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Specific Genes of Cytochrome P450 Monooxygenases Are Implicated in Biosynthesis of Caffeic Acid Metabolites in *rolC*-Transgenic Culture of *Eritrichium sericeum*

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Abstract—Expression of *rol* agrobacterial oncogenes in plant cell cultures is known to result in activation of secondary metabolite biosynthesis. In the present work, we show that *rolC* can activate expression of key genes of secondary metabolism using the *rolC*-transgenic culture of *Eritrichium sericeum* producing caffeic acid metabolites (rosmarinic acid and rabdosiin) as an example. Increased content of rosmarinic acid in the *rolC*-transformed callus culture resulted from transcriptional activation of members of the CYP98 family of plant cytochrome P450-containing monooxygenase genes. The *rolC* gene expression led to increased transcript abundance of the *CYP98A3* subfamily members, which are closely related homologs of *CYP98A6* of *Lithospermum erythrorhizon* and are known to be key genes in rosmarinic acid biosynthesis. In contrast, expression of other *CYP* genes, such as *CYP98A1* and *CYP98A2*, which are not implicated in rosmarinic acid biosynthesis, was not activated in the *rolC*-transformed calluses. These results are indicative of selective effect of *rolC* on transcription of particular genes implicated in secondary metabolism.

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Key words: *rolC* oncogene, cytochrome P450 monooxygenases, *CYP98*

The *rolC* gene, which is found in plasmids of the phytopathogen *Agrobacterium rhizogenes*, plays an important role in neoplastic transformation of plants, and its expression leads to significant morphological and biochemical modification of transformed tissues [1, 2]. It was found recently that the *rolC* gene is implicated in formation of plant cauline meristems and somatic embryoids [3]. Moreover, the *rolC* gene affected sea urchin development via formation of teratoma-like structures in the transformed embryos [4]. Despite numerous studies, the biochemical role of RolC remains unknown because of the absence of obvious homology with any known protein of pro- and eukaryotes [5].

The *rolC* gene expression in cultured plant cells results in elevated level of secondary metabolites in transformed cells. For instance, an increase was found in biosynthesis of ginsenosides in transformed cell cultures of *Panax ginseng* [6], indole alkaloids in transformed cultures of *Catharanthus roseus* [7], pyridine alkaloids in *Nicotiana tabacum* [8], tropane alkaloids in *Atropa belladonna* [9], and anthraquinones in *Rubia cordifolia* [10, 11]. The stimulating effect of the gene is caused by activation of expression of key genes implicated in biosynthesis of secondary metabolites, as recently demonstrated by the example of the biosynthesis of anthraquinones [12]. There are no data on the mechanism of biosynthetic activation of plant cells transformed with the *rolC* gene. All attempts to correlate effect of the gene with activation of known signaling pathways of secondary metabolite synthesis control in plants were unsuccessful. In particular, the *rolC*-mediated stimulation of secondary metabolism is independent of signaling pathways mediated by methyl jasmonate [13], salicylic acid, and ethylene [10], as well as of the calcium-dependent NADPH-oxidase pathway [11].

Meanwhile, a technique of plant cell transformation with *rolC* gene expressed from a strong promoter is one of the most powerful methods to date for elevation of biosynthetic capability of plant cell cultures, which is used when common biotechnological approaches have a restricted effect [14].

Abbreviation: CAMs, caffeic acid metabolites.

