Chapter 1
Resveratrol and Its Derivatives as Phytoalexins

1.1 General Introduction

In this chapter we begin by providing an overview of the biochemistry of phytoalexin synthesis in *Vitis vinifera* and the significance of these molecules in plant physiology. We then discuss the concentrations of these molecules in wines, the main dietary source of phytoalexins produced by *Vitis vinifera*. Our goal in this first chapter is to cultivate an appreciation for red wine polyphenols that extends beyond their effects in mammalian cells.

1.2 Grapevine Phytoalexins

Phytoalexins are secondary metabolites produced by plants in response to biotic and abiotic stressors. Across plant species, an enormous range of phytoalexins is produced, and these generally have antifungal and antimicrobial activities (Ahuja et al. 2012). Phytoalexins, and related compounds termed phytoanticipins (constitutively produced secondary metabolites with similar properties), have been studied for their possible beneficial effects on human health. Amongst these molecules are biologically active compounds with the potential to be further developed into functional food ingredients or dietary supplements (Boue et al. 2009). In the grapevine *Vitis vinifera*, the predominant phytoalexins produced in response to stress belong to a family of compounds called stilbenes (Fig. 1.1), which are synthesized from the amino acid phenylalanine.

One of the main stilbenes produced in grapevines is *trans*-resveratrol (*trans*-3,5,4'-trihydroxystilbene), which has been the subject of intensive investigation over the past two decades owing to its apparent health promoting properties. Although red wines are perhaps the best known dietary sources of resveratrol and its derivatives, these molecules are produced by a variety of phylogenetically diverse plant species, including peanut (*Arachis hypogaea*), Japanese knotweed (*Fallopia*

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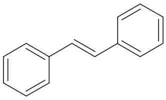

Fig. 1.1 Basic carbon skeleton structure of a stilbene

japonica), sorghum (*Sorghum bicolor*), and *Pinus* and *Picea* species (Parage et al. 2012). In instances where food or beverages are produced from these plants the biologically active phytoalexins are present in the consumed product, sometimes in negligible amounts and occasionally at concentrations sufficient to perhaps elicit biological responses.

1.3 Stilbene Synthesis

Trans-resveratrol is the initial stilbene product of *p*-coumaroyl-CoA and three molecules of malonyl-CoA in a reaction catalyzed by stilbene synthase (full pathway shown in Fig. 1.2). While most plants are capable of producing malonyl-CoA and CoA esters of cinnamic acid, the ability to synthesize stilbenes is limited to only a few plant species (reviewed in Chong et al. 2009). In *Vitis vinifera*, stilbene synthase is encoded by between 20 and 40 stilbene synthase genes, which is considerably more genetic diversity within this gene family than has been found in any other plant species (Chong et al. 2009; Parage et al. 2012). *Trans*-resveratrol serves as a precursor molecule that is further converted into a wide variety of compounds including piceid, pterostilbene, and viniferins by glycosylation, methoxylation, and oxidative oligomerization, respectively (Fig. 1.3). Some of these compounds, particularly piceid, are present at significantly higher concentrations than resveratrol in grapevine leaves (Boso et al. 2012), grape skins (Romero-Perez et al. 2001; Bavaresco et al. 2007), grape juices (Romero-Perez et al. 1999), and wines (Mark et al. 2005; Moreno-Labanda et al. 2004; Naugler et al. 2007).

Phytoalexins are produced in grapevines constitutively at low levels and are present in root, stem, leaf, and grape tissues (Wang et al. 2010). However, production and accumulation of phytoalexins within leaf and grape tissues is strongly induced by stressors such as UV light, heavy metals, ozone, and fungal infection (Jeandet et al. 2002). The expression of at least 20 stilbene synthase genes is induced by inoculation with the downy mildew fungus *Plasmopara viticola* (reviewed in Chong et al. 2009), and increased abundance of stilbene synthase protein on the leaf epidermis following UV exposure has been demonstrated using immunolocalization (Pan et al. 2009). *Trans*-resveratrol levels measured in leaf tissue vary with *Vitis vinifera* variety, but in all varieties they are increased substantially (up to 30-fold) within several days of inoculation with *P. viticola* (Boso et al. 2012). Like *trans-resveratrol*, the production of pterostilbene, piceid, and viniferins is also strongly enhanced by stress. Pterostilbene levels increase from undetectably low levels to a few μmol/mg

