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# Resveratrol Content and Expression of Phenylalanine Ammonia-lyase and Stilbene Synthase Genes in Cell Cultures of *Vitis amurensis* Treated with Coumaric Acid

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**Abstract** The present study examines the effect of *p*-coumaric acid (CA), a precursor of stilbenes and isoflavonoids, on biosynthesis of resveratrol in cell cultures of *Vitis amurensis*. Earlier, we transformed *V. amurensis* with the *rolB* gene of *Agrobacterium rhizogenes* and showed increased level of resveratrol production in the *rolB* transgenic cell culture. We used control and the *rolB*-transgenic cell culture of *V. amurensis* as a model system in this study. CA was capable of increasing resveratrol production in the control and the *rolB*-transgenic cell cultures in 10.3 and 1.5 times, respectively. The CA-treated control and *rolB* transgenic calli produced up to 0.06% and 1.1% DW of resveratrol. Using quantitative real-time RT-PCR, we characterized the expression of phenylalanine ammonia-lyase (*PAL*) and stilbene synthase (*STS*) genes in the CA-treated control and *rolB* transgenic cell cultures. The expression of *PAL* genes remained essentially unchanged under 0.1 mM of CA, while expression of *VaPAL1*, *VaPAL2*, *VaPAL3*, and *VaPAL5* genes was considerably decreased under 0.5 and 2 mM CA compared with the untreated cells. In the CA-treated calli, expression of *VaSTS2* and *VaSTS3* was increased, while expression of *VaSTS5*, *VaSTS8*, *VaSTS9*, and *VaSTS10* was significantly decreased. These results indicate that CA increased resveratrol accumulation in *V. amurensis* calli via selective enhancement of expression of individual *STS* genes.

**Keywords** *p*-Coumaric acid · Callus culture · Resveratrol · *STS* · *Vitis amurensis*

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**Abbreviations**

| | |
|---|---|
| CA | *p*-Coumaric acid |
| DW | Dry weight |
| MeJa | Methyl jasmonate |
| Phe | Phenylalanine |
| PAL | Phenylalanine ammonia-lyase |
| SA | Salicylic acid |
| STS | Stilbene synthase |

## Introduction

Resveratrol, 3,4′,5-trihydroxy-*trans*-stilbene, is a plant-derived polyphenol compound that displays a wide range of intriguing biological properties [1–5]. Resveratrol has been found to possess anti-inflammatory, antioxidant, and platelet antiaggregatory properties. In addition, resveratrol exhibits chemopreventive properties against certain cancers and cardiovascular diseases and has positive effects on age longevity [2, 4].

Grapevine is the main source of resveratrol [6]. Cell cultures of a variety of plant species contain low resveratrol levels (up to 0.01% dry wt.), and the use of inductors of secondary metabolism and elicitors does not result in a considerable increase in resveratrol production. The levels of resveratrol in *Vitis vinifera*, *Vitis amurensis*, and *Arachis hypogaea* cell cultures treated with various compounds including methyl jasmonate (MeJa), salicylic acid (SA), and sodium nitroprusside did not exceed 0.1% DW or 10 mg/l [6–11]. Results presented in [12] demonstrate that treatment of a *V. amurensis* cell culture with 5-azacytidine, an inhibitor of DNA methylation, resulted in a 2-fold increase in resveratrol production (up to 5.1 mg/l). The most significant success in increasing resveratrol content in plant cells has been reached while using cyclodextrins (CDs). CDs are a family of compounds made up of sugar molecules bound together in a ring (cyclic oligosaccharides). β-cyclodextrin, a seven sugar ring molecule, was often used to increase resveratrol content in various plant cell cultures [13–15]. The treatments with CDs resulted in high levels of resveratrol, more than 3,000 mg/l, after elicitation with the DIMEB, RAMEB, and CAVASOL®W7. However, most researchers have used high amounts of CDS, e.g. 50 mM~67 g/L of DIMEB, while the price of CDs is high. Therefore, it appears that the cost of resveratrol production using this approach would also be high.