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An unusual case of secondary metabolite synthesis inhibition was reported for cell cultures of *Eritrichium sericeum* transformed with the *rolC* gene [15]. Both plants and callus cultures of *E. sericeum* synthesize caffeic acid metabolites, namely rosmarinic acid and rabdosiin [16]. Rosmarinic acid is a caffeic acid dimer, whereas rabdosiin, which is produced from rosmarinic acid, is a tetramer. Both rosmarinic acid and rabdosiin inhibit a lymphocyte-specific kinase, thus weakening signals from T-cells to develop immunosuppression [17, 18], and also inhibit activity of HIV-1 integrase [19, 20]. The caffeic acid metabolite complex (rosmarinic acid/rabdosiin, 3 : 1) from *E. sericeum* callus culture significantly reduced symptoms of severe glomerulonephritis induced in rats by injection of nephrotoxic serum [21]. So, elevation of biosynthetic activity of cell cultures producing caffeic acid metabolites and elucidation of their biosynthetic pathways is an important problem. Recently, the key enzyme of rosmarinic acid biosynthesis was characterized, and the corresponding gene, *CYP98A6*, was cloned. The CYP98A6 protein catalyzes 3-hydroxylation of 4-coumaroyl-4′-hydroxyphenyllactic acid, an immediate precursor of rosmarinic acid [22]. *CYP98A6* is a member of a wide family of cytochrome P-450-containing monooxygenases that play important roles in both plant and animal metabolism [23]. *CYP98* is a family of cytochrome P-450 genes encoding 3′-hydroxylases of coumaric acid esters. These enzymes are involved in biosynthesis of lignin monomers and chlorogenic acid and are necessary for biosynthesis of various phenolic compounds [24]. The goal of the present work was identification of the *CYP98A6* gene or its homolog in the culture of *rolC*-transgenic *E. sericeum* cells and study of its expression.

MATERIALS AND METHODS

Transgenic *Eritrichium sericeum* (Lehm.) A. DC (Boraginaceae) callus cell cultures were prepared in 2003 as described in [15]. *Agrobacterium tumefaciens* GV3101 carrying either the pMP90RK (vector) or pPCV002-CaMVC plasmids was used for transformation. The pPCV002-CaMVC plasmid contains the *rolC* gene under the control of cauliflower mosaic virus (CaMV) 35S-promoter. The transformed cultures were designated as Es-vector (control) and Es-*rolC*, respectively. The cultures were grown in Erlenmeyer flasks, 100 ml in volume, on the $W_{B/A}$ medium [10] in the dark at 24°C for a subculture period of 30 days. Phytohormone concentrations were: 6-benzylaminopurine, 0.5 mg/liter; α-naphthylacetic acid, 2 mg/liter.

Qualitative and quantitative composition of polyphenols in dry tissue samples was determined by HPLC as described in [16].

RNA was isolated from 30-day-old *E. sericeum* Es-vector and Es-*rolC* callus cultures by the method using lithium chloride [25] optimized for secondary metabolite-rich tissues. The first DNA chain was synthesized using the M-MLV reverse transcriptase (Sileks, Russia) according to the manufacturer's protocol.

Expression of *PAL* genes was examined as follows: the degenerate primers 5′-ARGCYGCYGCYATYATGGA-3′ and 5′-GGRGTGCCYTGRAARTT-3′ against conservative regions were designed on the basis of known amino acid sequences of PAL (phenylalanine ammonia-lyase) from thale cress *Arabidopsis thaliana*, lettuce *Lactuca sativa*, raspberry *Rubus idaeus*, redroot gromwell *Lithospermum erythrorhizon*, soybean *Glycine max*, garden pea *Pisum sativum*, chickpea *Cicer arietinum*, and common bean *Phaseolus vulgaris* (GenBank accession Nos. NM_129260, NM_115186, AF411134, AF299330, AF237955, X52953, Q01861, Q04593, Q9SMK9, and P19143). The amplification protocol included initial denaturation at 96°C for 2 min followed by 40 cycles of 96°C for 15 sec, 53°C for 10 sec, and 72°C for 20 sec and a completion of synthesis at 72°C for 15 min. The length of PCR product was 266 bp (GenBank accession No. EU494974).

Expression of *CYP* genes was examined as follows: the degenerate primers 5′-GARTGGGCWATGGCNGA-3′ and 5′-RCACRTTMACATRMAC-3′ were designed on the basis of known amino acid sequences of CYP from thale cress *A. thaliana*, sorghum *Sorghum bicolor*, soybean *G. max*, redroot gromwell *L. erythrorhizon*, tobacco *Nicotiana tabacum*, and petunia *Petunia* x *hybrida* (GenBank accession Nos. O48956, O48922, NP_850337, NP_177594, NP_177595, BAC44836, BAB71716, AAK62346, and AAD56282). The amplification protocol included initial denaturation at 96°C for 2 min followed by 30 cycles of 96°C for 15 sec, 54°C for 10 sec, and 72°C for 15 sec and a completion of synthesis at 72°C for 15 min. The amplification product, a 256-bp fragment, is a part of the interdomain region of the gene.

