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EXORDIUM regulates brassinosteroid-responsive genes☆

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Abstract In a screen for potential mediators of brassinosteroid (BR) effects, the EXORDIUM (EXO) protein was identified as a regulator of BR-responsive genes. The *EXO* gene was characterized as a BR-up-regulated gene. *EXO* overexpression under the control of the 35SCaMV promoter resulted in increased transcript levels of the BR-up-regulated *KCS1*, *Exp5*, *δ-TIP*, and *AGP4* genes, which likely are involved in the mediation of BR-promoted growth. 35S::EXO lines grown in soil or in synthetic medium showed increased vegetative growth in comparison to wild-type plants, resembling the growth phenotype of BR-treated plants. Thus, the EXO protein most likely promotes growth via the modulation of gene expression patterns.

Key words: Brassinosteroid; EXORDIUM; Growth

1. Introduction

Brassinosteroid (BR)-deficient or -insensitive mutants show dwarf phenotypes [1–5] and reveal the pivotal role of BRs for growth and development. The BR signal transduction pathway starts at the plasma membrane. BR binding promotes active heterodimer formation of the leucine-rich repeat receptor kinases BRI1 [6] and BAK1 [7,8]. This leads to activation of both receptor kinases via transphosphorylation. The active kinases then phosphorylate downstream targets. The BIN2/UCU1 GSK3/SHAGGY-like kinase [9,10] functions as a negative regulator of BR signal transduction. Potentially, the BRI1 receptor complex inhibits BIN2/UCU1, which otherwise phosphorylates its substrates BZR1 and BES1. BZR1 and BES1 are closely related proteins, which contain multiple consensus sites for phosphorylation by GSK3 kinases. The *bes1-D* mutation leads to a BR-hypersensitive phenotype, which does not require BRs and BRI1 [11], and causes constitutive expression of BR-regulated genes. The *bzr1-1D* mutation suppresses both *det2* and *bri1* mutants [12,13]. In the presence of BRs, BRI1/BAK1 is active, BIN2 is inactivated and does not phosphorylate BES1/BZR1. Unphosphorylated BES1/BZR1 activates expression of BR-induced genes and growth responses. Within the nucleus, BIN3 and BIN5 constitute a putative *Arabidopsis* topoisomerase VI that modulates expression of many BR-regulated genes [14].

(Putative) components of the BR signalling pathway and mediators of BR responses (such as TRIP-1, BEE1, BEE2, BEE3, and BRH1) are characterized by BR-regulated transcript levels [15–17].

In this article, we provide evidence that the *EXO* gene [18] is a mediator of BR responses. The *EXO* gene was identified as BR-up-regulated. A 'development macroarray' (representing 190 genes) was established for assessment of the phytohormone (in particular BR) status of transgenic lines with altered *EXO* mRNA levels. 35S::EXO plants showed increased transcript levels of growth-related, BR-up-regulated genes. We conclude that the EXO protein is a component in BR signalling mediating (BR-promoted) growth.

2. Materials and methods

2.1. Plant materials and growth conditions

Except for the Affymetrix expression profiling experiment, *Arabidopsis thaliana* accession C24 was the wild-type. Transgenic plants were in the C24 background. For monitoring root and shoot growth, plants were grown for 19 days in vertical plates as described before [19]. Alternatively, plants were grown in soil under long day conditions as described before [20]. For the phytohormone treatments with 24-epibrassinolide (EBL; CIDtech Research, Cambridge, ON, Canada), 2,4-dichlorophenoxyacetic acid (2,4-D; Duchefa, Haarlem, The Netherlands), gibberellin (GA) (61.2% GA_4, 30.1% GA_7; Duchefa), and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Sigma, Taufkirchen, Germany), wild-type plants (C24) were grown in liquid culture (one-half-concentrated Murashige and Skoog (MS) medium supplemented with 1% (w/v) sucrose) under continuous shaking. After 7 days of culture the medium was changed. Phytohormones were added 3 days later. For the expression profiling experiment, wild-type plants (accession Col-0) were grown in one-half-concentrated MS medium supplemented with 1% (w/v) sucrose and solidified with 0.7% (w/v) agar under a 16-h day (140 μmol/s/m^2, 22°C)/8-h night (22°C) regime.