Genetic transformation of plant cells with various genes can significantly increase the production of secondary metabolites [16] and, therefore, is considered as a possible method to enhance production of various biologically active compounds by plant cell cultures. Many researchers have used overexpression of the stilbene synthase or resveratrol synthase (*STS*) genes to increase resveratrol content in plant cells. In *STS* transgenic plants, the content of resveratrol and piceid varied and did not exceed 0.05% DW [17, 18]. Plant tissues can be transformed by transferring the bacterial Ri plasmid T-DNA from *Agrobacterium rhizogenes*, which often promote production of various biological active compounds by the cells [19]. For the production of resveratrol, hairy root cultures of peanut *A. hypogaea* were elicited with sodium acetate [20] which resulted in a 60-fold increase in resveratrol production 24 h post treatment. Resveratrol represented 99% of the secreted stilbenes, and its concentration reached 35 mg/l. It has been shown that transformation of *V. amurensis* with *rolB* and *rolC* genes of *A. rhizogenes* enhance resveratrol production [8, 21]. A high level of *rolB* expression resulted in a considerable increase in resveratrol production, up to 3.15% DW or 150 mg/l, in the transformed cell line compared to the

control. However, a 3-year cultivation of the *rolB*-transgenic calli resulted in a gradual loss of the high levels of resveratrol production [3].

Another approach to increase resveratrol content in plant cell cultures is feeding the cells with biosynthetic precursors of resveratrol. Stilbenes, including resveratrol, are synthesized via the phenylpropanoid pathway [5]. Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) is the first enzyme in this pathway which catalyzes monooxidative deamination of phenylalanine (Phe) leading to cinnamate production [1]. Phe is an important precursor of the plant secondary metabolites synthesized via phenylpropanoid pathway. Another important precursor of stilbenes, including resveratrol, is *p*-coumaric acid (CA). CA is synthesized from cinnamic acid by the action of the P450-dependent enzyme cinnamic acid 4-hydroxylase (C4H; EC 1.14.13.11). The next step is ligation of CoA to CA by 4-coumarate:CoA ligase (4CL; EC 6.2.1.12). In the last step, STS (EC 2.3.1.95) condenses three molecules of malonyl-CoA and one molecule of coumaryl-CoA to form resveratrol [22].

Addition of Phe to the culture medium did not increase resveratrol production by a callus culture of *V. amurensis* [8]. 4CL from tobacco and STS from grapes were heterologiously expressed in yeast and bacterial systems [23]. The authors detected resveratrol in the culture medium of both the yeast and bacteria, 1.5–6 mg/l in yeast *Saccharomyces cerevisiae* [24, 25] and 16–171 mg/l in bacteria *Escherichia coli* [26, 27]. However, CA was never used to feed plant cell cultures in order to induce accumulation of plant secondary metabolites, including resveratrol. The present study aims to analyze the impact of CA on the biosynthesis and accumulation of resveratrol in cell cultures of *V. amurensis*. Using control (VV) and *rolB*-transgenic (VB2) callus cultures of *V. amurensis*, we showed that CA significantly increased the content and production of resveratrol by the calli via activation of the *VaSTS2* and *VaSTS3* genes. Interestingly, the *rolB* transgenic cell line exhibited lower inducibility to the CA treatment compared to the control cell line. The level of resveratrol production by the CA-treated calli of *V. amurensis* was higher than in SA-, MeJa-, and Phe-treated plant cells but lower than in *E. coli* transformed with the *STS* gene and *V. vinifera* cell cultures treated with CDs.

## Material and Methods

### *Vitis amurensis* Cell Cultures

The V2 callus culture of wild-growing grape *Vitis amurensis* Rupr. (Vitaceae) was established in 2002 as described previously [8]. The VV culture was obtained in 2004 by co-cultivation of the V2 cell suspension with *Agrobacterium tumefaciens* GV3101/pMP90RK strains containing pPCV002 plasmid vector, which contained only the kanamycin resistance gene (*nptII*), as described previously [8]. The *rolB* transgenic *V. amurensis* callus culture (designated VB2) were obtained in 2004 by transformation of the V2 cell suspension with *A. tumefaciens* strains GV3101 containing binary plasmid vector pPCV002-CaMVB/pMP90RK [28] as described earlier [8]. In the pPCV002-CaMVB construction, the *rolB* gene was under the control of cauliflower mosaic virus (CaMV) 35S promoter [28]. High level of *rolB* expression transformed callus culture of *V. amurensis* resulted in more than a 100-fold increase in resveratrol production (up to 3.15% dry wt.) compared to the control. Unfortunately, the high level of resveratrol content in the *rolB* transgenic callus culture of *V. amurensis* was not stable during long-term cultivation. The high resveratrol content decreased to 0.2–1.1% dry wt. after 2.5 years of cultivation; however, it is still significantly higher compared to the control cell culture VV. Therefore,

we used the control VV and *rolB* transgenic cell culture VB2 of *V. amurensis* as a model system to investigate the influence CA on resveratrol metabolism.

