RESEARCH PAPER

Gene profiling of the red light signalling pathways in roots

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Abstract

Red light, acting through the phytochromes, controls numerous aspects of plant development. Many of the signal transduction elements downstream of the phytochromes have been identified in the aerial portions of the plant; however, very few elements in red-light signalling have been identified specifically for roots. Gene profiling studies using microarrays and quantitative Real-Time PCR were performed to characterize gene expression changes in roots of Arabidopsis seedlings exposed to 1 h of red light. Several factors acting downstream of phytochromes in red-light signalling in roots were identified. Some of the genes found to be differentially expressed in this study have already been characterized in the red-light-signalling pathway for whole plants. For example, PHYTOCHROME KINASE 1 (PKS1), LONG HYPOCOTYL 5 (HY5), EARLY FLOWER-ING 4 (ELF4), and GIGANTEA (GI) were all significantly up-regulated in roots of seedlings exposed to 1 h of red light. The up-regulation of SUPPRESSOR OF PHYTOCHROME A RESPONSES 1 (SPA1) and CONSTITUTIVE PHOTOMORPHOGENIC 1-like (COP1-like) genes suggests that the PHYA-mediated pathway was attenuated by red light. In addition, genes involved in lateral root and root hair formation, root plastid development, phenylpropanoid metabolism, and hormone signalling were also regulated by exposure to red light. Interestingly, members of the RPT2/NPH3 (ROOT PHOTOTROPIC 2/NON PHOTOTROPIC HYPOCOTYL 3) family, which have been shown to mediate blue-light-induced phototropism, were also differentially regulated in roots in red light. Therefore, these results suggest that red and blue light pathways interact in roots of seedlings and that many elements involved in red-light-signalling found in the aerial portions of the plant are differentially expressed in roots within 1 h of red light exposure.

Key words: Arabidopsis, gene profiling, microarray, photomorphogenesis, red light, roots.

Introduction

Acquiring information about the surrounding environment is crucial to the survival of all living organisms. For plants, light is one of the most important environmental signals for optimal growth and survival. Seed germination, hypocotyl growth and inhibition, cotyledon expansion, chloroplast development, time-to-flowering, and plant architecture are all light-regulated processes (for a review see Chen et al., 2004). Light can also regulate many aspects of root growth and development such as gravitropism (Lu and Feldman, 1997; Oyama et al., 1997; Kiss, 2000), root hair formation (Oyama et al., 1997; De Simone et al., 2000a, b), orientation and growth of lateral roots (Bhalerao et al., 2002; Kiss et al., 2002), primary root elongation (Lariguet et al., 2003; Correll and Kiss, 2005), negative (Okada and Shimura, 1992) and positive phototropism (Ruppel et al., 2001; Kiss et al., 2003), root greening (Oyama et al., 1997; Usami et al., 2004), and secondary metabolite production (Hemm et al., 2004).

To detect and respond to the varying fluence, wavelength, and direction of light, plants have evolved several types of photoreceptors. These molecules include the blue/ UVA photoreceptors, i.e. the cryptochromes and phototropins, and the red/far-red photoreceptors, i.e. the phytochromes (Sharrock and Quail, 1989; Briggs et al., 2001; Lin and Shalitin, 2003). The phytochromes, designated PHYA to PHYE in Arabidopsis, play important roles in regulating many of the light-induced responses. For red-light-induced responses, PHYB is the primary photoreceptor in transducing the signal with other phytochromes playing more minor roles. In roots, both PHYA and PHYB are involved in regulating light-induced gravitropism,
positive red-light-induced phototropism, chloroplast development, secondary metabolite production, and root hair development (Feldman and Briggs, 1987; Johnson et al., 1994; De Simone et al., 2000b; Kiss et al., 2003; Hemm et al., 2004). The roles of the other phytochromes, PHYC to PHYE, in light-regulated processes in roots are largely unknown. In addition, very few of the downstream molecules of the photoreceptors in the light-signalling cascade have been characterized for roots. Most of what is known about light-signal transduction comes from studies using hypocotyls, stems, or leaves, even though very different gene expression profiles have been found between upper portions (hypocotyls and leaves) and roots exposed to different light treatments (Jiao et al., 2005; Ma et al., 2005). Therefore, roots provide a unique system to characterize light signalling in the whole plant.

Many signal transduction elements have recently been identified in red-light-induced responses. For example, once the light signal is detected by phytochromes, these photoreceptors can interact in the cytosol with proteins like PKS1 (PHYTOCHROME KINASE SUBSTRATE 1), a possible negative regulator of phytochrome-based responses (Fankhauser et al., 1999), or they can be translocated into the nucleus where they interact with other proteins (Ni et al., 1998). In addition to PKS1, PHYB can bind with ARR4 (ARABIDOPSIS RESPONSE REGULATOR 4), PIF3 (PHYTOCHROME INTERACTING FACTOR3), PIF4, and COP1 (CONSTITUTIVE PHOTOMORPHOGENESIS 1) to transduce the red-light signal (Sweere et al., 2001; Yang et al., 2001; Huq and Quail, 2002). ARR4 binds preferentially to the active form of PHYB (Pfr) and appears to stabilize this form by acting as a positive regulator of red-light-induced responses (Sweere et al., 2001). Although originally thought to be a positive regulator (Holland et al., 1999; Martinez-Garcia et al., 2000), PIF3 now appears to be a negative regulator in PHYB signalling, similar to PIF4 (Huq and Quail, 2002; Bauer et al., 2004; Kim et al., 2004; Park et al., 2004). COP1 is involved in the ubiquitin-dependent degradation of PHYA in the light. In addition, COP1 interacts with PHYB in a yeast two-hybrid assay (Yang et al., 2001). A positive correlation between COP1 abundance and PHYB-mediated responses has also been reported (Boccalandro et al., 2004), although the precise roles of COP1 in PHYB-mediated signalling and protein turnover are unclear. CONSTANT LIKE 3 (COL3) is a COP1-interacting protein acting downstream of phytochromes and COP1 in the red-light-signalling pathway (Datta et al., 2006). It seems that COL3 is a positive regulator of photomorphogenesis and can promote lateral root development in red light (Datta et al., 2006).

