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RESEARCH PAPER

Strigolactones are positive regulators of light-harvesting genes in tomato

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Abstract

Strigolactones are newly identified plant hormones, shown to participate in the regulation of lateral shoot branching and root development. However, little is known about their effects on biological processes, genes, and proteins. Transcription profiling of roots treated with GR24, a synthetic strigolactone with proven biological activity, and/or indole acetic acid (IAA) was combined with physiological and transcriptional analysis of a tomato mutant (*Sl-ORT1*) deficient in strigolactone production. GR24 treatment led to markedly induced expression of genes putatively involved in light harvesting. This was apparent in both the presence and absence of exogenously applied IAA, but not with IAA treatment alone. Following validation of the microarray results, transcriptional induction by light of the GR24-induced genes was demonstrated in leaves exposed to high or low light intensities. *Sl-ORT1* contained less chlorophyll and showed reduced expression of light harvesting-associated genes than the wild type (WT). Moreover, perfusion of GR24 into WT and *Sl-ORT1* leaves led to induction of most of the examined light harvesting-associated genes. Results suggest that GR24 treatment interferes with the root's response to IAA treatment and that strigolactones are potentially positive regulators of light harvesting in plants.

Key words: Auxin, chlorophyll, gene transcription, light harvesting, mutant, strigolatones.

Introduction

Strigolactones have been recently defined as a new group of plant hormones or their biosynthetic precursors (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). Strigolactone production has been demonstrated in many plant species (e.g. Sato *et al.*, 2005; Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008; Koltai *et al.*, 2010*b*), synthesized mainly in the roots and lower part of the stem (Foo *et al.*, 2001; reviewed by Dun *et al.*, 2009) and then moving towards the shoot apex (Foo *et al.*, 2001; Brewer *et al.* 2009; Ferguson and Beveridge 2009). Strigolactones have been shown to play a role in inhibition of shoot branching (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008; Brewer *et al.*, 2009; Ferguson and Beveridge, 2009) and thus to affect shoot architecture; they have also been shown to affect root growth (Koltai *et al.*, 2010*a*) and root system architecture (HK and YK, unpublished results). Strigolactones have been suggested to be derived from the carotenoid pathway (Matusova *et al.*, 2005) via the activity of various oxygenases (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008, and references therein).

Some studies have suggested key hypotheses regarding the role strigolactones may play in restraining lateral shoot bud outgrowth, thereby determining shoot architecture. It was suggested that strigolactones inhibit polar auxin transport from the buds by reducing the capacity for polar auxin transport from the apical meristem, resulting in restrained bud outgrowth (e.g. Bennett *et al.*, 2006; Mouchel and Leyser, 2007; Ongaro and Leyser, 2008; Leyser, 2009). Another possibility is that strigolactones serve as auxin-promoted secondary messengers that move up into the buds to repress their outgrowth (Brewer *et al.*, 2009; Ferguson and Beveridge, 2009; reviewed by Dun *et al.*, 2009), or that

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both auxin and strigolactones have the ability to change each other's levels and distribution in a dynamic feedback loop, which is required for the coordinated control of axillary branching (Hayward *et al.*, 2009).

Using a synthetic strigolactone (GR24) previously shown to have strigolactone-like biological activity (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008), an effect of strigolactones on tomato root development was recently found. GR24 was shown to interfere with the inhibitory effect of exogenously applied auxin on root elongation, conveyed via an increase in root cell length. Auxin efflux carriers were involved in this effect of strigolactone on root growth and root hair elongation (Koltai *et al.*, 2010*a*).

However, only little is known about the biological processes, genes, and proteins affected by strigolactones. For a better understanding of the effects of strigolactones on plant development and their cross-talk with auxin, gene expression was profiled following tomato root exposure to GR24 and indole acetic acid (IAA). Of the GR24-induced genes, many were putatively involved in light harvesting. Following validation of the microarray results, light-induced transcription of the GR24-induced genes was demonstrated in leaves exposed to high or low light intensities. Expression of these genes was reduced in a strigolactone-deficient tomato mutant (*Sl-ORT1*; Koltai *et al.*, 2010*b*), which was also found to possess reduced levels of chlorophyll. Moreover, exposure of both wild-type (WT) and *Sl-ORT1* leaves to GR24 led to induction of the expression of these genes. Together, the results suggest that strigolactones are potentially positive regulators of plant light-harvesting components.

