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MINI-REVIEW

# Perspectives for production and application of resveratrol

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**Abstract** The polyphenol *trans*-resveratrol (3,5,4′-trihydroxy-trans-stilbene) is one of the best known plant secondary metabolites. The number of articles devoted to *trans*-resveratrol has been steadily increasing. *Trans*-resveratrol is a molecule that is beneficial to human health; this explains the high level of interest in *trans*-resveratrol among different research groups. Therefore, it is important to develop an effective method to produce this compound commercially. The applicability of biotechnology for *trans*-resveratrol extraction is still uncertain. This review describes and compares the available biotechnological methods of *trans*-resveratrol production, focusing on their advantages and disadvantages.

**Keywords** Cell cultures · Resveratrol · Microorganisms

## Resveratrol is one of the most promising molecules from plants

Seventy years have passed since the first isolation of resveratrol from white hellebore, *Veratrum grandiflorum* O. Loes (Takaoka 1940). The name presumably comes from the fact that it is a resorcinol derivative coming from a *Veratrum* species. Currently, there are thousands of articles describing the metabolism and bioactive properties of *trans*-resveratrol (3,5,4′-trihydroxy-trans-stilbene) and its close derivatives. Most of this work has appeared in the past 20 years. In the past decade, there was a fivefold increase in the number of articles describing different properties of *trans*-resveratrol, according to a Web of Science search. The number of articles devoted to *trans*-resveratrol has been increasing by 40% each year.

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## Application of *trans*-resveratrol in nutrition and medicine

*Trans*-resveratrol is a beneficial molecule to human health, which explains its high appeal to different research groups. *Trans*-resveratrol has been associated with the "French paradox" because its daily consumption (e.g., in the form of red wine) helps prevent the development of cardiovascular diseases (Gronbaek et al. 2000; Jeandet et al. 1995; Stervbo et al. 2007). Red wine contains between 0.2 and 5.8 mg/L (Gu et al. 1999) depending on the grape variety, while white wine has much less. Resveratrol exerts its protective actions through the regulation of nitric oxide production (Giovannini et al. 2001), and it prevents thrombocyte aggregation through regulation of the synthesis of eicosanoids (Olas et al. 2001). In addition to red wine, other foods may represent valuable sources of *trans*-resveratrol, such as Itadori tea, peanuts, pistachios, peanut butter, and chocolate (Burns et al. 2002; Tokusoglu et al. 2005; Counet et al. 2006; Hurst et al. 2008).

It has been shown that *trans*-resveratrol, which possesses powerful antioxidant properties, interferes with oxidative modifications of lipids (Frankel and Waterhouse 1993; Leighton et al. 1999). Due to its antioxidant properties, *trans*-resveratrol has been hypothesized to play a role in the prevention of cardiovascular diseases (Bradamante et al. 2004) and may also provide some protection against certain types of cancer (Aggarwal et al. 2004; Kundu and Surh 2008). The anticancerogenic properties of this substance were first found in 1997 (Jang et al. 1997); since then, there have been numerous publications on the ability of *trans*-

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resveratrol to influence cellular mechanisms, including the regulation of cellular proliferation and growth (Roemer and Mahyar-Roemer 2002; Pervaiz 2003; Aggarwal et al. 2004). *Trans*-resveratrol possesses antineoplastic activity at the stages of establishment and growth of a tumor and in the formation of metastases (Aziz et al. 2003a). The anticarcinogenic effects of *trans*-resveratrol reside in its capabilities to inhibit cellular proliferation and have a direct cytotoxic effect in tumor cells (Shankar et al. 2007). Resveratrol halts both cellular growth and apoptosis by regulating numerous signaling pathways. Moreover, *trans*-resveratrol appears to protect against diabetes (Sameer et al. 2007; Sharma et al. 2007) and neurodegenerative diseases such as Alzheimer's disease (Anekonda 2006; Vingtdeux et al. 2008; Wang et al. 2006) through its induction of the Sirtuin 1 gene. Resveratrol has been proposed to extend the lifespan of lower and higher organisms by mimicking the caloric restriction effect, thus decreasing age-related maladies (Wood et al. 2004; Valenzano et al. 2006; Barger et al. 2008; Pearson et al. 2008).