The VV and VB2 callus cultures were cultivated with 35-day subculture intervals in the dark at 24–25°C in test tubes with 15 ml $W_{B/A}$ medium [29]. Reagents for the cell culture medium were purchased from Sigma Chemical Co (MO, USA) and Serva Feinbiochemica GmbH & Co (Heidelberg, Germany). Samples were harvested from 35-day cultures (linear phase of growth and the highest resveratrol content), weighed, and used for quantitative real-time RT-PCR. The calli were dried under hot air flow (60°C for 2 h) and used for resveratrol determination.

## Total RNA Isolation and Quantitative Real-Time RT-PCR

Total RNA isolation was performed using CTAB (hexadecyltrimethylammoniumbromide)-based extraction protocol developed by Bekesiova et al. [30]. Complementary DNAs were synthesized as described previously [8, 31–33]. Previously, by sequencing of cloning RT-PCR products as described [34] at the Instrumental Centre of Biotechnology and Gene Engineering of IBSS FEBRAS using an ABI 310 and 3130 Genetic Analyzers (Applied Biosystems, Foster City, USA), we obtained cDNA sequences of five *PAL* and ten *STS* genes of *V. amurensis*. The gene-specific primer pairs and TaqMan probes for *PAL1–PAL5*, *STS1–STS10*, *rolB*, and *Actin1* genes of *V. amurensis* are presented in Kiselev et al. [29, 35] and in Electronic supplementary material Table 1.

For TaqMan real-time RT-PCR, cDNAs were amplified as described [36] in 20-μl reaction mixtures containing 1× TaqMan Buffer B, 2.5 mM $MgCl_2$, 250 μM of each deoxynucleotide, 1 U Taq DNA polymerase, 0.5 μl (15 ng) cDNA sample, and 0.25 μM of each primer and probe (real-time PCR Kit, Syntol, Russia). The amplification conditions consisted of one cycle of 2 min at 95°C followed by 50 cycles of 10 s at 95°C and 25 s at 62°C. The TaqMan PCR essays were performed in an iCycler thermocycler supplied with iQ5 Multicolor Real-Time PCR detection system (Bio-Rad Laboratories, Inc., USA), and data were analyzed with the iQ5 Optical system software v.2.0 according to the manufacturer's instructions (normalized expression (relative quantification by the $2^{-\Delta\Delta CT}$ method)). Scaling options: highest (the highest expressing sample accrued the value 1 in the relative mRNA calculation). *V. amurensis* actin gene (GenBank ac. no. DQ517935) was used as endogenous control to normalize variance in the quality and the amount of cDNA used in each real-time RT-PCR experiment. A no-template control was included in every assay, and no-cycle threshold (Ct) values were consistently obtained after 50 cycles of PCR. Each TaqMan probe for *V. amurensis* actin gene was labeled with an FAM reporter dye at the 5′-end and a RTQ-1 quencher dye at the 3′-end, and TaqMan probe for *PAL* and *STS* genes was labeled with an ROX reporter dye at the 5′-end and a BHQ-2 quencher dye at the 3′-end (Syntol, Russia). The data were summarized from five independent experiments.

## CA Treatments

CA was obtained from Sigma (St. Louis, USA). Sterile solutions of CA (as ethanol solutions) were added to the autoclaved media aseptically in the desired concentrations (0.1, 0.5, and 2 mM).

## High-Performance Liquid Chromatography

*V. amurensis* cell cultures were analyzed for the presence of *trans*-resveratrol by HPLC analysis as described [21].

### Statistical Analysis

Statistical analysis employed the Statistica 8.0 program. The data are presented as mean± standard error (SEM) and were tested by paired Student's *t* test. The 0.05 level was selected as the point of minimal statistical significance in all analyses.

## Results and Discussion

### Effects of CA on Growth and Resveratrol Accumulation in Callus Cultures of *V. amurensis*

Figure 1a demonstrates that CA at a concentration of 0.1 mM significantly increased the growth of the VV cell culture of *V. amurensis*, while at concentrations 0.5 and 2 mM, it inhibited the growth of the culture. We observed similar effect of CA treatment on growth of the *rolB* transgenic VB2 cell culture. However, its growth was affected to a lesser degree (Fig. 1a).

