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ORIGINAL PAPER

Downregulation of Cinnamoyl CoA Reductase Affects Lignin and Phenolic Acids Biosynthesis in *Salvia miltiorrhiza* Bunge

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Abstract The biosynthesis of salvianolic acid B shares the phenylpropanoid pathway with lignin, and cinnamoyl CoA reductase (CCR; EC 1.2.1.44) is a specific enzyme in the lignin pathway. In this study, a CCR gene (*SmCCR1*) from *Salvia miltiorrhiza* Bunge was cloned using DNA walking technology (GenBank ID: JF798634). The full-length *SmCCR1* is 2,489 bp long and consists of four introns and five exons encoding a polypeptide of 324 amino acid residues. Sequence alignment revealed that *SmCCR1* shares 83 % identity with *CCR* sequences reported in *Camellia oleifera* and other plant species. Expression pattern analysis indicated that expression of *SmCCR1* can be induced by exposure to *Xanthomonas campestris* pv. *Campestris* or methyl jasmonate. To demonstrate its functioning, we selected a 296-bp fragment and established an RNA interference construct that was introduced into *S. miltiorrhiza* by *Agrobacterium tumefaciens*-mediated gene transfer. Transgenic plants exhibited dwarfing phenotypes, and both syringyl and guaiacyl lignin monomers were decreased more than 60 %. In contrast, biosynthesis of phenolic acids—danshensu, rosmarinic acid, and salvianolic acid B—was strongly induced by 2.03-, 1.41-, and 1.45-fold, respectively, in the roots of transgenic plants from line CCR-10. Consistent with these phytochemical changes, downregulation of *SmCCR1* also affected the expression of related genes in the phenolics and lignin biosynthetic pathways. Our results also provide potential opportunities for engineering danshensu and salvianolic acid B production in *S. miltiorrhiza*.

Keywords Cinnamoyl CoA reductase (CCR) · Functional analysis · Lignin · Phenolic acid · *Salvia miltiorrhiza* Bunge

Introduction

Salvia miltiorrhiza Bunge, called “danshen” in Chinese, is a well-known medicinal plant that is widely planted in China and other Asian countries. Its active constituents are divided into two main groups: lipid-soluble tanshinones and water-soluble phenolic acids, including rosmarinic acid, salvianolic acid B, and danshensu (3, 4-dihydroxyphenyllactic acid). Those water-soluble constituents now attract more attention because they are the main components of water decoction, the most common form of dosing administered to patients in Chinese clinics (Hu et al. 2005; Liu et al. 2006; Ma et al. 2006). Most of the phenolic acids in *S. miltiorrhiza* have been suggested to be derived from rosmarinic acid, a product of the phenylpropanoid pathway (Petersen et al. 1993).

Cellulose and lignin, the first and second most abundant compounds on earth, are major factors in the successful colonization of plants. Deposited in the secondary cell wall of specific cells, both provide additional structural reinforcement during defense responses to environmental cues such as wounding, mechanical stress, or pathogen attack (Vance et al. 1980; Capeleti et al. 2005; Escamilla-Treviño et al. 2010). In most angiosperm species, lignin is mainly composed of guaiacyl (G) and syringyl (S) monolignol subunits that are derived from coniferyl and sinapyl alcohol core monolignols, respectively (Vanholme et al. 2008).

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Biosynthesis of lignin begins with the common phenylpropanoid pathway, starting from phenylalanine and leading to cinnamoyl-CoA esters (Fig. 1). Lignin content can be reduced or altered by modifying genes such as *PAL*, *C4H*, *HCT*, *CCoAOMT*, *CCOMT*, *CCR*, and *CAD* (Leple et al. 2007; Shadle et al. 2007; Mir Derikvand et al. 2008; Bi et al. 2011). However, such manipulations can also affect plant growth and development.

As the first committed step in the lignin pathway, cinnamoyl CoA reductase is considered a potential control point in regulating the overall carbon flux toward lignin, while catalyzing *p*-coumaroyl-CoA, caffeoyl-CoA, feruloyl-CoA, 5-hydroxyferuloyl-CoA, and sinapoyl-CoA. For example, down-regulation of CCR can decrease lignin contents and limit carbon flow along the monolignol pathway, possibly enhancing the availability of coumaroyl-CoA esters, which are universal substrates of lignin, phenolics, and flavonoids (Rest et al. 2006).

