induced GUS expression was suppressed with both NPA concentrations tested (Fig. 4c and e compared with a). In addition, both NPA treatments inhibited lateral root formation normally induced by GB03 VOCs so that on day 8 only the primary root had developed. Total leaf surface area of plants with GB03 exposure was also compromised by NPA treatment, suggesting VOC-mediated growth promotion is interrupted by blocking auxin transport (Fig. 5). At the 10 μM NPA dose, there was a total loss of plant growth promotion whereas with 1 μM NPA growth promotion was reduced.

GB03 VOCs induce leaf cell enlargement

Genes related to cell wall modifications accounted for 28 and 32 genes differentially regulated at 48 and 72 h, respectively (Table 3), and accounted for ca. 5% of the total differentially regulated genes. Genes coding for cell expansion including expansins and UDP-glucose 4-epimerase (*UGE4*), exhibited an expression pattern favorable for cell expansion. Genes for seven of the eight differentially regulated expansin-related proteins known to have cell-wall loosening activity and to be involved in cell expansion and other developmental events during which cell-wall modifications occur, were up regulated. The gene encoding UDP-glucose 4-epimerase that channels UDP-D-galactose into cell wall polymers was down regulated with GB03 exposure.

Several other cell wall modifying genes encoding enzymes important to reduced cell-wall rigidity were predominantly up regulated including: pectate lyases, pectinases, and pectin methyl esterase inhibitors (Table 3). Pectate lyases and pectinases depolymerize the cell wall constituent pectate, causing cell wall degradation (Andro et al. 1984). In addition to direct pectin depolymerization,

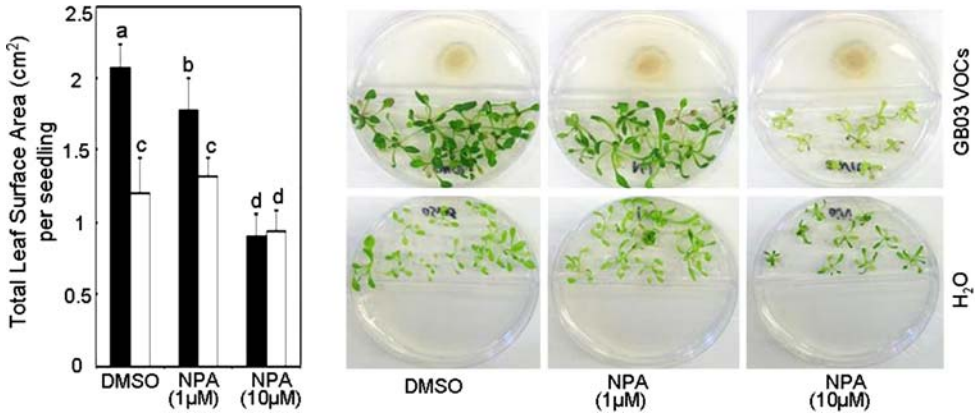

Fig. 5 VOC-induced growth promotion reduced by the auxin transport inhibitor NPA. *Different letters* indicate significant difference ($P \leq 0.05$) between treatments; three replicates were performed with a total seedling population of 27. Representative images are shown