Currently, *trans*-resveratrol is primarily consumed in the form of numerous biological additives. However, the described valuable properties of the *trans*-resveratrol are promising to find application in pharmaceutical industries.

### Application of *trans*-resveratrol in agriculture

The influence of *trans*-resveratrol on plant physiology is also interesting especially for agriculture. *Trans*-resveratrol shows antifungal activity (Jeandet et al. 2002; Adrian and Jeandet 2006); in leaves and berries, it acts as a phytoalexin that is produced in response to stresses, such as wounding or pathogen attack (Langcake and Pryce 1976). In wood, *trans*-resveratrol is produced constitutively and acts as a phytoanticipin (VanEtten et al. 1994). In addition, some plants have been genetically modified by transferring stilbene synthase or resveratrol synthase (*STS*) gene to produce *trans*-resveratrol for the assessment of its potential roles in health promotion and disease control in plants, such as in tobacco, tomato, and poplar (Giorcelli et al. 2004; Halls and Yu 2008; Delaunois et al. 2009). Transgenic plants producing resveratrol by the overexpression of *STS* genes have shown increased resistance against various fungal infections (Thomzik et al. 1997).

## Methods to obtain *trans*-resveratrol commercially

Because *trans*-resveratrol is an interesting molecule in terms of human health, it is important to develop an effective method to obtain it commercially. At the present time, *trans*-resveratrol is primarily produced for the derivation of its biologically active additives by field cultures of *Polygonum cuspidatum* (syn. *Fallopia japonica*). China is the main producer of *trans*-resveratrol from *Polygonum* root extracts. Several Chinese companies (e.g., Wuxi Gorunjie Technology Co. and Shanghai DND Pharm-Technology Co.) produce *trans*-resveratrol from *Polygonum* root extracts, with different degrees of purity. However, using recombinant microorganisms and plant cell suspensions is particularly promising because these biotechnologically engineered systems represent a reliable alternative method of *trans*-resveratrol production under controlled conditions. The use of biotechnology for *trans*-resveratrol extraction is still uncertain. This review describes and compares the available biotechnological methods of *trans*-resveratrol production, focusing on their advantages and disadvantages.

### Perspectives on trans-resveratrol production in plant cell cultures

Currently, cell and tissue cultures of different plant species can routinely be established under sterile conditions from explants, such as plant leaves, stems, roots, and meristems (Sahai and Knuth 1985). Strain improvement, methods for the selection of high-producing cell lines, and medium optimizations can produce an enhancement in secondary metabolite production. Although cultures of plant cells are also capable of producing resveratrol derivatives, this work will only discuss studies in which resveratrol itself was isolated. The level of *trans*-resveratrol production in plant cells is often represented by different units; in the present paper, *trans*-resveratrol production is indicated by mg/L or % of the dry weight (DW) or fresh weight (FW) (Table 1). The production of *trans*-resveratrol in plant cell cultures, e.g., cell cultures of *Vitis* spp. and *Arachis hypogaea*, has been analyzed by several groups (Table 1). Analysis of *trans*-resveratrol production in untreated plant cell cultures (Teguo et al. 1996; Krisa et al. 1999; Ku et al. 2005; Tassoni et al. 2005) revealed a low level of *trans*-resveratrol accumulation, less than 0.01% DW or 2–3 mg/L. Therefore, various strategies, such as pathogen attack, environmental stresses, biotransformation using an exogenous supply of biosynthetic precursors or chemical elicitors and genetic manipulation must be considered to improve the production of *trans*-resveratrol. Many efforts have been made to overcome the inability of cultured plant cells to synthesize high levels of *trans*-resveratrol (Table 1).

### The effect of pathogen attack on *trans*-resveratrol content in plant cells

It is well-known that the invasion of microbes such as fungi, bacterium, or insects in some plants can induce the biosynthesis of stilbenoids such as *trans*-resveratrol. For example, Yang et al. (2010) showed a significant increase (0.002%

