Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 579089, 14 pages
doi:10.1155/2012/579089

Review Article

Metabolic Engineering of Yeast and Plants for the Production of the Biologically Active Hydroxystilbene, Resveratrol

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Received 11 February 2012; Accepted 4 March 2012

Academic Editor: Alberto Inga

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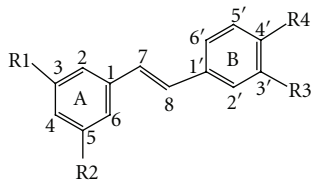
Resveratrol, a stilbenic compound deriving from the phenyalanine/polymalonate route, being stilbene synthase the last and key enzyme of this pathway, recently has become the focus of a number of studies in medicine and plant physiology. Increased demand for this molecule for nutraceutical, cosmetic and possibly pharmaceutic uses, makes its production a necessity. In this context, the use of biotechnology through recombinant microorganisms and plants is particularly promising. Interesting results can indeed arise from the potential of genetically modified microorganisms as an alternative mechanism for producing resveratrol. Strategies used to tailoring yeast as they do not possess the genes that encode for the resveratrol pathway, will be described. On the other hand, most interest has centered in recent years, on *STS* gene transfer experiments from various origins to the genome of numerous plants. This work also presents a comprehensive review on plant molecular engineering with the *STS* gene, resulting in disease resistance against microorganisms and the enhancement of the antioxidant activities of several fruits in transgenic lines.

1. Introduction

Hydroxystilbenes (hereafter referred to as stilbenes) are natural phenolic compounds occurring in a number of plant families including Vitaceae, Dipterocarpaceae, Gnetaceae, Pinaceae, Poaceae, Fabaceae, Leguminoseae, and Cyperaceae [1]. Although polyphenolic compounds display an enormous chemical diversity, stilbenes seem to constitute a rather restricted group of molecules, the skeleton of which is based on resveratrol especially in Vitaceae and Fabaceae (Figure 1) [2]. Resveratrol is one of the most extensively studied natural products, doubtless for its large spectrum of biological activities in human health as a cardioprotective, an antitumor, a neuroprotective, and an antioxidant agent. Some of the resveratrol's properties have been associated with the benefits of a moderate consumption of red wine. Many roles have also been ascribed to resveratrol and its derivatives (Figure 1) in plants; namely, they constitute antimicrobial, deterrent or repellent compounds acting as allelochemicals or phytoalexins, protecting them from attacks by fungi, bacteria, nematodes, or herbivores [3–5].

According to these potential activities in plants and humans, the interest for resveratrol has increased. Currently, rising demand for resveratrol and derivatives for nutraceutical, cosmetic, and putatively pharmaceutical uses makes their production a necessity. Metabolic engineering for resveratrol production thus has significant commercial value. As all the genes encoding enzymes responsible for resveratrol biosynthesis have been cloned and characterized in detail, this makes molecular engineering of this compound relatively straightforward.

Most interest has now centered upon metabolic engineering of resveratrol in plants with the objectives of increasing tolerance of the latter to pathogenic microorganisms and



	R1	R2	R3	R4
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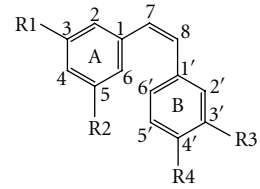
2	OH	GlcO	H	OH



	R1	R2	R3	R4
3	OH	OH	H	OH
4	OH	GlcO	H	OH

Figure 1: Molecular structures of *trans*- and *cis*-resveratrol 1 and 3, and *trans*- and *cis*-piceid, 2 and 4, respectively (GlcO = β-D-glucose).

improving the nutritional quality of food products through the expression of pharmaceutically active compounds in plants incapable of synthesizing resveratrol.

Microorganisms can also be used to heterologously express the resveratrol biosynthetic pathway to obtain this compound in valuable amounts. In this paper, we will discuss the potential of tailored microorganisms, specifically yeast, and plants for resveratrol production.

2. Resveratrol in Health and Disease

Resveratrol was identified in 1940 as a constitutive compound of the roots of white hellebore (*Veratrum grandiflorum*) [6]. Resveratrol is also present in high amounts in the roots of *Polygonum cuspidatum* used in traditional Chinese and Japanese medicine, and this compound was acclaimed for its wondrous effects for the treatment of human fungal diseases (suppurative dermatitis, gonorrhea favus, and athlete's foot), hyperlipidemia, atherosclerosis, or inflammations. Moreover, resveratrol recently has been shown to be a potent therapeutic agent [7–10].

First, there is considerable evidence that resveratrol acts both as a free radical scavenger [11, 12] and a potent antioxidant doubtless for its ability to promote the activities of numerous antioxidative enzymes [12, 13]. Resveratrol inhibits lipid peroxidation which is an indicator of possible free radical damage to cellular membranes [14, 15]. Resveratrol may operate in a number of antioxidant mechanisms leading to the development of atherosclerosis, protecting, for example, LDL molecules against peroxidation [16–18] or reactive oxygen species production by blood platelets [19]. There is however some evidence that resveratrol exhibits prooxidant activity under certain experimental conditions (in the presence of copper ions) [20], causing oxidative DNA damage that may lead to cell cycle arrest or apoptosis [21].