2.2. Generation of transgenic plants

The *EXO* coding sequence and short flanking regions were amplified from a rapid amplification of cDNA ends (RACE)-cDNA library (FirstChoice RLM-RACE kit, Ambion, Texas, USA) using the primers 5′-cgt taa att ata gtc caa gcg aca a-3′ and 5′-cca atc taa aca tca aag aca aaa-3′. The polymerase chain reaction (PCR) product was cloned into the pCR2.1-TOPO vector (Invitrogen, Karlsruhe, Germany). The *EXO* cDNA was then inserted into a modified pGREEN vector using the *Eco*RI restriction sites ([21]; Isabell Witt, personal communication [manuscript in preparation]), in order to allow constitutive overexpression under the control of the 35SCaMV promoter. The resulting construct was termed 35S::EXO. Sequence analysis revealed 100% identity to the *EXO* cDNA sequence. The construct was transformed

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Abbreviations: BR, brassinosteroid; EBL, 24-epibrassinolide

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into wild-type and *dwf1-6* plants. Transgenic T_1 generation plants were selected using BASTA.

35S::EXO T_2 plants (wild-type background) were used for phenotypic characterization. Plants were grown in soil without selection but were checked individually for increased *EXO* mRNA levels (Fig. 3B and Table 2). Plants grown in synthetic medium (i.e. on vertical plates) were selected for BASTA resistance (Fig. 3A). A pool of transgenic plants (derived from several independent T_1 plants) carrying the empty vector was used as control (shortly termed WT). Control plants (i.e. WT plants) were treated in all experiments in the same way as the *EXO* overexpression (i.e. with or without BASTA selection).

In order to insert EXO fragments into the pJawohl8-RNAi vector (accession number AF408413), PCR was performed on genomic DNA using the primers *att*B1:ccaaacataacaaaagaattacgct and ggcttgctctactatggtctgaata:*att*B2. The fragment was inserted into an entry vector using the BP clonase enzyme mix (Invitrogen). Fragments were transferred into the pJawohl8-RNAi vector using the LR clonase enzyme mix (Invitrogen). Sequence analysis confirmed the proper insertion of two fragments in inverse orientation. The resulting construct was termed EXO-RNAi. Transgenic lines were selected using BASTA. Several independent T_1 generation plants with clearly reduced *EXO* mRNA levels were identified. However, the resulting T_2 lines did not show a Mendelian segregation. Reduced *EXO* mRNA levels were observed only occasionally in single T_2 plants. Expression analysis of EXO-RNAi plants was performed with rosette leaves of BASTA-selected T_1 plants. BASTA-selected WT plants (see above) were used as control.

2.3. Gene expression analysis

Real-time reverse transcription (RT) PCR and Northern blot analysis were carried out as previously described [19,20]. Primer sequences for amplification of gene-specific fragments (used for synthesis of labelled probes for Northern blot hybridizations) were as follows: KCS1_fw: 5′-agc gat gat cgt gaa cca tta caa g-3′, KCS1_rev: 5′-gta acg ctt tcc aaa ccg cac tat t-3′, EXP5_fw: 5′-ata gaa ggg ttc ggt gca aga gaa g-3′, EXP5_rev: 5′-cgt tcc cac aca tat att cgc tac a-3′, AGP4_fw: 5′-ggg ttc caa gat tgt cca agt ttt c-3′, AGP4_rev: 5′-agc cct tcc aat ggt aaa tta cac g-3′. Primer sequences for real-time RT-PCR were as follows: KCS1_fw: 5′-acc gaa gct aag ggt cgg gtt a-3′, KCS1_rev: 5′-gta acg ctt tcc aaa ccg cac t-3′, EXP5_fw: 5′-cca ttt cga tct ttc tca gcc g-3′, EXP5_rev: 5′-cac ttc tct tgc acc gaa ccc t-3′, AGP4_fw: 5′-gga cct tca gat gca tcc cct-3′, AGP4_rev: 5′-ggc gta cat aat agc ggc gaa g-3′, *δ-TIP*_fw: 5′-cgc cat tgg tct tat cgt tgg t-3′, and *δ-TIP*_rev: 5′-gca aca gct ggt cca aag gaa c-3′. The *eEF1*, *CPD* and *DWF4* primer pairs are given in Müssig et al. [19]. Production and hybridization of nylon filter arrays ('development macroarrays'), reverse transcription and radiolabeling was largely carried out as described before [22]. The represented genes and primers used to amplify gene-specific fragments are described in the supplementary information available via the *FEBS Letters* web site. Hybridization of Affymetrix ATH1 arrays and data analysis was performed as described previously [19].