In this study, we cloned *SmCCR1* from *S. miltiorrhiza* and analyzed its sequence and expression pattern. We also focused on its functioning in lignin and salvianolic acid B biosynthesis, with the goal of proposing it as a new candidate gene for engineering phenolics production in *S. miltiorrhiza*.

Materials and Methods

Plant Material and Culture Conditions

Seeds of *S. miltiorrhiza* Bunge were surface-sterilized with 0.1 % mercuric chloride ($HgCl_2$) and germinated on an MS basal medium. Cultures were maintained at 25±2 °C under a 16-h photoperiod provided by cool-white fluorescent lamps (25 μmol m^{-2} s^{-1}).

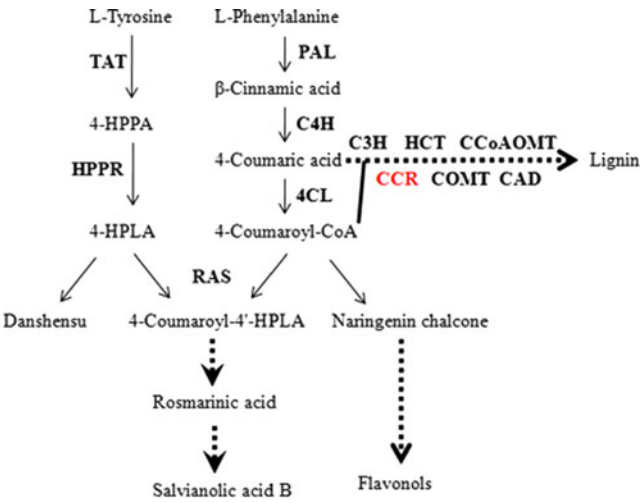

Fig. 1 Danshensu, salvianolic acid B and lignin biosynthesis pathway in *S. miltiorrhiza*. *CAD* cinnamyl alcohol dehydrogenase, *CCR* cinnamoyl-CoA reductase, *C3H* coumarate 3-hydroxylase, *C4H* cinnamate 4-hydroxylase, *4CL* hydroxycinnamate CoA ligase, *CCoAOMT* caffeoyl-coenzyme A 3-*O*-methyltransferase, *COMT* caffeic acid *O*-methyltransferase, *HCT* hydroxycinnamoyl CoA shikimate hydroxycinnamoyl transferase, *HPPR* hydroxyphenylpyruvate reductase, *PAL* phenylalanine ammonia lyase, *RAS* rosmarinic acid synthase, *TAT* tyrosine aminotransferase

DNA and RNA Isolations

Genomic DNA was isolated from young leaves by the CTAB method (Doyle and Doyle 1987). Total RNA was extracted using Trizol reagent (BioFlux, China) according to the manufacturer's instructions, and was subjected to cDNA synthesis by reverse-transcription with oligo-dT primers (Takara, Japan).

Cloning of *SmCCR1* and Sequence Analysis

By screening an EST library, we obtained a 687-bp sequence for *SmCCR1* (cinnamoyl CoA reductase from *S. miltiorrhiza*), located at the 3′ end of the coding region (GenBank ID: CV167323). Alignment analysis revealed that it shares 81 % identity with CCR sequences reported in other plant species (data not shown).

The 5′ sequence end of *SmCCR1* was cloned by DNA walking technology using a Universal Genome Walker Kit (Clontech, USA) according to the manufacturer's instructions. Amplified fragments were then purified and cloned into a pDM19-T Easy vector (Takara). After sequencing of the 5′ end, we obtained the entire coding sequence DNA and RNA of *SmCCR1* through PCR.

The amino acid sequence of SmCCR1 was deduced and analyzed using the ProtParam tool (http://cn.expasy.org/tools/protparam.html). A Basic Local Alignment Search Tool (BLAST) search was conducted on the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple-alignment analysis was performed by Clustal W (http://www.ebi.ac.uk/Tools/clustalw/index.html), and the transcription start was predicted via Neural Network Promoter Prediction software (http://www.fruitfly.org/seq-tools/promoter.html). Promoter motifs were analyzed according to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare.html), and a maximum likelihood tree was built using PhyML (Guindon and Gascuel 2003).