Resveratrol is also considered as an antiproliferative agent for cancer [22], exerting an antitumor activity either as a cytostatic or as a cytotoxic agent in various types of cancer. Resveratrol induces cell death through positive induction of death receptor-mediated apoptosis [23–25], mitchondria-mediated apotosis [26], and nuclear (transcription) factor-mediated apotosis [27, 28].

Refractory disease and poor prognosis in many tumors have been related with high expression of antiapoptotic molecules such as survivin; resveratrol was proven to act at this level through survivin depletion [29]. Similarly, numerous works showed that resveratrol is a potent inhibitor of cell cycle progression, causing G1 phase arrest [30], inhibiting G0-G1 transition in human lymphocyte [31] or perturbing progression through S and G2 phases in cultured bovine artery endothelial cell proliferation [32]. Since the pioneering work of the group of Pezzuto [33], affording for the very first time evidence for the cancer chemoprotective activity of resveratrol on HL-60 and Hepa 1clc7 cells, many other models of human cancers were used, confirming these findings. The most frequently described mode of action for resveratrol concerns apoptosis (see above), the response depending on the expression of the tumor suppressor gene p53. A number of related factors can be modulated by resveratrol, such as activation of caspases, decreases in the antiapoptotic proteins Bcl-2 and Bcl-x^L [34, 35], increases in the proapoptotic proteins Bax [36], inhibition of cyclins and cyclin-dependent kinases, that is, proteins implicated in cell cycle progression [32, 37], and interference with nuclear transcription factor kappa B (NF-κB)—and activator protein 1 (AP-1)—mediated cascades.

Most importantly, there are several works concerning both the antitumor effects and the antitumor mechanisms of resveratrol *in vivo*. In several studies, it was shown, namely, that resveratrol inhibits the development of skin cancer *in vivo* by topical applications, causing a significant reduction of the tumor diameter and the tumor incidence [33, 38, 39]. Moreover, resveratrol significantly inhibits the UV-B-mediated increase in skin thickness and skin edema [40]. On the other hand, resveratrol was proven not to be very effective in inhibiting the progression of leukemia *in vivo*, even when high doses were used [41], despite its antileukemic activity *in vitro* and though resveratrol can be converted to the known antileukemic related stilbene, piceatannol [42]. In regard to breast cancer, resveratrol was shown to reduce N-methyl-N-nitrosourea-induced mammary tumorigenesis in female rats at high doses (100 mg/kg body weight) though being uneffective at lower doses (10 mg/kg body weight) [43]. Otherwise, some authors have shown that resveratrol glucosides, namely, piceid (a 3-O-β-D-resveratrol glucoside), administered orally or intraperitoneally to mice, reduced tumor volume, tumor weight, and metastasis in lung carcinoma [44, 45]. A few investigations about the antitumor effects

of resveratrol and derivatives against liver cancers have been reported to date, showing that this compound administered orally or intraperitoneally to rats caused a 25% reduction in tumor cell numbers and restrained hepatoma cell invasion but not proliferation [46, 47]. Finally, orally administration of resveratrol to mice was shown to prevent colonic tumor formation and reduce small intestinal tumors by 70% [48].

Neurologic benefits of resveratrol described experimentally concern the following diseases: cerebral ischemia in rats [49, 50], amyotrophic lateral sclerosis [51], Parkinson's disease in rats [52], spinal cord lesions in rabbits [53], brain edema and tumors in human cells [54], seizure in rats [55–57], pain and cognitive impairment in rats [58, 59]. According to Doré [60], the neuroprotective effects of resveratrol could be mediated by regulation of the heme oxygenase antioxidant systems in neurons, especially in case of age-related vascular dementia and Alzheimer's disease [61].

There are several mechanisms to support resveratrol as a cardioprotective agent such as inhibition of LDL peroxidation, reduction of the degree of neointimal hyperplasia and restenosis, inhibition of platelet aggregation together with chemoprevention of atherosclerogenesis. Resveratrol was proven to inhibit LDL peroxidation *via* antioxidant and free scavenging activities in *ex vivo* rat heart studies [62] and circumstantial evidence of its potent role in preventing atherosclerogenesis could thus be given [63, 64]. Resveratrol was shown to inhibit neointimal hyperplasia in several animal studies and vascular smooth muscle cell proliferation in a rabbit model of restenosis [65, 66] and to block platelet aggregation from high-cholesterol-fed rabbits [67].

Resveratrol exerts some activities against a range of bacteria affecting humans (*Chlamydia pneumonia*, the cause of human acute respiratory tract infections [68], and *Helicobacter pylori*, the main agent responsible for chronic gastritis and peptic ulcer disease [69, 70]). Resveratrol also inhibited the growth of several bacteria known to be major agents of human skin infections such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* [71]. Similarly, resveratrol completely inhibits the growth of *Neisseria gonorrhoeae* (responsible for the sexually transmitted disease gonorrhoea) [72]. Stilbenes generally also have biocidal activities against plant fungal pathogens (namely, *Botrytis cinerea*, the causal organism for gray mold, *Pyricularia oryzae*, the agent of pyriculariosis, *Plasmopara viticola*, the causal agent for downy mildew [73–77], fungi associated with esca of grapevine [78]), and human fungal pathogens such as *Candida albicans*, an agent of candidiasis [79].