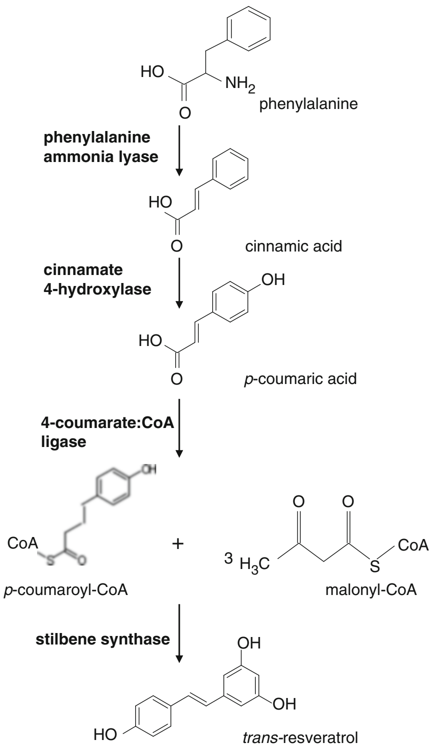

Fig. 1.2 Synthesis of *trans*-resveratrol from *p*-coumaroyl-CoA and malonyl-CoA by stilbene synthase

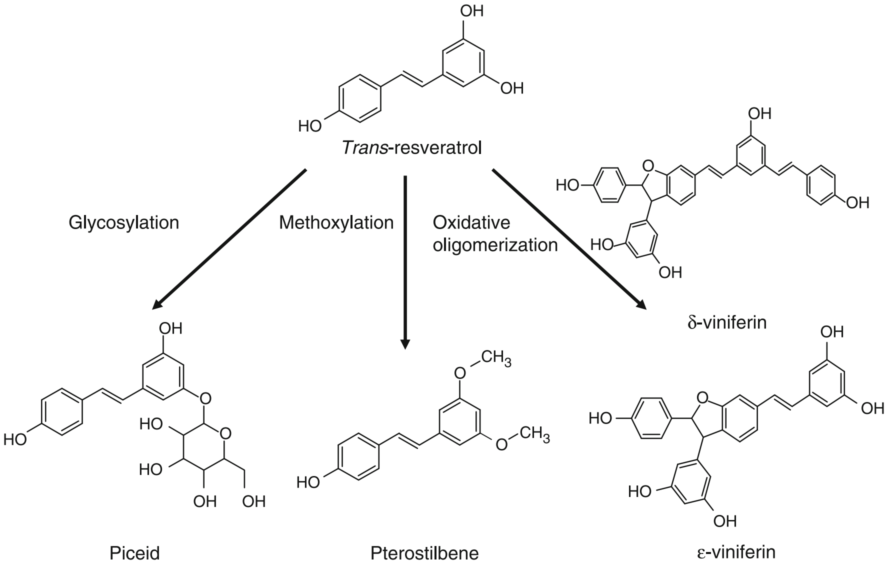

Fig. 1.3 *Trans*-resveratrol is a precursor molecule that may be converted into piceid, pterostilbene, and viniferins by glycosylation, methoxylation, and oxidative oligomerization, respectively

free weight within several days following infection with *P. viticola* (Boso et al. 2012), in parallel with an approximately fivefold increase in the expression of resveratrol O-methyltransferase, the enzyme catalyzing *trans*-pterostilbene synthesis from *trans*-resveratrol (Schmidlin et al. 2008). Piceid, ε-viniferin, and δ-viniferin undergo similar increases of up to 100-fold within 3 days following *P. viticola* infection. Although many of the measurements of polyphenol levels following infection have been made in grapevine leaves, similar increases have been shown also to occur in grape skins, which is relevant to the abundance of these molecules in red wines (Romero-Perez 2001; Montero et al. 2003).