Materials and methods

In vitro plant growth

Tomato (*Solanum lycopersicum*) cv. M82 (WT; Eshed *et al.*, 1992) seedlings were surface-sterilized, immersed in sterile distilled water for 1 h, and placed on half-strength Murashige and Skoog (MS) agar medium supplemented with 1.5% (w/v) sucrose, in Petri dishes (50 seeds per Petri dish). Dishes were placed horizontally in the dark at 25 °C for 72 h to induce germination. Germinated seeds were gently transferred to half-strength MS agar medium supplemented with 1.5% (w/v) sucrose in Petri dishes. Petri dishes included the following hormone concentrations (designated as treatments): IAA at a concentration of 10^{-8} M; IAA at a concentration of 10^{-8} M supplemented with the synthetic strigolactone GR24 (Johnson *et al.*, 1981) at a concentration of 13.5 μM; GR24 at a concentration of 27 μM; and non-supplemented control. Hormone concentrations were determined, in a previous study, to have significant effects on root growth under the same growth conditions (Koltai *et al.*, 2010*a*). The plates were placed vertically to allow gravitropic root growth along the surface of the agar under 16/8 h (day/night) fluorescent lighting (100 μmol m^{-2} s^{-1}) at 25 °C. Roots were marked after 24 h of incubation; following an additional 24 h, roots were sectioned from the 24 h mark to the root tip and were frozen in liquid nitrogen.

Plant growth under greenhouse conditions

WT and *Sl-ORT1* seeds were surface-sterilized and allowed to germinate and grow in styrofoam seedling trays in soil:vermiculite (1:1, v/v). Four-week-old seedlings were transferred to 3.0 l pots (one plant per pot) with a 1:1 mixture of soil and vermiculite. For each WT or *Sl-ORT1* plant, 12 pots with plants were grown under full light intensity (August–September 2009) and another 12 pots under shaded light, the latter provided with black 50% shading nets. Plants were grown in the greenhouse under natural light conditions supplemented with artificial light (100 μmol m^{-2} s^{-1}) to maintain a 16/8 h (day/night) photoperiod at 28 °C/24 °C (day/night). Plants were fertilized with 7:1:7 (NPK) solutions. The dry weight (DW) of shoots was determined for each plant after 45 d; 12 plants were examined for each WT and *Sl-ORT1* strain, for each shading treatment. DW was determined following 72 h incubation in an air forced oven at 70°C. The experiment was repeated twice. Means ±SE were determined for all replicates; means of replicates were subjected to statistical analysis by multiple-range test ($P \leqslant 0.05$), using the JMP statistical package (SAS, Cary, NC, USA).

Leaf injection with GR24

The first fully expanded leaves of M82 or *Sl-ORT* plants grown under greenhouse conditions as described above were injected with 1 ml of either GR24 (2.7 μM) or water as a control. Five spots on the leaf were injected with 1 ml of fluid into the lower epidermis, such that the injected fluid spread throughout the leaf tissue; injection sites were marked. Marked areas were collected 48 h after injection, and were frozen in liquid nitrogen.

RNA extraction

RNA extraction was performed, for each biological replicate, from 150 mg of roots or leaves of plants grown under *in vitro* and greenhouse conditions, respectively, as described above. Total RNA was extracted using TRI reagent (MRC, Cincinnati, OH, USA) and treated with Turbo DNase enzyme (Ambion) as per the manufacturer's instructions. For the microarray experiments, no DNase treatment was performed. Rather, RNA was purified and concentrated using an RNeasy MiniElute Cleanup Kit (Qiagen, Valencia, CA, USA) as per the manufacturer's instructions, and was taken for hybridization.

cDNA synthesis

For cDNA synthesis, 2.5 μg of total RNA and 0.1 μM of random hexamer primers were heated for 5 min at 65 °C and snap-chilled on ice. The following components were added to the reaction mixture: 0.2 mM dNTP mixture (Fermentas, Glen Burnie, MD, USA), M-MuLV-reverse transcriptase (RT) buffer (1× final concentration), 40 U of RNase M-MuLV inhibitor (Fermentas), 200 U of M-MuLV-RT enzyme (Fermentas), and diethylpyrocarbonate (DEPC)-treated water to a reaction volume of 21 μl. The reaction was incubated at 42 °C for 60 min following an incubation at 70 °C for 10 min.

Microarray experiments

Microarray chip description and hybridization: The Affymetrics GeneChip® Tomato Genome Array that was used consists of 10 227 *S. lycopersicum* probe sets for examination of ~9200 *S. lycopersicum* transcripts. Sequence information for this array was selected from public data sources including *S. lycopersicum* UniGene Build #20 and GenBank mRNAs (http://www.affymetrix.com/products/arrays/specific/tomato.affx); the total number of genes in the tomato euchromatin is ~40 000 (Mueller *et al.*, 2009).