RT-PCR linearity was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The reaction was linear during 30 cycles for *CYP* and 40 cycles for *PAL*.

Both the *CYP* and *PAL* gene expressions were normalized to the expression of *E. sericeum* actin gene. The primers 5′-TAYAAYGAGCTTCGTGTTGC-3′ and 5′-ACACCATCWCCAGARTCCA-3′ were used for the actin gene amplification. The amplification protocol included initial denaturation at 96°C for 2 min followed by 40 cycles of 96°C for 15 sec, 53°C for 10 sec, and 72°C for 20 sec and a completion of synthesis at 72°C for 15 min. The product of PCR from cDNA was 206 bp in length (GenBank accession No. EU293968), and from DNA it was 290 bp in length. The same cDNA sample was used as a template both for PCR with primers specific to actin gene and with degenerate primers designed for the *CYP* and *PAL* genes. Following gel electrophoresis, the amplicons synthesized in PCR were isolated from the

Table 1. Number of clones (% of overall number of sequenced clones) of each *CYP* gene subfamily in control and *rolC*-transgenic *E. sericeum* cultures

Gene	Es-vector, %	Es-*rolC*, %
CYP98A1	70.45	40.74
CYP98A2	27.27	29.63
CYP98A3a	2.27	14.11
CYP98A3b	0	15.52

Note: The table is drawn on the basis of cloned amplicons of *CYP* genes obtained using degenerate primers.

gel using the Glass Milk kit (Sileks) and cloned in the pTZ57R/T vector according to the manufacturer's (MBI Fermentas, Lithuania) protocol.

RT-PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, USA) according to the manufacturer's protocol on an ABI 310 Genetic Analyzer (USA) at the Institute of Biology and Soil Sciences, Far East Division of the Russian Academy of Sciences. The *CYP* and *PAL* gene regions constrained by degenerate primers were sequenced and converted into amino acid sequences using the Gene runner 3.05 software. These amino acid sequences were then compared with known amino acid sequences of CYP and PAL from other plant species using the NCBI BLAST software. The phylogenetic tree was constructed using the ClustalX software.

In all, 27 clones of Es-*rolC* containing the *CYP* gene insert and 44 clones of Es-vector were sequenced for gene expression analysis. The sequences were numbered in the sequencing order. The qualitative clone compositions are presented in Table 1. A total of 200 clones of both cell cultures were analyzed for *PAL* gene expression. Then expression of each *CYP98* gene subfamily was estimated (in relative units) from the data of Table 1, as well as data on overall expression of the genes obtained using degenerate primers. The relative units were calculated from the equation: overall expression of *CYP* genes normalized to the expression of *E. sericeum* actin gene × % clones of each *CYP* gene/100.

The data were processed using Statistica v.5.5 software. All data are presented as the mean value ± standard deviation and verified with Student's *t*-test assuming significance level $p = 0.05$ to be minimal in all experiments.

RESULTS

Growth and biosynthetic characteristics of *E. sericeum* callus cultures transformed with the *rolC* gene. The callus cultures Es-vector (control) and Es-*rolC* were obtained in 2003 [15]. Two-year monitoring of these cultures for contents of CAMs (caffeic acid metabolites) showed a three-fold decrease in level of CAMs in *rolC*-calluses, that is, the mean production of CAMs was 1.40 ± 0.40% CAMs of dry tissue weight [15]. Subsequent monitoring revealed increase in biosynthetic activity of *rolC*-cells. In November 2006, the *rolC*-calluses produced 4.56% CAMs, whereas the vector culture produced 2.18% (Table 2). These were the cultures we used for experiments described in this work.