**Table 1** Resveratrol production in plant cell cultures

| Plant | Type of culture | Inducer/elicitor/ precursor | Maximal quantity of *trans*-resveratrol | References |
|---|---|---|---|---|
| *Arachis hypogaea* L. cv. Andru II | Hairy roots | Sodium acetate | 0.1–0.35% DW[b], 0.005–0.018% FW[b], 18–35 mg/L[a] | Medina-Bolivar et al. 2007 |
| *Arachis hypogaea* L. | Hairy roots | Control | 0.15% DW[b], 0.008% FW[b], 11.4 mg/L[a] | Kim et al. 2008 |
| *Arachis hypogaea* L. cv. Tainan no. 14 | Callus | UV irradiation | 0.02% DW[b], 0.001% FW[a], 0.24 mg/L[b] | Ku et al. 2005 |
| | Cell suspension culture | UV irradiation | 0.01% DW[b], 0.001% FW[a], 0.14 mg/L[b] | Ku et al. 2005 |
| *Gossypium hirsutum* L. | Cell suspension culture | Control | <0.001% DW[a], <0.001% FW[b], 0.01 mg/L[b] | Kouakou et al. 2006 |
| *Vitis amurensis* Rupr. | Callus | Phenylalanine | 0.05% DW[a], 0.003% FW[a], 2.4 mg/L[a] | Kiselev et al. 2007 |
| | | SA | 0.05% DW[a], 0.003% FW[a], 4.7 mg/L[a] | Kiselev et al. 2007 |
| | | MeJA | 0.06% DW[a], 0.003% FW[a], 2.3 mg/L[a] | Kiselev et al. 2007 |
| | | Transformation with *rolB* gene | 3.15% DW[a], 0.195% FW[b], 200–315 mg/L[b] | Kiselev et al. 2007 |
| | | Transformation with *rolC* gene | 0.14% DW[a], 0.006% FW[a], 14.3 mg/L[a] | Dubrovina et al. 2010 |
| | | 5-azacytidine, block DNA methylation | 0.10% DW[a], 0.004% FW[a], 5.1 mg/L[a] | Kiselev et al. 2010 |
| *Vitis labrusca* L. cv. "Washington Concord" | Cell suspension culture | L-Alanine | 0.22% DW[b], 0.011% FW[b], 2.2 mg/L[b] | Chen et al. 2006 |
| *Vitis vinifera* L. cv. Barbera | Cell suspension culture | MeJA | <0.01% DW[a], <0.001% FW[b], 0.11 mg/L[b] | Tassoni et al. 2005 |
| | | Control | 0.02% DW[b], 0.001% FW[b], 1.6 mg/L[b] | Ferri et al. 2009 |
| *Vitis vinifera* L. cv Gamay Freaux var. Teinturier | Cell suspension culture | MeJA and sucrose | 0.06% DW[b], 0.003% FW[a], 5.5 mg/L[b] | Belhadj et al. 2008 |
| *Vitis vinifera* L. var. Gamay | Cell suspension culture | DIMEB (5 mM) | 0.51% DW[b], 0.025% FW[b], 100.3 mg/L[a] | Morales et al. 1998 |
| | | DIMEB and incubation with *Xylophilus ampelinus* | 1.02% DW[b], 0.051% FW[b], 202.9 mg/L[a] | Morales et al. 1998 |
| *Vitis vinifera* cv. Gamay rouge | Cell suspension culture | DIMEB (50 mM)<br>RAMEB (50 mM) | 15.3% DW[b], 0.765% FW[b], 3,060 mg/L[a]<br>16.6% DW[b], 0.83% FW[b], 3,320 mg/L[a] | Bru and Pedreno 2006 |
| *Vitis vinifera* cv. Monastrell albino | | DIMEB (50 mM) | 23.4% DW[b], 1.17% FW[b], 4,680 mg/L[a] | |
| | | RAMEB (50 mM) | 25.2% DW[b], 1.26% FW[b], 5,027 mg/L[a] | |
| *Vitis vinifera* L. cv. Monastrell albino | Cell suspension culture | DIMEB (50 mM)<br>DIMEB and MeJA | 5.02% DW[b], 0.251% FW[a], 753 mg/L[a]<br>36.51% DW[b], 1.823% FW[b], 3,651 mg/L[b] | Lijavetzky et al. 2008 |
| *Vitis vinifera* L. cv. Barbera | Cell suspension culture | Sucrose | 0.01% DW[b], <0.001% FW[b], 6.7 mg/L[b] | Ferri et al. 2011 |

*DW* dry weight, *FW* fresh weight, *UV* ultraviolet, *MeJA* methyl jasmonate, DIMEB 2,6-di-O-methyl-β-cyclodextrin