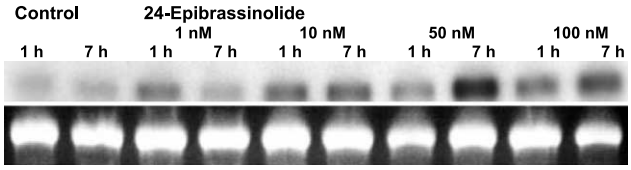

Fig. 1. BR induction of *EXO* gene expression. Wild-type plants were grown for 10 days in half-concentrated MS medium supplemented with 1% sucrose. Plants were treated with 1 nM, 10 nM, 50 nM, 100 nM EBL, or a control solution. Lower panels show ethidium bromide-stained rRNA bands to demonstrate that equal amounts of total RNA were loaded in each lane.

3. Results

3.1. EXO modulates expression of BR-responsive genes

Previous expression profiling experiments indicated the BR induction of *EXO* gene expression ([20]; data not shown). BR-deficient *dwf1-6* and *CPD* antisense plants showed weaker *EXO* expression, and 1-h BR treatments resulted in increased *EXO* transcript levels in both wild-type and *dwf1-6* plants. An independent expression profiling experiment was carried out with Affymetrix ATH1 microarrays. Wild-type plants were treated for 7 h with either 300 nM EBL or a control solution. Large pools of plants grown under aseptic conditions were used for RNA isolation and target synthesis. The qualitative and quantitative outcome of the Affymetrix Microarray Suite Version 5.0 software was used to identify genes with altered transcript levels upon BR treatment. The information used included the detection *P* values calculated through single array analysis, the change *P* values, and signal log ratios determined through comparison analysis.

In agreement with our previous data, increased *EXO* mRNA levels were found in BR-treated plants (Table 1). Northern blot analysis confirmed the BR induction (Fig. 1). GA (0.1 μM and 1 μM GA_4/GA_7; 1 h and 3 h), 2,4-D (10 nM, 0.1 μM, and 1 μM; 1 h and 3 h), and the ethylene precursor ACC (0.1 μM, 1 μM; 1 h and 3 h) did not alter *EXO* transcript levels (data not shown).

In order to test a putative role of the EXO protein in the mediation of BR effects, macroarrays (termed 'development macroarrays') with 190 gene-specific fragments spotted in duplicate were established. The DNA fragments on nylon membranes represented 50 BR-regulated genes and numerous other genes required for growth and development (see supplementary information). The filters were hybridized with ^{33}P-labelled first-strand cDNAs derived from BASTA-selected soil-grown T_1 generation plants.

Table 1
BR regulation of *EXO*, *KCS1*, *AGP4*, *EXP5*, *δ-TIP*, *CPD*, and *DWF4* gene expression

Gene	Wild-type (Col-0): 7 h 300 nM EBL vs. 7 h control		
	Detection (detection *P* value) Experiment/Baseline	Fold change	Change (change *P* value)
EXO At4g08950	P (0.000244)/P (0.000244)	3.5	I (0.00002)
KCS1 At1g01120	P (0.000244)/P (0.023926)	2.0	I (0.000046)
AGP4 At5g10430	P (0.000244)/P (0.030273)	7.0	I (0.00002)
EXP5 At3g29030	P (0.000244)/P (0.037598)	2.3	I (0.000023)
δ-TIP At3g16240	P (0.000244)/P (0.000244)	2.1	I (0.00003)
CPD At5g05690	P (0.000244)/P (0.000244)	−2.8	D (0.99998)
DWF4 At3g50660	P (0.037598)/P (0.001953)	−2.8	D (0.99998)

Expression profiles of the above-ground part of wild-type plants were established using Affymetrix ATH1 arrays. Plants were treated for 7 h with either 300 nM EBL or a control solution. Data were analyzed using standard parameters of the Affymetrix Microarray Suite Version 5.0 software.