Table 3 Cell wall modification genes differentially regulated by GB03 VOCs

Expressed genes (GB03/control)	48 h	72 h	Expressed genes (GB03/control)	48 h	72 h
Up-regulation promotes cell expansion					
At2g20750, expansin, EXPB1	**2.0 ***	**1.7**	**At2g28950, expansin, EXP6**	**1.2**	**1.4 ***
At4g28250, expansin, EXPB3	**1.7 ***	**2.1 ***	**At1g26770, expansin, EXP10**	**1.2 ***	**1.7 ***
At2g39700, expansin, EXP4	**1.3**	**1.7 ***	**At4g17030, expansin related**	**1.7 ***	**1.4**
At3g29030, expansin, EXP5	**1.8 ***	**2.1 ***	At2g40610, expansin, EXP8	0.5 *	1.0
Down-regulation promotes cell expansion					
At1g12780, UDP-glucose 4-epimerase	**0.42 ***	**0.53**			
Up-regulation reduces cell wall rigidity					
At5g63180, pectate lyase	**1.6 ***	**1.9 ***	**At3g17130, pectin methylesterase inhibitor**	**1.7 ***	**1.8 ***
At1g67750, pectate lyase	**1.6**	**1.8 ***	**At3g62820, pectin methylesterase inhibitor**	**1.7**	**1.5 ***
At1g04680, pectate lyase	**1.5 ***	**1.3**	**At1g23205, pectin methylesterase inhibitor**	**1.6 ***	**1.8**
At3g53190, pectate lyase	**1.4 ***	**1.7 ***	**At5g20740, pectin methylesterase inhibitor**	**1.4 ***	**1.6 ***
At3g54920, pectate lyase	**1.2**	**1.7 ***	At1g47960, pectin methylesterase inhibitor	0.6 *	0.5 *
At5g14650, pectinase	**1.7 ***	**0.4**	**At5g26670, pectinacetylesterase**	**1.1**	**1.5 ***
At2g43870, pectinase	**1.6 ***	**1.5 ***	At4g19420, pectinacetylesterase	0.8 *	0.6 *
At2g43860, pectinase	**1.6 ***	0 *	**At5g19730, pectinesterase**	**1.4**	**1.5 ***
At3g16850, pectinase	**1.2**	**1.5***	**At3g43270**, pectinesterase	0.7 *	0.6 *
Down-regulation reduces cell wall rigidity			**At2g33790, pollen allergen/extensin**	**0.5 ***	**0.3 ***
At2g25300, galactosyltransferase	**0.5 ***	**0.4**	At5g45880, pollen allergen/extensin	1.2	1.6 *
At4g38080, putative extensin	**0.8**	**0.6 ***	**At3g10340, phenylalanine ammonia-lyase**	**0.5 ***	**0.4**
At5g09530, putative extensin	**0.4 ***	**0.3 ***	At1g24735, O-methyltransferase	1.4 *	0. 6
At5g21280, putative extensin	1.5 *	2.0 *	**At1g21100, O-methyltransferase**	**0.4 ***	**0.5**
Down-regulation promotes cell wall rigidity			At3g13750, beta-galactosidase	0.3 *	0.3 *
At5g56870, beta-galactosidase	0.2 *	0.2 *	At4g26140, beta-galactosidase	0.5 *	0.4 *
Uncertain consequence on cell wall architecture			At4g30270, xyloglucan endotransglycosylase	0.4 *	0.4 *
At3g10740, glycosyl hydrolase family 51	0.3 *	0.3 *	At3g44990, xyloglucan endotransglycosylase	1.5 *	1.8 *
At2g32990, glycosyl hydrolase family 9	1.9 *	2.0 *	At5g57560, xyloglucan endotransglycosylase	0.6 *	0.6
At5g08000, glycosyl hydrolase family 17	1.4 *	1.6 *	At5g65730, xyloglucan endotransglycosylase	0.6 *	0.6 *

Bold font indicates gene regulation considered favorable for cell expansion. Asterisk indicates P values ≤ 0.05 between GB03 and control treatments. Values are means of ratios of three biological replicates. For the arithmetic scale used, values greater than 1 are up regulated while values less than 1 are down regulated. At least one of the two time points meets all of the threshold requirements (see Materials and methods)

four pectin methylesterase inhibitors were also up regulated (Table 3). Since pectin methylesteases (PME) cleave methyl groups from highly esterified pectin to initiate binding of carboxyl ions with Ca^{2+} and cause walls to become more rigid, inhibition of PMEs may well allow for a reduction in cell-wall rigidity. Two pectin acetyl esterases

(PAEs) were identified as well, with one being up regulated while the other down regulated, as were two pectin esterase genes. PAEs, which likely act together with a range of other pectin-degrading enzymes, may well be involved in softening and loosening the primary cell wall in nematode-infected plant roots (Vercauteren et al. 2002).

Down regulation of a galactosyltransferase, as well as several extensin genes also suggest enzymatic activity directed towards lower cell-wall rigidity (Table 3). Galactosyltransferases have been identified as a key factor in the regulation of galactose-substitution in galactomannan biosynthesis (Reid et al. 1995) with galactomannans being a dominant constituent in legume-cell wall assembly (Reid 1997). And extensin molecules can interlock separated microfibrils to reinforce cell wall architecture subsequent to cell growth (Buchanan et al.2000). Although phenylalanine ammonia lyase (PAL) is often categorized as a regulatory enzyme of flavonoid biosynthesis, it is included in the cell wall modification grouping (Table 3) because *PAL* functions to catalyze the biosynthesis of the plant cell wall constituents lignin (Humphreys and Chapple 2002; Boerjan et al. 2003) and suberin (Bernards et al. 2000); and *PAL* down regulation appears contrary to the gene expression patterns of all other identified flavonoid synthesis enzymes with plant exposed to GB03 VOCs (supplementary data). The down regulation of *PAL* (At3g10340) may well result in reduced lignin (and in turn cell wall rigidity) as has been reported by Anterola and Lewis (2002). *O*-Methyltransferases (OMTs) are also associated with enhanced lignification (Vincent et al. 2005) and *omt1* knockout mutants in *Arabidopsis* lack syringyl units of lignin (Goujon et al. 2003). Both of the differential regulation OMTs were down regulated with GB03 exposure at 72 h while one was up regulated at the 48 h time point (Table 3). In a transgenic alfalfa line deficient in caffeoyl CoA 3-*O*-methyltransferase, lignin was found to be structurally similar to lignin in the corresponding wild type line, however a ca. 20% decrease in lignin content was observed in the transgenic line (Marita et al. 2003).