[a] Indicates the quantity of resveratrol obtained in the cited article

[b] Indicates the quantity of resveratrol calculated from data on resveratrol content in terms of fresh or dry biomass accumulation given that plant cells usually accumulate 10–20 g/L dry and 200–300 g/L fresh biomass of cultivated cells

FW) in the level of *trans*-resveratrol production after the fungal invasion of *Botryodiplodia theobromae*. Also, young plants of *Vitis vinifera* cv. (cultivar) Chardonnay, Touriga Nacional and two clones of cv. Aragonez were infected using two strains of *Phaeomoniella chlamydospora* and were harvested after 5 months from inoculation. Resveratrol was found to augment significantly in tested plants, to 0.012% FW (Martin et al. 2009). Berries of *V. vinifera* L. cv. Barbera were infected during ripening by a conidial suspension of *Aspergillus* spp. to control stilbene-induced synthesis (Bavaresco et al. 2003). All the tested fungi, except *Aspergillus fumigatus*, significantly increased *trans*-resveratrol synthesis over the control (4.3 μg/g FW or 0.0004% FW).

## The effect of ultraviolet (UV) irradiation and wounding on *trans*-resveratrol content in plants and plant cell cultures

Accumulation of resveratrol in leaves of peanut *A. hypogea* increased over 60-fold (0.006% FW or 56 μg/g FW) in response to UV light after 12 h and threefold (0.6 μg/g FW) in response to wounding after 24 h after treatment (Chung et al. 2003). Also, postharvest UV C-treatment was used to induce stilbene biosynthesis in grapes (Guerrero et al. 2010). After UV irradiation, the Syrah variety reached a maximum 20 μg/g FW or 0.002% FW of *trans*-resveratrol in the grape plants.

Cell cultures of *A. hypogaea* responded to UV irradiation with an increase in *trans*-resveratrol production up to

12 μg/g FW or 0.001% FW, which equals to 0.03% DW; however, the *trans*-resveratrol content decreased up to 2 μg/g FW within 24 h of the treatment (Ku et al. 2005). A higher level of *trans*-resveratrol production (0.006% FW or 63 μg/g FW) was detected in 12-day-old cell cultures of *V. vinifera* L. after 48-h UV treatment (Keskin and Kunter 2008).

## Increasing *trans*-resveratrol content by genetic manipulations in plants and plant cell cultures

### *STS genes*

The genetic transformation of plant cells with various genes can significantly increase the production of secondary metabolites (Gomez-Galera et al. 2007); therefore, genetic transformation is now considered a possible means of increasing the production of secondary metabolites. Stilbenes, including *trans*-resveratrol, are synthesized via the phenylpropanoid pathway (Langcake and Pryce 1977). Stilbene synthase (STS, EC 2.3.1.95) condenses three molecules of malonyl-CoA and one molecule of coumaroyl-CoA to form resveratrol (Rupprich et al. 1980). Therefore, many researchers have used overexpression of the *STS* genes to increase the *trans*-resveratrol content in plant cells (Kobayashi et al. 2000; Coutos-Thevenot et al. 2001; Giorcelli et al. 2004; Husken et al. 2005; Schijlen et al. 2006; Schwekendiek et al. 2007). Plants are usually genetically modified to produce *trans*-resveratrol to assess its role in human health promotion and plant disease control. Unfortunately, there is generally only information about the *trans*-resveratrol content of the transgenic plants, not the cell cultures. It is interesting to compare the *trans*-resveratrol levels in different plant cells overexpressing the *STS* genes. For example, the transformation of various plants with the *Vst1* gene resulted in an increase in the levels of *trans*-resveratrol and its derivatives. The highest *trans*-resveratrol levels (up to 350 μg/g DW or 0.035% DW) were registered after the transformation of grape cells with *Vst1* under the control of the PR10 promoter (Coutos-Thevenot et al. 2001). In this case, the levels of *trans*-resveratrol increased only after elicitor treatment (UV light or *Botrytis* infection). Furthermore, high *trans*-resveratrol levels were seen in only one of 50 transgenic strains. This increase might thus be caused by mutations of the genes involved in *trans*-resveratrol biosynthesis, provoked by the integration of exogenous sequences. In *STS* transgenic plants, the *trans*-resveratrol and piceid levels varied but were not more than 0.05% DW (Kobayashi et al. 2000; Giorcelli et al. 2004; Husken et al. 2005; Schijlen et al. 2006; Schwekendiek et al. 2007). The creation of *STS* transgenic plants has been attempted in a number of studies (Kobayashi et al. 2000; Coutos-Thevenot et al. 2001; Giorcelli et al. 2004; Husken et al. 2005; Schijlen et al. 2006; Schwekendiek et al. 2007). However, none of the attempts resulted in high *trans*-resveratrol levels. It is possible that the enzymes encoded by the *STS* genes used in these studies do not actively participate in *trans*-resveratrol biosynthesis or have less specific activities (Kiselev et al. 2009a).