Expression Analysis of *SmCCR1*

Two-month-old plants were soaked in solutions of either methyl jasmonate (MeJA, 5 μM) or *Xanthomonas campestris* pv. *Campestris* (XC-1). Samples were collected at 0, 1, 3, 6, 12, 24, or 48 h after treatment and were frozen immediately in liquid nitrogen for storage at −80 °C. The growth condition of XC-1 was perviously described (Lummerzheim et al. 1993).

Real-time quantitative PCR was used to analyze the expression pattern of S*mCCR1*, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) being amplified as a control. Conditions in the iQ5 thermocycler (Bio-Rad, USA) included 1 min of predenaturation at 94 °C; then 35 cycles of

10 s denaturation at 94 °C, 20 s annealing at 60 °C, and 15 s of collection fluorescence at 82 °C. Expression was quantified by the comparative CT method.

RNAi Construct and Plant Transformation

A 296-bp fragment corresponding to the 3′ region of *SmCCR1* cDNA was amplified by PCR, using upstream primer 5′-CGGGATCCTCGAGGCTGGGCTGATGAGGAATACTG CAAG-3′ (sites underlined for *Bam*H I and *Xho* I, respectively) plus downstream primer 5′-CCATCGATGGTACCCCTTG CAGATGGGTTCTCATACACCAG-3′ (sites underlined for *Cla* I and *Kpn* I, respectively). The fragment was then introduced in different orientations upstream or downstream of the intron in vector pKANNIBAL. The recombinant pKANNIBAL and pART27 (Gleave 1992) were digested by *Not* I and ligated to generate the final RNAi vector pART27-CCRi, which was then introduced into *Agrobacterium tumefaciens* strain EHA105 by the freeze-thaw method (Holsters et al. 1978).

Gene transfer was performed as described by Yan and Wang (2007). Transformed plants were cultured on an MS medium before being grown in the greenhouse and allowed to self-pollinate. Primers PDK F (5′-GTGATGTGTAAGACGAA GAAG-3′) and PDK R (5′-GATAGATCTTGCGCTTTG-3′) were used to amplify a 427-bp fragment from the intron for testing these putative transgenic plants.

Lignin Analysis

Air-dried stems from 2-month-old plants (three per sample) were ground to pass through a 2-mm diameter mesh. This powder was then exhaustively extracted for 24 h with acetone in a Soxhlet apparatus. The main G and S lignin monomers were analyzed via gas chromatography–mass spectrometry (GC–MS). Qualitative and quantitative analyses of individual lignin monomers, per their trimethylsilylated derivatives, were performed from specific ion chromatograms that were reconstructed (at 269 for G monomers and 299 for S) after making an appropriate calibration relative to the tetracosane internal standard (Hoffmann et al. 2004).

Extraction of Total Phenolics

Roots were collected from plants grown in the greenhouse for 60 days. Dried tissues were ground to a fine powder in a mechanical grinder with a 2-mm diameter mesh. The samples (100 mg exactly) were extracted with 500 μL of 75 % methanol under sonication for 20 min, and then centrifuged at 12,000×*g* for 6 min. The supernatant was collected and the residual pellet was re-extracted twice with 500 μL of solvent. All supernatants were combined as a total extract for evaluating their phenolic compounds. Extractions were performed in triplicate.

HPLC Analysis of Phenolic Compounds

Extracts were analyzed on a SHIMADZU C_{18} column (5 μm, 150×4.6 mm) connected to an LC-2010AHPLC system equipped with an SPD-M10 V photodiode array (PDA) detector and LC-Solution software (SHIMADZU, Kyoto, Japan). The mobile phase comprised 0.1 % acetic acid in water (A) and methanol (B). The solvent gradient was as follows: 0–5 min, B 98–90 %; 5–15 min, B 90–80 %; 15–25 min, B 80–75 %; 25–30 min, B 75–60 %; 30–40 min, B 60–50 %; and 40–60 min, B 50–35 %. Conditions included a flow rate of 1.0 mL min^{-1}, column temperature of 30 °C, injection with 15 μL of each sample, and PDA detector wavelengths ranging from 200 to 800 nm, with samples measured at 280 nm. Samples and mobile phases were put through a 0.22-μm filter before injection. Major peaks from these extracts were identified by comparing them with UV spectra of authentic standards under identical conditions.