Finally, resveratrol has been shown to extend the lifespan of lower organisms, yeast [80], and metazoans [81], *via* the sirtuin/Sir2 family. More recently, a study has confirmed the conservation of these preventive and protective mechanisms controlling lifespan extension in higher organisms such as mice [82]. Using a similar approach, other works reached the same conclusions with regard to the protective effects of resveratrol against diet-induced obesity and insulin resistance [7, 82].

Otherwise, DNA-damaging products can induce premature senescence in cancer cells, limiting tumor development. However, senescent cancer cells may reenter the cell cycle and lead to tumor relapse. Recently, resveratrol was remarked by its ability to induce DNA damage in cancer cells. In fact, resveratrol suppressed viability and induced DNA damage in human head and neck squamous carcinoma cells [83]. Similarly, human squamous cancer cells treated with resveratrol were shown to express oxidative stress-mediated DNA damage [84].

3. Biosynthesis of Resveratrol

Stilbene phytoalexins, as flavonoid-type phytoalexins, are formed on the phenylalanine/polymalonate route, being the last step of this biosynthesis pathway catalyzed by stilbene synthase (STS) (Figure 2). *Trans*-resveratrol can be synthesized either starting with phenylalanine or from tyrosine, both pathways giving rise to *para*-coumaric acid through, respectively, the phenylalanine ammonia lyase (PAL) and the cinnamate 4 hydroxylase (C4H), or directly through the tyrosine ammonia lyase (TAL). A *para*-coumaric-acid coenzyme A ligase (4CL) transforms the *para*-coumaric acid into *para*-coumaroyl-CoA, which then leads to *trans*-resveratrol after condensation with three molecules of malonyl-CoA through STS activity [85]. STS belongs to the so-called type III of the polyketide synthase enzyme superfamily, a class of enzymes which carry out iterative condensation reactions with malonyl-CoA. [86, 87]. Resveratrol synthesis is induced in plants as a response to fungal infection [73, 74, 88], abiotic stresses (UV irradiation, metallic salts, methyl jasmonate) [89–93] as well as to natural compounds eliciting plant defense responses [94, 95] or nonpathogenic rhizobacteria [96, 97].

STS is encoded by a multigene family mainly comprising the resveratrol-forming *STS* genes from grapevine (*pSV21*, *pSV25*, *pSV696*, *pSV368*, and *StSy*) [98], (*Vst1*, *Vst2*, *Vst3*) [99], the *AhRS* gene from *Arachis hypogea* [100], and an STS-encoding gene from *Parthenocissus henryana* [101]. Other *STS* genes have also been isolated from pine [102–104], together with a stilbene synthase gene from *Vitis riparia* cv Gloire de Montpellier [105]. The *Sb STS1* gene isolated from *Sorghum* is at present the only one *STS* gene described in a Monocotyledonous plant [106]. Knowledge of the resveratrol biosynthetic route thus paves the way for metabolic engineering in microorganisms or plants.

4. Tailoring Yeast for Resveratrol Biosynthesis

Microorganisms are widely used biological systems whose engineering may be useful for the production of numerous valuable molecules and can lead to interesting applications for example in the wine industry [107–109]. Engineering bacteria or yeast for resveratrol might thus represent a valuable means of its production in large quantities. Their tailoring is necessary because microorganisms do not possess the genes that encode for the resveratrol pathway. In this paper, we will focus only on yeast tailoring for resveratrol production. Two main strategies have already been used to that end: (i) introducing the entire biosynthetic pathway