RNA, purified as described above, was subjected to Affymetrics microarray hybridization, as instructed by the manufacturer using a GeneChip® 3′ IVT Express Kit (Affymetrix).

Quantification and data analysis: GeneChips were scanned using the Affymetrix GeneChip Scanner 3000 7G. Data were quantified with the Affymetrix Expression Console using the MAS 5.0 algorithm

with Affymetrix default analysis settings and global scaling as the normalization method. Filtration on confidence was performed based on one-sample *t*-test ($P \leqslant 0.05$), and the resulting gene lists were filtered using GeneSpring GX (Agilent) for differentially expressed genes (>2-fold and <0.5-fold change between treatment versus control). Three biological replicates were performed for each examined treatment (i.e. IAA, IAA+13.5 μM GR24, and 27 μM GR24).

Gene annotation

Annotation of part of the array-represented gene sequences is based on Gene Ontology nomenclature (GO; http://www.geneontology.org/) and was supplied by Affymetrix Inc. Additional gene annotations were found by BLAST function for comparisons between array-represented gene sequences and the NCBI non-redundant protein and TAIR databases. Functional classifications were performed by MapMan software (Thimm *et al.*, 2004).

Quantitative real-time PCR

Quantitative real-time (qPCR) was performed on RNA extracted from either roots or leaves, as described above. For microarray validation, three additional (to those of the microarray) and independent experiments were examined. The qPCR was performed using components supplied in the KAPA SYBR FAST qPCR kits (Kapa Biosystems, Woburn, MA, USA) and gene-specific primers (Table 1). The reaction mixture consisted of the following components: 2× Master Mix with integrated antibody-mediated hot start, SYBR® Green I fluorescent dye, $MgCl_2$, dNTPs, stabilizers, 2 μl of the template, and PCR-grade water to a final volume of 10 μl. The qPCR analysis was carried out on a Rotor gene 6000 instrument (Corbett-Qiagen, Valencia, CA, USA) according to the following program: 3 min at 95 °C, followed by 49 cycles of 95 °C for 3 s, 60 °C for 20 s, and 72 °C for 1 s. Primers used for qPCR are presented in Table 1. The threshold cycle (Ct) was calculated by the Rotor gene 6000 instrument software. The level of expression of the target genes was calculated relative to that of the reference mRNA; tomato 18S rRNA (accession no. AY552528) served as the reference gene for the amount of RNA, and was amplified using the forward primer 5′TTGATTACGTCCCTGCCCTTTGTACAC3′ and the reverse primer 5′AGGTTCACACCTACGGAAACCTTGTTAC3′. Values of the steady-state level of gene transcripts were determined as a ratio between two conditions (e.g. GR24 treatment versus control, or *Sl-ORT1* versus WT) using the $2^{\Delta\text{-}\Delta Ct}$ method (Arocho *et al.*, 2006). A value above or below 1 represents an increase or decrease, respectively, in the steady-state level of gene transcripts for the examined conditions (i.e. that of the nominator versus that of the denominator). Means ±SE were calculated for three biological replicates for each examined treatment. Means of replicates were subjected to statistical analysis by multiple-range test ($P \leqslant 0.05$), using the JMP statistical package.

Measurements of chlorophyll levels

Chlorophyll was measured using a hand-held chlorophyll meter (SPAD-502 chlorophyll meter, Minolta Camera Co., Ltd, Osaka, Japan), according to the manufacturer's instructions. The measurements were performed on eight mature (fully exposed) leaves from each of four (45 d old) plants per variant in the morning (10:00 to 11:00 h) under natural sunlight (1200 μmol m^{-2} s^{-1}, ~12 h of daylight) in the greenhouse.

Results

Transcription profiling of roots treated with IAA and GR24

Roots grown on plates supplemented with different hormones: IAA (10^{-8} M), IAA (10^{-8} M)+GR24 (13.5 μM), and GR24 (27 μM), and non-treated controls were profiled for gene expression using tomato Affymetrix microarrays (Supplementary Table S1 available at *JXB* online). Hierarchical clustering of the expression results suggested that the two treatments with GR24, namely IAA+GR24 and GR24, were more similar to each other in terms of gene expression than the IAA+GR24 and IAA treatments (Supplementary Fig. S1).