Statistical analysis was conducted with SPSS 16.0 software. Analysis of variance was followed by Tukey's pairwise comparison tests ($P<0.05$) to determine significant differences between means.

Results

Isolation and Sequence Analysis of *SmCCR1*

The full-length *SmCCR1* was isolated with gene-specific primers (Electronic Supplementary Material (ESM) Table 1), designed according to the sequence screened from the EST

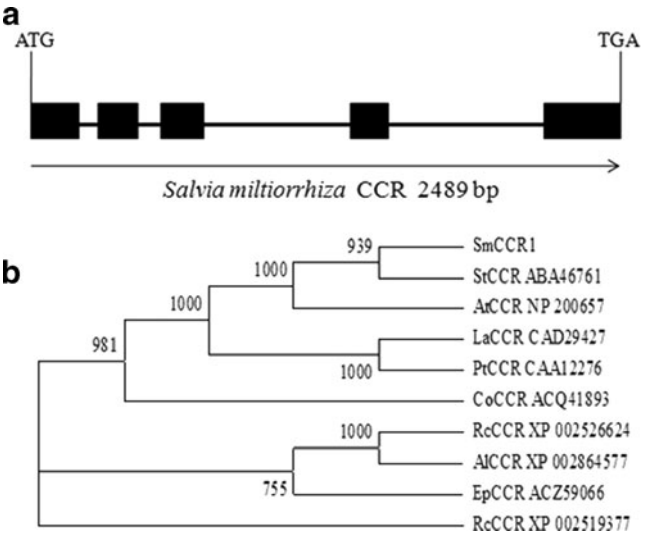

Fig. 2 **a** Structure of *SmCCR1*; exons denoted by *black boxes*, introns by *lines*. Length of exons in base pairs. **b** Phylogenetic tree of SmCCR1 and nine CCRs characterized from dicots and monocots. Tree was built using PhyML; neighbor-joining tree, with MEGA4

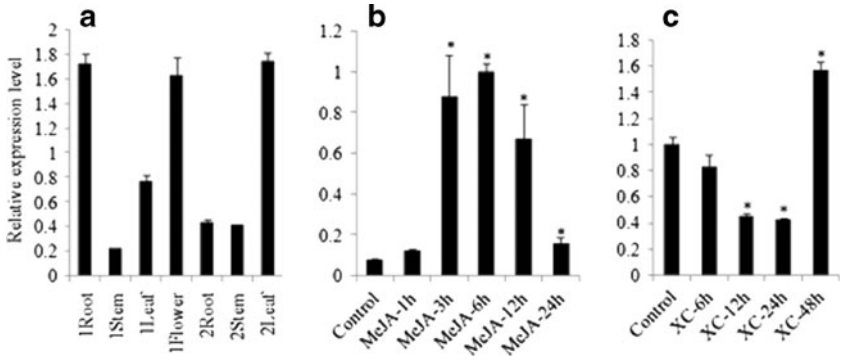

Fig. 3 Expression pattern of *SmCCR1* in different organs (**a**) and under treatment with MeJA (**b**) or XC-1 (**c**). *1* Overwintering *S. miltiorrhiza* plant; *2*, 2-month-old plants. Data represent average of three experiments; *error bars* standard deviations. *Asterisk* difference is significant at $P<0.05$ compared with untransformed control

library, and sequenced. Using RT-PCR, we also obtained a 975-bp cDNA sequence with an ORF encoding a 324 amino acid protein, designated as *SmCCR1*. The full-length *SmCCR* DNA was 2,489 bp long and comprised five exons and four introns in sizes of 86, 101, 644, and 683 bp (Fig. 2a). Those introns had characteristics typical of plant introns, being rich in A + T and having a standard GT/AG splicing site. A BLAST P search in NCBI (http://www.ncbi.nlm.nih.gov/BLAST) indicated that *SmCCR1* has an NADB binding domain and shares 83 % similarity with *Camellia oleifera* and other *CCR* genes.