A total of 30 of the 38 differentially regulated genes with a known function associated with cell-wall structure were shifted in favor to promote cell-wall expansion or reduced cell-wall rigidity. In opposition to this apparent transcriptional trend of cell-wall loosening, several genes were identified. The three beta-galactosidases with enzymatic capability to degrade cell-wall galactans under specific developmental conditions such as during pollen expansion (Hruba et al. 2005) and fruit ripening (Carey et al. 2001), were unexpectedly down regulated. Since juvenile plants were employed in this study, down regulation of reproduction-associated genes may not be relevant to the observed trend of cell wall loosening observed in vegetative tissues.

Several genes with uncertain consequence on cell wall architecture were also differentially regulated including: glycosyl hydrolases, and xyloglucan endotransglycosylases (*XET*s) (Table 3). *XET*s are a class of wall architecture enzymes responsible for the cleaving and reattachment of xylogulcan chains; the role of these enzymes in both wall loosening and wall strengthening has been reported (reviewed by Nishitani 1997; Campbell and Braam 1999).

Plant cell size modifications, suggested by changes in cell-wall transcript patterns were examined by studying the cross section of leaves at three defined distances from the leaf tip (Fig. 6a). An increase in leaf cell size for GB03 treated seedlings compared to water control tissue was observed at each leaf cross section (Fig. 6b). To quantify and catalogue leaf cell size distribution, a donut shaped region centered but excluding the central vein region and that had a diameter defined by the abaxial and adaxial leaf epidermis was used to take cell size counts. Cell size measurement in this local leaf tissue containing epidermis-, palisade- parenchyma-, spongy parenchyma- and mesophyll-cells revealed a shift to larger cell size populations for GB03 treated samples compared to control treatments (Fig. 6c). The most abundant cell area in GB03-treated leaf tissue was 1,000–1,500 μm^2 (ca. 22 cells) versus 200–440 μm^2 (ca. 15 cells) for control samples. In making cell count measurements of whole leaves, GB03 treated samples contained 659 ± 16 cells compared to 556 ± 14 for water-treated controls. In contrast to the larger cell sizes observed in leaf tissue, GB03 VOCs did not trigger observable differences in root cell size either in the primary or lateral roots (Fig. 6d, e). The most abundant root cell area is within a range of 200–400 μm^2 for both VOC-treated and water-treated control samples. Numbers of outer-layer cells in primary root and lateral roots were not statistically different with 35 ± 1 and 29 ± 3 cells, respectively in GB03 exposed plants and 34 ± 1 and 25 ± 1, respectively in controls. In terms of lateral root numbers, GB03-exposed plants consistently developed greater numbers of lateral roots than water controls (Fig. 6a).

Discussion

Previous models of rhizobacterial-stimulated plant growth promotion suggest that soil microbes produce auxin and/or other phytohormones that drive plant growth promotion (Loper and Schroth 1986; Timmusk et al. 1999; MacDonald et al. 1986; Glick et al. 1999, Lin et al. 1983), however in the case of biologically active bacterial VOCs, blends of volatile chemicals devoid of traditional auxins can alone trigger plant growth promotion mediated by auxin synthesis and transport in the plant. The abundance of transcripts encoding enzymes involved in auxin synthesis was up-