CA at a concentration of 0.1 mM significantly increased content and production of resveratrol in the VV cell culture by 6.9- and 10.3-fold, respectively (Fig. 1c). We propose that the increase is the result of the faster growth capacity of the calli (Fig. 1a, b). Since CA at 0.5 and 2 mM concentrations inhibited the growth of the VV cell culture, the level of resveratrol accumulation was also decreased (Fig. 1c).

CA at a concentration of 0.5 mM significantly increased the resveratrol content and production in the VB2 cell culture, by 1.6- and 1.5-fold, respectively (Fig. 1b, c). The highest level of resveratrol content and production was detected at 0.5 mM of CA—1.1% dry weight (DW) or 40.1 mg/l. Cultivation in the presence of 0.1 and 2 mM CA did not significantly change resveratrol content in the VB2 cell culture. However, 2 mM CA significantly decreased the level of resveratrol production due to the inhibited growth capacity of the culture. The treatment with more than 0.5 mM of CA was associated with necrotic phenotype of the VV and VB2 calli.

Interestingly, we detected trace amounts of CA along with resveratrol in the 2 mM CA-treated VV and VB2 calli and in the culture medium (less than 0.3% DW, data not shown). The data indicate that CA at a concentration of 2 mM was in excess for the grape cells.

### Expression of the *rolB*, *PAL*, and *STS* Genes

The levels of *rolB*, *PAL*, and *STS* gene expression were analyzed by means of quantitative real-time RT-PCR (Fig. 2). The level of *rolB* gene expression in the CA-treated cells of *V. amurensis* was not considerably changed compared to the untreated cells (Fig. 2).

The total expression of *PAL* genes in the calli of *V. amurensis* treated with 0.1 mM CA remained essentially unchanged compared with the untreated cells. In the control VV cells, 2 mM CA considerably decreased expression of *VaPAL1*, *VaPAL2*, and *VaPAL5* genes, while 0.5 mM CA decreased *VaPAL3* expression (Fig. 2). In the *rolB* transgenic VB2 cell culture, 0.5 and 2 mM CA considerably decreased expression of the *VaPAL3* gene. We suggest that the decrease in *VaPAL1*, *VaPAL2*, and *VaPAL5* gene expression for the VV and *VaPAL3* for the VB2 cell cultures under 2 mM CA treatment is a result of cell necrotic phenotype under high concentrations of CA. Presumably, the total *PAL* gene expression has not changed considerably because PAL acts upstream to 4-coumaroyl-CoA in the phenylpropanoid pathway, and therefore CA cannot affect expression of *PAL* genes.

In the VV and VB2 calli treated with CA, expression of *VaSTS2* was significantly increased compared with the untreated cells (Fig. 2). Expression of *VaSTS3* was increased