Statistical and 2-fold cut-off were used to identify genes that were significantly and differentially expressed for each of the treatments (Supplementary Table S2 at *JXB* online): most differentially expressed genes were found in the GR24 treatment, less in the IAA+GR24 treatment, and even less in the IAA treatment. Moreover, intersection by a Venn

Table 1. Lists of primers used for quantitative PCR

Affymetrix probe ID	Gene accession no.	Gene annotation	Forward primer 5′→3′	Reverse primer 5′→3′
Les.2668.1.S1_at	AW224185	Auxin- and ethylene-responsive GH3-like protein (GH3)	CCCGCAGTTCCATTTTGTC	TACTCAACCACGCTGGTGTT
LesAffx.48947.1.S1_at	AW626006	F-box domain-containing protein	CTTTAGGTCCACGGGGTACA	CCCCAACAATATTCCCATGT
LesAffx.63489.1.S1_at	BI921137	Transmembrane BAX inhibitor motif-containing protein 4	TCAAAGAGAGGGCAGGACTT	TACGCGCAGAAAACAATAGC
Les.376.1.S1_at	BG627516	Ribulose-1,5-bisphosphate carboxylase, small subunit precursor	ACTTGGTCGGAATCGAAGAA	TGCCTACAAGCCAGAAGGAT
Les.147.1.S1_at	BG629070	Chlorophyll *a*/*b*-binding protein precursor	GTGGTCGGAAAGGTTCTCAA	GAGGCATTTGCTGAGTTGAA
Les.4345.2.A1_x_at	AI781554	Lhcb1*1 gene for light-harvesting chlorophyll *a*/*b*-binding protein (homologue)	GTCTGCAAGGTGATCAGCAA	TGGAAGCTTCGACCCATTAG
Les.2168.1.S1_at	BT013274.1	Photosystem I PSI-N mRNA, nuclear gene encoding chloroplast protein (homologue)	GCTGCTGCACTTTTCACATC	GCACCACTTGTAGCCAACCT
Les.608.1.S1_at	BG628276	Chloroplast pigment-binding protein CP26 (CP26) (homologue)	TGGAATGAAGGACGAATGTG	TTTGGCCTGGAGGAATTGTA

diagram of each of the treatments versus non-treated controls suggested that more genes were shared between the GR24 treatments (IAA+GR24 and GR24) than between the IAA treatments (IAA+GR24 and IAA), or between IAA and the GR24 treatments (Fig. 1).

Expression of eight significantly and differentially regulated genes was validated by qPCR. All eight genes showed expression patterns with the same tendency as those detected in the microarray experiments, confirming the accuracy of the microarray data (Table 2).

These results suggested that under the examined conditions, treatment of roots with GR24 interferes with the effects on roots of simultaneous treatment with IAA.

Functional classification of genes significantly and differentially expressed in roots treated with IAA and GR24

The significantly and differentially expressed genes (Supplementary Table S2 at *JXB* online) in the three root treatments were divided according to their putative association with biological processes. Significantly, in the GR24 treatments (i.e. IAA+GR24 and GR24), many of the up-regulated genes were involved in light harvesting (Supplementary Fig. S2). Notable among these were genes of photosystem I (PSI) and PSII (Fig. 2). However, in the IAA-only treatment, the light harvesting-associated genes were not induced (Supplementary Fig. S2, Fig. 2).

Expression profiles of light harvesting-associated genes in WT leaves grown under full and reduced light intensities

To examine the association between the GR24-induced, putative light harvesting-associated genes and plant light reactions, the light regulation of the transcription of these genes was examined in leaves of WT tomato plants grown under full and reduced light intensities.

Under 50% shading, total primary shoot DW was reduced by ~66% (8.9±0.4 g for full light intensity versus 3.0±0.5 g for shaded plants) and chlorophyll levels were reduced by ~20% (47.9±0.7 SPAD units for full light intensity versus 39.2±0.5 SPAD units for shaded plants).

Under these shaded, reduced-chlorophyll conditions, transcription levels of the GR24-induced putative light harvesting-associated genes were reduced in comparison with their transcription levels under full-light conditions (Table 2). Hence, these genes are regulated transcriptionally and are induced by high-light conditions, these latter conditions being associated with increased content of chlorophyll.

Determination of chlorophyll content and level of transcription of genes associated with light harvesting in a mutant deficient in strigolactone production

The above results suggested that exposure to strigolactones induces light harvesting-associated genes. To validate this notion, the levels of chlorophyll were examined in a mutant, *Sl-ORT1*, which is deficient in strigolactone production (Dor *et al.*, 2010; Koltai *et al.*, 2010*b*) in comparison with WT plants.

Under full-light conditions, *Sl-ORT1* had a significantly reduced level of chlorophyll relative to the WT (41.5±0.8 SPAD units and 47.9±0.7 SPAD units, respectively).

