

# Stilbene Biosynthesis

Subjects: Plant Sciences | Biochemistry & Molecular Biology | Nutrition & Dietetics

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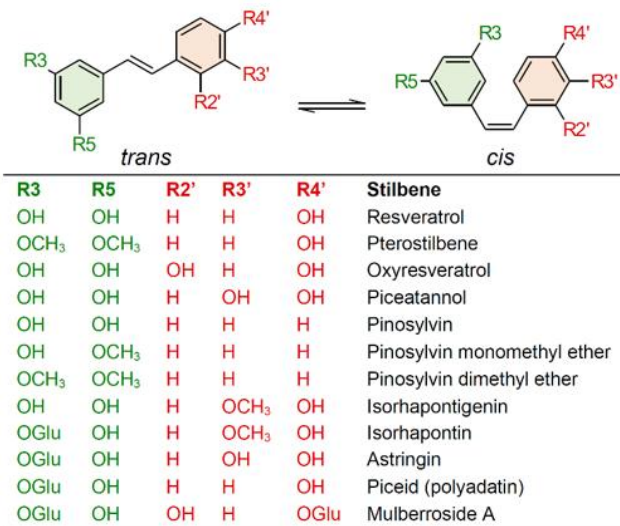
Stilbenes are a small family of polyphenolic secondary metabolites that can be found in several distantly related plant species. These compounds act as phytoalexins, playing a crucial role in plant defense against phytopathogens, as well as being involved in the adaptation of plants to abiotic environmental factors. Among stilbenes, *trans*-resveratrol is certainly the most popular and extensively studied for its health properties. However, many other stilbenes, both monomeric and oligomeric, are currently under intensive investigation due to their biological role and bioactivity.

Keywords: stilbenes ; secondary metabolites ; polyphenols ; phytoalexins ; phenylpropanoid pathway ; stilbene biosynthesis ; stilbene synthase ; resveratrol synthase ; pinosylvin synthase

## 1. Introduction

Stilbenes are a small yet important class of non-flavonoid polyphenols, sharing a common structure characterized by a 14-carbon skeleton composed of two benzene rings linked by an ethylene bridge (Figure 1). Due to the presence of the central ethylene moiety between the aromatic rings, stilbenes exist as the two possible stereoisomers *cis* and *trans*. However, the naturally occurring stilbenes are usually in the *trans* form [1]. Plant stilbenes, together with other polyphenols such as flavonoids, isoflavonoids, curcuminoids, and xanthenes, belong to the class of polyketides. Over 400 different stilbene compounds are currently known [2], mostly derived from *trans*-resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) (Figure 1), although different structures can be found in specific plant families [3].

Stilbenes have been identified in at least 72 plant species belonging to 31 genera and 12 distantly related families, including Pinaceae (e.g., *Picea abies* (L.) Karst. and *Pinus nigra* J.F. Arnold), Gnetaceae (e.g., *Gnetum parvifolium* (Warb.) W.C. Cheng and *G. africanum* Welw.), Fabaceae (*Arachis hypogaea* L. and *Robinia pseudoacacia* L.), Vitaceae (e.g., *Vitis vinifera* L. and *V. amurensis* Rupr.), Moraceae (e.g., *Morus alba* L. and *M. macroura* Miq.), and Polygonaceae (e.g., *Polygonum cuspidatum* Sieb. et Zucc. and *P. multiflorum* Thunb.) [4][5]. Given their nutraceutical value, stilbene content and composition have mainly been investigated in food plants, and the knowledge of stilbene distribution in nature is still poor. This is partially related to the complexity of the quali-quantitative analysis of stilbenes, which is in turn related to the unavailability of standards and the detection limits of analytical methods [2]. For these reasons, most of the studies carried out to date have been focused on simple stilbenes, such as resveratrol, piceid, pterostilbene, and piceatannol (Figure 1). Current knowledge on the distribution of stilbenes in the plant kingdom will not be presented in this review, as this topic is covered by excellent recent reviews [4][5].