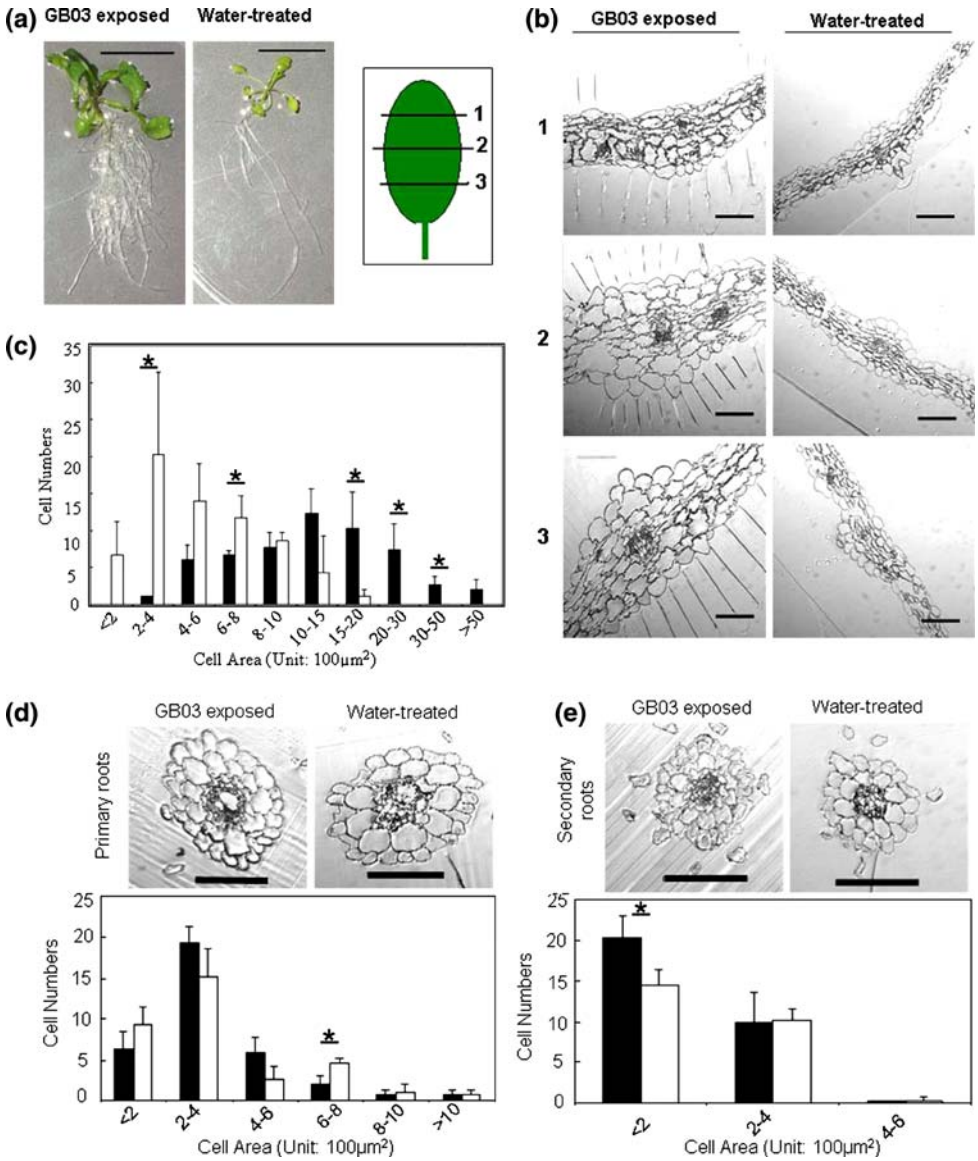

Fig. 6 Cell size comparisons for GB03 and water control plants. Sixteen-day-old plants (line = 1 cm) and leaf cuts (**a**); leaf cross sections with central vein centered (line = 100 μm) (**b**); shifted cell-size population (cross section 2) with GB03 (*black bars*) and water treatment (*white bars*) (mean ± SD, $n = 3$) (**c**). Cell size for primary (**d**) and secondary (**e**) root with GB03 (*black bars*) and water (*white bars*) (line = 100 μm) (mean ± SD, $n = 5$). Asterisks indicate significant difference ($P \leq 0.05$) between treatments

regulated in aerial regions of VOC-treated plants (Fig. 3), while auxin-dependent *DR5::GUS* expression revealed that VOC-treated plants had less auxin in leaves and more auxin in roots, compared with water-treated controls (Fig. 2). Although the role auxin plays in controlling leaf expansion remains unclear, increased auxin levels have been reported to have negative effects on leaf expansion. *Arabidopsis* mutants *sur1* and *sur2*, which overproduce auxin, have reduced leaf expansion (Boerjan et al. 1995). Transgenic petunia (*Petunia hybrida*), which overproduces auxin, develops leaves that are epinastic as well as smaller and narrower than wild type plants (Klee et al. 1987). And application of exogenous auxin to bean (*Phaseolus vulgaris*) and *Arabidopsis* leaf blades inhibits long-term blade elongation (Keller et al.2004). Application of the auxin transport inhibitor NPA to petioles traps auxin within the leaf and selectively increases leaf auxin content thereby inhibited leaf growth (Keller et al. 2004). On the other hand, low auxin concentrations drive cell elongation, cell enlargement and cell differentiation in tobacco cell culture (Winicur et al. 1998; Zazimalova et al. 1995). Although NPA-induced reduction of plant growth due to diminished plant perception of VOCs and/or downstream interference of critical non-auxin signaling pathways can not be ruled out, the spatial and temporal redistribution of auxin observed with GB03 VOCs (Fig. 2) as well as exogenous NPA treatment (Fig. 4) demonstratively implicates auxin participation in plant growth promotion.