HY5, a bZIP (BASIC REGION-LEUCINE ZIPPER) transcription factor, acts downstream of phytochromes in light-signalling pathways and is a positive regulator of photomorphogenic responses. HY5 is targeted for degradation to the COP9 signalosome through COP1 in both the dark and light (Bauer et al., 2004). In roots, HY5 is involved in the inhibition of both root hair and primary root elongation, inhibition of lateral root development, and the promotion of gravitropic orientation, secondary wall thickening, the phenylpropanoid pathway, and chloroplast development (Oyama et al., 1997; Hemm et al., 2004). Two other elements in root development and light-regulated responses include the ATP-binding cassette proteins, MDR1 (MULTIDRUG RESISTANCE 1) and PGP1 (PLEIOTROPIC DRUG RESISTANCE 1). These proteins are involved in both photomorphogenesis and auxin-based responses in roots such as elongation and gravitropism (Lin and Wang, 2005). Recently, a gene involved in phytochrome-regulated gravitropism in hypocotyls was identified, GIL1 (GRAVITROPIC IN THE LIGHT 1); however, the role that this plays in root responses is unknown (Allen et al., 2006). Interestingly, it appears that MDR1 acts upstream of PHYA in attenuating these responses. Other light-signalling proteins have also been identified in roots such as PKS1, LAF6, (LONG AFTER FAR-RED 6; Lariguet et al., 2003; Moller et al., 2001), although the roles of these proteins in the light-regulated processes of roots are largely unknown.

Gene expression profiling using microarrays has shown that light regulates more than 30% of the genome in seedlings of Arabidopsis, including more than 26 cellular pathways (Ma et al., 2001). In whole seedlings and leaves, microarray analyses have identified many of the genes involved in red- and far-red-light signalling (Tepperman et al., 2001, 2004; Wang et al., 2002; Jiao et al., 2005; Ma et al., 2005). For whole seedlings, as the duration of light treatment increased, a greater number of genes was differentially expressed. For example, only 1.7% of the genome was differentially expressed in seedlings after 1 h of red light treatment, but 11% of the genome was differentially expressed after 24 h of red light treatment (Tepperman et al., 2004). Therefore, many more downstream elements are regulated after a longer duration of light treatment.

Although there have been large numbers of microarray studies comparing light-regulated gene expression patterns in whole seedlings, leaves, and hypocotyls, there are only a few studies that have analysed the gene expression changes in roots with different light treatments (Ma et al., 2001, 2005; Tepperman et al., 2001, 2004; Wang et al., 2002). One study compared the gene expression profiles of roots (18-d-old seedlings) that were exposed to 4 h of far-red light from dark-adapted (4 d dark-adapted) plants to roots from dark-adapted plants (Sato-Nara et al., 2004). Surprisingly, no genes (out of 7000) were significantly regulated after the 4 h of far-red light treatment (Sato-Nara et al., 2004). However, these authors used microarrays with only 7000 elements represented and had a conservative 3-fold threshold to define significant regulation (Sato-Nara et al., 2004). Therefore, using arrays with more elements or
lower thresholds for significance may identify more significantly regulated genes in response to far-red light.

Another recent study compared the gene expression between roots from dark- and white-light-grown seedlings of both *Arabidopsis* and rice (Jiao et al., 2005; Ma et al., 2005). These authors found that approximately 40% of the genome was expressed in *Arabidopsis* roots, and that approximately 3.5% of the total genome was differentially expressed in roots from dark- versus light-grown seedlings of *Arabidopsis* (≥2-fold change; Jiao et al., 2005; Ma et al., 2005). Of these genes, only a small portion overlapped with light-regulated genes from hypocotyls and cotyledons, suggesting that roots have very different light-regulated processes (Ma et al., 2005).

Despite these recent studies, limited information about the early light-signalling events in roots is available. In this paper, the gene expression profiles of roots from dark-grown seedlings exposed to 1 h of red light were compared with controls. Through microarray studies with Affymetrix Genechips®, the differential expression of genes involved in the phenylpropanoid pathway, root plastid development, auxin and ethylene signalling, lateral root development, and transcription have been identified. In addition, the up-regulation of some of the elements in blue-light-signalling pathways was identified with the red-light treatment used here, suggesting an interaction of pathways induced by red and blue light.

**Materials and methods**

**Plant material and growth condition**

Seeds of *Arabidopsis thaliana* ecotype Landsberg erecta (Ler) were sterilized in 70% (v/v) ethanol for 5 min, two rinses of 90% (v/v) ethanol and four rinses in sterile ddH₂O. Seeds were sown onto a presterilized cellophane that was placed on top of an agar growth medium (Kiss and Swatzell, 1996) consisting of ½ MS medium with 1% (w/v) sucrose, 1.2% (w/v) agar in square (100 mm × 15 mm) Petri dishes. Seeds were stratified for 2 d at 4 °C in the dark and then exposed to white light for 2 h at room temperature to synchronize germination. Seedlings were grown for 7 d in the dark at 21 °C before being transferred to either red light (1 h) or continued darkness. Seedlings were collected in RNA later® (Ambion), and roots were excised and stored at −80 °C. Samples were pooled from several plates for each biological replicate. Three biological replicates were used for microarray analysis.

Red light was obtained by passing light from fluorescent bulbs through Plexiglas filters (Rhône and Hass No. 2423, Dayton Plastics, Columbus, OH). Fluence rate through the red filter was 12–14 μmol m⁻² s⁻¹ with a transmission maximum of 630 nm. The fluence rate was measured with a Li-Cor LI-189 Quantum Radiometer Photometer equipped with a LI-190SA Quantum sensor.

**Microarray procedures**

**Preparation of labelled copy RNA:** Total RNA was extracted from each sample and prepared for hybridization according to the Affymetrix GeneChip® Expression Analysis Technical Manual. Briefly, RNA was extracted from frozen tissue using the RNeasy® Mini Kit (Qiagen Inc, Valencia, Ca), and residual DNA was removed by performing an on-column digestion using a DNA-free kit (Ambion).