### *rol genes*

Plant tissues can be transformed by transferring the bacterial Ri plasmid T-DNA from *Agrobacterium rhizogenes*, which causes a genetic modification that leads to the development of hairy roots. For the production of *trans*-resveratrol, hairy root cultures of peanut *A. hypogea* were induced when elicited with sodium acetate (Medina-Bolivar et al. 2007), and *trans*-resveratrol production was increased 60-fold after 24 h, with *trans*-resveratrol representing 99% of the secreted stilbenes, at concentrations from 18 to 35 mg/L (Table 1). Because bacterial infection frequency, growth, and production of stilbenes depend on the strain of *A. rhizogenes* used (Kim et al. 2008), strain selection is crucial for successful applications of hairy root technology to stilbene production.

The integration of individual *rol* genes of *A. rhizogenes* into the plant genome and transformation with wild strains of *Agrobacterium* may enhance the biosynthesis of certain groups of secondary metabolites (Palazon et al. 1998; Bonhomme et al. 2000). Recently, it was shown that *rolB* and *rolC* from *A. rhizogenes* enhance *trans*-resveratrol production in cell cultures of *Vitis amurensis* Rupr (Kiselev et al. 2007; Dubrovina et al. 2009; Dubrovina et al. 2010). A high level of *rolB* expression resulted in a more than 100-fold increase in *trans*-resveratrol production (up to 3.15% DW or 250 mg/L) in the transformed culture compared to the control culture (Table 1). *RolB* may increase *trans*-resveratrol production via selective enhancement of the expression of particular genes of the *PAL* and *STS* families in *V. amurensis* (Kiselev et al. 2009a). Unfortunately, these transformed calli showed a compact and globular morphology, as compared to the friable nature of normal calli; this was not compatible with the establishment of cell suspensions.

## Increasing *trans*-resveratrol content in plant cell cultures using its biosynthetic precursors or chemical elicitors

*V. vinifera* suspension cultures responded to methyl jasmonate (MeJA) and sodium orthovanadate by a five to sixfold increase in *trans*-resveratrol production up to 0.01% DW (Tassoni et al. 2005). Other cell suspensions, such as those from *V. vinifera* cv. Gamay, produced up to 0.01% DW of *trans*-resveratrol following MeJA elicitation and an increase in the sugar concentration of the culture medium

(Belhadj et al. 2008). Experiments with MeJA, salicylic acid (SA), and sodium nitroprusside (a nitric oxide donor), using *V. amurensis* cell cultures (Kiselev et al. 2007) support the results from other studies that high *trans*-resveratrol production cannot be initiated by simple elicitor treatments. The possibility that a low pool of *trans*-resveratrol precursors was the cause of the low *trans*-resveratrol yield is unlikely because manipulations, such as the addition of phenylalanine to the culture medium, were not effective (Kiselev et al. 2007). The levels of *trans*-resveratrol in plant cell cultures after treatment with compounds including MeJA and SA (Teguo et al. 1996; Krisa et al. 1999; Ku et al. 2005; Tassoni et al. 2005; Kiselev et al. 2007) did not exceed 0.05% DW.

The most significant success in increasing *trans*-resveratrol content in plant cells has been reached using cyclodextrins (CDs). CDs are a family of compounds made up of sugar molecules bound together in a ring (cyclic oligosaccharides). CDs are produced from starch by means of enzymatic conversion. They are used in the food, pharmaceutical, and chemical industries, as well as in agriculture and environmental engineering. A typical CD contains several glucose monomers ranging from six to eight units in a ring, forming a cone shape. β-cyclodextrin, a seven sugar ring molecule, was often used for increasing *trans*-resveratrol content in plant cell cultures (Morales et al. 1998; Bru and Pedreno 2003; Bru and Pedreno 2006; Lucas-Abellan et al. 2007).