In addition to decreasing auxin levels in aerial portions of *Arabidopsis*, GB03 exposure increased root auxin content and auxin accumulation at sites of lateral root primordia (Fig. 2e, h). Auxin is required at several developmental stages to facilitate lateral root formation. Lateral root primordia that are unable to divide if excised from the primary

root, can be rescued if supplemented with exogenous auxin (Dubrovsky et al.2001). Removal of shoot apical tissues abolishes the IAA pulse that promotes the emergence of lateral root primodia, thereby blocking emergence of lateral roots (Bhalerao et al.2002). Root systems may become independent of shoot-generated auxin with both auxin content and the biosynthetic capacity of root systems increasing substantially with age (Ljung et al. 2002). Nevertheless, the initial dependence of lateral root development on shoot-derived auxin allows coordination of root development with leaf development, enabling a balance between carbon and nitrogen metabolism to be established. Early developmental branching of roots contributes to water uptake, and facilitates the extraction of nutrients essential for robust plant growth and development. GB03 VOCs may trigger sustained plant growth promotion by an optimized coordination between root and leaf development.

Although IAA biosynthesis remains incompletely defined in plants, two IAA biosynthetic pathways, tryptophan-dependent and tryptophan-independent, have been identified by stable isotope labeling studies (reviewed in Ljung et al.2005; Woodward and Bartel 2005). It is envisioned that GB03 VOCs induce IAA biosynthesis via a Trp-dependent pathway based on our transcript profiles. Transcripts of three nitrilase (NIT1, NIT2, and NIT3) that catalyze the terminal step in the Trp-dependent IAA pathway are induced by GB03 exposure; GB03 VOCs also induce gene expression of tryptophan synthase (TSB2) and anthranilate synthase (ASA1), the latter catalyzing the rate-limiting step of tryptophan biosynthesis.

Down-regulation of the putative auxin efflux carrier At2g17500 with GB03 treatment was observed at transcriptional level and may be associated with localized auxin in the roots (Fig. 3). Genes encoding flavonoid biosynthesis were found to be coordinately up-regulated (supplementary data) along with downstream glycosylated flavonoids including: Rha-Glc-Rha-quercetin, Rha-Glc-Rha-kaempferol, Glc-Rha-quercetin, Glc-Rha-kaempferol, Rha-Rha-kaempferol (data not shown). Interestingly flavonoids can serve as negative regulators of auxin transport, albeit there is little data for the role of glycosylated flavonols *in planta* in contrast to their aglycone counterparts (Faulkner and Rubery 1992; Bernasconi 1996; Murphy et al. 2000, 2002; Peer et al. 2004). With a GB03-triggered accumulation of flavonoids, an inhibition of acropetal auxin transport could result, contributing to an increase in root auxin levels. An alternative scenario that endogenous increases in flavonoids trigger the same response as exogenous NPA applications is unlikely since flavonoid increases induced by GB03 VOCs are correlated with growth promotion, while the introduction of NPA inhibits growth (Fig. 5). Differential localization of flavonoids compared with unregulated NPA distribution could account for different patterns of auxin accumulation observed in leaves and roots with and without NPA treatment. For example, supplements of NPA to the media inhibited basipetal auxin transport (Fig. 4) and did in fact compromise growth promotion triggered by GB03 VOCs, while basipetal auxin transport with GB03-triggered flavonoid increases were compatible with plant growth promotion (Fig. 5).

Auxin may not be the only hormone responsible for plant growth promotion. Other non-auxin related phytohormone genes have also been identified as differentially expressed from the microarray experiments (supplementary data). These include two gibberellin-responsive proteins, two ABA-responsive proteins, and several ethylene-responsive proteins, together with enzymes involved in gibberellin biosynthesis, cytokinin biosynthesis, and ethylene biosynthesis. A cytokinin primary responsive gene, ARR5 (*Arabidopsis* response regulator 5) was down regulated by GB03 exposure. *Arabidopsis* response regulators (ARRs) have been found to be involved in signal transduction triggered by cytokinin, ethylene, and light (Sweere et al. 2001; To et al. 2004; Hass et al. 2004) and are thereby suggested to be important components of hormonal cross-talk. Down regulation of ARR5 in response to GB03 VOCs may prove to serve an important role in hormone regulation of growth promotion. Previous mutant line data indicate that GB03 VOCs promote plant growth through a cytokinin receptor or ethylene signal by using *cre1* (cytokinin receptor deficient) and *ein2* (ethylene insensitive) mutants, respectively (Ryu et al. 2003). Microarray results did not show altered transcriptional activity of the receptor protein *cre1* nor the integral membrane protein *ein2* (Guo and Ecker 2004).