**Target labelling and array hybridization:** Total RNA samples were submitted to the University of Florida’s Interdisciplinary Center for Biotechnology Research (ICBR) Gene Expression Core Facility (Gainesville, FL). The quality of each of the RNA samples was determined by evaluating the relative amounts of 28S and 18S ribosomal peaks using a Bioanalyzer (Agilent Technologies, Palo Alto, CA). Five μg of total RNA was used as a template for complementary RNA (cRNA) synthesis with the GeneChip® One-Cycle Target Labeling Kit (Affymetrix, Santa Clara, CA). First strand synthesis was primed with a T7-dT₃₄ oligonucleotide primer containing a T7 RNA polymerase promoter sequence on the 5’ end. Second strand products were cleaned and used as a template for in vitro transcription (IVT) with biotin-labelled nucleotides. IVT reactions were cleaned, and 20 μg of the product was heated at 94 °C for 35 min in fragmentation buffer provided with the labelling kit in order to produce fragments that are 35–200 base pairs in length. A 15 μg aliquot of fragmented cRNA was hybridized for 16 h at 45 °C to an Affymetrix GeneChip® ATH1 genome array. After hybridization, each microarray was stained with a streptavidin–phycocyanin conjugate, washed, and visualized with a GeneChip® Scanner 3000 (Affymetrix, Santa Clara, CA). Images were inspected visually for hybridization artefacts. Quality assessment metrics were generated for each scanned image. Samples that did not pass quality assessment were eliminated from further analyses. All expression data was submitted to GEO (Gene Expression Omnibus database) under the series accession number GSE4933.

**Generation of expression values and data analysis:** Microarray Suite Version 5 software (Affymetrix, Santa Clara, CA), was used to convert intensity data into quantitative estimates of gene expression. All expression values were globally scaled to 500. A probability statistic associated with each gene’s presence or absence was also generated. Genes not expressed in any of the samples were considered absent. Absent genes were removed from the data set and not included in further analyses. The natural log-transformed expression values were subjected to an analysis of variance (ANOVA) for a complete block design (Steel and Torrie, 1960) where the different treatment times served as blocks. P-values were calculated using Tukey HSD test. Statistical analyses were performed with Analytical Tools, software developed by ICBR.

**Real time RT-PCR**

Total RNA was extracted from roots as described above, and cDNA was synthesized according to SuperScript II RNaseH⁻ kit protocol (Invitrogen) on 500 ng of RNA and oligo dT oligonucleotide as a primer. PCR primers were designed using OligoAnalyzer 3.00 (http://biotools.idtdna/biotools) to create amplicons 100–180 bp in length. Primers used had the following characteristics: melting temperature between 55–60 °C, 40–60% G/C content, 3° end content overall and matching only desired tentative consensus sequence in The Arabidopsis Information Resource (TAIR). Actin8 was used as housekeeping control and the primers were: F_ACTIN8 CTTTCC-GGTGTAACGCATTACGGCT; R_PTR2 TGCCAAGTCTGTCTACGGTG, R_ACAACCZGCAGAACATACCA; F_SPA1 TTGCAGAGGAGATATAATG, R_AGATGTA-GAAACCGAGAC; F_PKS1 TGCGCCAAGTGAAGTAAGCGT, R_AGGATGTATGCTGC; F_GAAACGCGGATTAGTGCCT; F_RPT2 TGCCAAGTCTGTCTACGGTG, R_ACAACCZGCAGAACATACCA; F_SPA1 TTGCAGAGGAGATATAATG, R_AGATGTA-GAAACCGAGAC; F_PKS1 TGCGCCAAGTGAAGTAAGCGT, R_AGGATGTATGCTGC; F_GAAACGCGGATTAGTGCCT; F_COPI FAMILY AT5p52250 TACGACGTGAGGAAACAAATGGC, R_AACGCGTATGGGAAGATCCACCG; F_GI AACCATTTTCTGTGGGGGACT; R_AAGA-CCCTGCGGTCTACTA.

Real-Time PCR was performed using Rotor Gene RG 3000 (Corbett Research) and the experimental conditions were: activation...
of 95 °C for 15 min, denaturing 40 cycles at 95 °C for 10 s, and annealing and extension 56–60 °C for 1 min. The following reagents were combined with each sample and control: 20 μl of SYBR Green PCR master mix (Qiagen), 2.5 μl of primers (0.2 μM) and 3 μl of template. Data were analysed using Rotor Gene 6.0 software and gene expression data were calculated using Standard Curve methods (Livak, 1997).

Results

Overall effects of red light on gene expression in roots

A total of 661 genes were significantly differentially expressed in roots of 7-d-old seedlings exposed to 1 h of red light compared with dark-grown controls (P <0.05). Of these, 351 genes were regulated at least 2-fold (log₂ ratio of red/dark signal >1) with 128 that were induced, and 223 that were repressed (Fig. 1; see supplementary Table 1 at JXB online). This corresponds to ~1.4% of the genome as being differentially expressed within 1 h or red light treatment in roots of Arabidopsis seedlings. A preliminary overview using the Functional Catalog from the Munich Information Center for Protein Sequences (MIPS) classified the majority of genes regulated in response to red light in the metabolism category (11 induced and 21 repressed, Fig. 2) with only a smaller portion in the transcription category (8 induced and 5 repressed). However, by grouping genes in their putative functional categories, the expression changes in 21 induced (≥2-fold) transcription factors and 17 repressed transcription factors (see supplementary Table 2 at JXB online) were found. The largest group of transcription factors, with nine members, was from the zinc finger family where six were up-regulated and 3 three down-regulated (see supplementary Table 2 at JXB online).

Other elements that were significantly differentially expressed (≥2-fold) were classified in the following categories: photomorphogenesis (Table 1), transcriptional regulation, protein turnover, phenylpropanoid metabolism, root growth, chloroplast and light harvesting, cell wall development, hormone signalling, transporters, and transcription (see supplementary Table 2 at JXB online). In addition to these genes, 87 of the 351 differentially expressed genes were classified as hypothetical or expressed (see supplementary Table 1 at JXB online).

PHYA-mediated signal transduction pathway is repressed and PHYB-mediated pathway is induced

The red-light signalling pathway in plants is primarily mediated by PHYB with other phytochromes playing lesser roles in regulating the response. In this study, we found that some genes involved in either PHYA-mediated (i.e.

![Fig. 1](https://academic.oup.com/jxb/article-abstract/57/12/3217/450176/1231745076)

Fig. 1. Number of genes defined as responding to 1 h of red light in roots of Arabidopsis seedlings. Venn diagrams show the percentage (%) and number of genes (grey) regulated by red light (left) and those genes that are up- and down-regulated ≥2-fold (right).