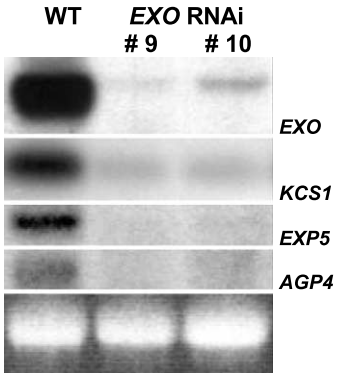

Fig. 2. Northern blot analysis of *EXO*, *KCS1*, *AGP4*, and *EXP5* gene expression in rosette leaves of 4-week-old EXO-RNAi plants grown in soil. Lower panels show ethidium bromide-stained rRNA bands to demonstrate that equal amounts of total RNA were loaded in each lane.

35S::EXO plants (with verified elevated *EXO* mRNA levels) were compared to EXO-RNAi plants (with verified reduced *EXO* transcript levels). The *KCS1* [23], *δ-TIP* [24], *Exp5*, and *AGP4* genes showed altered transcript levels (data not shown). Northern blot analysis confirmed elevated *KCS1*, *Exp5*, and *AGP4* mRNA levels in T_1 generation 35S::EXO plants (data not shown), and decreased mRNA levels in T_1 generation EXO-RNAi plants (Fig. 2). Real-time RT-PCR analysis confirmed increased *KCS1*, *Exp5*, *AGP4*, and *δ-TIP* transcript levels in soil-grown 35S::EXO T_2 generation plants (Table 2). The BR up-regulation of these genes was previously detected in expression profiling experiments [20]. The *KCS1*, *δ-TIP*, *Exp5*, and *AGP4* genes were induced upon 1-h BR treatments in wild-type and *dwf1-6* plants. The *KCS1* gene showed weaker expression in untreated *dwf1-6* and *CPD* antisense plants in comparison to untreated wild-type plants. The BR induction was confirmed in the present study in wild-type plants treated with 300 nM EBL for 7 h. The genes showed stronger expression upon the BR treatment (Table 1). The *KCS1*, *Exp5*, and *AGP4* genes encode cell wall (modifying) proteins. The KCS1 protein is involved in wax biosynthesis. Phenotypic changes in the *kcs1-1* mutant included thinner stems and less resistance to low humidity stress [23]. Expansins are involved in cell wall loosening and break non-covalent bonding between cellulose and hemicellulose. Arabinogalactan proteins (such as AGP4) are extracellular proteoglycans that are implicated in many plant growth and developmental processes. The δ-TIP protein is a plant aquaporin [24]. The transcriptional and/or post-translational regulation of aquaporins determines changes in membrane water permeability [25]. Thus, the KCS1, Exp5, AGP4, and δ-TIP proteins likely are involved in (BR-promoted) growth.

The expression data indicate altered transcript levels of BR-regulated genes in 35S::EXO and EXO-RNAi plants. Thus, *EXO* overexpression could induce BR biosynthesis, enhance BR sensitivity, or trigger BR responses. However, only a subset of BR-regulated genes showed altered transcript levels according to the macroarray data and *EXO* overexpression barely affected the *CPD* and *DWF4* transcript levels (Table 2). These observations suggest that BR levels and BR sensitivity are not affected by *EXO* overexpression and indicate a role of EXO in the regulation of a subset of BR-inducible genes downstream of BR perception. *EXO* overexpression in the BR-deficient *dwf1-6* mutant did not result in elevated

Table 2
Real-time RT-PCR analysis of *EXO*, *KCS1*, *Exp5*, *AGP4*, *δ-TIP*, *CPD*, and *DWF4* gene expression in 35S::EXO plants (wild-type background)