1.4 Roles of Stilbenes in Grapevines

Stilbene production in plants is a central component of their response to stress. Resistance of *Vitis* species to fungal infection is generally correlated with their ability to produce stilbene phytoalexins (Douillet-Breuil et al. 1999; Schnee et al. 2008; Malacarne et al. 2011). Exogenous application of *trans*-resveratrol to grape berries is also an effective means of enhancing their resistance to fungal infection (Gonzalez Urena et al. 2003). Transgenic expression of stilbene synthase genes in non grapevine species is being utilized to enable stilbene production and in turn capture the potent antifungal properties of these molecules (Thomzik et al. 2001; Coutos-Thevenot et al. 2001; Zhu et al. 2004; Liu et al. 2011).

Indeed, there is good experimental evidence for potent antibacterial, antifungal, and anti-nematodal activities of *trans*-resveratrol and its derivatives (Chong et al. 2009). In laboratory experiments, *trans*-resveratrol inhibits *P. viticola* growth and development, and exhibits similar suppressive activity against a number of other fungal pathogens, including *Cladosporium cucumerinum, Botrytis cinerea, Oidium tuckeri, Pyricularia oryzae,* and *Sphaeropsis sapinea* at concentrations that are roughly equivalent to those produced by grapevine (reviewed in Jeandet et al. 2002). Pterostilbene is actually a more potent inhibitor of fungal growth than *trans-resveratrol* (Jeandet et al. 2002), though it may not accumulate to levels required for this activity in grape skin. ε-viniferin has antifungal activity similar to that of pterostilbene, suggesting that *trans*-resveratrol may be the least effective as an antifungal agent of this family of stilbene compounds.

1.5 Approaches to Increasing Stilbene Levels in Grapevines

Increased stilbene synthesis in *Vitis vinifera* is beneficial on two levels: (1) elevated stilbene levels, particularly in grape skin, will lead to higher concentrations of these apparent health-promoting compounds in wine, and (2) the increased stilbene concentrations will impart greater resistance to environmental stressors. Strategies to achieve this goal are therefore the focus of much research.

Application of exogenous stressors may be used to promote the endogenous production of *trans*-resveratrol and its derivatives in grapevine leaves and grapes, and this is of interest as a means of enhancing the levels of these compounds in red wines. One approach to enhancing stilbene levels in grapes has been to stimulate the signalling pathways involved in upregulating stilbene production. The plant hormone jasmonic acid is an effective inducer of phenylalanine ammonia lyase and stilbene synthase expression that significantly enhances stilbene synthesis. Repeated application of methyljasmonate to growing vines substantially increases the levels of *trans*-resveratrol and ε-viniferin in berries (Larronde et al. 2003; Vezzulli et al. 2007). In grapevine cell cultures, both methyljasmonate, and also cyclodextrins, stimulate stilbene synthesis (Lijavetzky et al. 2008; Zamboni et al. 2009). Interestingly, brief exposure to anoxia also appears to stimulate *trans*-resveratrol synthesis in harvested grapes (Jimenez et al. 2007). Thus, several non-genomic approaches have proven effective in enhancing stilbene levels in wine grapes and wine grape cells.

Another experimental method being used to increase stilbene levels is transgenic engineering to promote the synthesis of *trans*-resveratrol and its derivatives in plant species that do not naturally produce these molecules. Since all plant species produce the immediate precursors of *trans*-resveratrol, 4-coumaroyl-CoA and malonyl-CoA, transgenic overexpression of stilbene synthase alone is sufficient to instigate *trans*-resveratrol synthesis. Transgenic lines overexpressing stilbene synthase and/or O-methyltransferase have been engineered in a number of plant species, including tomato (*Solanum lycopersicum*), *Arabidopsis thaliana,* and tobacco (*Nicotiana tabacum*). Typically, when just stilbene synthase is overexpressed, the predominant polyphenol accumulating is *trans*-piceid rather than *trans*-resveratrol (Liu et al. 2006; Ingrosso et al. 2011). In transgenic plants co-expressing stilbene synthase and O-methyltransferase, pterostilbene accumulates as the major product, again with relatively low levels of *trans*-resveratrol (Rimando et al. 2012; Xu et al. 2012). Although these studies indicate that engineered plants could be used to produce stilbenes, it is interesting to note that there is evidence that excessive levels of these molecules interferes also with normal plant development (Ingrosso et al. 2011). The application of transgenic engineering to enhance stilbene levels is an active and developing area of research.