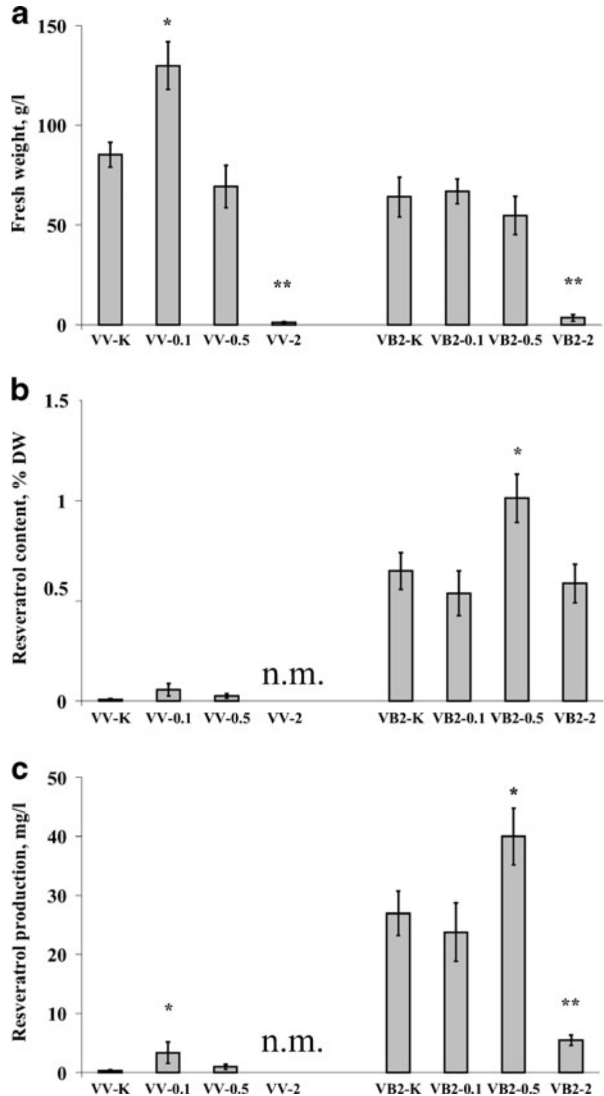


**Fig. 1** Biomass accumulation **a**, resveratrol content **b**, and resveratrol production **c** in VV and VB2 callus cultures of *V. amurensis* treated with CA. Abbreviations: *VV-K* VV cell culture growing on standard medium; *VV-0.1* on medium with 0.1 mM CA; *VV-0.5* on medium with 0.5 mM CA; *VV-2* on medium with 2 mM CA; *VB2-K* VB2 cell culture growing on standard medium; *VB2-0.1* on medium with 0.1 mM CA; *VB2-0.5* on medium with 0.5 mM CA; *VB2-2* on medium with 2 mM CA. The data are presented as mean±SEM obtained from two independent experiments, *n. m.* not measured, *$p<0.05$, **$p<0.01$ vs. values of the VV or VB2 cultures without CA

by 2.6-fold compared with the untreated cells, but the increase was not significant. Also, we detected a significant decrease in expression of several *STS* genes: the expression of *VaSTS8* and *VaSTS10* genes was significantly decreased in the VV calli under 2 mM CA, and the expression of *VaSTS5*, *VaSTS8*, *VaSTS9*, and *VaSTS10* genes was significantly decreased in the VB2 calli under 2 mM CA (Fig. 2). We propose that the decrease in *STS* gene expression is a result of necrotic phenotype under high concentrations of CA.

## Conclusion

In this study, we examined the influence of CA, a resveratrol precursor, on the levels of resveratrol accumulation and *PAL* and *STS* gene expression in the control and *rolB*