Through DNA walking, we obtained a 1,215-bp 5′ flanking sequence of *SmCCR1*. The putative transcription start site was −191 bp upstream from the start codon ATG, as predicted by Neural Network Promoter Prediction Analysis software. Promoter motifs in this region were also predicted. CAAT and TATA boxes were common *cis*-acting elements in the promoter regions. According to the results, the most probably TATA box was located at −34, which was observed in the majority of eukaryotic genes (Minami et al. 1989). There were 24 CAAT boxes found in the 5′ flanking region of *SmCCR1*. We also found MYB binding site, which was a potential special transcription factor binding sites, and a CGTCA motif in the *SmCCR1* promoter. It has been reported that overexpression of transcription factor *AtPAP1* could induce the expression of SmCCR1 in *S. miltiorrhiza* (Zhang et al. 2010), which was consistent with our prediction.

The Deduced SmCCR1 Protein

SmCCR1 encoded a protein with a calculated molecular mass of 35.7 kDa and a calculated pI of 6.057. But it did not contain the signature motif NWYCY, which was thought to be a feature of the catalytic site (Lacombe et al. 1997). Putative conserved domains, including NADH–flavin reductase, and an NAD-dependent epimerase/dehydratase, for binding cofactors, were characterized by BLAST P. These findings demonstrated that SmCCR1 can be classified as a member of the oxido-reductase family.

To study the evolutionary relationships among different CCR proteins from various plant species, we constructed a phylogenetic tree based on the deduced amino acid

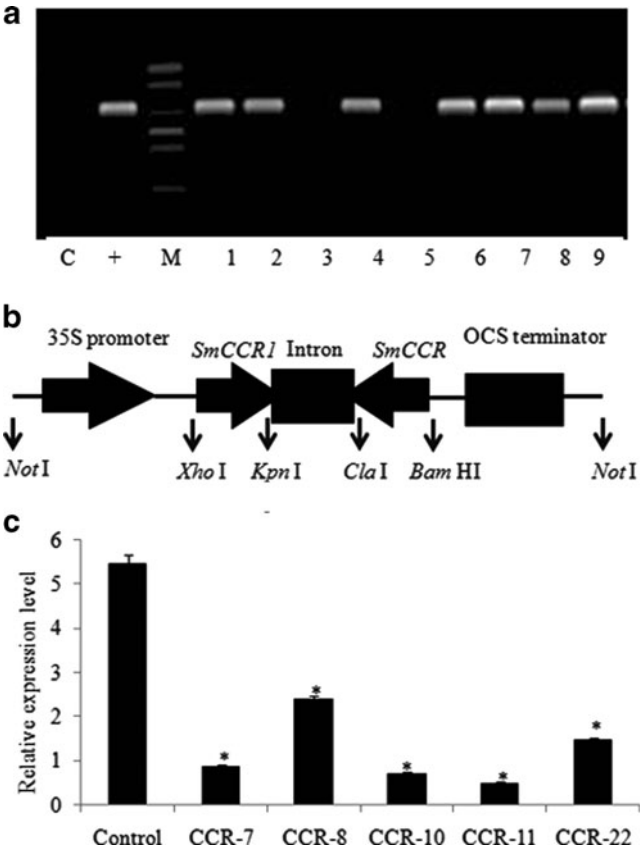

Fig. 4 Vector construct and molecular analysis of transgenic plant. **a** PCR-screening of SmCCR1-suppressed S. miltiorrhiza. Pyruvate orthophosphate dikinase (PDK) intron was amplified to screen positive transgenic lines. M DL2000 DNA marker, band sizes from top: 100, 250, 500, 750, 1,000, and 2,000 bp; + positive control; C no template control; 1–9 different transgenic lines. **b** Sketch map of interfering box. Restriction sites and target gene are marked. **c** Real-time quantitative PCR analysis of *SmCCR1* in RNAi *S. miltiorrhiza*. All data are means of three replicates, with *error bars* showing standard deviations. *Asterisk* difference is significant at $P<0.05$ compared with untransformed control

Fig. 5 Phenotype of CCR downregulated transgenic plants and control

sequences of SmCCR1 and other CCRs (Fig. 2b). Among the ten members in that protein family, SmCCR1 was most closely related to CCRs from *Solanum tuberosum* (Solanaceae) and *Arabidopsis thaliana* (Cruciferae).