**Figure 1.** Chemical structures of common stilbene monomer derivatives. (OGlu) O-β-D-glucopyranoside.

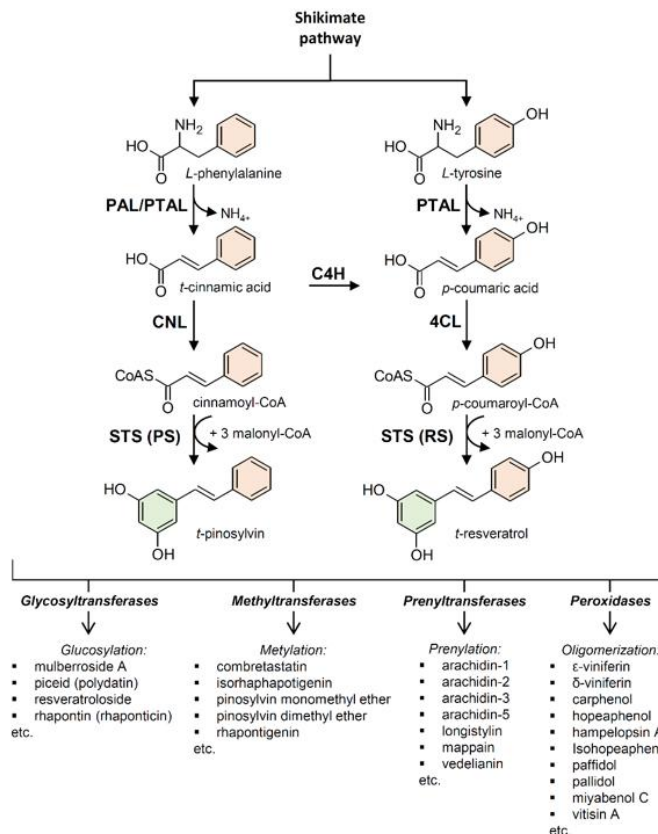
Stilbenes are mainly involved in constitutive and inducible protection of the plant against biotic (phytopathogenic microorganisms and herbivores) and abiotic (e.g., UV radiation and tropospheric ozone) stress [3][6]. On one side they counteract the aggression exerting a direct toxic effect on the pathogen, while on the other they act as antioxidants, protecting the cells from oxidative damage [7][8][9]. Stilbenes possess several antipathogenic properties including antibacterial, antifungal [10][11], nematocidal [12], and insecticidal [13][14]. They could also act as a deterrent towards vertebrate herbivory [15], as a possible negative effect of stilbenes has been reported on snowshoe hares (*Lepus americanus* Erxleben) [16][17] and field voles (*Microtus agrestis* L.) [18]. The role of stilbenes, among other polyphenols, in counteracting oxidative stress is just as important, as the plant response to pathogen attack involves the production of reactive oxygen species (ROS), which both act as signals for the activation of stress and defense pathways and as toxic substances capable of directly damaging the pathogen. Oxidative stress may also be induced by many abiotic conditions, such as drought, thermal stress, ultraviolet radiation, mechanical stress, heavy metals, salts, and air pollutants such as ozone [19]. Unsurprisingly, many of these factors also affect stilbene production [20].

Over the past 20 years, the bioactivities of stilbenes have been intensively investigated due to their impact on human health. Among stilbenes, resveratrol is the best known and the most studied. Basic scientific research and over 240 clinical studies have demonstrated the multiplicity of *trans*-resveratrol pharmacological effects, including antioxidant [21], anti-inflammatory [22], anticancer [23][24], estrogenic [25], neuroprotective [26], cardioprotective [27], anti-atherosclerotic [28], anti-aging [29], anti-diabetic [30], anti-osteoporosis [25], and anti-obesity properties [31]. In recent years, considerable attention has also been paid to other monomeric stilbenes, including pterostilbene [32], pinosylvin [33], and piceatannol [34], as well as to oligomeric stilbenes such as viniferins [35][36], which have been shown to possess similar and often more pronounced health-promoting properties than resveratrol.

Due to their potential use in the nutraceutical, cosmeceutical, and pharmaceutical fields, great interest is directed at the methods for large-scale production of stilbenes. For instance, it has been estimated that the global market for *trans*-resveratrol will almost double in the next 6 years, from 58 million USD in 2020 to 99.4 million USD by 2026 [37]. Methods for obtaining stilbenes can be grouped into three categories: direct extraction from plants, chemical synthesis, and the use of biotechnologies. The chemical synthesis of stilbenes has been reported, but this method is not economically feasible, in addition to being difficult in terms of stereospecific synthesis [38][39]. Considerable efforts have been devoted to the development of biotechnological methods for stilbene production, which broadly include tissue culture techniques [40], biotransformation [41], and metabolic engineering [42]. Nevertheless, the major way of supplying stilbenes is the direct extraction from plants such as *P. cuspidatum* and *V. vinifera* [43].