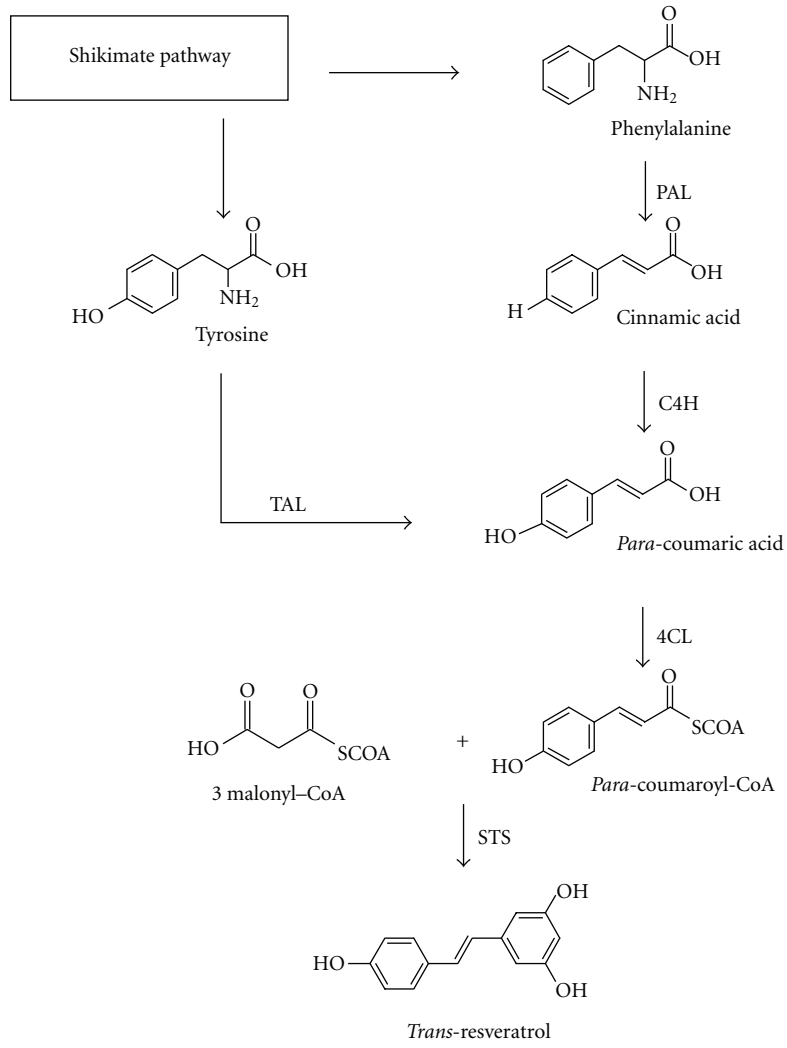


Figure 2: Biosynthesis of resveratrol via the phenylalanine/polymalonate pathway. PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamate-4-hydroxylase; 4CL, *para*-coumaric acid: coenzyme A ligase; STS, stilbene synthase.

using aromatic amino acids as substrates (L-phenylalanine or L-tyrosine) [110–113] and (ii) introducing specific genes, such as *para*-coumaroyl-CoA ligase and stilbene synthase, starting with *para*-coumaric acid as a substrate (Figure 2) [114–120].

4.1. Engineering the Entire Pathway. To obtain resveratrol from its precursors L-phenylalanine or L-tyrosine on the phenylpropanoid route appears to be the most promising option in terms of production cost. The entire resveratrol pathway has been introduced successfully into the oleaginous yeast *Yarrowia lipolytica* (ATCC 20362 strain) with the genes encoding for phenylalanine/tyrosine ammonia lyase (PAL/TAL), cinnamate-4-hydroxylase (4CH), *para*-coumaroyl-CoA ligase (4CL), and stilbene synthase (STS) activities [110] (Table 1). Metabolic engineering was completed by constitutive expression of malonyl CoA, a precursor for both naringenin-chalcone, the first C15 intermediate in the flavonoid route, and stilbene synthesis. Genes that encode for each required enzyme from different plant species (grapevine, peanuts) have been tested. In the best performing yeast, genes that encode for the PAL and TAL from *Rhodotorula glutinis*, the 4CL from *Streptomyces coelicolor*, and an STS from *Vitis* sp. have been used, and the resulting production of resveratrol reached in this system 1.46 mg/L [110].

Current efforts to increase resveratrol bioproduction in yeast have focused, so far not surprisingly, on heterologous expression of the genes encoding for the enzymes involved in the phenylpropanoid pathway and for STS, in baker yeast *Saccharomyces cerevisiae* [111, 113–120]. Namely, the entire resveratrol pathway has been introduced in *S. cerevisiae* (CEN.PK113-5D strain), but also in molds such as *Aspergillus niger* (FGSC A913 strain) and *A. oryzae* (MG1363 strain). In these cases, pathway expression started with the PAL enzyme. The ability of tailored *S. cerevisiae* to produce resveratrol was characterized, but it was not synthesized in measurable amounts in this system. [111]. More recently,

Table 1: Metabolic engineering of resveratrol in yeast.

Microorganisms/species	Introduced gene(s)	Origin of genes	Resveratrol quantity	References
Yeast *Yarrowia lipolytica*	*PAL/TAL, C4H*, *4CL*, *STS*	*Rhodotorula glutinis* (*PAL/TAL*), *Streptomyces coelicolor* (*4CL*), *Vitis* sp. (*STS*)	1.46 mg/L	[110]
Yeast *Saccharomyces cerevisiae*	*PAL, C4H*, *4CL*, *STS*	*Arabidopsis thaliana* (*PAL, C4H, 4CL*), *Rheum tataricum* (*STS*)	not detectable	[111]
Yeast *Saccharomyces cerevisiae*	*TAL, 4CL*::*STS fusion protein*	*Rhodobacter sphaeroides* (*TAL*), *Arabidopsis thaliana* (*4CL*), *Vitis vinifera* (*STS*)	5.25 mg/L	[116]
Yeast *Saccharomyces cerevisiae*	*4CL*, *STS*	*Populus trichocarpa*×*Populus deltoides* (*4CL*), *Vitis vinifera* (*STS*)	1.45 mg/L	[114]
Yeast *Saccharomyces cerevisiae*	*4CL*, *STS*	*Nicotiana tabacum* (*4CL*), *Vitis vinifera* (*STS*)	5.8 mg/L	[115]
Yeast *Saccharomyces cerevisiae*	*4CL1*, *STS*	*Arabidopsis thaliana* (*4CL*), *Vitis vinifera* (*STS*)	262–391 mg/L	[117]
Yeast *Saccharomyces cerevisiae* with phenylalanine	*PAL, CPR, C4H, 4CL*, *STS*	*Populus trichocarpa*×*P. deltoides* (PAL, CPR[a]) *Glycine max* (*C4H, 4CL*), *Vitis vinifera* (*STS*)	0.29 mg/L	[113]
Yeast *Saccharomyces cerevisiae* with *para*-coumaric acid	*PAL, CPR, C4H, 4CL*, *STS*	*Populus trichocarpa*×*P. deltoides* (*PAL, CPR*) *Glycine max* (*C4H, 4CL*), *Vitis vinifera* (*STS*)	0.31 mg/L	[113]
Yeast *Saccharomyces cerevisiae*	*TAL, 4CL*::*STS fusion protein, araE transporter*	*Rhodobacter sphaeroides* (*TAL*), *Escherichia coli* (*araE*), *Arabidopsis thaliana* (*4CL*), *Vitis vinifera* (*STS*)	3.1 mg/L 1.27 mg/L (without the araE transporter)	[119]
Yeast *Saccharomyces cerevisiae*	*TAL, 4CL*::*STS fusion protein*	*Rhodobacter sphaeroides* (*TAL*), *Arabidopsis thaliana* (*4CL*), *Vitis vinifera* (*STS*)	14.4 mg/L	[120]