Expression of *PAL* genes. Figure 1 represents a scheme of rosmarinic acid biosynthesis in plants. Initially, we estimated the expression level of *PAL* genes, whose activities determine the levels of metabolite influxes into the plant phenylpropanoid pathway. PAL catalyzes conversion of phenylalanine to cinnamic acid. Using PCR with degenerate primers, we prepared amplicons of expected size (266 bp), both for control and *rolC*-cultures (Fig. 2). Cloning and sequencing of these fragments showed their homology with known *PAL* genes of plants. Nucleotide sequences determined from three or more independent sequencings of these fragments are deposited in GenBank. Analysis of 60 clones demonstrated expression of the *PAL1* gene (Acc. No. EU494974) in Es-vector and Es-*rolC* cultures. The resulting data are indicative of non-elevated expression level of *PAL1* in the *rolC*-transgenic culture compared with the control (Fig. 2). These are in agreement with the data on the absence of metabolite flow limitations in *E. sericeum* calluses imposed by PAL-catalyzed reaction, because addition of phenylalanine had no activating effect on biosynthesis of CAMs [21].

Expression of *CYP* genes. Expression of the *CYP* genes was analyzed in the same way. The primary data also showed no difference in the *CYP* gene expression levels between the control and Es-*rolC* cultures (Fig. 3a). Moreover, the overall expression level of *CYP* genes in the Es-*rolC* culture tended to decrease, but its values did not transcended the limits of statistical error and did not significantly differ from those of control culture (Fig. 3b). Analysis of 256-bp fragments revealed close homology with known cytochrome P-450-containing plant monooxygenases belonging to the CYP98 family (Fig. 4).

Table 2. Contents of caffeic acid metabolites in control and *rolC*-transgenic *E. sericeum* cell cultures (% of dry tissue weight)

Culture	Rosmarinic acid	Rabdosiin	Total level of CAMs
Es-vector	0.89	1.29	2.18
Es-*rolC*	3.73	0.83	4.56

Note: Calluses were grown on the agarized $W_{B/A}$ medium for 30 days.