Cell suspension cultures of *V. vinifera* cv. Gamay Rouge were treated with CDs, and the production of *trans*-resveratrol was analyzed (Bru and Pedreno 2006). The treatment resulted in high levels of *trans*-resveratrol, more than 3,000 mg/L (Table 1), after elicitation with the CDs 2,6-di-O-methyl-β-cyclodextrin (DIMEB), randomly methylated-beta-cyclodextrins (RAMEB), and CAVASOL®W7 (the trade name of WACKER's cyclodextrin derivatives and covers a wide portfolio of α, β, and γ derivatives). Similarly, a *V. vinifera* cv. Monastrell albino cell suspension culture accumulated high amounts of *trans*-resveratrol: up to 4,963 mg/L after CAVASOL®W7 treatment and up to 5,027 mg/L after RAMED treatment (Bru and Pedreno 2006). One of the limiting factors in resveratrol production in plant cell cultures is the series of post-biosynthetic steps. In plant cells, there are several enzymes that metabolize *trans*-resveratrol to single compounds or resveratrol derivatives (Chong et al. 2009). Such high *trans*-resveratrol concentrations are possible because CDs not only act as elicitors but also protect *trans*-resveratrol from post-biosynthetic modifications in the medium by forming a complex with it (Bru and Pedreno 2006; Lucas-Abellan et al. 2007). DIMEB, RAMEB, and CAVASOL®W7 are the strongest inducers of *trans*-resveratrol biosynthesis in plant cells (Table 1). However, most researchers have only used 50 mM (~67 g/L) of DIMEB. According to www.cyclolab.hu, the price of CDs is high. Therefore, it appears that the cost of *trans*-resveratrol production using this method of biosynthesis induction in plant cell culture would also be high.

## The past, present, and perspectives of employed methods for increasing trans-resveratrol content in plant cells

Previously, it was common to treat various plants and plant cell cultures with well-known elicitors and resveratrol precursors to increase *trans*-resveratrol content. In the experiments, the resulting level of *trans*-resveratrol accumulation was less than 100 mg/L. Recently, significant progress in increasing *trans*-resveratrol content in plant cells has been reached using transformation of grape cells with the *rolB* gene of *A. rhizogenes* and treatment various plant cell cultures with CDs (250–5,027 mg/L). An effective method to obtain resveratrol commercially appears to be based on combination of different approaches and requires searching more effective and cheap elicitors and genes for plant transformation.

Also, the production of secondary metabolites through plant cell cultures presents an additional challenge because the level of secondary metabolite production during long-term cultivation is often unstable and unpredictable. It has been demonstrated that cultivating plant cells *in vitro* for a long time induces various mutations, and genetic anomalies have been observed in both cultured cells and plants regenerated from cultured cells. These mutations include cytological abnormalities such as ploidy changes and chromosome rearrangements, single base substitutions, changes in the copy numbers of repeated sequences, and alterations in DNA methylation patterns (Rani and Raina

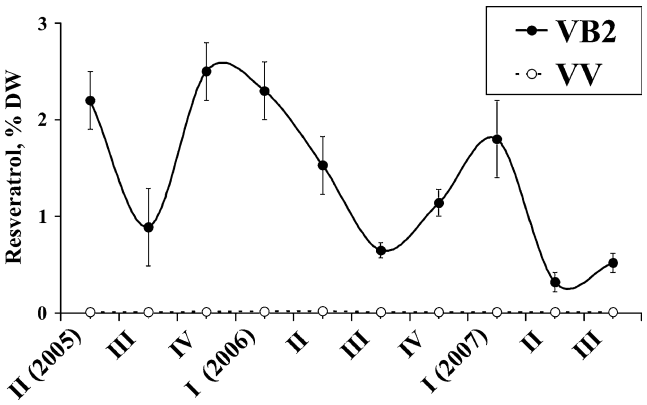


**Fig. 1** A long-term analysis of resveratrol accumulation in control (VV) and *rolB*-transgenic (VB2) callus cultures of *V. amurensis* (Kiselev et al. 2007) in continuous 35-day subcultures. I, II, III, and IV indicate quarter of years in which measurements of the resveratrol content were made. There were at least two HPLC measurements (Kiselev et al. 2007) in every quarter