[a] CPR: Cytochrome P450 Reductase.

Trantas et al. [113] constructed the complete resveratrol biosynthetic pathway in *S. cerevisiae* to produce resveratrol from phenylalanine. When the medium was supplemented with 10 mM of phenylalanine, the strain produced 0.29 mg/L *trans*-resveratrol after about 120 h of cultivation. One can thus admit that the introduction of the complete resveratrol pathway in yeast leads to a low production of this compound when compared to some other data obtained in engineered bacteria where resveratrol synthesis can reach up to 100–170 mg/L [121, 122].

4.2. Introducing Selective Genes. An alternative strategy to engineering the entire pathway is directed towards transforming microorganisms with selective genes [114–120] (Table 1). The yeast strain *S. cerevisiae* FY23 was transformed with both the *4CL* and *STS* genes under utilization of *para*-coumaric acid as a precursor (added to the culture medium). However, in this system, resveratrol production remained low (1.45 mg/L) [114]. Interestingly, in another study, authors reported that transformation of *S. cerevisiae* (CEN.PK113-3b strain) with the *4CL* gene from tobacco and the *STS* gene from grapevine enabled it to produce resveratrol in higher quantities (5.8 mg/L) [115]. A *S. cerevisiae* (WAT11 strain) cotransformed with 4CL and STS constructs and fed with *para*-coumaric acid only produced 0.65 mg/L resveratrol, but, when the 4CL and the STS-encoding genes that were added to the yeast genome were submitted to protein fusion, yeast expressing 4CL::STS fusion protein exhibited a 10-fold increase in resveratrol production (5.25 mg/L resveratrol) compared to the coexpression of 4CL and STS [116]. This underlines the importance of the spatial localization of these two related enzymes [116]. In another study of the same group [120], authors have utilized a synthetic scaffold to recruit the 4CL1 and STS enzymes of the resveratrol pathway to improve resveratrol production in *S. cerevisiae*. A 5-fold improvement of the resveratrol production was obtained over the nonscaffolded control, and a 2.7-fold increase (14.4 mg/L within 96 h incubation) was finally observed over the previous reported study with protein fusion [116]. This work clearly demonstrated that synthetic scaffolds can be used for the optimization of engineered metabolic pathway.

Most importantly, it has recently been shown that yeast cells expressing *4CL*, *STS* genes together with the *araE* gene encoding for a high-capacity *Escherichia coli* transporter, but with no affinity for resveratrol itself, could enhance resveratrol accumulation. Yeast cells carrying the *araE* gene produced up to 3.1 mg/L, that is, 2.44-fold higher resveratrol than the control cells [119]. Such an engineered yeast was also proven to increase the resveratrol content in a white wine during the fermentation process [119].

It should be noted that the efficacy of recombinant microorganisms for resveratrol production depends on various factors, such as the species and the strain, the origin of the transferred genes, culture conditions as well as other parameters such as plasmids or precursors used (Table 1). A recent study [117] has shown that fermenting yeast expressing the *para*-coumaroyl-coenzyme A ligase (4CL1) from *Arabidopsis thaliana* and the stilbene synthase from *Vitis vinifera* in a rich medium could considerably improve resveratrol production, rising from a few milligrams per liter to 262 mg/L in rich medium using a laboratory strain.

Moreover, resveratrol amounts reached up to 391 mg/L when fermentation was achieved with an industrial Brazilian sugar cane yeast [117].

Taken together, these data indicate that the levels of resveratrol that can be produced by microorganisms remain low, although optimization of the processes might be possible.