Transcription Profile of *SmCCR1*

SmCCR1 expression varied in different tissues of *S. miltiorrhiza*. Real-time quantitative PCR indicated that expression was strongest in old roots, flowers, and young leaves. For old roots in particular, this expression was correlated with more active lignification at that stage of development (Fig. 3a). This pattern was similar to that of *StCCR1* in potato (Larsen 2004). Lignin content has also been demonstrated to increase significantly in the leaves of resistant potato plants upon infection by *Phytophthora infestans* (Larsen 2004).

Expression of *CCR* genes can be induced by various environmental factors, e.g., abiotic stress, light, mechanical wounding, and bacterial infection. For example, *ZmCCR2* is highly induced by water deficiency in the root elongation zone (Fan et al. 2006), whereas *AtCCR2* is induced in response to pathogenesis (Lauvergeat et al. 2001). Therefore, to characterize the expression pattern of *SmCCR1*, we exposed 2-month-old seedlings to MeJA or XC-1. Transcription of *SmCCR1* responded to varying degrees according to treatment. For MeJA, transcription was up-regulated after 1 h, reaching a peak at 6 h (Fig. 3b). Such treatment has also been shown to induce strong *IiCCR* expression in *Isatis indigotica* (Hu et al. 2010), whereas expression of *AtCCR2* is not increased. For XC-1 exposure, transcription levels decreased at 24 h before rising again at hour 48 (Fig. 3c), the same as noted with *A. thaliana*. The level of *AtCCR2* mRNA was increased strongly and transiently after inoculation by the pathogen, suggested that *SmCCR1* could have a function in the plant defense mechanism.

Phenotype of *SmCCR1*-Suppressed *S. miltiorrhiza*

To generate RNAi-mediated *SmCCR1*-suppressed plants, we designed a construct that produced dsRNA under the control of the cauliflower mosaic virus (CaMV) 35S promoter (Fig. 4b). It was then introduced into *S. miltiorrhiza* via *A. tumefaciens*-mediated gene transfer. Leaves from 1-month-old transgenic plantlets were used for DNA extraction and PCR-screening. An expected 427-bp fragment of the PDK intron was amplified in the positive control and RNAi lines (especially lines 7, 8, 10, 11, 17, and 22;

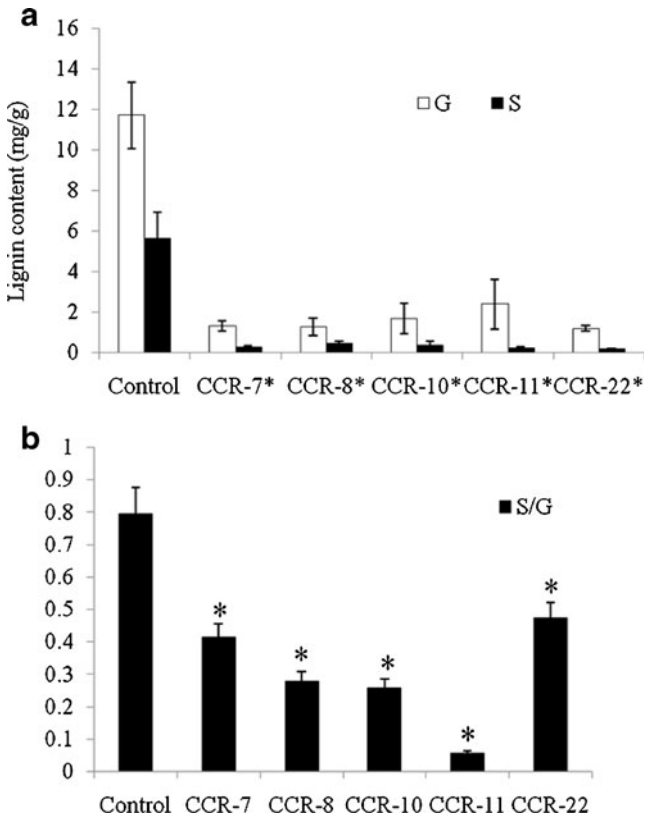

Fig. 6 Content of lignin monomer (**a**) and ratio of S/G (**b**). Data represent average of three experiments; *error bars* standard deviations. *Asterisk* difference is significant at $P<0.05$ compared with untransformed control