Gene	35S::EXO # 8 vs. WT Fold change C_T values	35S::EXO # 9 vs. WT Fold change C_T values	35S::EXO # 16 vs. WT Fold change C_T values
EXO	15.1	17.0	10.8
	$C_{T\,WT}$ 22.82 ± 0.22	$C_{T\,WT}$ 22.82 ± 0.22	$C_{T\,WT}$ 20.77 ± 0.32
	$C_{T\,\#8}$ 19.38 ± 0.15	$C_{T\,\#9}$ 19.05 ± 0.03	$C_{T\,\#16}$ 17.83 ± 0.41
eIF1α	$C_{T\,WT}$ 19.67 ± 0.37	$C_{T\,WT}$ 19.67 ± 0.37	$C_{T\,WT}$ 18.66 ± 0.16
	$C_{T\,\#8}$ 20.15 ± 0.10	$C_{T\,\#9}$ 19.99 ± 0.09	$C_{T\,\#16}$ 19.15 ± 0.17
KCS1	5.9	5.5	3.3
	$C_{T\,WT}$ 23.59 ± 0.44	$C_{T\,WT}$ 23.59 ± 0.44	$C_{T\,WT}$ 24.98 ± 0.86
	$C_{T\,\#8}$ 21.82 ± 0.47	$C_{T\,\#9}$ 21.83 ± 0.68	$C_{T\,\#16}$ 23.74 ± 0.24
Exp5	7.4	9.9	3.6
	$C_{T\,WT}$ 23.33 ± 0.12	$C_{T\,WT}$ 23.33 ± 0.12	$C_{T\,WT}$ 24.36 ± 0.10
	$C_{T\,\#8}$ 21.24 ± 0.18	$C_{T\,\#9}$ 20.72 ± 0.30	$C_{T\,\#16}$ 22.99 ± 0.10
AGP4	9.7	6.2	5.1
	$C_{T\,WT}$ 26.84 ± 0.35	$C_{T\,WT}$ 26.84 ± 0.35	$C_{T\,WT}$ 27.24 ± 0.22
	$C_{T\,\#8}$ 24.37 ± 0.20	$C_{T\,\#9}$ 24.91 ± 0.44	$C_{T\,\#16}$ 25.36 ± 0.74
δ-TIP	2.7	6.9	1.5
	$C_{T\,WT}$ 21.76 ± 0.16	$C_{T\,WT}$ 21.76 ± 0.16	$C_{T\,WT}$ 22.98 ± 0.27
	$C_{T\,\#8}$ 21.10 ± 0.25	$C_{T\,\#9}$ 19.67 ± 0.17	$C_{T\,\#16}$ 22.88 ± 0.52
CPD	1.3	−1.1	−1.5
	$C_{T\,WT}$ 20.48 ± 0.18	$C_{T\,WT}$ 20.48 ± 0.18	$C_{T\,WT}$ 20.81 ± 0.36
	$C_{T\,\#8}$ 21.61 ± 0.12	$C_{T\,\#9}$ 21.11 ± 0.09	$C_{T\,\#16}$ 21.91 ± 0.25
DWF4	1.5	1.5	−1.1
	$C_{T\,WT}$ 24.34 ± 0.13	$C_{T\,WT}$ 24.34 ± 0.13	$C_{T\,WT}$ 25.52 ± 0.59
	$C_{T\,\#8}$ 24.54 ± 0.20	$C_{T\,\#9}$ 24.42 ± 0.46	$C_{T\,\#16}$ 26.16 ± 0.46
eIF1α	$C_{T\,WT}$ 17.67 ± 0.18	$C_{T\,WT}$ 17.67 ± 0.18	$C_{T\,WT}$ 18.66 ± 0.16
	$C_{T\,\#8}$ 18.47 ± 0.06	$C_{T\,\#9}$ 18.37 ± 0.26	$C_{T\,\#16}$ 19.15 ± 0.17

RNA was extracted from rosette leaves of 43-day-old T_2 generation plants. Fold change values were calculated from average C_T (threshold cycle) values (see Section 2). The table also lists the average C_T values (± S.D.) from three replicates per experiment for the genes of interest and *eIF1α*. Similar results were obtained in independent experiments using 28-day-old plants (data not shown).

Table 3
Real-time RT-PCR analysis of *EXO*, *KCS1*, *Exp5*, *AGP4*, and *δ-TIP* gene expression in 35S::EXO plants (*dwf1-6* background, plants shown in Fig. 3C)