The level of transcription of the GR24-induced light-responsive genes was determined in WT and *Sl-ORT1* leaves grown under full-light conditions. All examined light harvesting-associated genes showed reduced levels of transcription in *Sl-ORT1* in comparison with the WT (Table 2).

Together, these and the above results of the chlorophyll level in *Sl-ORT1* leaves suggest that lack of strigolactones is associated with a reduced level of light harvesting in plants, reflected as both reduced transcription of light harvesting-associated genes and a decrease in chlorophyll level.

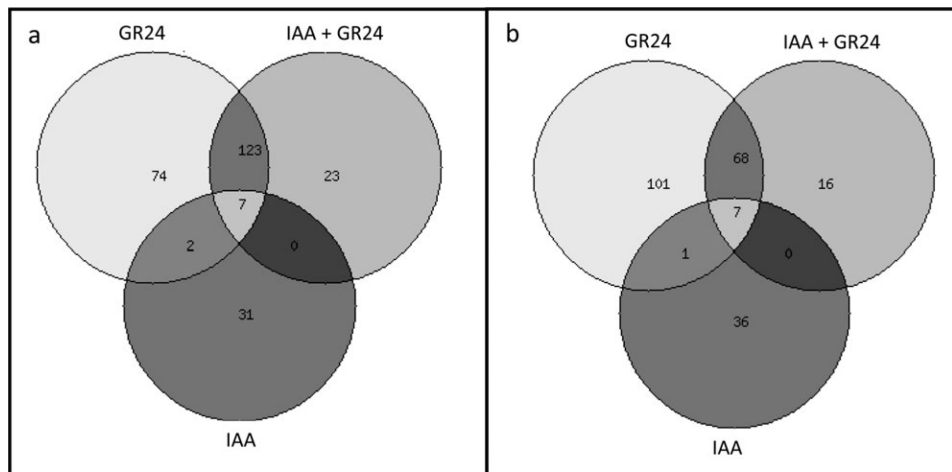

Fig. 1. Intersection of significantly and differentially regulated gene lists. Differentially regulated genes were identified from hybridization data of roots exposed to GR24 and IAA treatments [IAA (10^{-8} M), IAA (10^{-8} M)+GR24 (13.5 μM), and GR24 (27 μM)] versus non-treated controls. The intersection area presents the number of genes differentially regulated for each of the treatments. (a) Up-regulated genes, (b) down-regulated genes.

Table 2. Gene transcription levels of GR24-induced and repressed genes

Presented are gene transcription levels from microarray results of roots treated with GR24 (27 μM) versus control and roots treated with IAA (10^{-8} M)+GR24 (13.5 μM) versus control, from qPCR of WT roots treated with GR24 (27 μM) versus control, and of WT leaves from plants grown under reduced (50%) versus those grown under full-light intensities, from qPCR of WT leaves versus *Sl-ORT1* leaves and of WT and *Sl-ORT1* leaves 48 h after of injection with GR24, versus water-injected controls.

Gene annotation	Accession no.	Microarray result[a]GR24 (27 μM)/ control	Microarray result IAA (10^{-8} M)+GR24 (13.5 μM)/ control	qPCR WT roots treated with GR24 (27 μM)/ control	qPCR WT leaves 50% light/full light	qPCR leaves of *Sl-ORT1*/ WT	qPCR WT leaves 48 h after GR24 injection/ control	qPCR *Sl-ORT1* leaves 48 h after GR24 injection/ control
Auxin- and ethylene-responsive GH3-like protein (GH3)	AW224185	0.43	0.36	0.31±0.49	ND	ND	ND	ND
F-box domain-containing protein	AW626006	0.34	0.44	0.04±0.02	ND	ND	ND	ND
Transmembrane BAX inhibitor motif-containing protein 4	BI921137	0.31	0.33	0.05±0.04	ND	ND	ND	ND
Ribulose-1,5-bisphosphate carboxylase, small subunit precursor	BG627516	2.45	2.94	10.84±5.14	0.02±0.03	0.2±0.03	2.94±0.46	2.71±0.91
Chlorophyll *a*/*b*-binding protein precursor	BG629070	4.39	4.65	9.40±3.61	0.004±0.006	0.06±0.02	3.15±0.39	2.85±1.04
Lhcb1*1 gene for light-harvesting chlorophyll *a*/*b*-binding protein (homologue)	AI781554	4.49	5.06	11.82±1.44	0.04±0.07	0.13±0.14	2.58±0.30	2.82±1.06
Photosystem I PSI-N mRNA, nuclear gene-encoding chloroplast protein (homologue)	BT013274.1	2.04	2.77	9.00±1.73	0.005±0.006	0.09±0.03	1.29±0.04	3.28±1.09
Chloroplast pigment-binding protein CP26 (CP26) (homologue)	BG628276	2.79	3.48	11.05±8.03	0.001±0.008	0.05±0.04	2.87±0.30	2.21±0.44

[a] Microarray results are significant (P <0.05).
ND, not determined.