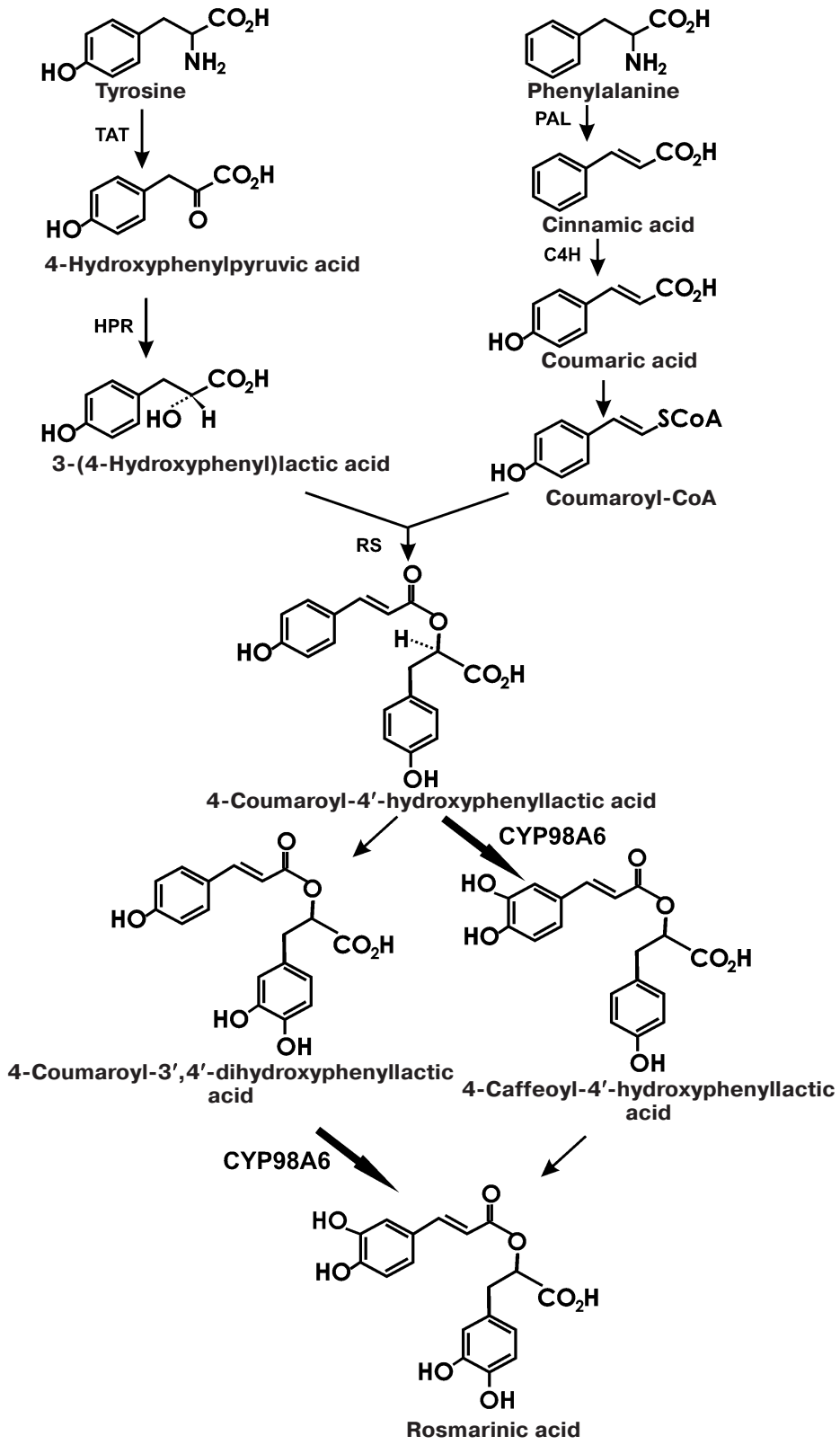

Fig. 1. Biosynthetic pathway leading to rosmarinic acid production (according to [22]). PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA-ligase; TAT, tyrosine aminotransferase; HPR, hydroxyphenylpyruvate reductase; RS, rosmarinic acid synthase.

The amino acid sequences, 74 amino acid residues in length, constructed on the basis of nucleotide fragments of *E. sericeum CYP98* genes represent the interdomain region. The *E. sericeum* CYP98 amino acid fragments aligned using the ClustalX software split into three families named *CYP98A1*, *CYP98A2*, and *CYP98A3*. The amino acid sequences of these proteins showed no less than 71% identity among them. Several gene subfamilies were found in each family. We assigned literal markings (a, b, c) to those subfamilies, whose amino acid sequences differed by one or more amino acids and whose sequencings were repeated several times. In this way, we identified the *CYP98A3a* and *CYP98A3b* subfamilies differing from each other by three amino acid residues. The nucleotide sequences of *CYP98A1*, *CYP98A2*, *CYP98A3a*, and *CYP98A3b* are deposited in GenBank (Acc. Nos. EU494971, EU494972, EU494973, and EU494975).

Alignment and comparison of *E. sericeum* CYP98 amino acid fragments with known plant cytochrome P-450-containing monooxygenase sequences have shown 80% homology between CYP98A1 representatives and soybean CYP98A2 (Acc. No. O48922), an enzyme with unknown substrate specificity [26]. The *E. sericeum* CYP98A2 is the most homologous to CYP98A13 (Acc. No. AAL99200) from sweet basil *Ocimum basilicum* (94% homology), which catalyzes 3′-hydroxylation of *p*-coumaroyl shikimate [27].