Fig. 2. Overview of induced and repressed biological processes in roots of dark-grown seedlings exposed to 1 h of red light. Classification of genes (at least 2-fold differential regulation) was based on the Functional Catalogue of the Munich Information Center for Protein Sequences (MIPS). Genes of unknown function or classification are not shown. Numbers of genes found in each category are identified on the x-axis.
Table 1. Differentially regulated expression (≥2-fold) of genes involved in photomorphogenesis in roots of 7-d-old dark-grown seedlings exposed to 1 h (~12 μmol m⁻² s⁻¹) of red light

<table>
<thead>
<tr>
<th>AGI no.</th>
<th>Description</th>
<th>Gene name</th>
<th>Log₂ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>At5g11260</td>
<td>bZIP transcription factor</td>
<td>HY5</td>
<td>3.8</td>
</tr>
<tr>
<td>At5g48800</td>
<td>BTB/POZ domain and coiled-coil domain</td>
<td>NPH3-family</td>
<td>1.0</td>
</tr>
<tr>
<td>At2g46340</td>
<td>Coiled-coil domain</td>
<td>SPA1</td>
<td>2.7</td>
</tr>
<tr>
<td>At2g02950</td>
<td>Soluble protein involved in red, far-red phototransduction</td>
<td>PKS1</td>
<td>1.9</td>
</tr>
<tr>
<td>At2g30520</td>
<td>BTB/POZ domain and coiled-coil domain</td>
<td>RPT2</td>
<td>1.7</td>
</tr>
<tr>
<td>At5g52250</td>
<td>WD-40 repeat family protein</td>
<td>PnCOP1 similar</td>
<td>1.2</td>
</tr>
<tr>
<td>At2g40080</td>
<td>Circadian rhythm regulator</td>
<td>ELF4</td>
<td>2.3</td>
</tr>
<tr>
<td>At1g03010</td>
<td>BTB/POZ domain and coiled-coil domain</td>
<td>NPH3 family</td>
<td>-1.0</td>
</tr>
<tr>
<td>At1g64240</td>
<td>Similar to light repressor protein kinase</td>
<td>LRRKK</td>
<td>-2.0</td>
</tr>
<tr>
<td>At1g 22770</td>
<td>Gigantea protein</td>
<td>GI</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* Arabidopsis Genome Initiative number.
** log₂ ratio (red-light/dark-grown roots).
*** log₂ ratio <1.0 but 1.0 for quantitative PCR (see Fig. 3).

SPA1, SUPPRESSOR OF PHYTOCHROME A 1) or PHYB-mediated-signalling (i.e. ELF4, EARLY FLOWERING 4 and GI, GIGANTEA) pathways were significantly regulated as well as some genes involved in both pathways (e.g. HY5, PKS1; Table 1). SPA1 is a nuclear-localized repressor of PHYA-mediated signalling (Hoecker et al., 1999) and was significantly up-regulated in roots exposed to 1 h of red light. In addition, a member of the COP1 family (At5g52250; COP1-like) was also significantly induced. COP1 has been shown to be involved in suppressing PHYA-mediated responses (Saigo et al., 2003; Seo et al., 2004).

Elements in the PHYB-mediated pathway were also induced in response to red light. ELF4 and GI are specifically implicated in PHYB-regulated responses, and the genes encoding these proteins were both induced (GI 2-fold in qRT-PCR treatment only). In addition, two light-inducible genes related to both PHYA- and PHYB-signal transduction, PKS1 and HY5, were up-regulated.

**Overlap in blue and red-light-signal transduction pathways**

Blue light induces a wide range of physiological responses including phototropism, stomatal opening, and chloroplast movement. Recent molecular genetic studies have shown that PHOT1 (phototropin 1) and PHOT2 function as photoreceptors for phototropism (Briggs et al., 2001), and RPT2 and NPH3 transduce signals downstream of photoreceptors to induce the phototropic response (Inada et al., 2004).

Interestingly, three elements involved in the phototropin or UVA/blue-light-signalling pathway were found significantly differentially expressed in roots following red-light treatment. These included RPT2/NPH3-family genes, two of which were up-regulated (NPH3-like At5g48800 and RPT2) and the other down-regulated (another NPH3-like At1g03010). These genes belong to the novel NPH3/RPT2 family, which is intimately involved in blue-light-signalling pathways, including phototropism (Motchouliski and Liscum, 1999; Liscum and Stowe-Evans, 2000).

**Chloroplast genes are differentially regulated**

Chloroplast development in roots exposed to light has been well-documented for a variety of species. In Arabidopsis, red light can promote, although less effectively than blue or white light, chloroplast development and greening in root tissues (Usami et al., 2004). Eight genes were found up-regulated (see supplementary Table 2 at JXB online) associated with the chloroplast or in light harvesting elements including HY5, CBL-INTERACTING PROTEIN KINASE 13 (CIKP13); PHOTOSYSTEM II OXYGEN-EVOLVING COMPLEX 2 (PSBO2); PROTEIN SA (PSAB); PsaC subunit of photosystem I (PASC), and six genes down-regulated including a DIFFERENTIATION AND GREENING (DAG)-similar (At1g72530), a Psb subunit of photosystem II (PSBP family, At4g15510) and an oxidoreductase (At4g10500). Some of these differentially expressed elements, although not directly involved in light harvesting or chloroplast development, are known to be localized in the chloroplast (see supplementary Table 2 at JXB online).

CIKP13 is a protein located in the chloroplast and involved in protein amino acid phosphorylation and signal transduction (Harmon et al., 2000; Sanders et al., 2002). PSBO2, a protein that localizes in the thylakoid, is an extrinsic subunit of photosystem II and has been proposed to play a central role in stabilization of the catalytic manganese cluster. PSAB, the gene of which is part of the chloroplast genome, encodes the D1 subunit of photosystem I and II reaction centres and it is involved in light harvesting and photosynthesis (Klein et al., 1988). PSBP encodes a 23 kDa extrinsic protein that is part of photosystem II and participates in the regulation of oxygen evolution. DAG is a plastid developmental protein required for...
chloroplast differentiation (Chatterjee et al., 1996). Overall, differential regulation of the aforementioned genes add to the growing body of evidence reporting root greening and indicate that this process starts within 1 h of red light.