Genes differentially regulated with exposure to GB03 emissions and associated with cell wall modifications were examined for possible regulatory control of cell enlargement. The induction of a group of pectin-related genes, including pectin methylesterase inhibitor, pectinase, and pectate lyase (Table 3) suggested that cell-wall loosening contributes to cell expansion. Pectins, a mixture of heterogeneous, branched, and highly hydrated polysaccharides rich in D-galacturonic acid, are thought to perform several cell-wall functions including determination of wall porosity, provide charged surfaces that can modulate wall pH and ion balance, and serve as recognition molecules that alert plant cells to the presence of symbiotic organisms, pathogens, and insects (review in Buchanan et al.2000). Pectin methylesterases hydrolyze methyl ester of pectins and thus create free carboxyl groups that can link with Ca^{2+} ions and stabilize two polygalacturonan (PGA) chains, thereby making the pectic network more rigid. Moreover, binding of GalA units in PGA to Ca^{2+} results in calcium pectate, which is the cementing substance for cell adhesion. Besides the action of cell wall reassembly enzymes, enzymes involved in the biosynthesis of cell walls also

seemed to be altered. UDP-glucose 4-epimerase is involved in channeling UDP-D-galactose into cell wall polymers and was down regulated with plant exposure to GB03 VOCs. Mutation in *UGE4* has been reported to weaken cell structure as observed with a dramatic bulging of root epidermal cells (Schiefelbein and Somerville 1990; Baskin et al. 1992).

In a previous transcriptome study with *Arabidopsis* colonized by the PGPR *Pseudomonas thivervalensis* (strain MLG45) that triggers induced systemic resistance while photosynthesis rates were repressed (Cartieaux et al. 2003), transcripts associated with defense were up regulated and photosynthesis genes were reduced relative to un-colonized plants. This coupled transcriptional/physiological regulation in *Arabidopsis* in contact with MLG45 links observed transcriptional regulation with phenotype responses mediated by PGPR. By utilizing microarray technology, the effects of emitted VOCs from *B. subtilis* GB03 on *Arabidopsis* gene expression have been identified and used to unravel a mechanism for plant growth promotion mediated by soil microbes. From the approximately 2% of the *Arabidopsis* genome differentially regulated with plant exposure to bacterial VOCs, genetic and physiological studies reported here establish that cell expansion and auxin signaling participate in plant growth promotion. From a whole plant perspective, the coordinated regulation of carbon and nutrient metabolism to provide an appropriate leaf-to-root ratio is essential for robust plant growth. The observation that GB03 VOCs regulate plant auxin homeostasis tissue-specifically to promote leaf expansion as well as lateral roots appears to successfully provide such a balance.

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References

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science 284:2148–2152

Andro T, Chambost JP, Kotoujansky A, Cattaneo J, Bertheau Y, Barras F, Van Gijsegem F, Coleno A (1984) Mutants of *Erwinia chrysanthemi* defective in secretion of pectinase and cellulase. J Bacteriol 160:1199–1203

Anterola AM, Lewis NG (2002) Trends in lignin modification: a comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. Phytochemistry 61:221–294

Baskin TI, Betzner AS, Hoggart R, Cork A, Williamson RE (1992) Root morphology mutants in *Arabidopsis thaliana*. Aust J Plant Physiol 19:427–437

Bernards MA, Susag LM, Bedgar DL, Anterola AM, Lewis NG (2000) Induced phenylpropanoid metabolism during suberization and lignification: a comparative analysis. J Plant Physiol 157:601–607

Bernasconi P (1996) Effect of synthetic and natural protein tyrosine kinase inhibitors on auxin efflux in zucchini (*Cucurbita pepo*) hypocotyls. Physiol Plant 96:205–510

Bhalerao RP, Eklof J, Ljung K, Marchant A, Bennett M, Sandberg G (2002) Shoot-derived auxin is essential for early lateral root emergence in *Arabidopsis* seedlings. Plant J 29:325–332

Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Van Onckelen H, Van Montagu M, Inze D (1995) Superroot, a recessive mutation in *Arabidopsis*, confers auxin overproduction. Plant Cell 7:1405–1419