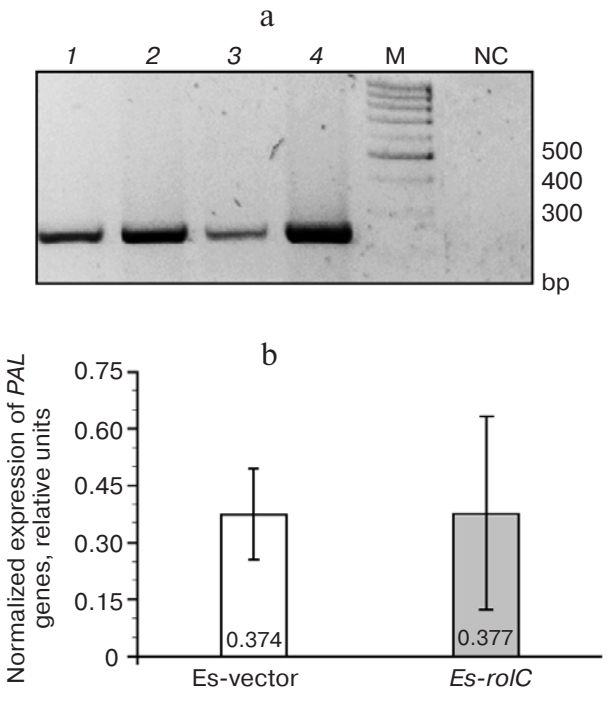

Fig. 2. Expression of the *PAL* gene in the control culture Es-vector and *rolC*-transgenic callus culture Es-*rolC*. a) Gel electrophoresis of PCR products of *E. sericeum PAL* cDNA. Degenerate primers were used. Lanes: *1*, *2*) callus culture Es-vector, cDNA template dilutions 1 : 3 and 1 : 1, respectively; *3*, *4*) callus culture Es-*rolC*, cDNA template dilutions 1 : 3 and 1 : 1, respectively; M, molecular mass marker; NC, negative control. b) Quantitative estimation of the *PAL* gene expression.

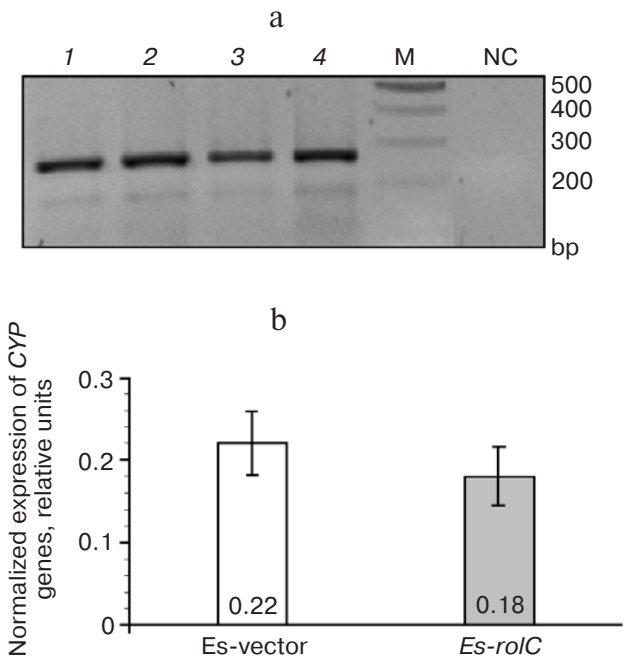

Fig. 3. Expression of *E. sericeum CYP* genes in the control culture Es-vector and *rolC*-transgenic callus culture Es-*rolC*. a) Gel electrophoresis of PCR products of *E. sericeum CYP* cDNA. Degenerate primers were used. The product size was 256 bp. Lanes: *1*, *2*) callus culture Es-vector, cDNA template dilutions 1 : 3 and 1 : 1, respectively; *3*, *4*) callus culture Es-*rolC*, cDNA template dilutions 1 : 3 and 1 : 1, respectively; M, molecular mass marker; NC, negative control. b) Quantitative estimation of the *CYP* gene expression.

Both CYP98A3a and CYP98A3b display 93-94% homology with CYP98A6 (Acc. No. BAC44836) from cell culture of redroot gromwell *L. erythrorhizon* [22]. CYP98A6 is a 4-coumaroyl-4′-hydroxyphenyllactate hydroxylase, that is, the enzyme catalyzing the final reaction of the rosmarinic acid biosynthesis pathway (Fig. 1). A quantitative expression analysis of these isoforms in the vector culture and *rolC*-culture gave intriguing results. In particular, the level of *CYP98A3a* and *CYP98A3b* transcripts in Es-*rolC*-calluses about 5-fold exceeded that in the control culture (Fig. 5). At the same time, the expression level of *CYP98A2* was unchanged, whereas the expression level of *CYP98A1* was about twofold decreased.