5. Tailoring Plants for Resveratrol Biosynthesis

As resveratrol is a potent phytoalexin against plant pathogens and can enhance plant resistance to microbial disease, earlier applications of resveratrol engineering focused primarily on this antimicrobial potential [123, 124]. Tailoring plants for resveratrol synthesis thus constitutes the second aspect of this paper. Particular attention will be given in this section to the choice of the promoters and the enhancer elements used to improve STS transcriptional activity in the transgenes as well as the biological benefits of resveratrol production in terms of enhancement of antioxidant activity in fruits and legumes.

We have seen that tailoring yeast needs the introduction of the entire resveratrol pathway (requiring at least 4 or 5 genes) or the introduction of selective genes (requiring at least 2 genes) depending on the precursor used, phenylalanine or *para*-coumaric acid. Tailoring plants for resveratrol synthesis appear to be very simple, since STS is a key enzyme of resveratrol synthesis utilizing as substrates precursor molecules that are present throughout the plant kingdom. The introduction of a single gene is thus sufficient to synthesize resveratrol in heterologous plant species. A lot of transformations were then operated to investigate the potential of stilbene biosynthetic genes to confer resistance to pathogens or to increase their nutritional values [123, 124].

5.1. Production of Resveratrol in Transgenic Plants: Gene and Promoter Options. The first gene transfer experiment was performed by the group of Kindl with a complete *STS* gene from *Arachis hypogea* introduced into tobacco [100], leading to resveratrol accumulation in response to short-wavelength ultraviolet light. This experiment was then continued by the same group by the transfer of two grapevine *STS* genes, *Vst1* and *Vst2*, in tobacco, conferring to the plant a higher resistance to *Botrytis cinerea* infection. This work constitutes the first report of a disease resistance resulting from foreign phytoalexin expression in a novel plant [125]. Since this pioneering work, *STS* genes have been transferred to a number of plants, including rice [126], barley and wheat [127–130], alfalfa [131], kiwifruit [132], grapevine [133, 134] apple [135, 136], aspen [137], papaya [138], white poplar [139], oilseed rape [140], banana [141], *Rehmannia* [142] tomato [143–147], *Arabidopsis* [148], lettuce [101], pea [149], and hop [150] (Table 2).

In grapevine, genome sequencing has revealed a large array of *STS* genes, with 43 genes identified and 20 of these being shown to be expressed [151]. But to date, only a few *STS* genes from grapevine are used for the metabolic engineering of plants, being *Vst1* and *Stsy* the most commonly genes chosen (Table 2). STS-encoding genes from other plants have also been used, notably the *AhRS* gene from *Arachis hypogea* [100, 131], the *SbSTS1* gene from *Sorghum bicolor* [106, 148], and an STS-encoding gene from *Parthenocissus henryana* [101]. Chimeric genes or a combination of two STS encoding genes, based on *Vst1* and *Vst2*, can increase significantly resveratrol production in the transformed lines [128, 152].

On a practical point of view, the modulation of gene expression is mainly controlled by the promoter chosen to drive the transgene. By now, a limited number of promoters (the constitutive promoter pCaMV35S, its own stress-responsive promoter pVst1, the fungus-inducible promoter pPR10.1 or the tissue specific promoter p-nap) have been used for the expression of STS-encoding genes upon plant transformation (Table 2 and references therein). As expected, the pCaMV35S promoter, which is the most commonly used to overexpress a transgene in plants [152], triggered strong and constitutive stilbene accumulation (Table 2). In this case, stilbene synthesis is higher than that observed with inducible promoters, but, as a consequence, causes a drastic depletion of the endogenous pools of precursors. In grapevine, the pVst1 promoter which is induced either by biotic factors (pathogens, elicitors) or abiotic stresses (wounding, UV light), allows high stilbene production without interfering with secondary biosynthetic pathways. Generally, it appears thus preferable to transform plants with a construct having a pathogen-inducible promoter in order to avoid depletion of other pathways.

Expression of the *STS* gene may be optimized by the utilization of enhancer elements [127, 130] and/or heterologous promoters [131–135, 140, 152] to improve STS transcription. Combination of the pVst1 promoter with the 35S enhancer element [127, 130] or use of a chimeric promoter resulting, for example, from a fusion between the alfalfa pPR10.1 promoter and the pVst1 promoter can lead to higher expression of the transgene without affecting promoter inducibility or specific expression patterns [127], or to an increased production of resveratrol upon fungal infection (reaching 5–100-fold the levels found in nontransgenic leaves [133]). Finally, tissue-specific promoters such as the p-nap seed-specific napin promoter can be used to induce stilbene production [140].

At this stage of the discussion, it appears important to underline that the choice of the promoter for *STS* gene expression should be performed depending on the expected results: when the enhancement of plant resistance against pathogens through resveratrol production is searched, thus one can recommend the choice of a strong constitutive promoter or a pathogen-inducible promoter (see above). As far as may concern this review, when the improvement of food products is sought through, for example the increase of the antioxidant activity of the transformed plants, a tissue-specific [140, 147] or inducible promoter would be a better option.

STS genes were transferred to plants by means of *Agrobacterium* spp. in tomato [143], grapevine [133], kiwifruits [132] or particle bombardment in barley and wheat [127–129]. However, as epigenetic modifications may occur and lead to expression variability, decreasing the ability

Table 2: Metabolic engineering of stilbene synthase in plants, and resulting effects on stilbene levels, resistance to pathogens, and antioxidant activities.