## 2. Biosynthesis of Stilbenes

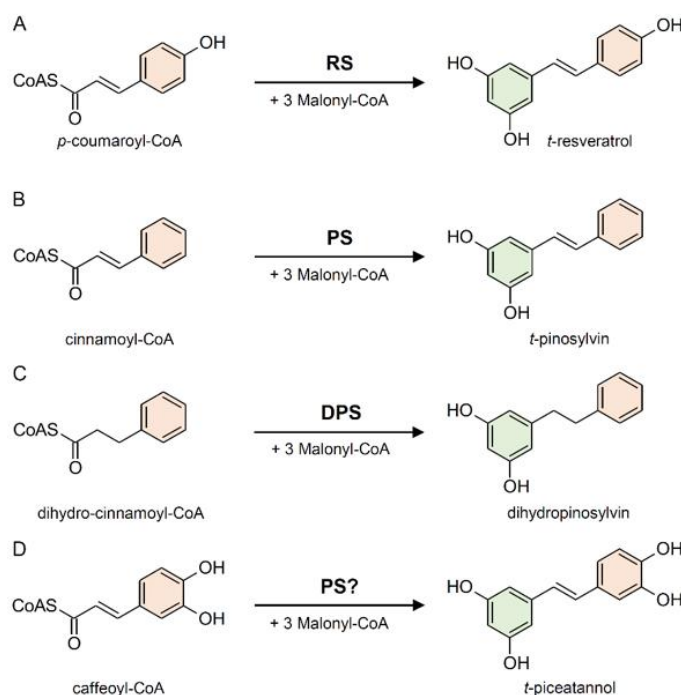
Stilbenes are biosynthesized through the phenylpropanoid pathway, which is also responsible for the biosynthesis of numerous primary and secondary metabolites including flavonoids, coumarins, hydrolyzable tannins, monolignols, lignans, and lignins [44]. Generated by the shikimate pathway, the aromatic amino acid *L*-phenylalanine is the primary starting molecule of the phenylpropanoid pathway (Figure 2). The non-oxidative deamination of *L*-phenylalanine to form *trans*-cinnamic acid, catalyzed by phenylalanine ammonia-lyase (PAL; EC 4.3.1.24), is the entry step for the carbon channeling from primary metabolism into the phenylpropanoid secondary metabolism. PAL is ubiquitous in plants [45], and it is undoubtedly the most studied enzyme involved in plant secondary metabolism [46]. Cinnamic acid can be bound to a coenzyme A (CoA) molecule by cinnamate:CoA ligase (CNL; EC 6.2.1.-) to form cinnamoyl-CoA. Alternatively, cinnamic acid can be hydroxylated by cinnamate 4-hydroxylase (C4H), a cytochrome P450 enzyme (EC 1.14.14.91), to form *p*-coumaric acid. Some plants (mainly monocots but also dicots) possess a bifunctional phenylalanine/tyrosine ammonia-lyase (PTAL, EC 4.3.1.25) that efficiently deaminates both *L*-phenylalanine (PAL activity) and *L*-tyrosine (TAL activity) [47][48][49][50]. These plants can directly produce *p*-coumaric acid using *L*-tyrosine as a substrate, bypassing the requirement for *L*-phenylalanine and C4H. A molecule of CoA is then bound to *p*-coumaric acid by 4-coumarate: CoA ligase (4CL; EC 6.2.1.12), generating *p*-coumaroyl-CoA, which provides an active intermediate in numerous branches of the general phenylpropanoid pathway [51].



**Figure 2.** Stilbene biosynthesis in plants. (PAL) phenylalanine ammonia-lyase; (PTAL) bifunctional L-phenylalanine/L-tyrosine ammonia-lyase; (C4H) cinnamate 4-hydroxylase; (4CL) 4-coumarate:CoA ligase; (CNL) cinnamate:CoA ligase; (STS) stilbene synthase; (RS) resveratrol synthase; (PS) pinosylvin synthase.