DISCUSSION

RolC can selectively activate expression of specific secondary metabolism gene isoforms. It is known that the *rolC* gene expressed in plant cell cultures can activate

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Es  CYP98A3a   --------LVRNPRVQQKAQEELDRVVGSDRIMTEADVSKLPYLQCIVKESLRLHPPTPLMLPHKASANVKLGGYDIPKGSI------    74
Le  CYP98A6    STEWAMAELVRNPRVQRKAQEELDRVVGPDRIMTEADVPKLPYLQCIVKESLRLHPPTPLMLPHRASANVKIGGYDIPKGSIVHVNVW   440
Es  CYP98A3b   --------LVRNPRVQQKAQEELDRVIGSDRIMTEADFPKLPYLQCIVKESLRLHPPTPLMLPHKASANVKLGGYDIPKGSI------    74
Es  CYP98A2    --------LIKNPRVQQKAQEELDRVIGYERVMTEADFSSLPYLQCVAKEALRLHPPTPLMLPHRANANVKIGGYDIPKGSN------    74
Ob  CYP98A13   SVEWAMAELIKNPRVQQKAQEELDKVIGFERVMTETDFSSLPYLQCVAKEALRLHPPTPLMLPHRANTNVKIGGYDIPKGSNVHVNVW   444
Gm  CYP98A2p   SVEWAMAELIRNPRVQQKVQEELDRVIGLERVMTEADFSNLPYLQCVTKEAMRLHPPTPLMLPHRANANVKVGGYDIPKGSNVHVNVW   444
Es  CYP98A1    --------LLRHPRVIKKAQEELDRVIGTERFMTEADFPNLPYLQAMTKEALRLHPSTPLMLPHRASTNVKVGGYDIPKDTI------    74
At  CYP98A8    VIEWAMAEMIKCPTVQEKAQQELDSVVGSERLMTESDIPILPYLQCVVKEALRLHPSTPLMLPHKASETVWVGGYKVPKGATVYVNVQ   436
                       ::: * * .*.*:*** *:* :*.***:*.. *****.:.**::****.*******:*. .* :***.:**.:
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Fig. 4. Alignment of *E. sericeum* CYP98A1, CYP98A2, CYP98A3a, and CYP98A3b amino acid sequences deduced from nucleotide sequences (Acc. Nos. EU494971, EU494972, EU494973, and EU494975) with amino acid sequences of CYP98 family representatives: CYP98A13 (Acc. No. AAL99200) from *O. basilicum*, CYP98A2 (Acc. No. O48922) from *G. max*, CYP98A6 (Acc. No. BAC44836) from *L. erythrorhizon*, and CYP98A8 (Acc. No. NP_177594) from *A. thaliana*. Es, *E. sericeum*; Le, *L. erythrorhizon*; At, *A. thaliana*; Ob, *O. basilicum*; Gm, *G. max*.

secondary metabolism processes, but the activation mechanism remains unknown. RolC seems to induce enhanced expression of genes encoding the key secondary metabolism enzymes. In manjishtha (*Rubia cordifolia*) cell cultures, the *rolC* expression level determined the expression level of isochorismate synthase gene (*ICS*), whose product is crucial in biosynthesis of anthraquinones [12]. No specificity of the gene effect was drawn out, using transgenic manjishtha cells as a model, because *ICS* genes were present as two highly homologous forms. In our case, the key enzyme of rosmarinic acid biosynthesis is encoded by *CYP98A6* representing a broad family of cytochrome P-450-containing monooxygenases [22]. A search for analogs of this gene in *E. sericeum* cell cultures has in fact displayed their presence (Fig. 4). Prevalence of *CYP98A3a* and *CYP98A3b* transcripts in Es-*rolC*-calluses compared with the controls might suggest selective activation of individual forms of this gene family by *rolC*, which channelizes the metabolite stream into biosynthesis of specific secondary metabolites. Thus, it is very likely that the agrobacterial *rolC* gene mediates activation of phenylpropanoid secondary metabolite biosynthesis by means of enhancement of *CYP98A3* expression.

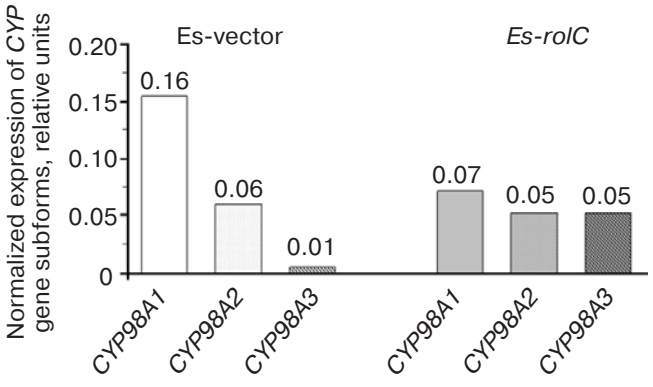

Fig. 5. Normalized expression of *CYP* gene subfamily in control culture Es-vector and *rolC*-transgenic callus culture Es-*rolC*. The values were obtained from the data on relative overall expression of *CYP* genes and quantitative distribution of sequenced clones.