Plant/species	Introduced gene(s)	Promoter	Produced stilbene(s)	Stilbene quantity (mg/kg of FW)	Biological activity	References
Tobacco (*Nicotiana tabacum L.*)	*Arachis hypogea STS*	Stress-induced promoter	Resveratrol	—	—	[100]
	Grapevine Vst1 and Vst2	Stress responsive pVst1	Resveratrol	400	Resistance to *Botrytis cinerea*	[125]
	Chimeric *STS* gene	Constitutive CaMV 35S	Resveratrol	50 to 290	Altered flower morphology, male sterility	[152]
Rice (*Oryza sativa L.*)	*Grapevine Vst1*	Stress responsive pVst1	—	—	Resistance to *Pyricularia oryzae* ?	[126]
Wheat (*Triticum aestivum L.*)	*Grapevine Vst1*	Combination pVst1 +35S enhancer	—	—	Resistance to *Botrytis cinerea*	[127, 129]
	Chimeric *STS* gene	Maize ubiquitin promoter	Resveratrol	2	—	[128]
	Grapevine Vst1 and *Vst2*	Combination pVst1 +35S enhancer	Unknown derivative stilbene compounds	35 to 190	Resistance to *Puccinia recondita* and *Septoria nodorum*	[130]
Barley (*Hordeum vulgare L.*)	*Grapevine Vst1*	Combination pVst1 +35S enhancer	—	—	Resistance to *Botrytis cinerea*	[127]
Alfalfa (*Medicago sativa L.*)	*Arachis hypogea* STS gene *(AhRS)*	Constitutive CaMV 35S	Piceid	0.5 to 20	Resistance to *Phoma medicaginis*	[131]
Arabidopsis thaliana L.	*Sorghum SbSTS1*	Constitutive CaMV 35S	Piceid	584	—	[106, 148]
Kiwi (*Actinidia deliciosa*)	*pSV25*	Constitutive CaMV 35S	Piceid	20 to 182	No resistance to *Botrytis cinerea*	[132]
Grapevine (*Vitis vinifera L.*)	*Grapevine Vst1*	Fungus inducible *ms* PR 10.1	Resveratrol		*In vitro* resistance to *Botrytis cinerea*	[133]
	Vitis pseudoreticulata STS	Constitutive CaMV 35S	Resveratrol	2.586	Not determined	[134]
Apple (*Malus domestica*)	*Grapevine Vst1*	Stress responsive pVst1	Unknown resveratrol-glycoside	—	—	[135]
	Grapevine Vst1	Stress responsive pVst1	Piceid	3 to 7 for non-UV-irradiated fruit and 23 to 62 for UV-irradiated fruit	No influence on other phenolic compounds	[136]
Tomato (*Lycopersicon esculentum Mill.*)	*Grapevine Vst1* and *Vst2*	Stress responsive pVst1	Resveratrol	—	Resistance to *Phytophthora infestans* No resistance to *Botrytis cinerea* and *Alternaria solani*	[143]
	Grapevine StSy	Constitutive pCaMV 35S	Resveratrol and piceid	4 to 53	Antioxidant primary metabolism and increase in total antioxidant activity	[144]

Table 2: Continued.

Plant/species	Introduced gene(s)	Promoter	Produced stilbene(s)	Stilbene quantity (mg/kg of FW)	Biological activity	References
	Grapevine StSy	Constitutive pCaMV 35S	Resveratrol and piceid	0.1 to 1.2	Enhancement of natural antiradical properties	[145]
	Grapevine StSy	Constitutive pCaMV 35S	Resveratrol and piceid	0.42 to 126 depending on the stage of ripening and fruit samples	Differences in rutin, naringenin, and chlorogenic acid contents	[146]
	Grapevine StSy	Fruit-specific promoter TomLoxB	Resveratrol and piceid		Increases in total antioxidant capability and ascorbic acid content	[147]
Rehmannia glutinosa Libosch.	*Arachis hypogea AhRS3*	Constitutive pCaMV 35S	Resveratrol and piceid	22 to 116 up to 650 with stress treatment	Antioxidant capabilities Resistance to *Fusarium oxysporum*	[142]
Lettuce (*Lactuca sativa L.*)	*Parthenocissus henryanaSTS*	Constitutive pCaMV 35S	Resveratrol	56.4	Effect on Hela cell morphology	[101]
Pea (*Pisum. sativum L.*)	*Grapevine Vst1*	Stress responsive pVst1	Occurrence of two resveratrol-glucoside compounds	0.53 to 5.2	—	[149]
White poplar (*Populus alba L.*)	*Grapevine StSy*	Constitutive pCaMV 35S	Piceid	309 to 615	No *in vitro* resistance to *Melampsora pulcherrima*	[137, 139]
Papaya (*Carica papaya L.*)	*Grapevine Vst1*	Stress responsive pVst1	Resveratrol glucoside	54	Resistance to *Phytophthora palmivora*	[138]
Oilseed rape (*Brassica napus L.*)	*Grapevine Vst1*	Tissue specific p-nap	Resveratrol glucoside	361 to 616	Food quality improvement: high piceid rate content and reduction of sinapate esters	[140]
Hop (*Humulus lupulus L.*)	*Grapevine Vst1*	Constitutive pCaMV 35S	Piceid, unknown stilbene astringin, resveratrol	490 to 560	Higher amounts of flavonoids and acids	[150]

of the transgenes for stilbene synthesis, the selection of plants with a single gene insertion will be more appropriate. As a consequence, the use of *Agrobacterium*-mediated transformation, which leads to lower transgene insertion numbers, should preferentially be chosen [124].