**Phenylpropanoid metabolism is induced**

In plants, large amounts of carbon from aromatic amino acid metabolism are diverted into the biosynthesis of natural products based on a phenylpropane skeleton. These diverse phenylpropanoid compounds, which include flavonoids, lignin, coumarins, and many small phenolic molecules, have a multiplicity of functions in structural support, pigmentation, defence, and signalling. The phenylpropanoid metabolism was induced by red light in this study (see supplementary Table 2 at JXB online) with the up-regulation of key genes in phenylpropanoid metabolism i.e. *PAL3* (*PHENYLALANINE AMMONIA LYASE 3; At5g04230*), two *CAD* class III genes (*CINNAMYL ALCOHOL DEHYDROGENASE; At2g2170* and *At2g21890*), and *COMT*-like II genes (*CAFFEIC ACID O-METHYLTRANSFERASEs, At3g53140/At5g37170*).

**ABC transporters are differentially regulated**

Six ABC (ATP-Binding Cassette) transporters differentially regulated by red light were found (see supplementary Table 2 at JXB online). Two genes that encode members of the non-intrinsic ABC proteins (NAPs) family, *NAP2* (*At5g44110*) and *NAP9* (*At5g02270*) and a gene encoding a white-brown complex homologue (WBCs) family member *WBC4* (*At4g25750*) were up-regulated. In addition, one member of the multidrug resistance associated protein (MRP) family, *MDR7* (*At5g46540*) and two pleiotropic drug resistance (PDR) subfamily (*PDR12* and *PDR5*; At1g15520 and At2g37280, respectively) genes, were down-regulated (see supplementary Table 2 at JXB online). *NAP2* and *NAP9* were highly induced, with NAP2 being among the highest induced genes in this study (NAP2/POP1).

**Overlap in gene expression from red- and white-light-induced pathways**

A comparison of these results from dark-grown roots exposed to 1 h of red light (7-d-old seedlings) with studies that compared roots from dark- and light-grown seedlings (6-d-old seedlings) indicated that only 26 out of the 351 differentially expressed genes (≥2-fold) from the 1 h red-light studies overlapped with 883 of differentially expressed genes from roots grown in continuous white light (Table 2; Ma et al., 2005). The differences in gene regulation between these two studies may be associated with

**Table 2. Comparison of gene expression profiles from roots grown in continuous white light with roots of seedlings exposed to 1 h of red light relative to dark-grown roots**

<table>
<thead>
<tr>
<th>AGI no.</th>
<th>Description</th>
<th>Gene name</th>
<th>Continuous white light*</th>
<th>1 h red light</th>
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<tbody>
<tr>
<td><strong>Up-regulated in both</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At5g11260</td>
<td>bZip transcription factor</td>
<td>HYS</td>
<td>4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8</td>
</tr>
<tr>
<td>At5g2250</td>
<td>Transducin family</td>
<td>PtCOP1-like</td>
<td>2.9</td>
<td>1.2</td>
</tr>
<tr>
<td>At2g40080</td>
<td>Expressed (circadian rhythm associated)</td>
<td>ELF4</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>At5g48880</td>
<td>Acetyl-CoA-acyl transferase</td>
<td>FKT1</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>At3g21560</td>
<td>UDP-glucosyltransferase</td>
<td>2.4</td>
<td>1.2</td>
<td></td>
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<tr>
<td>At5g44110</td>
<td>ABC transporter</td>
<td>POP1; NAP2</td>
<td>3.8</td>
<td>4.4</td>
</tr>
<tr>
<td>At5g02270</td>
<td>ABC transporter NBD-like</td>
<td>NAP9 (POP like)</td>
<td>2.6</td>
<td>2.3</td>
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<tr>
<td>At3g22830</td>
<td>Heat Shock Protein Transcription Factor glycine rich</td>
<td>Similar to GRP2</td>
<td>1.1</td>
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<tr>
<td>At2g46340</td>
<td>Suppressor of phytochrome responses</td>
<td>SPA1</td>
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<td>RAP2/6</td>
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<td>1</td>
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<td>At5g54470</td>
<td>Zn Finger (B-box)</td>
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<td>3.1</td>
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<tr>
<td>At3g45160</td>
<td>Expressed</td>
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<td>3.2</td>
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<td></td>
<td></td>
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<tr>
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<tr>
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<td>Similar to resistance complex protein Expressed</td>
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<td>–5.7</td>
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</table>

* Gene expression results are from seedlings grown in continuous white-light (150 μmol m<sup>−2</sup> s<sup>−1</sup>) for 6 d compared to roots from seedlings grown in the dark. Results are from 70 mer >26 k oligo custom arrays (Ma et al., 2005).

<sup>b</sup> HY5 was marked as absent (Ma et al., 2005).
the different ecotypes (Columbia versus Landsberg), different plant ages (6- versus 7-d-old roots), different light fluence rates (150 versus 14 μmol m⁻² s⁻¹) and quality (white versus red light), the duration of illumination (continuous versus 1 h), and the factors associated with microarray platforms (custom slide arrays versus Affymetrix Genechips®). In addition, due to the sensitivity of light-induced genes even to dim-green light, roots from the dark-grown seedlings were collected in absolute darkness using RNAlater (Ambion). The method used to collect 6-d-old dark root samples from the previous study was not described (Ma et al., 2005). Due to all of the differences between red-light and continuous-white-light studies with roots, it is not surprising that there were only a few genes overlapping in the expression profiles (Table 2).

Of the 26 overlapping genes from roots in the red-light and continuous white light treatments, 12 were up-regulated in both treatments, 10 were down-regulated in both treatments, and four were differentially regulated between the two treatments relative to dark controls (Table 2). HYS, PnCOP1-like, SPA1, RAP2.6 (RAN-BINDING 2.6) and two ABC transporter proteins (POP; POP1), all light-regulated genes in whole seedlings (Tepperman et al., 2004), were induced under both conditions in roots (Table 2). Because these genes were up-regulated after 6 d of white-light treatment, it appears that these genes may remain induced in roots even after a long duration of light treatment. Repressed genes from the two light conditions included a gibberellin-regulated protein, GI-STIMULATED TRANSCRIPT 2 (GASA2; At4g09610), a gene encoding a heavy metal associated protein (At3g07600), and a gene encoding a disease resistant protein (At5g45070).