2000; Kaeppler et al. 2000; Kiselev and Bulgakov 2009; Kiselev et al. 2009b). The mechanisms of both somatic and meiotic inheritance of variations can contribute to a decline in the vigor and regenerability of cultures over time (Kaeppler et al. 2000). For example, a three-year cultivation of *rolB*-transgenic grape cells (Kiselev et al. 2007) resulted in a gradual loss of the high level of *trans*-resveratrol production (Fig. 1). Currently, the cultivated cells synthesize no more than 1% DW *trans*-resveratrol (Fig. 1). However, this value is significantly above that observed in the control cell culture of *V. amurensis* (Fig. 1). The addition of MeJA and SA, which are well-known inducers of plant secondary metabolism, increased *trans*-resveratrol accumulation to 2% DW. However, it is not known how long cells can synthesize high amounts of *trans*-resveratrol in response to treatment with MeJA or SA. Studying mutagenesis in plant cell culture may promote an understanding of how to manipulate plant cell cultures for the profitable commercial production of biologically active compounds.

## Bioproduction of *trans*-resveratrol in microorganisms

Microorganisms are widely used as model organisms for the production of various valuable molecules (Chemler and Koffas 2008). Engineering bacteria or yeast for *trans*-resveratrol production may represent an efficient means of high-level production of *trans*-resveratrol. The issues involved in using microorganisms for large-scale production of various compounds have been described (Donnez et al. 2009). This review discusses only established results for *trans*-resveratrol production in yeast and bacteria.

The genes responsible for *trans*-resveratrol biosynthesis are not found in microorganisms; therefore, it is necessary to use recombinant microorganisms. A strategy for *trans*-resveratrol production in microorganisms might include transformation with the 4-coumaroyl-CoA ligase (*4CL*) and *STS* genes, which are required for *trans*-resveratrol biosynthesis when 4-coumaric acid is added to the culture medium as a precursor.

*Saccharomyces cerevisiae* (CEN.PK113-3b strain) transformed with the *4CL* gene from tobacco and the *STS* gene from grapevine produced 5.8 mg/L *trans*-resveratrol, the highest level of *trans*-resveratrol that has been obtained in yeast culture (Beekwilder et al. 2006). Other strains have produced less *trans*-resveratrol under similar conditions (Becker et al. 2003; Zhang et al. 2006; Katz et al. 2008). *Escherichia coli* transformation with these genes produced more *trans*-resveratrol than transgenic yeast. A metabolically engineered *E. coli* (JM109 strain), which was transformed with the *4CL* gene from *Arabidopsis thaliana* and the *STS* gene from *A. hypogea*, was able to convert 4-coumaric acid into *trans*-resveratrol, producing a 100 mg/L yield (Watts et al. 2006). When the same *E. coli* strain was modified with the *4CL* gene from *Lithospermum erythrorhizon* and the *STS* gene from *A. hypogea* and coumaroyl-CoA was used as a substrate, *trans*-resveratrol production reached 171 mg/L (Katsuyamal et al. 2007).

## Conclusions

This paper reviewed recent data on valuable biological activities of *trans*-resveratrol and its production using various biotechnological methods. Resveratrol is one of the most promising molecules synthesized by plants. The highest level and rate of *trans*-resveratrol production were detected in *Vitis* suspension cultures where *trans*-resveratrol production reached 1,257 mg/L per day (Bru and Pedreno 2006; Donnez et al. 2009). The highest rate of *trans*-resveratrol production in bacterial cells was ten times lower (126 mg/L per day) than in plant cell cultures (Katsuyamal et al. 2007; Donnez et al. 2009). However, it is necessary to use expensive elicitors to induce and maintain high levels of *trans*-resveratrol production in plant cell cultures. The high rates of accumulation of mutations in plant cells necessitate some limitations in the subsequent commercial use of these cell cultures. The efficiency of recombinant microorganisms in *trans*-resveratrol biosynthesis depends on factors such as the species and strains and the origin of the transferred gene as well as other parameters such as plasmids and resveratrol precursors. Analysis of the literary data indicates that the level of *trans*-resveratrol obtained in microorganisms remains low. Therefore, further investigation is required before we can confidently identify the best method of *trans*-resveratrol production.

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