Transcription profiles of genes associated with light harvesting following direct GR24 application

To demonstrate a direct connection between plant exposure to strigolactones (in the form of GR24) and transcription of the light harvesting-associated genes, light harvesting-associated gene transcription was profiled in *Sl-ORT1* and WT leaves 48 h after injecting leaves with GR24. All but one of the examined light-harvesting genes appeared to be induced upon GR24 injection, in both *Sl-ORT1* and WT leaves (Table 2).

Discussion

In this study, the understanding of plant responses to strigolactones is promoted by a demonstration that strigolactones have a positive effect on the plant's light harvesting.

Gene expression profiling suggested that GR24 treatments are similar in their effect on gene expression in treated roots in the presence and absence of IAA. Gene expression profiling allowed the identification of GR24-induced genes, activated upon GR24 treatment regardless of other conditions (i.e. treatment with IAA or different GR24 concentrations); these genes, many of which are putatively associated with light harvesting, are discussed further on. Gene expression profiling also suggested that treatment of roots with GR24 interferes with the effect of IAA treatment on roots.

Accordingly, unlike in the GR24 treatments, in the IAA-only treatment the light harvesting-associated genes were not induced. This lack of IAA induction of light harvesting-associated genes is in agreement with the results of Volfová *et al.* (1978), suggesting that IAA treatment reduces chlorophyll content in wheat. This lack of IAA induction of light harvesting-associated genes is also in agreement with the results of Zhong and Ye (2001), suggesting that inhibition of auxin transport in *Arabidopsis*, leading to reduced auxin polar flow along the inflorescence stems and in the hypocotyls, is associated with increased chlorophyll content.

This interference of strigolactones with auxin responses detected in the present system is reflected at the gene expression level; however, it may also be reflected at the root phenotypic level: exposure of roots to GR24 interfered with the inhibitory effect of exogenously applied auxin on root cell elongation in tomato plants (Koltai *et al.*, 2010*a*). Although the present results should be further validated,