5.2. Stilbene Production and Biological Benefits of Resveratrol Synthesis in STS Engineered Plants. Expression of *STS* genes resulted in resveratrol accumulation in transgenic lines. The obtained stilbene amounts are generally higher than those reached in engineered yeast, ranging from a few mg/kg fresh weight to hundreds of mg/kg fresh weight (see Table 2). Both free resveratrol and its glycosylated forms can be recovered in plant extracts [144]: piceid, a 3-O-β-D-resveratrol glucoside, occurred in different plant species transformed with *STS* genes (Table 2) [106, 132, 136, 139, 140, 142, 145, 146, 148, 150]. However, levels of accumulated stilbenes depend on the plant species (probably because of different endogenous pools of enzymes or precursors, as well as differences in secondary metabolism pathways), the promoter used (constitutive or inducible promoters), the ripening stage in case of transgenic fruits [136, 144] and the age of the organs [125, 132].

From a practical viewpoint, the ectopic production of resveratrol observed in transformed plants can lead to broad-spectrum resistance against fungi in transgenic plants, but

disparate effects were observed (Table 2). Some works indeed reported transformations improving the resistance of rice to *Pyricularia oryzae* [126], tomato to *Phytophthora infestans* [143], barley and wheat to *B. cinerea* [127, 129], wheat to *Oidium tuckeri* [127, 129], alfalfa to *Phoma medicaginis* [131] and papaya to *Phytophthora palmivora* [138]. Conversely, in some others, no resistance was observed after transforming the plants with stilbene synthase genes. For example, transformation of white poplar (*Populus alba*) with *STS*, leading to the accumulation of piceid, does not confer any increased resistance to rust disease (*Melaspora pulcherrima*) [137, 139]. Similarly, no increased resistance against *B. cinerea* has been observed in STS transgenic kiwi plants [132].

With regard to the improvement of the nutritional value of agricultural crops and fruits, there are already several studies reporting an increase of the antioxidant activities in transgenic tomatoes and apples overexpressing STS-encoding genes [136, 144, 147]. In tomato, for example, resveratrol accumulation in transgenic fruit increased their global antioxidant activities, as well as their contents in other well-known antioxidants such as ascorbic acid and glutathione [144]. Antioxidant activity, as a consequence of resveratrol accumulation, was also shown to suffer a two-fold increase in transgenic tomato fruit *versus* controls [145]. Moreover, a correlation was found between resveratrol concentrations and antioxidant activities in ripe and unripe fruits [145]. More recently [147], tomato plants expressing a stilbene synthase gene *(StSy)* under the control of a fruit-specific promoter (promoter TomLoxB) were shown to accumulate resveratrol and piceid in the skin of the mature fruits, being the resveratrol content of the plants transformed with the specific promoter TomLoxB 20-fold lower than that of plants previously transformed with the constitutive pCaMV35S promoter [144]. However, both the total antioxidant capability and the ascorbic acid content were increased in the transformed fruits. These results explain the higher capability of transgenic fruits to counteract the proinflammatory effects of phorbol ester in monocyte–macrophages *via* the inhibition of induced cyclooxygenase-2 enzyme [147]. This last example constitutes a nice illustration of what can be expected from molecular engineering of resveratrol in plants in terms of improvement of the nutritional value of fruits or food products.

6. Conclusions

Increased demand for resveratrol for nutraceutical, cosmetic and possibly pharmaceutic uses makes its production from sustainable sourcing a necessity. In this context, the use of biotechnology through recombinant microorganisms and plants is particularly promising [5, 112, 118]. Interesting results can indeed arise from the potential of genetically modified microorganisms as an alternative mechanism for producing resveratrol, as this compound can be synthesized directly in recombinant yeast (the subject of the present review) but also in bacteria, such as *Escherichia coli* [112, 118]. Use of recombinant bacteria or yeast is of interest for the food industry, which could produce resveratrol in large quantities in biofermentators. Tailoring yeast can also receive direct applications in winemaking, as for example, fermentation engineering to produce resveratrol in wine (or to increase the wine resveratrol content) [119]. Otherwise, a transgenic yeast expressing a gene for a glycosyl hydrolase capable of liberating free resveratrol from its glucoside form has been reported as well to increase resveratrol amounts in wine [153] or the wine-related antioxidant content [114]. Beside the fact that disease resistance can be obtained following expression of STS-encoding genes, molecular engineering of plants with resveratrol may also lead to food products comprising edible legumes, cereals, or fruits, which can be ingested with their potential clinical benefits, by humans. Taken together, these results suggest the overall relevance of metabolic engineering of resveratrol.

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