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol 54:519–546

Buchanan BB, Gruissem W, Jones RL (2000) The cell wall (in biochemistry and molecular biology of plants). American Society of Plant Physiologists, pp 52–89

Campbell P, Braam J (1999) Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall-modifying functions. Trends Plant Sci 4:361–366

Carey AT, Smith DL, Harrison E, Bird CR, Gross KC, Seymour GB, Tucker GA (2001) Down-regulation of a ripening-related beta-galactosidase gene (TBG1) in transgenic tomato fruits. J Exp Bot 52:663–668

Cartieaux F, Thibaud MC, Zimmerli L, Lessard P, Sarrobert C, David P, Gerbaud A, Robaglia C, Somerville S, Nussaume L (2003) Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. Plant J 36:177–188

Dubrovsky JG, Rost TL, Colon-Carmona A, Doerner P (2001) Early primordium morphogenesis during lateral root initiation in *Arabidopsis thaliana*. Planta 214:30–36

Faulkner IJ, Rubery PH (1992) Flavonoids and flavonoid sulphates as probes of auxin-transport regulation in *Cucurbita pepo* hypocotyl segments and vesicles. Planta 186:618–625

Glick BR, Patten CN, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promotion bacteria. Imperial College Press, London, pp 1–13

Goujon T, Sibout R, Pollet B, Maba B, Nussaume L, Bechtold N, Lu F, Ralph J, Mila I, Barriere Y, Lapierre C, Jouanin L (2003) A new *Arabidopsis thaliana* mutant deficient in the expression of *O*-methyltransferase impacts lignins and sinapoyl esters. Plant Mol Biol 51:973–989

Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr Opin Plant Biol 7:40–49

Halitschke R, Baldwin IT (2003) Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. Plant J 36:794–807

Hass C, Lohrmann J, Albrecht V, Sweere U, Hummel F, Yoo SD, Hwang I, Zhu T, Schafer E, Kudla J, Harter K (2004) The response regulator 2 mediates ethylene signaling and hormone signal integration in *Arabidopsis*. EMBO J 23:3290–3302

Hillebrand H, Bartling D, Weiler EW (1998) Structural analysis of the nit2/nit1/nit3 gene cluster encoding nitrilases, enzymes catalyzing the terminal activation step in indole-acetic acid biosynthesis in *Arabidopsis thaliana*. Plant Mol Biol 36:89–99

Hruba P, Honys D, Twell D, Capkova V, Tupy J (2005) Expression of beta-galactosidase and beta-xylosidase genes during microspore and pollen development. Planta 220:931–940

Humphreys JM, Chapple C (2002) Rewriting the lignin roadmap. Curr Opin Plant Biol 5:224–229

Inan G, Zhang Q, Li P, Wang Z, Cao Z, Zhang H, Zhang C, Quist TM, Goodwin SM, Zhu J, Shi H, Damsz B, Charbaji T, Gong Q, Ma S, Fredricksen M, Galbraith DW, Jenks MA, Rhodes D,

Hasegawa PM, Bohnert HJ, Joly RJ, Bressan RA, Zhu JK (2004) Salt stress. A halophyte and cryophyte *Arabidopsis* relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. Plant Physiol 135:1718–1737

Jones DL, Dennis PG, Owen AG, van Hees PAW (2003) Organic acid behavior in soils-misconceptions and knowledge gaps. Plant Soil 248:31– 41

Keller CP, Stahlberg R, Barkawi LS, Cohen JD (2004) Long-term inhibition by auxin of leaf blade expansion in bean and *Arabidopsis*. Plant Physiol 134:1217–1226

Klee HJ, Horsch RB, Hinchee MA, Hein MB, Hoffman NL (1987) The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. Genes Dev 1:86–96

Kloepper JW, Rodriguez-Kabana R, Zehnder GW, Murphy J, Sikora E, Fernandez C (1999) Plant root–bacterial interactions in biological control of soil borne diseases and potential extension to systemic and foliar diseases. Aust J Plant Pathol 28:27–33

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94:1259–1266

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006) Plant growth-promotion rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl Soil Ecol 31:91–100

Lin W, Okon Y, Hardy RWF (1983) Enhanced mineral uptake by *Zea mays* and sorghum bicolor roots inoculated with *Azospirillum brasilense*. Appl Environ Microbiol 45:1775–1779

Ljung K, Hull AK, Kowalczyk M, Marchant A, Celenza J, Cohen JD, Sandberg G (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. Plant Mol Biol 49:249–272

Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G (2005) Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. Plant Cell 17:1090–1104