Table 1 Concentrations of danshensu, rosmarinic acid, and salvianolic acid B

Line	Danshensu (mg g^{-1} DW)	Rosmarinic acid (mg g^{-1} DW)	Salvianolic acid B (mg g^{-1} DW)
Control	0.93±0.02	4.52±0.34	24.84±0.13
CCR-8	1.64±0.24[a]	5.85±0.20[a]	33.23±4.45
CCR-10	1.89±0.06[a]	6.39±0.23[a]	35.98±0.74[a]

[a]Significantly different (*t* test at $p<0.05$)

Fig. 4a). Seven positive lines of 18 independent transformants were screened by PCR and five were then selected for analysis of gene expression.

After total RNA was extracted, real-time PCR analysis showed that *SmCCR1* transcription in the transgenic lines was reduced about 60–90 % compared with the control (Fig. 4c). We also moved *SmCCR1*-suppressed plants into soil in a greenhouse for further observation. Compared with untransformed control lines, the RNAi lines exhibited a dwarfism phenotype (Fig. 5).

Lignin Modifications in Transgenic Plants

The G and S lignin-derived monomers were analyzed by GC–MS. Changes were observed in the lignin level and composition (relative proportions of S- and G-type monomers) of *SmCCR1* transgenic and control plants. G lignin was reduced more than 60 % in all transgenic plants while S lignin declined by about 90 % (Fig. 6a; ESM Fig. 1). At the same time, the ratio of S/G did not rise (Fig. 6b). In CCR-10, both S and especially G were dramatically diminished (Fig. 6a; ESM Fig. 1).

Phenolic Contents in Transgenic Plants

The impact of *SmCCR1* suppression on phenylpropanoid metabolic flow was further investigated by implementing HPLC. Following the downregulation of *SmCCR1* and lignin, analysis of phenolic acids showed increases in the levels of danshensu (2.03-fold higher), rosmarinic acid (1.41-fold), and salvianolic acid B (1.45-fold) in the roots of suppressed lines compared with the control (Table 1).

Effect of *SmCCR1* on the Expression of Genes in the Lignin and Phenolics Pathways

To understand the mechanisms of lignin and phenolic acids production, we examined transcript levels of eight key structural genes in those biosynthesis pathways by real-time PCR (Fig. 7). Compared with control plants, expression of *SmCOMT* was markedly depressed in the lignin pathway of suppressed lines. By contrast, expression of *PAL*, *C4H*, and *4CL* was markedly increased in the core phenylpropanoid pathway while that of *RAS* and *TAT*, two enzymes in the phenylpropanoid and tyrosine-derived branch pathways respectively, was slightly depressed.

Discussion

Downregulation of *SmCCR1* Decreases Lignin Biosynthesis in *S. miltiorrhiza*

We cloned *SmCCR1* from *S. miltiorrhiza*, a traditional Chinese medicinal plant. However, in contrast to the patterns of *CCR* genes in other species, such as wheat, maize, *A. thaliana*, and *Eucalyptus gunnii*, *SmCCR1* is expressed strongly in *Salvia* leaves, stems, and flowers (Lacombe et al. 1997; Pichon et al. 1998; Lauvergeat et al. 2001). Compared with those other genes, downregulation of *SmCCR1* caused a dramatic reduction in lignin contents and was associated with plant dwarfism, although it contains no NWYCY signature motif (Lacombe et al. 1997). This suggests that another signature motif may be active in *CCR* genes, a possibility that can be investigated with more experimentation.