**Confirmation of the results with Real-Time PCR**

Quantitative Real-Time PCR (qRT-PCR) was used to confirm the level of expression of six genes associated with photomorphogenesis (HYS, SPA1, COP1-like, At5g52250, PKS1, RPT2, and GI) whose expression was shown to be differentially regulated in red light using microarrays (Fig. 3). For GI, the gene expression was found only to be 1.7-fold increased (although significant \( P < 0.05; \) Table 1) with the microarrays. Because this gene is known to be involved in PHYB-mediated responses, it was decided to check expression levels using qRT-PCR and found the expression difference to be greater than 2-fold (Fig. 3). Using the Standard Curve Method (Livak, 1997), it was found that all six genes were induced within 1 h of red-light treatment (≥2-fold). Thus, in general, the qRT-PCR results confirm the microarray results. Interestingly, the RT-PCR method showed a greater level of gene expression than was found with the microarray studies for almost all the genes analysed, and this is probably due to the enhanced sensitivity of RT-PCR techniques. For example, HYS showed a 14-fold increase using microarrays whereas qRT-PCR detected 21-fold induction in red light compared with the dark-grown controls. Similar trends, although to a lesser extent, were found for RPT2, PKS1, and SPA1 whereas COP1-like showed little differences in expression using both methods. The qRT-PCR, along with microarray results, indicates that the red-light signalling cascade is being activated in roots of seedlings within 1 h of red-light treatment.

**Discussion**

In this study, we show that 1.4% of the genome is significantly differentially regulated (≥2-fold; \( P < 0.05 \)) in roots of Arabidopsis seedlings within 1 h of red-light treatment compared with control seedlings grown in continuous dark. Many of the genes identified to be differentially regulated are involved in photomorphogenesis, lateral and root hair development, phototropism, root greening, and phenylpropanoid metabolism (Table 1; see supplementary Table 2 at JXB online). These results provide an insight into the genetic mechanisms plants use to co-ordinate the many responses of roots to red light.

**Red light suppresses PHYA-mediated signalling while inducing both PHYB-mediated and blue/UVA pathways**

**Suppression of PHYA-mediated signal transduction:** In this study, up-regulation of PKS1 was found in roots within 1 h of red-light exposure. This may act as a possible mechanism to inhibit PHYA responses in red light. PKS1 acts as a negative regulator of phytochromes, particularly...
PHYA, by preventing nuclear import of the phytochrome upon light activation (Fankhauser et al., 1999). Although not found with gene expression data (Ma et al., 2005), PKS1 expression remained high in roots after several days in the light and localized to the region of root elongation (Lariguet et al., 2003).

In addition to PKS1, SPA1 and COP1-like (At5g52250) genes were found to be significantly up-regulated in red light. In the light, SPA1 interacts with COP1, and this complex can target HY5 for degradation, thereby attenuating PHYA-mediated signalling (Saijo et al., 2003). COP1, a member of the E3 ubiquitin ligase family, is involved in targeting a variety of proteins for degradation including PHYA, HY5, and LAF1 (LONG AFTER FAR RED 1; for a review see Chen et al., 2004). The specific role of the COP1-like gene that was up-regulated in these studies is unknown. However, it is known that the COP/DET/FUS group of proteins regulates HY5 activity at the level of protein stability (Oyama et al., 1997; Osterlund et al., 2000). Overall, up-regulation of PKS1, SPA1, and COP1-like genes suggests that the PHYA-mediated signalling pathway is repressed within 1 h of red-light exposure in roots of seedlings (Fig. 4).

SPA1 and PKS1 were also up-regulated in 1 h of red-light treatment in whole seedlings, and the expression levels of both genes decreased as the duration of light exposure increased (Tepperman et al., 2004). Therefore, it appears that PKS1 and SPA1 are similarly regulated in both roots and whole seedlings after 1 h of red-light exposure. Interestingly, these genes had only slightly reduced expression levels in whole seedlings from phyB mutants compared with WT seedlings, suggesting that other phytochromes are involved in regulating these genes in response to red light (Tepperman et al., 2004). Studies comparing expression levels of these genes in other phytochrome mutants may help to identify which specific phytochromes are involved in regulating the expression of these genes.

**Induction of PHYB-mediated signal transduction:** Several elements in the PHYB-mediated signalling pathway were up-regulated within 1 h of red-light exposure in roots, including HY5, ELF4, PKS1, and GI. Although the GI expression level was less than 2-fold for our microarray analysis (1.74-fold; $P=0.0037$), it was greater than 2-fold for our RT-PCR analysis (Fig. 3). Both ELF4 and GI act downstream of PHYB and are involved in circadian clock regulation and photoperiodism (Huq et al., 2000; Khanna et al., 2003). Up-regulation of these circadian clock-associated genes may be a result of a response or processes that occur distal to the roots. For example, genes involved in flowering can be expressed throughout the whole plant, including the roots (Wilson et al., 2005).

In addition to its role in circadian rhythms, GI is involved in oxidative stress tolerance, cold stress responses, and carbohydrate metabolism (Eimert et al., 1995; Kurepa et al., 1998; Fowler et al., 1999; Cao et al., 2005), and ELF4 is involved in seedling de-etiolation (Khanna et al., 2003). Consequently, the possibility cannot be ruled out that these genes may regulate other non-circadian functions in roots.

Interestingly, ELF4 was up-regulated at the same level in both 1 h of red-light treatment and in continuous white-light treatment in roots (Table 2). For whole seedlings, ELF4 and GI were early-induced genes in red light with expression levels returning approximately to the starting levels after 24 h of red-light exposure (Tepperman et al., 2004). These time-course studies indicated that ELF4 expression in WT seedlings had a brief minor peak 1 h after the dark-grown seedlings were exposed to continuous red light and a broader, second peak at approximately 9 h, returning to basal levels at 24 h of red-light treatment (Kikis et al., 2005). Therefore, it appears that roots regulate ELF4 expression in a manner similar to the regulation found in whole seedlings for the 1 h time point. However, for longer-term light exposure, the expression of ELF4 appears to be different between roots and whole seedlings. Not surprisingly, the expression levels of ELF4 and GI were reduced in phyB mutants compared with the wild type (Tepperman et al., 2004), suggesting that PHYB plays a significant role in the expression of these genes.

As described previously, it was found that a COP1-like gene (At5g52250) was significantly induced in roots of seedlings exposed to red light. In addition to the role of COP1 in attenuating PHYA-mediated signalling, COP1 may also be involved in regulating PHYB-mediated signalling (Boccalandro et al., 2004). These authors suggest that...
COP1 can act as a positive regulator of PHYB-controlled responses. Thus, they hypothesize that COP1 is involved in the degradation of negative regulators of photomorphogenesis or in the transcriptional activation of PHYB (Boccalandro et al., 2004) causing, in either case, a positive effect in PHYB-mediated downstream signalling.