Loper JE, Schroth MN (1986) Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology 76:386–389

Luschnig C, Gaxiola RA, Grisafi P, Fink GR (1998) EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. Genes Dev 12:2175–2187

MacDonald EMS, Powell GK, Regier DA, Glass NL, Roberto F, Kosuge T, Morris RO (1986) Secretion of zwatin, Ribosylzeatin, and ribosyl-1″-methylzeatin by *Pseudomonas savastanoi* plasmid-coded cytokinin biosynthesis. Plant Physiol 82:742–747

Marita JM, Ralph J, Hatfield RD, Guo D, Chen F, Dixon RA (2003) Structural and compositional modifications in lignin of transgenic alfalfa down-regulated in caffeic acid 3-*O*-methyltransferase and caffeoyl coenzyme A 3-*O*-methyltransferase. Phytochemistry 62:53–65

Mattsson J, Ckurshumova W, Berleth T (2003) Auxin signaling in *Arabidopsis* leaf vascular development. Plant Physiol 131:1327–1339

Murphy A, Peer WA, Taiz L (2000) Regulation of auxin transport by aminopeptidases and endogenous flavonoids. Planta 211:315–324

Murphy AS, Hoogner KR, Peer WA, Taiz L (2002) Identification, purification, and molecular cloning of N-1-naphthylphthalmic acid-binding plasma membrane-associated aminopeptidases from *Arabidopsis*. Plant Physiol 128:935–950

Nishitani K (1997) The role of endoxyloglucan transferase in the organization of plant cell walls. Int Rev Cytol 173:157–206

Paré PW, Farag MA, Krishnamachari V, Zhang H, Ryu CM, Kloepper JW (2005) Elicitors and priming agents initiate plant defense responses. Photosynth Res 85:149–159

Peer WA, Bandyopadhyay A, Blakeslee JJ, Makam SN, Chen RJ, Masson PH, Murphy AS (2004) Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in *Arabidopsis thaliana*. Plant Cell 16:1898–1911

Reid JSG (1997) Carbohydrate metabolism: structural carbohydrates. In: Dey PM, Harborne JB (eds) Plant biochemistry. Academic, San Diego, pp 205–236

Reid JSG, Edwards M, Gidley MJ, Clark AH (1995) Enzyme specificity in galactomannan biosynthesis. Planta 195:489–495

Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci USA 100:4927–4932

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Salzman RA, Brady JA, Finlayson SA, Buchanan CD, Summer EJ, Sun F, Klein PE, Klein RR, Pratt LH, Cordonnier-Pratt MM, Mullet JE (2005) Transcriptional profiling of Sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. Plant Physiol 138:352–368

Schiefelbein JW, Somerville C (1990) Genetic control of root hair development in *Arabidopsis thaliana*. Plant Cell 2:235–243

Sieberer T, Seifert GJ, Hauser MT, Grisafi P, Fink GR, Luschnig C (2000) Post-transcriptional control of the *Arabidopsis* auxin efflux carrier EIR1 requires AXR1. Curr Biol 10:1595–1598

Sweere U, Eichenber K, Lohrmann J, Mira-Rodado V, Baurle I, Kudla J, Nagy F, Schafer E, Harter K (2001) Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling. Science 294:1108–1111

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31:1847–1852

To JPC, Haberer G, Ferreira FJ, Deruere J, Mason MG, Schaller GE, Alonso JM, Ecker JR, Kieber JJ (2004) Type-A *Arabidopsis* response regulators are partially redundant negative regulators of cytokinin signaling in *Arabidopsis*. Plant Cell 16:658–671

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971

Vercauteren I, de Almeida Engler J, De Groodt R, Gheysen G (2002) An *Arabidopsis thaliana* pectin acetylesterase gene is upregulated in nematode feeding sites induced by root-knot and cyst nematodes. Mol Plant Microbe Interact 15:404–407

Vincent D, Lapierre C, Pollet B, Cornic G, Negroni L, Zivy M (2005) Water deficits affect caffeate *O*-methyltransferase, lignification, and related enzymes in maize leaves: a proteomic investigation. Plant Physiol 137:949–960

Winicur ZM, Zhang GF, Staehelin LA (1998) Auxin deprivation induces synchronous golgi differentiation in suspension-cultured tobacco BY-2 cells. Plant Physiol 117:501–513

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot 95:707–735

Zazimalova E, Opatrny Z, Brezinova A (1995) The effect of auxin starvation on the growth of auxin-dependent tobacco cell culture-dynamics of auxin binding activity and endogenous free IAA content. J Exp Bot 46:1205–1213