Gene	35S::EXO in *dwf1-6* vs. WT		
	Fold change C_T values Plant # 1	Fold change C_T values Plant # 2	Fold change C_T values Plant # 3
EXO	3.7	5.7	7.0
	$C_{T\,WT}$ 23.61 ± 0.25	$C_{T\,WT}$ 23.61 ± 0.25	$C_{T\,WT}$ 23.61 ± 0.25
	$C_{T\,\#1}$ 21.90 ± 0.21	$C_{T\,\#9}$ 21.30 ± 0.18	$C_{T\,\#9}$ 20.42 ± 0.15
KCS1	−4.1	−4.8	−1.9
	$C_{T\,WT}$ 23.15 ± 0.06	$C_{T\,WT}$ 23.15 ± 0.06	$C_{T\,WT}$ 23.15 ± 0.06
	$C_{T\,\#1}$ 25.37 ± 0.24	$C_{T\,\#9}$ 25.62 ± 0.35	$C_{T\,\#9}$ 23.69 ± 0.34
Exp5	−1.2	−2.1	−1.7
	$C_{T\,WT}$ 23.34 ± 0.07	$C_{T\,WT}$ 23.34 ± 0.07	$C_{T\,WT}$ 23.34 ± 0.07
	$C_{T\,\#1}$ 23.81 ± 0.21	$C_{T\,\#9}$ 24.61 ± 0.28	$C_{T\,\#9}$ 23.70 ± 0.03
AGP4	1.5	1.0	−1.7
	$C_{T\,WT}$ 22.79 ± 0.19	$C_{T\,WT}$ 22.79 ± 0.19	$C_{T\,WT}$ 22.79 ± 0.19
	$C_{T\,\#1}$ 22.40 ± 0.10	$C_{T\,\#9}$ 22.95 ± 0.29	$C_{T\,\#9}$ 23.16 ± 0.03
δ-TIP	−1.6	−1.5	−2.5
	$C_{T\,WT}$ 18.27 ± 0.15	$C_{T\,WT}$ 18.27 ± 0.15	$C_{T\,WT}$ 18.27 ± 0.15
	$C_{T\,\#1}$ 19.13 ± 0.13	$C_{T\,\#9}$ 19.04 ± 0.25	$C_{T\,\#9}$ 19.21 ± 0.17
eIF1α	$C_{T\,WT}$ 18.46 ± 0.05	$C_{T\,WT}$ 18.46 ± 0.05	$C_{T\,WT}$ 18.46 ± 0.05
	$C_{T\,\#1}$ 18.64 ± 0.46	$C_{T\,\#9}$ 18.65 ± 0.02	$C_{T\,\#9}$ 18.08 ± 0.28

RNA was extracted from 23-day-old T_1 generation plants (above-ground part of plants). Fold change values were calculated from average C_T (threshold cycle) values (see Section 2). The table also lists the average C_T values (± S.D.) from three replicates per experiment for the genes of interest and *eIF1α*.

KCS1, *Exp5*, *AGP4*, and *δ-TIP* transcript levels (Table 3). Thus, *EXO* overexpression is not sufficient to trigger the expression of these genes in the absence of normal BR levels.

3.2. EXO *overexpression promotes growth*

The increased *KCS1*, *Exp5*, *AGP4*, and *δ-TIP* mRNA levels suggested a role of the EXO protein in the regulation of growth processes. This hypothesis was further supported by the phenotype of 35S::EXO plants (wild-type background) which showed enhanced vegetative growth. Petiole length and rosette leaf area of 35S::EXO plants were increased in comparison to wild-type plants (Fig. 3A,B). In order to evaluate the effects of *EXO* overexpression on root growth, four independent 35S::EXO lines and WT plants (carrying the empty vector) were grown on vertical plates. *EXO* overexpression promoted root growth in all 35S::EXO lines (Fig. 3A). Thus, the EXO protein promotes growth in both shoots and roots.

EXO overexpression in the BR-deficient *dwf1-6* background did not normalize the dwarf phenotype in five T_1 generation plants (Fig. 3C, Table 3, and data not shown), further indicating that *EXO* overexpression is not sufficient to enhance growth in the absence of BRs.

4. Discussion

BR promotes growth in both shoots and roots. Molecular events underlying this effect include increased expression of cell wall-modifying enzymes (such as XTHs and expansins), stimulation of cell division, increased photosynthetic activity, and effects on carbon partitioning (reviewed in [26]).

35S::EXO plants showed elevated transcript levels of the BR-responsive *KCS1*, *AGP4*, *EXP5*, and *δ-TIP* genes. The effects of *EXO* overexpression on petiole elongation, leaf expansion, and root growth resemble the effects of BR treatments (e.g. [19,27]). In line with previous findings [18], plants with reduced *EXO* expression showed normal growth, suggesting functional redundancy of either homologs of the EXO protein or EXO-regulated genes.