Taken together, 1 h of red-light exposure in the roots of seedlings increase the transcription level of a subset of genes (i.e. GI, ELF4, COP1-like, PKS1, and HY5) that play a role in PHYB-mediated responses.

Blue/UV-light-induced pathway and phototropic response in roots: In this study, the induction of an NPH3-like (At5g48800) gene and the RPT2 gene, along with the repression of another NPH3-like gene (At1g03010), were found. These results strongly suggest that the red and blue light signalling pathways overlap. Phototropin 1 (PHOT1) and PHOT2, which are blue light receptor kinases, function in blue-light-induced phototropism with RPT2 (root phototropism 2) and NPH3 (nonphototropic hypocotyl 3) transducing the signal downstream of the blue light. Mutations in NPH3 and RPT2 genes of Arabidopsis apparently disrupt the function of proteins acting early in PHOT1- and PHOT2-phototropic signalling pathways. Loss-of-function rpt2 mutants retain nearly normal phototropism in hypocotyls, whereas the same response is impaired in roots, indicating that genetic regulation pathways mediating phototropism are different in roots and shoots (Okada and Shimura, 1992).

NPH3 is highly expressed in dark-grown seedlings and remains unaffected by light (Liscum, 2002; Sakai et al., 2000), while RPT2 mRNA is barely detectable in etiolated seedlings, but increases dramatically with increasing light exposure. Moreover, RPT2 mRNA levels are induced by blue, green, and red light (Sakai et al., 2000; Tepperman et al., 2004), which indicates that this regulator of phototropism is a light-inducible gene and may be a common element in light signalling between the red and blue light pathways.

Whole seedlings also had elements involved in the phototropin-mediated and blue/UVA-signalling pathway regulated within 1 h of red light. For example, RPT2, PHR2 (PHOTOLYASE/BLUE LIGHT PHOTORECEPTOR 2), and PHOT2 were induced in red light while PHOT1 expression was repressed (Tepperman et al., 2004). These genes, as well as the RPT2/NPH3 family, are potential targets for identifying the interacting affects of red-light enhancement of blue-light-induced phototropism or the red-light-induced phototropism found in roots.

Synergistic interactions between phytochromes and blue-light photoreceptors do occur during the phototropic response (Iino, 1990; Liscum and Stowe-Evans, 2000; Kumar and Kiss, 2006). For example, PHYA and PHYB are the predominant phytochromes regulating phototropic enhancement of hypocotyls pretreated with red light (Parks et al., 1996; Janoudi et al., 1997). In addition, phytochromes A, B, and D modulate blue-light phototropism in the absence of a red-light pretreatment (Whippo and Hangarter, 2004). Recently, PKS proteins were identified as elements linking phytochrome and phototropic curvature in hypocotyls (Lariguet et al., 2006). However, results from studies with Arabidopsis and maize suggest that phytochromes may alter the activity and/or abundance of elements downstream to enhance phototropic curvature in hypocotyls (Liu and Iino, 1996; Janoudi et al., 1997; Parks et al., 1997). On the other hand, the enhancement of blue-light phototropism by red light has not been established in roots. Nevertheless, phytochromes might modify the activity of intermediaries downstream of blue-light-induced phototropism. This hypothetical mode of action for photoreceptors is summarized in Fig. 5A.

Roots also have a red-light-induced positive phototropic response (Ruppel et al., 2001; Kiss et al., 2003), and this may be another explanation for the regulation of RPT2/NPH3 genes in Arabidopsis roots after 1 h of red light. Although it is known that PHYA and PHYB are the photoreceptors sensing the light signal (Kiss et al., 2003), elements operating downstream from the phytochromes have not been characterized. A potential model is illustrated in Fig. 5B.

It is also possible that both NPH3 and RPT2 genes are involved in responses other than phototropism in roots. RPT2 has recently been implicated in stomatal opening; however, NPH3 was not necessary for stomatal opening or chloroplast relocation (Inada et al., 2004). Further analysis

![Fig. 5](https://academic.oup.com/jxb/article-abstract/57/12/3217/450176)
on the role of these elements in root responses may identify what role they play in root development.

Phenylpropanoid metabolism signal-transduction is induced
Phenylpropanoid metabolism is an important metabolic pathway responsible for the synthesis of both developmentally required secondary compounds (such as lignin and flavonoids) and for the synthesis of defensive compounds. In roots, phenylpropanoid content depends on plant age, the quality and intensity of light treatment, and the source of root material (basal portions versus root tips; Hemm et al., 2004). The greatest amount of phenylpropanoids (coniferin, syringin, Rha-Glu-Quercetin) were found in roots that were grown in white light compared with roots from plants grown in the dark, red, or blue light (Hemm et al., 2004).

Although the roots from seedlings grown in red light had the lowest phenylpropanoid content of all treatments, roots from phyB had reduced amounts of phenylpropanoids compared with wild-type plants, suggesting that PHYB is involved in regulating phenylpropanoid metabolism in roots (Hemm et al., 2004).

For roots, the expression profiles of the genes from phenylpropanoid metabolism are just beginning to be explored. For instance, using RT-PCR, Hemm et al. (2004) identified several genes in the phenylpropanoid metabolism pathway of roots that were induced (>=2-fold) within 5 h of white-light treatment. These include PAL1 (PHENYLALANINE AMMONIA LYASE 1), PAL2, PAL3, CH (CHALCONE SYNTHASE), C4H (TRANS-CINNAMATE 4-HYDROXYLASE), 4CL3 (4-COUMARATE:CoA LI-GASE), C3'H (p-COUMARATE 3-HYDROXYLASE), CAD-C (CINNAMYL ALCOHOL DEHYDROGENASE), and HY5. Here, five genes associated with phenylpropanoid metabolism were found that were significantly up-regulated in 1 h of red-light treatment (HY5, PAL3, COMT-like 11; COMT-like 12; and CAD7/CAD8; see supplementary Table 2 at JXB online). PAL3 was not significantly up-regulated in roots from seedlings that have been grown continuously in white light, so it appears that there is a transient gene expression of PAL3 in roots (Hemm et al., 2004; Ma et al., 2005). Interestingly, cotyledons and hypocotyls from light-grown seedlings and roots with 5 h of white-light treatment had significant up-regulation of both PAL1 and PAL2 (Hemm et al., 2004; Ma et al., 2005), suggesting that these genes may be regulated during longer or different wavelength and fluence rates of light treatments than those performed here. In contrast to our results for roots where there was no significant regulation of PAL1, for whole seedlings, this gene was induced within 1 h of red light (Tepperman et al., 2004). Studies on the time-course regulation of the PAL genes in roots will improve our understanding of phenylpropanoid metabolism in plants.