Earlier, we hypothesized that *rolC* exerts control over rosmarinic acid, rather than rabdosiin, production [15]. The production of these two compounds in normal *E. sericeum* calluses either regulates differently or rabdosiin is nonenzymatically produced from rosmarinic acid [16]. The data of the present work meet this concept, because *rolC* activated synthesis of rosmarinic acid, but not rabdosiin (Table 2).

Expression of the *PAL* gene in *rolC*-transgenic *E. sericeum* culture did not differ from that in the control culture (Fig. 2). At the same time, activation of polyphenol biosynthesis in elicitor-stimulated cell cultures is known to be associated with enhanced expression of *PAL* gene [22]. The absence of *PAL* expression activation in the *rolC*-transgenic culture can be explained by *rolC*-mediated activation of secondary metabolism occurring via a pathway different from those induced by elicitors [11, 13, 28]. The data of this work meets the conception that the *rolC* gene has its own unique mechanism of secondary metabolism activation [29].

Slow host cell signaling pathway modifications in *rolC*-transgenic plant cultures. The *rolC* gene was earlier described as a secondary metabolism suppressor in transgenic cell and root cultures of *E. sericeum* and *L. erythrorhizon* [15]. In the present work, we have shown that, following a long period of growth, the *rolC*-calluses of *E. sericeum* come to accumulate considerable amounts of CAMs (Fig. 1). The cause of alteration in biosynthetic activity of *rolC*-calluses is not yet clear. However, it is worth noting that a similar effect was observed in ginseng cell cultures transformed with the *rolC* gene [30]. In this case, a period of reduced (as compared with control) productivity of the transgenic calluses was 6 months, followed by fast elevation of ginsenoid contents in *rolC*-cultures of ginseng with its stabilization at a level 2-3-fold exceeding that in control cultures [31]. As for *E. sericeum* cells, the period of secondary metabolite synthesis suppression was more prolonged (2 years). A similar situation was observed for other effects of the *rolC* gene, in particular, for formation of transgenic roots by ginseng *rolC*-calluses. Rhizogenesis occurred in five independently transformed ginseng callus cultures, but all of the cultures lost their ability for root formation after 16-60 months of

growth, and some callus cultures began to develop cauline meristems and somatic embryoids [3]. The simplest explanation of these processes is that expression of the transgene altered for a longstanding cultivation of calluses. However, such a mechanism is not supported by experimental data [3]. In general, expression of the *rolC* gene in transformed plant cell cultures is characterized by high stability. For example, the difference in the expression between individual clones was maintained for 12-year growth of *rolC*-transgenic ginseng calluses [32] and septenary growth of *rolC*-transgenic manjishtha calluses [12]. It was hypothesized that the *rolC* gene conditions slow and lasting processes of host cell signaling pathway modification [29]. These processes also seem to determine the phenomenology of secondary metabolite biosynthesis.

This kind of slow modification processes is described in literature for human tumor cell genomes [33]. Some somatic mutations in these cells were accidental, whereas significant number of them, "driver mutations", were in some way directed and can provide growth advantage to the tumor cell carrying this mutation. Directed mutations often occur in kinase domains of various protein kinase genes [33]. In connection with this, a fact of interest is that the *rolC* gene conditions excision of plant calcium-dependent protein kinase mRNA sequences corresponding to the VII catalytic subdomain of these kinases [34]. Such modification does not shift the reading frame, but theoretically has to alter substrate specificity of the protein. This analogy may be accidental, but might also reflect a certain global process of eukaryotic tumor cell modification. It was repeatedly noted that *rolC*-transformed cells, as time goes, come to predominate over normal cells in growth rate [29]. In general, a growing pool of data suggest functional analogy between the plant oncogene *rolC* and some animal oncogenes (the data are summarized in review [29]), and the recently found ability of *rolC* to induce teratoma-like structures in sea urchin embryos [4] reinforces these data.

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