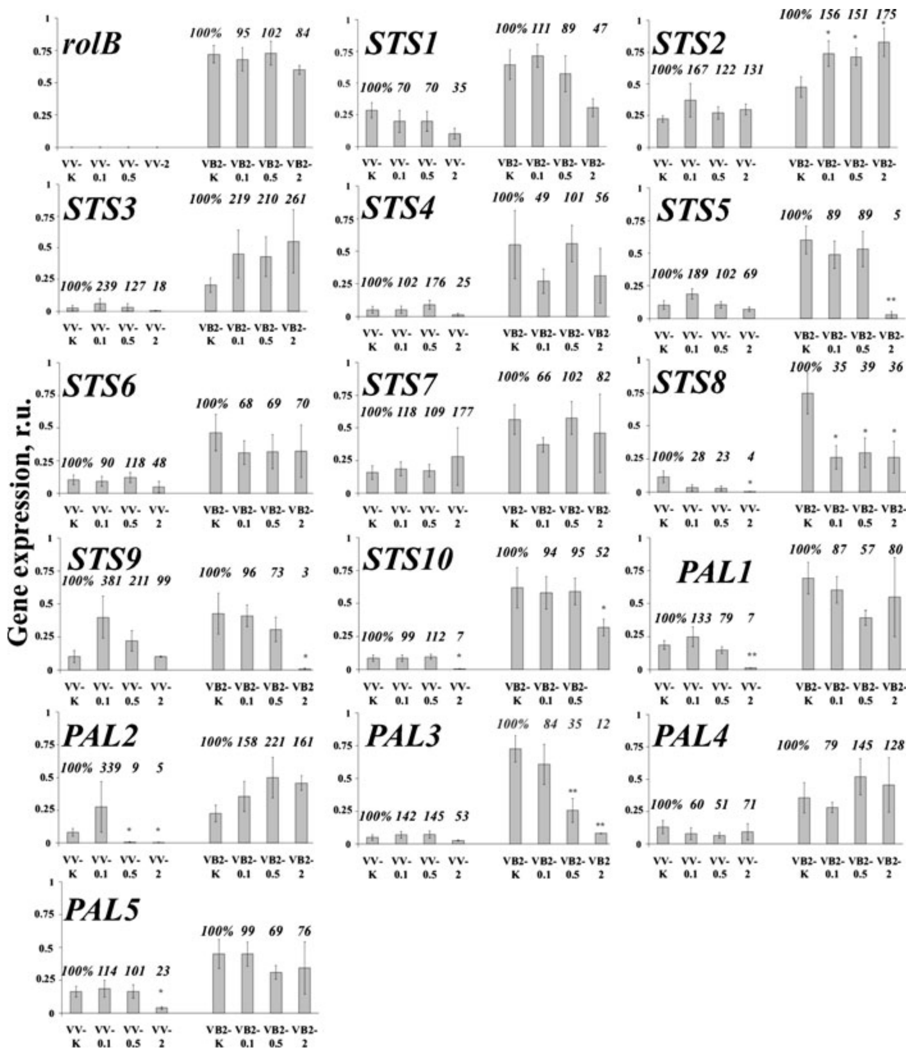


**Fig. 2** *rolB*, *VaPAL*, and *VaSTS* expression levels detected by real-time PCR in *V. amurensis* cell cultures VV and VB2 treated with CA. Note: *r.u.*—relative units. The data are presented as mean±SEM obtained from three independent experiments, *$p<0.05$, **$p<0.01$ vs. values of the VV or VB2 cultures without CA

transgenic cell cultures of *V. amurensis*. Feeding with CA considerably increased resveratrol production by the grape cell cultures. Interestingly, the *rolB* transgenic cell line exhibited lower inducibility to the CA treatment compared to the control cell line. Resveratrol production was increased in the VV and VB2 cell cultures in 10.2 and 1.5 times, respectively. The level of resveratrol production by the VV cell culture treated with CA was 1.5, 1.8, and 1.7 times higher than that in the SA-, MeJa-, and Phe-treated V2 cell culture which is the parent cell line for the VV cell line [8], but lower than in *E. coli* transformed with the *STS* gene [26, 27] and *V. vinifera* cell cultures treated with CDs [13–15]. The data suggest that activation of resveratrol biosynthesis is more efficient when feeding the cells with precursors which are closer than Phe. Analysis of *PAL* and *STS* gene expression under

CA treatment suggests that CA increased resveratrol accumulation in the calli of *V. amurensis* via selective enhancement of expression of individual *STS* genes (especially *VaSTS2* and *VaSTS3* genes). It is noteworthy that the influence of CA treatment on *PAL* and *STS* gene expression and expression of other genes important for plant secondary metabolism has not been studied before.

CA increased resveratrol production up to 2.9 (0.1 mM CA) and 13.1 mg/l (0.5 mM CA) in the VV and VB2 cell cultures, respectively. CA at 0.1 and 0.5 mM equals to 16.6 and 82 mg/l which means that no more than 15% of the CA pool in the calli converted to resveratrol. Efficiency of CA utilization in our system is lower than that in the *E. coli* cultures transformed with *STS* gene, wherein about 90% of CA added to the medium converted to resveratrol [23–25]. It is possible that there are some proteins which degraded CA or led to its release in the extracellular space and decreased its content in the cell cultures of *V. amurensis*. CA unavailability could be the rate limiting step of resveratrol accumulation in the control and *rolB* transgenic calli of *V. amurensis*. Further analysis of expression of the genes playing key roles in resveratrol biosynthesis and degradation pathways would help obtain plant cell cultures with high level of resveratrol production.