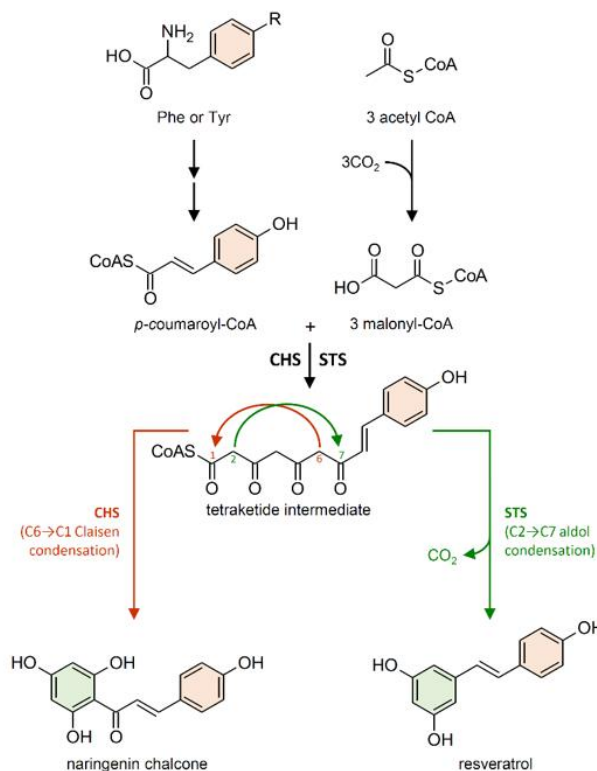
## 2.1. Stilbene Synthase

The enzyme stilbene synthases (STS) catalyze the direct formation of the stilbene skeleton through a single reaction from three units of malonyl-CoA and one CoA-ester of a cinnamic acid derivative (*p*-coumaroyl-CoA to form *trans*-resveratrol or cinnamoyl-CoA to form *trans*-pinosylvin) [52] (Figures 2 and 3). Malonyl-CoA is generated through a carboxylation reaction between acetyl-CoA and a bicarbonate ion ( $\text{HCO}_3^-$ ) catalyzed by acetyl-CoA carboxylase (EC 6.4.1.2) in the presence of ATP (Figure 4).



**Figure 3.** Examples of reactions catalyzed by stilbene synthase enzymes. (A) Conversion of *p*-coumaroyl-CoA into *t*-resveratrol by resveratrol (RS) synthase (or trihydroxystilbene synthase I). (B) Conversion of cinnamoyl-CoA into *t*-pinosylvin by pinosylvin synthase (PS). (C) Conversion of dihydro-cinnamoyl-CoA into dihydropinosylvin by dihydro-

pinosylvin synthase (DPS). (D) Conversion of caffeoyl-CoA into *t*-piceatannol, probably catalyzed by PS.



**Figure 4.** Reactions catalyzed by chalcone synthase (CHS) and stilbene synthase (STS) to produce naringenin chalcone and resveratrol, respectively. R = H phenylalanine (Phe); R = OH tyrosine (Tyr). Double arrows indicate multiple steps in the biosynthetic pathway.

Based on the preferred starting substrate, STS enzymes are classified into either a *p*-coumaroyl-CoA-specific type, such as trihydroxystilbene synthase I (also known as resveratrol synthase, EC 2.3.1.95), or a cinnamoyl-CoA-specific type, such as pinosylvin synthase (EC 2.3.1.146) (Figures 2 and 3). The former type has been mainly found in angiosperms like peanut [53], grapevine [54], and Tatar rhubarb (*Rheum tataricum* L.f) [55], while the latter type is typical in conifers and has been identified in several *Pinus* species like Scots pine (*P. sylvestris* L.) [56], Japanese red pine (*P. densiflora* Siebold & Zucc.) [57], and Eastern white pine (*P. strobus* L.) [58].

*Pinus* species can biosynthesize two types of stilbenes, i.e., pinosylvin and dihydropinosylvin, which are biosynthetically derived from cinnamoyl-CoA and dihydrocinnamoyl-CoA, respectively (Figure 3B,C). STS from *P. strobus* shows a clear preference for cinnamoyl-CoA and was therefore characterized as pinosylvin synthase [58]. Otherwise, STS from *P. sylvestris* shows an unusual preference for dihydro-cinnamoyl-CoA, identifying it as a dihydro-pinosylvin synthase [56]. STS does not exhibit absolute substrate specificity. While showing a preference for a given substrate, the same STS enzyme can accept different cinnamic acid derivatives as starting substrates catalyzing the biosynthesis of different stilbenes. For example, the enzyme responsible for the biosynthesis of piceatannol (3,5,3',4'-tetrahydroxystilbene) has not been identified yet, however, pinosylvin synthase from *P. strobus* proved to be active with caffeoyl-CoA in vitro (Figure 3D), suggesting that it could be responsible for piceatannol biosynthesis *in planta* [58].