1.6 Stilbenes in Wines

Although resveratrol and its derivative molecules are found in a variety of wines, they are typically present at much higher levels in red wines due to a long fermentation process that includes contact with the grape skins. This allows the highly hydrophobic stilbene compounds to be extracted from grape skin into the forming ethanol. Although red wines are a relatively rich dietary source of resveratrol and its derivatives, the absolute concentrations of these compounds are nonetheless low, ranging from undetectable to about 63 μM (Stervbo et al. 2007; Dourtoglou et al. 1999;

Naugler et al. 2007). Levels of resveratrol vary with wine variety, with relatively high levels identified in some Pinot Noir and Merlot wines (Stervbo et al. 2007). Regional variation is also evident, and indeed environmental stresses unique to a given region are likely to affect resveratrol and stilbene production in grape skin, and therefore the levels present in wines produced from these grapes.

There has been a great deal of focus on the levels of resveratrol alone in red wines; however, it is important to note that some resveratrol derivatives are actually present at higher levels than resveratrol itself in red wines. For example, in studies of Hungarian (Mark et al. 2005), Spanish (Moreno-Labanda et al. 2004), and Canadian (Naugler et al. 2007) wines, piceid levels were found to be as much as tenfold higher than those of resveratrol. Piceid is also present at higher concentrations than resveratrol in grape juices (Romero-Perez et al. 1999) and cocoa (Hurst et al. 2008). Several resveratrol oligomers similarly accumulate to quite high levels in grapevine leaves (Boso et al. 2012), particularly following fungal elicitation. However, the levels of ε-viniferin (Adrian et al. 2000a, b), hopeaphenol (Boutegrabet et al. 2011), and pallidol (Naugler et al. 2007) in red wines appear to be generally lower than those of resveratrol and piceid. Pterostilbene levels are particularly low in grape berries and in red wines (e.g., Adrian et al. 2000a, b; Boso et al. 2012). Thus, resveratrol and piceid appear to be the major stilbenes present in red wines.

1.7 Conclusions

Grapevines produce a variety of stilbene molecules in response to both biotic and abiotic stresses. Identification of the signalling pathways and genes involved has led to strategies for targeting these with the goal of enhancing stilbene production both in *Vitaceae* and other plant species. Many of these strategies have now been shown to be quite effective at boosting the concentrations of resveratrol and its derivatives in wine grapes and instigating their production in other plant species. Given the accumulating evidence for applications of these compounds in human health contexts, ongoing research in this area is likely to yield increasingly high resveratrol grapes for wine production. Recent work has indicated that red wine supplemented with up to 200 mg/L resveratrol is palatable and stable (Gaudette & Pickering 2011), and therefore, high resveratrol wines should be an effective means of increasing dietary intake of resveratrol and its stilbene derivatives.

References

Adrian M, Jeandet P, Breuil AC, Levite D, Debord S, Bessis R (2000a) Assay of resveratrol and derivative stilbenes in wines by direct injection high performance liquid chromatography. Am J Enol Viticult 51:37–41

Adrian M, Jeandet P, Douillet-Breuil AC, Tesson L, Bessis R (2000b) Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. J Agric Food Chem 48:6103–6105

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends Plant Sci 17:73–90

Bavaresco L, Pezzutto S, Gatti M, Mattivi F (2007) Role of the variety and some environmental factors on grape stilbenes. Vitis 46:57–61

Boso S, Alonso-Villaverde V, Martinez M-C, Kassemeyer H-H (2012) Quantification of stilbenes in *Vitis* genotypes with different levels of resistance to *Plasmopara viticola* infection. Am J Enol Viticult 63:419–423

Boue SM, Cleveland TE, Carter-Wientjes C, Shih BY, Bhatnagar D, McLachlan JM, Burow ME (2009) Phytoalexin-enriched functional foods. J Agric Food Chem 57:2614–2622