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## References

1. Jeandet, P., Douillt-Breuil, A. C., Bessis, R., Debord, S., Sbaghi, M., & Adrian, M. (2002). Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *Journal of Agricultural and Food Chemistry, 50*, 2731–2741.
2. Shankar, S., Singh, G., & Srivastava, R. K. (2007). Chemoprevention by resveratrol: molecular mechanisms and therapeutic potential. *Frontiers in Bioscience, 12*, 4839–4854.
3. Kiselev, K. V. (2011). Perspectives for production and application of resveratrol. *Applied Microbiology and Biotechnology, 90*, 417–425.
4. Aggarwal, B. B., Bhardwaj, A., Aggarwal, R. S., Seeram, N. P., Shishodia, S., & Takada, Y. (2004). Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Research, 24*, 2783–2840.
5. Langcake, P., & Pryce, R. J. (1977). A new class of phytoalexins from grapevines. *Experientia, 33*, 151–152.
6. Tassoni, A., Fornale, S., Franceschetti, M., Musiani, F., Michael, A. J., Perry, B., et al. (2005). Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. *The New Phytologist, 166*, 895–905.
7. Belhadj, A., Telef, N., Saigne, C., Cluzet, S., Barrieu, F., Hamdi, S., et al. (2008). Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. *Plant Physiology and Biotechnology, 46*, 493–499.
8. Kiselev, K. V., Dubrovina, A. S., Veselova, M. V., Bulgakov, V. P., Fedoreyev, S. A., & Zhuravlev, Y. N. (2007). The *rolB* gene-induced overproduction of resveratrol in *Vitis amurensis* transformed cells. *Journal of Biotechnology, 128*, 681–692.
9. Teguo, P. W., Decendit, A., Krisa, S., Deffieux, C., Vercauteren, J., & Merillon, J. M. (1996). The accumulation of stilbene glycosides in *Vitis vinifera* cell suspension cultures. *Journal of Natural Products, 59*, 1189–1191.
10. Krisa, S., Larronde, F., Budzinski, H., Decendit, A., Deffieux, G., & Merillon, J. M. (1999). Stilbene production by *Vitis vinifera* cell suspension cultures: methyl jasmonate induction and C-13 biolabeling. *Journal of Natural Products, 62*, 1688–1690.