Chloroplast development is differentially regulated
Roots, which are normally not exposed directly to light, express photoreceptors and can respond to light by developing chloroplasts in a process known as root greening. In Arabidopsis, root greening occurs most effectively in blue or white light although some greening can occur in red light (Usami et al., 2004). Phytochrome B is the main photoreceptor involved in regulating root greening in red light with HY5 playing an important downstream role in regulating the process (Oyama et al., 1997; Usami et al., 2004). COP1 and DET1 act downstream of photoreceptors and are negative regulators of photomorphogenesis (Hardtke and Deng, 2000). These two proteins are also negative regulators of the greening process in red light of roots (Usami et al., 2004). For roots of dark-grown seedlings exposed to 1 h of red light, seven genes were found significantly up-regulated and six down-regulated that are associated with chloroplasts or photosynthetic elements (see supplementary Table 2 at JXB online). A comparison of the changes in gene expression in roots exposed to blue light may indicate the differences associated with chloroplast development of roots exposed to different qualities of light.

Root hair formation is induced
In this study, two genes were identified that were closely involved in root hair cell differentiation CPC (CAPRICE) and root hair formation RHD3 (ROOT HAIR DEFECTIVE 3). CPC, a MYB protein, is a positive regulator of hair cell differentiation in Arabidopsis roots (Wada et al., 1997). Elevated levels of CPC transcript result in a high concentration of the CPC protein, which in turn repress WER (WEREWOLF) and GL2 (GLABRA2) gene transcription, thus permitting the hair cell type to differentiate (for a review see Birnbaum and Benfey, 2004).

The RHD3 gene encodes a putative GTP-binding protein required for appropriate cell enlargement in Arabidopsis. RHD3 expression is required for normal root hair growth (Schiefelbein and Somerville, 1990). Specifically, it has been established that RHD3 is critical at the very beginning of hair formation and during root hair elongation (Parker et al., 2000). In a physiological context, up-regulation of CPC and RHD3 seems plausible since the normal developmental pathway for root hair formation (i.e., not induced by external stimuli) starts as early as 4 d in seedlings. In addition, root hair formation is stimulated by red light and regulated by phytochromes A and B (De Simone et al., 2000b). Further studies on the gene expression of these genes in root tips should prove useful in understanding light-regulated root development.

Light-regulated genes in roots and seedlings
Previous studies have examined the differential expression of genes in roots from light- and dark-grown seedlings.
(Sato-Nara et al., 2004; Ma et al., 2005). In this study, very few genes (26) were found to be significantly differentially expressed in both 1 h of red-light treatment and continuous white-light treatment (Table 2). Despite the reduced number of genes overlapping in both studies, several major components in light signalling have been identified (i.e. HY5, ELF4, SPA1, PnCOP1-like, RAP2.6), suggesting that light signalling might involve similar elements in the early and later stages of transduction.

Gene expression studies of whole seedlings compared the time-course expression profiles from red-light treated plants with dark controls (Tepperman et al., 2004). These studies found that 138 genes were differentially expressed within 1 h of the red-light treatment (Tepperman et al., 2004). Of these genes, nine overlapping elements with the profiles from roots in red-light treatments were found. These genes include HY5, SPA1, PKS1, RPT2, STO, ELF4, POP1, and RAP2.6, which were induced, and bHLH (zeta gene) which was suppressed in the red-light treatments. RAP2.6 is a putative AP2 domain ethylene response factor (ERF) that is up-regulated later in whole seedlings exposed to red light, suggesting it is an indirect target in light regulation (Tepperman et al., 2004). Also, RAP2.6 regulation appears not to be directly regulated by PHYB but perhaps by other phytochromes (Tepperman et al., 2004). RAP2.6 has also been shown to be regulated by cold treatment, and this, along with GI, may be an interacting element in circadian clock rhythms and temperature regulation (Fowler and Thomashow, 2002). In addition, RAP2.6 is also induced by bacterial infection and to a lesser extent by jasmonic acid (He et al., 2004). Induction of this gene correlates with increased susceptibility to disease, but the role of RAP2.6 in red-light regulated processes in roots is unknown.

Gene expression analysis of dark-grown whole seedlings exposed to 1 h of red light has also been investigated by T Krestch and coworkers. This microarray data was deposited in the NASCAArray database (Nottingham Arabidopsis Stock Centre, http://arabidopsis.info/) with experiment reference number NASCAARRAYS-124. Comparison of our results with roots of seedlings exposed to 1 h of red-light treatment with dark-grown seedlings (mainly hypocotyls and cotyledons) show 15 overlapping genes (14 up-regulated and 1 down-regulated genes). Major components in light signal transduction pathways are included in this group of genes, such as HY5, PKS1, ELF4, COP1-like (At5g52250), and RPT2. In addition, two ABC transporters (i.e. POP1 and NAP9), a heat shock transcription factor (i.e. similar to GRP2), and four expressed proteins with unknown function were up-regulated, and a homeobox–leucine zipper family protein was down-regulated in both studies.

Interestingly, HY5, POP1, SPA1, RAP2.6, and ELF4 were up-regulated in roots of light-grown seedling, in roots of seedlings exposed to 1 h red light, and in whole seedlings exposed to 1 h red light. This evidence reinforces the idea that elements involved in the first steps of light signal transduction are conserved in roots.

Light regulates many processes in roots, although the extent that these processes are controlled by light in normal (i.e. underground) conditions is largely unknown. There are several ways in which roots can be exposed to light. For example, roots that are uncovered from the soil surface will be exposed directly to illumination, light can diffuse through the soil to the roots (Tester and Morris, 1987; Mandoli et al., 1990), or light can be transmitted through tissues from the aerial tissues to the roots (Lauter, 1996; Sun et al., 2003). Therefore, roots may respond directly to the light signal even when they are buried in soil. Future experiments comparing expression profiles from roots of seedlings grown in different light quality and quantity will help identify wavelength specific pathways and the roles of these genes in light-regulated responses in roots.

**Supplementary data**

Supplementary data can be found at JXB online.

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**Gene profiling of the red light signalling pathways in roots**

3227


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