STS enzymes belong to the type III polyketide synthase superfamily (PKSs), which also includes chalcone synthase (CHS; EC 2.3.1.74) [59]. STS and CHS share a high degree of similarity both in their amino acid sequence identity (which reaches 75–90% depending on the species) and in their crystallographic structures [51][60]. *CHS* genes are present in the genome of all plants analyzed so far, while *STS* have been identified in a limited number of plant species, often phylogenetically unrelated. Converging lines of evidence indicate that CHS is the archetypal enzyme from which STS evolved multiple times independently in stilbene-producing plants, through gene duplication followed by functional divergence [60][61][62]. CHS and STS are the most investigated enzymes among PKSs and, due to their high sequence similarity, they are often referred to as the CHS/STS family [63][64].

Although it employs the same substrates as STS, CHS is responsible for the first committed step in the biosynthesis of flavonoid-type compounds. Both enzymes generate the same linear tetraketide intermediate. However, CHS catalyzes a C6→C1 Claisen condensation of the intermediate to produce naringenin chalcone, while STS catalyzes an alternative C2→C7 aldol condensation of the intermediate to form a stilbene backbone (Figure 4) [59][65][66].

STS was first extracted and purified from suspension cultures of peanut cells elicited with UV radiation [53]. Cloning of two peanut *STS* genes revealed a high sequence identity with *CHS* throughout the coding region and the presence of an intron at the same position as a conserved intron in *CHS* [67]. *STS* genes and cDNAs were subsequently cloned and characterized from grapevine cell suspension cultures [68] and Scots pine plantlets [56], both induced by fungal elicitors. At present, *STS* genes have been cloned from several plant species including mulberry (*Morus notabilis* C.K. Schneid and *M. atropurpurea* Roxb.) [42][66], Scots pine [69], white spruce (*Picea glauca* (Moench) Voss) [70], Norway spruce (*Picea abies* (L.) H. Karst.) [74], Japanese red pine [57], and sorghum (*Sorghum bicolor* (L.) Moench) [72]. To the best of our knowledge, sorghum is the only monocot plant in which an *STS* gene (*SbSTS1*) has been identified.

In most stilbene-producing plants, *STS* exists as a small family consisting of 1–10 closely related paralogs. For example, the *STS* multigene family is represented by two members in white spruce [70] and Norway spruce [74], three members in Japanese red pine [57], almost five members in Scots pine [69], six members in peanut [73], and ten members in mulberry [66]. Remarkable exceptions to this rule are sorghum, in whose genome only one *STS* gene has been identified [74][75], and grapevine, which possesses an uncommonly large number of *STS* genes. Both grapevine and sorghum genomes have been entirely sequenced [74][76]. Early Southern-blot analysis suggested that the grapevine *STS* gene family consisted of 15–20 members [77]. Genome-wide analysis carried out on the *V. vinifera* PN40024 genome led to the identification of 48 putative *STS* genes, designated *VvSTS1* to *VvSTS48*, with at least 33 potentially coding for functional *STS* proteins [60] [62].

To date, there is no evidence regarding the different substrate specificity and enzymatic activity of different *VvSTS*s. Functional characterization of nine *VvSTS*s confirmed that they encode for functional *STS* enzymes [62]. Since these nine genes were specifically chosen to represent the diversity of the *VvSTS* gene family, it is most likely that all grapevine *VvSTS*s encode enzymes with similar activity and specificity. Despite the high similarity between *STS* genes which makes it difficult to accurately distinguish the individual transcripts, gene expression studies revealed different transcriptional responses of distinct *VvSTS*s during development and in response to environmental stresses [60][78][79]. The expression of some *VvSTS*s was also found to be tissue-specific [60][79]. It is therefore likely that the large quantity of members in the grapevine *STS* gene family has evolved to allow for fine spatial and temporal regulation of stilbene biosynthesis under both normal and stress conditions.

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