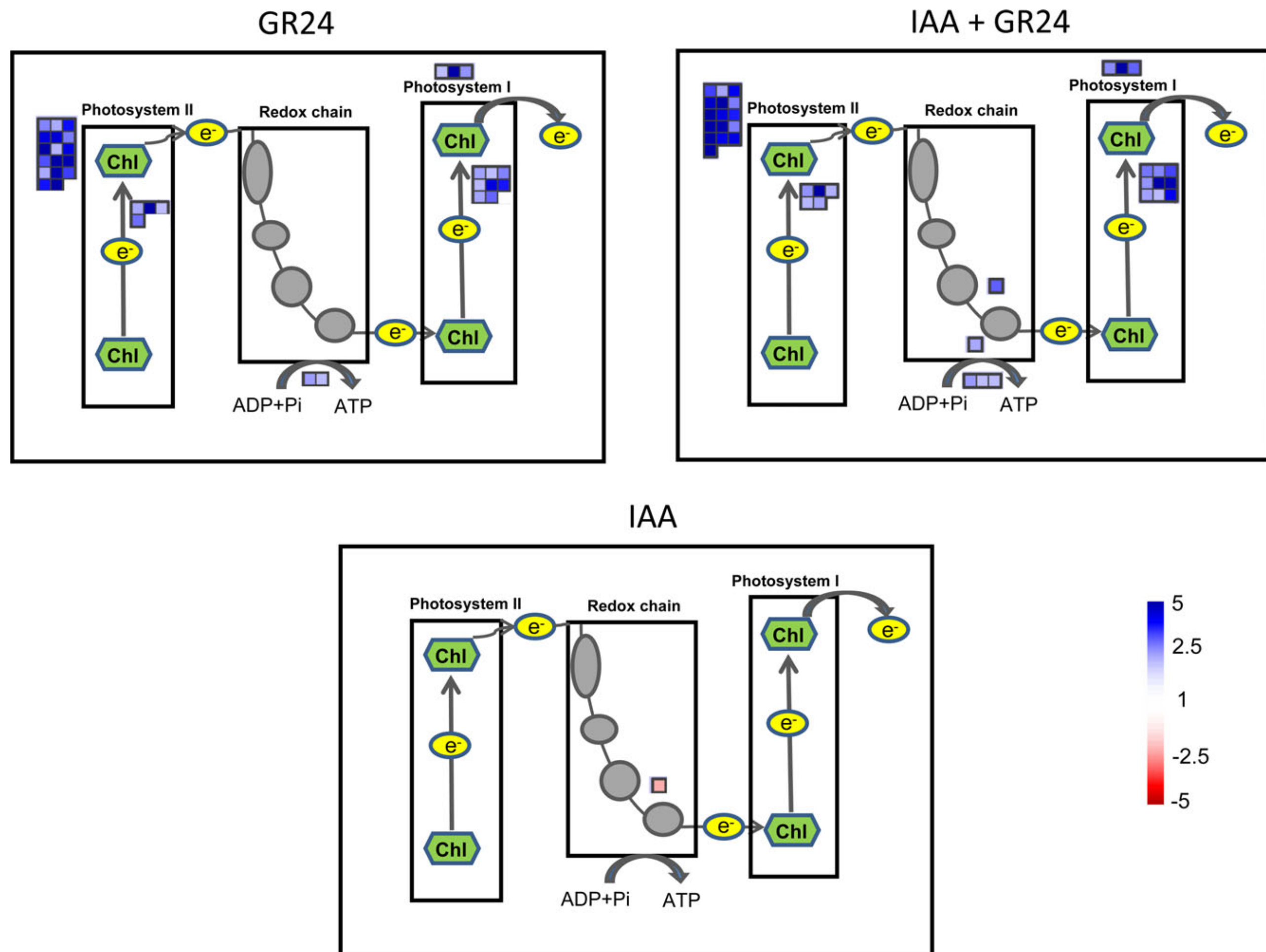

Fig. 2. Illustration of the light reaction-associated biological pathways in which differentially regulated genes putatively participate. Differentially regulated genes were identified from hybridization data of roots exposed to GR24 and IAA treatments [IAA (10_8 M), IAA (10_8 M)+GR24 (13.5 IM), and GR24 (27 IM)] versus non-treated controls. Blue or red squares represent individual genes. The colour within the squares represents fold change in gene expression in treatments versus controls; values of fold change are as indicated in the colour scale. Chl signifies chlorophyll. The figure was adapted from MapMan software (Thimm *et al.*, 2004).

they support the suggestion that strigolactones may interfere with the capacity for polar auxin transport from the apical meristem (e.g. Bennett *et al.*, 2006; Mouchel and Leyser, 2007; Ongaro and Leyser, 2008; Leyser, 2009): exogenous application of strigolactones may interfere with polar auxin transport thereby leading to increased chlorophyll content (see also Zhong and Ye, 2001).

Several lines of evidence in the present study suggest that strigolactones are positive regulators of plant light harvesting. The first is the list of genes induced by GR24, which is enriched in genes putatively associated with light harvesting. These include components of PSI and PSII, which are multisubunit membrane–protein complexes that constitute, as the principal converter of sunlight into chemical energy (reviewed by Nelson and Yocum, 2006), precursors of chlorophyll *a*/*b*-binding proteins, which serve as light-harvesting antennae for the capture of light energy and its transfer to the photosynthetic reaction centers (reviewed by Koziol *et al.*, 2007), and ribulose-1,5-bisphosphate carboxylase (Rubisco), the primary CO_2-fixing enzyme (reviewed by Portis and Parry, 2007).

Light regulation at the transcription level of these GR24-induced genes was demonstrated in leaves of plants exposed to different light regimes, and is in agreement with multiple studies showing light induction at the transcription level of light harvesting-associated genes (e.g. Guo *et al.*, 2008). Moreover, transcription of some of these genes was induced upon direct GR24 application to leaves. Hence, it is suggested that plant exposure to strigolactones activates light harvesting-related genes.

The second piece of evidence for the association between strigolactones and light harvesting came from analysis of *Sl-ORT1*, a tomato mutant deficient in strigolactone biosynthesis (Dor *et al.*, 2010; Koltai *et al.*, 2010*b*). Notably, in *Sl-ORT1*, all examined GR24- and light-induced genes were transcriptionally down-regulated in comparison with the WT. Accordingly, in *Sl-ORT1*, a reduced level of chlorophyll was detected in leaves relative to the WT. Together, these results further support the hypothesis that strigolactones are inducers of light harvesting; this induction is associated, at least partially, with effects on chlorophyll levels.