11. Ku, K. L., Chang, P. S., Cheng, Y. C., & Lien, C. Y. (2005). Production of stilbenoids from the callus of *Arachis hypogaea*: a novel source of the anticancer compound piceatannol. *Journal of Agriculture and Food Chemistry, 53*, 3877–3881.
12. Kiselev, K. V., Tyunin, A. P., Manyakhin, A. Y., & Zhuravlev, Y. N. (2011). Resveratrol content and expression patterns of stilbene synthase genes in *Vitis amurensis* cells treated with 5-azacytidine. *Plant Cell Tissue and Organ Culture, 105*, 65–72.
13. Morales, M., Bru, R., Garcia-Carmona, F., Barcelo, A. R., & Pedreno, M. A. (1998). Effect of dimethyl-*b*-cyclodextrins on resveratrol metabolism in Gamay grapevine cell cultures before and after inoculation with *Xylophilus ampelinus*. *Plant Cell Tissue and Organ Culture, 53*, 179–187.
14. Bru, M.R., & Pedreno, G.M.L.D.E. (2006). Method for the production of resveratrol in cell cultures. *US patent* 2006/0205049.
15. Lucas-Abellan, C., Fortea, I., Lopez-Nicolas, J. M., & Nunez-Delicado, E. (2007). Cyclodextrins as resveratrol carrier system. *Food Chemistry, 104*, 39–44.
16. Gomez-Galera, S., Pelacho, A. M., Gene, A., Capell, T., & Christou, P. (2007). The genetic manipulation of medicinal and aromatic plants. *Plant Cell Reports, 26*, 1689–1715.
17. Giorcelli, A., Sparvoli, F., Mattivi, F., Tava, A., Balestrazzi, A., Vrhovsek, U., et al. (2004). Expression of the stilbene synthase (*StSy*) gene from grapevine in transgenic white poplar results in high accumulation of the antioxidant resveratrol glucosides. *Transgenic Research, 13*, 203–214.
18. Schwekendiek, A., Spring, O., Heyerick, A., Pickel, B., Pitsch, N. T., Peschke, F., et al. (2007). Constitutive expression of a grapevine stilbene synthase gene in transgenic hop (*Humulus lupulus* L.) yields resveratrol and its derivatives in substantial quantities. *Journal of Agriculture and Food Chemistry, 55*, 7002–7009.
19. Veena, V., & Taylor, C. G. (2007). *Agrobacterium rhizogenes*: recent developments and promising applications. *In Vitro Cellular and Development Biology-Plant, 43*, 383–403.
20. Medina-Bolivar, F., Condori, J., Rimando, A. M., Hubstenberger, J., Shelton, K., O'Keefe, S. F., et al. (2007). Production and secretion of resveratrol in hairy root cultures of peanut. *Phytochemistry, 68*, 1992–2003.
21. Dubrovina, A. S., Manyakhin, A. Y., Zhuravlev, Y. N., & Kiselev, K. V. (2010). Resveratrol content and expression of phenylalanine ammonia-lyase and stilbene synthase genes in *rolC* transgenic cell cultures of *Vitis amurensis*. *Applied Microbiology and Biotechnology, 88*, 727–736.
22. Rupprich, N., Hildebrand, H., & Kindl, H. (1980). Substrate specificity in vivo and in vitro in the formation of stilbenes—biosynthesis of rhaponticin. *Archives of Biochemistry and Biophysics, 200*, 72–78.
23. Donnez, D., Jeandet, P., Clement, C., & Courot, E. (2009). Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. *Trends in Biotechnology, 27*, 706–713.
24. Beekwilder, J., Wolswinkel, R., Jonker, H., Hall, R., de Vos, C. H. R., & Bovy, A. (2006). Production of resveratrol in recombinant microorganisms. *Applied and Environmental Microbiology, 72*, 5670–5672.
25. Becker, J. V. W., Armstrong, G. O., Van der Merwe, M. J., Lambrechts, M. G., Vivier, M. A., & Pretorius, I. S. (2003). Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of the wine-related antioxidant resveratrol. *FEMS Yeast Research, 4*, 79–85.
26. Watts, K. T., Lee, P. C., & Schmidt-Dannert, C. (2006). Biosynthesis of plant-specific stilbene polyketides in metabolically engineered *Escherichia coli*. *BMC Biotechnology, 6*, 22.
27. Katsuyama, Y., Funa, N., Miyahisa, I., & Horinouchi, S. (2007). Synthesis of unnatural flavonoids and stilbenes by exploiting the plant biosynthetic pathway in *Escherichia coli*. *Chemistry & Biology, 14*, 613–621.
28. Spena, A., Schmulling, T., Koncz, C., & Schell, J. S. (1987). Independent and synergistic activity of *rol* A; B and C loci in stimulating abnormal growth in plants. *EMBO Journal, 6*, 3891–3899.
29. Kiselev, K. V., Dubrovina, A. S., & Bulgakov, V. P. (2009). Phenylalanine ammonia-lyase and stilbene synthase gene expression in *rolB* transgenic cell cultures of *Vitis amurensis*. *Applied Microbiology and Biotechnology, 82*, 647–655.
30. Bekesiova, I., Nap, J. P., & Mlynarova, L. (1999). Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. *Plant Molecular Biology Reporter, 17*, 269–277.
31. Dubrovina, A. S., Kiselev, K. V., Veselova, M. V., Isaeva, G. A., Fedoreyev, S. A., & Zhuravlev, Y. N. (2009). Enhanced resveratrol accumulation in *rolB* transgenic cultures of *Vitis amurensis* correlates with unusual changes in *CDPK* gene expression. *Journal of Plant Physiology, 166*, 1194–1206.
32. Kiselev, K. V., Turlenko, A. V., & Zhuravlev, Y. N. (2009). *PgWUS* expression during somatic embryo development in a *Panax ginseng* 2c3 cell culture expressing the *rolC* oncogene. *Plant Growth Regulation, 59*, 237–243.
33. Kiselev, K. V., & Dubrovina, A. S. (2010). A new method for analysing gene expression based on frequency analysis of RT-PCR products obtained with degenerate primers. *Acta Physiologiae Plantarum, 32*, 495–502.

34. Kiselev, K. V., Kusaykin, M. I., Dubrovina, A. S., Bezverbny, D. A., Zvyagintseva, T. N., & Bulgakov, V. P. (2006). The *rolC* gene induces expression of a pathogenesis-related beta-1,3-glucanase in transformed ginseng cells. *Phytochemistry, 67*, 2225–2231.
35. Kiselev, K. V., Dubrovina, A. S., Isaeva, G. A., & Zhuravlev, Y. N. (2010). The effect of salicylic acid on phenylalanine ammonia-lyase and stilbene synthase gene expression in *Vitis amurensis* cell culture. *Russian Journal of Plant Physiology, 57*, 415–421.
36. Kiselev, K. V., Turlenko, A. V., & Zhuravlev, Y. N. (2010). Structure and expression profiling of a novel calcium-dependent protein kinase gene *PgCDPK1a* in roots, leaves, and cell cultures of *Panax ginseng*. *Plant Cell Tissue and Organ Culture, 103*, 197–204.