Boutegrabet L, Fekete A, Hertkorn N, Papastamoulis Y, Waffo-Téguo P, Mérillon JM, Jeandet P, Gougeon RD, Schmitt-Kopplin P (2011) Determination of stilbene derivatives in Burgundy red wines by ultra-high-pressure liquid chromatography. Anal Bioanal Chem 401:1513–1521

Chong J, Poutaraud A, Hugueney P (2009) Metabolism and roles of stilbenes in plants. Plant Sci 177:143–155

Coutos-Thevenot P, Poinssot B, Bonomelli A, Yean H, Breda C, Buffard D, Esnault R, Hain R, Boulay M (2001) In vitro tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase *Vst*1 gene under the control of a pathogen-inducible PR10 promoter. J Exp Bot 52:901–910

Douillet-Breuil AC, Jeandet P, Adrian M, Bessis R (1999) Changes in the phytoalexin content of various Vits spp. In response to ultraviolet C elicitation. J Agric Food Chem 47:4456–4461

Dourtoglou VG, Makris DP, Bois-Dounasand F, Zonas C (1999) Trans-resveratrol concentration in wines produced in Greece. J Food Comps Anal 12:227–233

Gaudette NJ, Pickering GJ (2011) Sensory and chemical characteristics of trans-resveratrol fortified wine. Aust J Grape Wine Res 17:249–257

Gonzalez Urena A, Orea JM, Montero C, Jimenez JB, Gonzalez JL, Sanchez A, Dorado M (2003) Improving the post-harvest resistance in fruits by external application of trans-resveratrol. J Agric Food Chem 51:82–89

Hurst WJ, Glinski JA, Miller KB, Apgar J, Davey MH, Stuart DA (2008) Survey of the trans-resveratrol and trans-piceid content of cocoa-containing and chocolate products. J Agric Food Chem 56:8374–8378

Ingrosso I, Bonsegna S, De Demonico S, Laddomada B, Blando F, Santino A, Giovinazzo G (2011) Over-expression of a grape stilbene synthase gene in tomato induces parthenocarpy and causes abnormal pollen development. Plant Physiol Biochem 49:1092–1099

Jeandet P, Douillet-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. J Agric Food Chem 50:2731–2741

Jimenez JB, Orea JM, Urena AG, Escribano P, de la Osa PL, Guadarrama A (2007) Short anoxic treatments to enhance trans-resveratrol content in grapes and wine. Eur Food Res Technol 224:373–378

Larronde F, Gaudilliere JP, Krisa S, Decendit A, Deffieux G, Merillon JM (2003) Airborne methyl jasmonate induces stilbene accumulation in leaves and berries of grapevine plants. Am J Enol Viticult 54:63–66

Lijavetzky D, Almagro L, Belchi-Navarro S, Martinez-Zapater JM, Bru L, Pedreno MA (2008) Synergistic effect of methyljasmonate and cyclodextrin on stilbene biosynthesis pathway gene expression and resveratrol production in Monastrell grapevine cell cultures. BMC Res Notes 1:132

Liu SJ, Hu YL, Wang XL, Zhong J, Lin ZP (2006) High content of resveratrol in lettuce transformed with a stilbene synthase gene of Parthenocissus henryana. Journal of Agricultural and Food Chemistry 54:8082–8085

Liu Z, Zhuang C, Sheng S, Shao L, Zhao W, Zhao S (2011) Overexpression of a resveratrol synthase gene (*PcRS*) from *Polygonum cuspidatum* in transgenic *Arabidopsis* causes the accumulation of *trans*-piceid with antifungal activity. Plant Cell Rep 30:2027–2036

Malacarne G, Vrhovsek U, Zulini L, Cestaro A, Stefanini M, Mattivi F, Delledonne M, Velasco R, Moser C (2011) Resistance to Plasmopara viticola in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. BMC Plant Biol 11:114

Mark L, Nikfardjam MS, Avar P, Ohmacht R (2005) A validated HPLC method for the analysis of *trans*-resveratrol and *trans*-piceid in Hungarian wines. J Chromatogr Sci 43:445–449

Moreno-Labanda JF, Mallavia R, Pérez-Fons L, Lizama V, Saura D, Micol V (2004) Determination of piceid and resveratrol in Spanish wines deriving from Monastrell (*Vitis vinifera L.*) grape variety. J Agric Food Chem 52:5396–5403