Two other studies have indicated a connection between strigolactones and light reactions. One (Mashiguchi *et al.*, 2009) found that light signalling-related genes are induced in *Arabidopsis* seedlings upon exposure to GR24. The second (Shen *et al.*, 2007) examined light responses of *max2* mutant seedlings (*pps*). *max2* plants are mutated in an F-box protein suggested to be a strigolactone receptor and display an increased level of shoot branching (Stirnberg *et al.*, 2002; Bennett *et al.*, 2006; Umehara *et al.*, 2008). *max2* seedlings were shown to have longer hypocotyls and slightly smaller cotyledons under continuous red, far-red, and blue light compared with those of the WT (Shen *et al.*, 2007). Moreover, several genes, including Rubisco small subunit and chlorophyll *a*/*b*-binding protein precursors, were found to exhibit a slower rate of induction upon red light exposure in *max2* mutants relative to the WT (Shen *et al.*, 2007). However, it was not determined whether MAX2 was involved in light signalling separately or as part of the shoot branching-regulatory pathway.

In the present study, Rubisco and chlorophyll *a*/*b*-binding protein precursor were among the GR24-induced genes whose expression was reduced in *Sl-ORT1* in comparison with the WT. These results, and the reduced chlorophyll level detected in *Sl-ORT1*, suggest that mutants flawed in strigolactone perception or synthesis (i.e. *max2* and *Sl-ORT1*, respectively) are also flawed in their light harvesting, in turn suggesting a direct connection between strigolactones and light harvesting, and co-regulation of strigolactones and light on light-harvesting components.

Other plant hormones are also known to be connected with light-harvesting pathways. Brassinosteroids were demonstrated to increase chlorophyll levels in plants (e.g. Krizek and Mandava, 1983); cytokinin co-regulates, along with light, many plant processes (reviewed by Werner and Schmulling, 2009), whereas specifically regarding light harvesting it delays chlorophyll destruction (Riefler *et al.*, 2006; Sergiev *et al.*, 2007). Cytokinin was also shown to affect chloroplast organization during photosynthetic acclimation to canopy density (Boonman *et al.*, 2009). Hence, possible interaction of strigolactones with other hormones such as cytokinin (Ferguson and Beveridge, 2009) or brassinosteroids may be a mediator of the detected connection between strigolactones and plant light harvesting.

To conclude, there are several lines of evidence suggesting a role for strigolactones as positive regulators of light harvesting. Further studies are needed to determine the junction points of the co-regulation of strigolactones and light on light-harvesting components. Moreover, since carotenoid biosynthesis has been shown to be light dependent (e.g. Cazzonelli *et al.*, 2009), and since strigolactones are thought to be derived from this pathway (Matusova *et al.*, 2005), it might be that the cross-talk between strigolactones and light-associated pathways follows a feedback loop which is required for the plant's coordinated growth and development.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Raw microarray data of signal intensity for each of the probes on the microarray. Affymetrix probe set ID and signal intensity for each of the probes in each treatment [GR24 (27 μM), IAA (10^{-8} M)+GR24 (13.5 μM), and IAA (10^{-8} M)], for each of the biological replicates (a–c) is shown.

Table S2. Lists of genes that are significantly and differentially expressed. Genes were selected based on their expression ratio in roots exposed to GR24 and IAA treatments [IAA (10^{-8} M), IAA (10^{-8} M)+GR24 (13.5 μM), and GR24 (27 μM)] versus non-treated controls. Annotation is based on Gene Ontology (GO) nomenclature and derived from Affymetrix data and BLAST searches. Genes are divided into up- and down-regulated; within each category; lists are divided into numbers of genes differentially regulated, based upon the intersection between treatments of significantly and differentially regulated gene lists (Fig. 2). Probe set ID, gene symbol, gene title, GO biological process term, GO molecular function term, GO cellular component term, and gene accession numbers are presented. Red letters of Probe ID represent probes with no known annotation.

Figure S1. Hierarchical clustering of microarray hybridization gene expression data of roots exposed to GR24 and IAA treatments: IAA (10^{-8} M), IAA (10^{-8} M)+GR24 (13.5 μM), GR24 (27 μM), and non-treated controls. The colour scale represents fold change in gene expression in treatments versus controls.

Figure S2. Illustration of the general metabolism biological pathways in which differentially regulated genes putatively participate. Differentially regulated genes were identified from hybridization data of roots exposed to GR24 and IAA treatments [IAA (10^{-8} M), IAA (10^{-8} M)+GR24 (13.5 μM), and GR24 (27 μM)] versus non-treated controls. Blue or red squares represent individual genes. The colour within the squares represents fold change in gene expression in treatments versus controls; values of fold change are as indicated in the colour scale. The figure was adapted from MapMan software (Thimm *et al.*, 2004).

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