Downregulation of *CCR* expression commonly results in a significant decline in lignin contents and the alteration of cell wall structure (Goujon et al. 2003; Rest et al. 2006; Wadenbäck et al. 2008). For example, this downregulation in tobacco drastically affects the plant phenotype and causes the S/G ratio

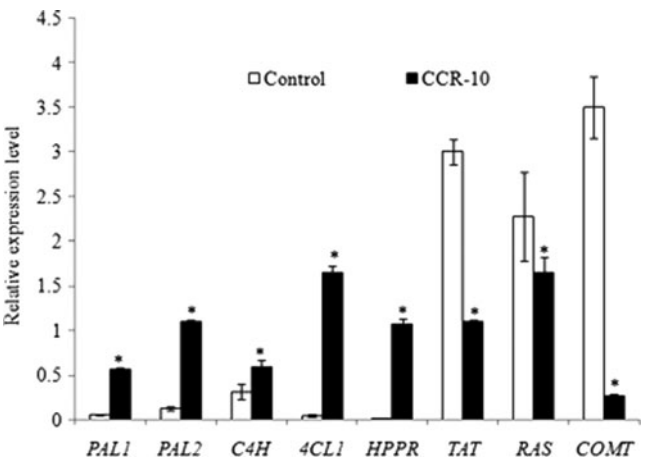

Fig. 7 Relative expression levels of lignin biosynthesis genes in control and CCR-10 plants. Data represent average of three experiments; *error bars* standard deviations. *Asterisk* that difference is significant at $P<0.05$ compared with untransformed control

to increase from 0.91 to 3.16 (Pincon et al. 2001). However, downregulation of *SmCCR1* in our transgenic *S. miltiorrhiza* was associated with a reduction in both S and G subunits (especially S lignin), and decrease in the S/G ratio from 0.80 to 0.06 in CCR-11. This suggested that SmCCR1 had a preference for sinapoly-CoA than feruloyl-CoA.

Downregulation of *SmCCR1* Improves Phenolic Contents

A decrease in flux through competitive pathways can be exploited to enhance or modify the production of hypothetical plant phenolics (Kirsi et al. 2004). In fact, downregulation of *CCR* genes in several species has been shown to redirect metabolic flux away from developmentally related lignification in *CCR1*-deficient plants. Silencing of those genes in tobacco, tomato, poplar, and perennial ryegrass decreases the supply of caffeoyl-CoA and feruloyl-CoA to G and S units, respectively, and leads to the greater accumulation of cinnamic and ferulic acids (Tu et al. 2010; Rest et al. 2006). By contrast, the level and composition of flavonols and anthocyanins are also enhanced in those transgenic lines.

As a result of success in decreasing lignin synthesis in RNAi transgenic lines, concentrations of rosmarinic acid and salvianolic acid B were enhanced. Surprisingly, the level of danshensu was doubled in roots of transgenics compared with the control. This active pharmaceutical ingredient antagonizes lipid peroxidation and eliminates oxygen free radicals (Wang et al. 2005; Wu et al. 2007). When combined with emodin, it alleviates the effect of severe acute pancreatitis in rats (Wang et al. 2010). For the expression of *PAL*, *C4H*, and *4CL* was elevated in *SmCCR1*-suppressed *S. miltiorrhiza*, therefore, the accumulation of rosmarinic acid and salvianolic acid B was improved. Hydroxyphenylpyruvate reductase (HPPR) catalyzes the specific biosynthetic step in the biosynthesis of rosmarinic acid from the aromatic amino acids phenylalanine and tyrosine (Kim et al. 2004). However, the function of HPPR in danshensu biosynthesis remains unclear. Nevertheless, our study data imply that the accumulation of danshensu in transgenic *S. miltiorrhiza* may be due to higher transcription of that gene.

For the constantly increasing demand of *S. miltiorrhiza* for its varied pharmacologic functions, using modern biotechnology to improve the contents has become the basic content (Li et al. 2009). This research is of great significance for further studies of the secondary metabolite regulation and molecular breeding of *S. miltiorrhiza* and provides a promising strategy for genetic engineering of other medicinal plants.

In conclusion, we have cloned a *CCR1* gene, *SmCCR1*, from *S. miltiorrhiza*. This gene is mainly expressed in the roots and young leaves, and can also be induced by treatment with MeJA or XC-1. Transgenic plants exhibit a dwarfing phenotype and their concentrations of lignin, especially S subunits, are suppressed. Unexpectedly, levels of salvianolic acid B and danshensu were increased in our transgenic plants. This elevated accumulation of phenolic acids and danshensu was caused by downregulation of *SmCCR1*. Our results also may provide a method and potential opportunities for engineering danshensu and salvianolic acid B production in *S. miltiorrhiza*.

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