The observed effects of *EXO* overexpression on the *KCS1*, *AGP4*, *EXP5*, and *δ-TIP* genes and on growth in conjunction with the lack of change in *CPD* and *DWF4* expression (these genes are negatively feedback controlled by BRs) indicate that the EXO protein most likely represents a downstream component in the BR-signalling pathway, which mediates a specific subset of genomic BR effects. *EXO* overexpression in the BR-deficient *dwf1-6* mutant did not result in elevated *KCS1*, *AGP4*, *EXP5*, and *δ-TIP* mRNA levels, and did not normalize the dwarf phenotype. Thus, cellular responses to *EXO* overexpression are BR-dependent and EXO action apparently requires other BR-dependent components.

The *EXO* gene is a member of a small gene family. Sequence comparisons barely hinted at the function of the gene product [18]. In the Col-0 background *EXO* was expressed in embryos and in regions of active cell division such as the apical meristem and young leaves. *EXO* promoter activity was also detected in rosette leaves and roots. These findings and the maximal mRNA abundance during M phase of the cell cycle suggested a cell cycle phase-dependent regulation of *EXO* gene expression [18]. Re-evaluation of tissue-specific expression in the C24 background by means of Northern blot analysis revealed expression in all organs analyzed (data not shown). Flowers and stems had the highest transcript levels, moderate expression was found in seedlings, bracts, roots, and siliques. The strong expression in stems suggests that *EXO* gene expression is not restricted to dividing cells and is not expressed in a cell cycle-dependent manner. The phenotypic alterations and the changes in gene expression patterns observed in 35S::EXO plants hint at stimulation of cell elongation rather than cell division. Detailed microscopic analyses of 35S::EXO plants will clarify alterations at the cellular level. The determination of the subcellular localization and the identification of interacting proteins hold the potential to elucidate the molecular basis of the EXO-mediated growth promotion.

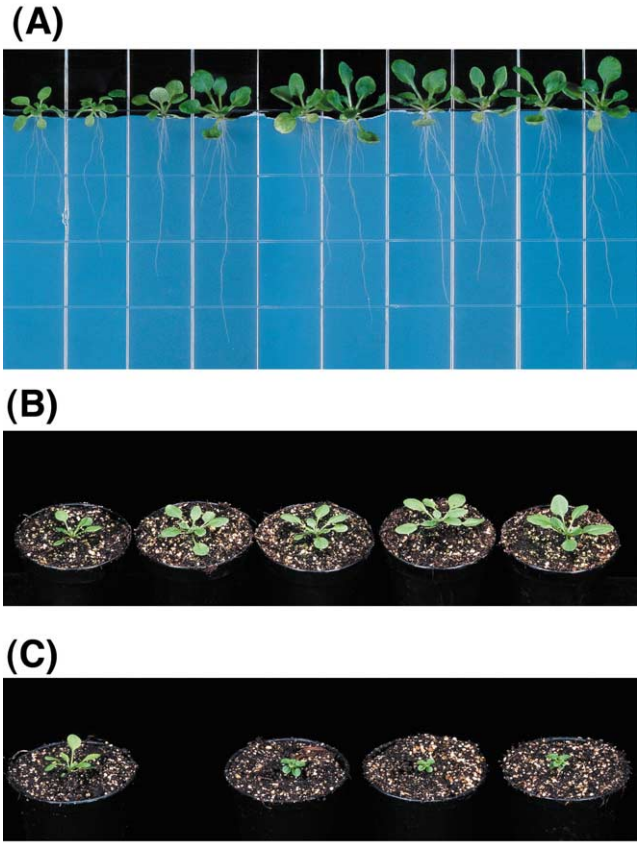

Fig. 3. *EXO* overexpression in the wild-type but not in the *dwf1-6* background promotes growth. In A and B left to right: WT and plants of the *EXO*-overexpressing lines # 8, # 9, # 14, and # 16 (wild-type background). A: WT and 35S::EXO plants were grown on vertically oriented plates on synthetic medium for 19 days. Two plants per genotype are shown. B: WT and 35S::EXO plants were grown in soil for 23 days under long-day conditions. C: *EXO* overexpression does not restore the dwarf phenotype of the *dwf1-6* mutant. Plants were grown in soil for 21 days under long-day conditions. Left to right: WT and plants of the *EXO*-overexpressing lines # 1, # 2, and # 3 (*dwf1-6* background).

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