Montero C, Cristescu SM, Jime'nez JB, Orea JM, te Lintel Hekkert S, Harren FJM, Gonza'lez Uren A (2003) Trans-resveratrol and grape disease resistance. A dynamical study by high-resolution laser-based techniques. Plant Physiol 131:129–138

Naugler C, McCallum JL, Klassen G, Strommer J (2007) Concentrations of *trans*-resveratrol and related stilbenes in Nova Scotia wines. Am J Enol Vit 58:117–119

Pan Q-H, Wang L, Li J-M (2009) Amounts and subcellular localization of stilbene synthase in response of grape berries to UV irradiation. Plant Sci 176:360–366

Parage C, Tavares R, Rety S, Baltenweck-Guyot R, Poutaraud A, Renault L, Heintz D, Lugan R, Marais G, Aubourg S, Hugueney P (2012) Structural, functional and evolutionary analysis of the unusually large stilbene synthase gene family in grapevine (*Vitis vinifera*). Plant Physiol 160(3):1407–1419

Rimando AM, Pan Z, Polashock JJ, Dayan FE, Mizuno CS, Snook ME, Liu C-J, Baserson SR (2012) *In planta* production of the highly potent resveratrol analogue pterostilbene via stilbene synthase and O-methyltransferase co-expression. Plant Biotech J 10:269–283

Romero-Pérez AI, Ibern-Gómez M, Lamuela-Raventós RM, de La Torre-Boronat MC (1999) Piceid, the major resveratrol derivative in grape juices. J Agric Food Chem 47:1533–1536

Romero-Pérez AI, Lamuela-Raventós RM, Andrés-Lacueva C, de La Torre-Boronat MC (2001) Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. J Agric Food Chem 49:210–215

Schmidlin L, Poutaraud A, Claudel P, Mestre P, Prado E, Santos-Rosa M, Wiedemann-Merdinoglu S, Karst F, Merdinolglu D, Hugueney P (2008) A stress-inducible resveratrol O-methyltransferase involved in the biosynthesis of pterostilbene in grapevine. Plant Physiol 148:1630–1639

Schnee S, Viret O, Gindro K (2008) Role of stilbenes in the resistance of grapevine to powdery mildew. Physiol Mol Plant Pathol 72:128–133

Stervbo U, Vang O, Bonnesen C (2007) A review of the content of the putative chemopreventive phytoalexin resveratrol in red wine. Food Chem 101:449–457

Thomzik JE, Stenzel K, Stocker R, Schreier PH, Hain R, Stahl DJ (2001) Synthesis of a grapevine phytoalexin in transgenic tomatoes (*Lycopersicon Esculentum* Mill.) conditions resistance against *Phytophthora infestans*. Physiol Mol Plant Pathol 51:265–278

Vezzulli S, Civardi S, Ferrari F, Bavaresco L (2007) Methyl jasmonate treatment as a trigger of resveratrol synthesis in cultivated grapevine. Am J Enol Viticult 58:530–533

Wang W, Tang K, Yang HR, Wen PF, Zhang P, Wang HL, Huang WD (2010) Distribution of resveratrol and stilbene synthase in young grape plants (*Vitis vinifera* L. cv. Cabernet Sauvignon) and the effect of UV-C on its accumulation. Plant Physiol Biochem 48:142–152

Xu Y, Xu TF, Zhao XC, Zou Y, Li ZQ, Xiang J, Li FJ, Wang YJ (2012) Co-expression of VpROMT gene from Chinese wild Vitis pseudoreticulata with VpSTS in tobacco plants and its effects on the accumulation of pterostilbene. Protoplasma 249:819–833

Zamboni A, Gatto P, Cestaro A, Pilati S, Viola R, Mattivi F, Moser C, Velasco R (2009) Grapevine cell early activation of specific responses to DIMEB, a resveratrol elicitor. BMC Genomics 10:363

Zhu YJ, Agbayani R, Jackson MC, Tang CS, Moore PH (2004) Expression of the grapevine stilbene synthase gene VST1 in papaya provides increased resistance against diseases caused by *Phytophthora palmivora*. Planta 220:241–250