15

MOLECULAR ASPECTS OF GRAPEVINE-PATHOGENIC FUNGI INTERACTIONS

E. Gomès[1] & P. Coutos-Thévenot[2]

[1] Institut des Sciences de la Vigne et du Vin,
UMR INRA 1287 Ecophysiologie et Génomique Fonctionnelle de la Vigne,
Domaine de la Grande Ferrade, 33883 Villenave d'Ornon, FRANCE

[2] FRE CNRS 3091 Physiologie Moléculaire des Transports de sucres
Université de Poitiers, Bâtiment de Botanique,
40 Av. du Recteur Pineau, 86022 Poitiers Cedex, FRANCE

1. INTRODUCTION

Grapevine is a major and highly valuable fruit crop with roughly 2.25 mil ha grown worldwide in 2007 (source: U.S. Food and Agriculture Organization). Unfortunately, most of the premium cultivars used for winemaking, including the widely used European *Vitis vinifera* cultivars, are highly susceptible to several pathogenic microorganisms including fungi, oomycetes, bacteria, phytoplasma and viruses. In the past 15 years, the understanding of grapevine-pathogen interactions has entered the molecular era and will most certainly constitute a basis for future improvement of grapevine disease tolerance. After a brief presentation of the main fungal- or oomycete-induced diseases, this chapter aims to give an overview of some aspects of grapevine-pathogenic fungi or oomycete interactions, at the molecular level. It includes an overview of resistance gene analogs, elicitors that induce defense reactions in grapevine, signalling pathways and gene activation.

2. MAIN GRAPEVINE FUNGAL OR OOMYCETE-INDUCED DISEASES

2.1. Foliage and berry diseases

2.1.1. Powdery mildew

Powdery mildew, caused by the ascomycete *Uncinula necator* (syn.

K.A. Roubelakis-Angelakis (ed.), *Grapevine Molecular Physiology & Biotechnology*, 2nd edn.,
DOI 10.1007/978-90-481-2305-6_15, © Springer Science+Business Media B.V. 2009

Erysiphe necator), an obligate biotrophic parasite of grapevine, is considered to be one of the most important fungal diseases in viticulture worldwide. Symptoms appear as grayish powdery or dusty patches of fungus growth on the upper side of the leaves and on other green parts of the vines, leading to a decrease in photosynthetic activity. In infected clusters, berries turn hard, brown, are smaller than uninfected ones, and may split open. Besides direct loss of yield, infected berries fail to properly mature and significantly alter wine quality (Calonnec et al. 2004). Almost no *V. vinifera* cultivar is immune to *U. necator*, but other grapevine species such as *Vitis labrusca*, *Vitis aestivalis* or *Vitis berlandieri* as well as *Muscadinia rotundifolia* possess various levels of resistance (Mullins et al. 1992).

2.1.2. Downy mildew

Downy mildew is caused by the oomycete *Plasmopara viticola*, also an obligate biotrophic parasite of grapevine. It still is one of the most destructive grapevine diseases in Europe and in the eastern half of the United States. Downy mildew affects the leaves, fruit, and shoots of grapevines. First symptoms occur as yellowish oily lesions on the leave upper surfaces; they rapidly give rise to white, felt-like "downy" fungal mass on the corresponding lower sides of the leaves. Infected berries first appear grayish then turn "downy" during pathogen sporulation. Yield losses occur through death of leaf tissue, low-quality fruit, and weakened young shoots.

2.1.3. Grey mould

Grey mould is the third major fungal disease affecting grapevine foliage and berries, particularly severe in areas where wet weather occurs between véraison and harvest. It is due to the broad host-range necrotrophic ascomycete *Botrytis cinerea*, which causes necrotic spots on leaves, total or partial destruction of the bunches before flowering and later on, rotting of berry clusters. Besides losses of fruit yield, infection of berries by *B. cinerea* also deteriorates wine quality by inducing the appearance of mushroom earthy off-odors (La Guerche et al. 2006).

Grey mould, powdery and downy mildews are controlled at the vineyard mostly by chemical spraying, sterol demethylation inhibitors or quinone outside inhibiting fungicides. However, besides negative environmental impacts, pathogens develop resistances towards these pesticides (Délye et al. 1997, Leroux et al. 1998, Chen et al. 2007).

2.2. Wood decay diseases

2.2.1. *Eutypa* dieback

Eutypa dieback is a wood decay disease caused by the ascomycete *Eutypa lata*. Symptoms do not usually appear until vines are at least six years old. Shoot symptoms are most evident during the beginning of the spring, with shoot arising from infected trunks being stunted with small chlorotic leaves (Moller and Kasimatis 1978). Berries fail to develop or develop very poorly, inducing yield losses ranging from 30 to 60% on highly susceptible cultivars (Munkvold and Marois 1994). *Eutypa* dieback shoot symptoms are always accompanied by a canker, which often appears V-shaped in a cross-section of the perennial wood. Cankers progress toward the trunk, killing the distal portions of the vine, and eventually, the entire vine may die in an average period of 10 years after the initial infection (Pascoe 1999). Currently, there is no cure for *Eutypa* dieback.

2.2.2. Esca

Esca, a.k.a. 'apoplexy' or 'lack measles' is a complex trunk disease involving at least five fungi, *Fomitiporia punctata*, *Stereum hirsutum*, *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, and *E. lata*, that obstruct the vascular system (Larignon and Dubos 1997). It affects both young and older vines. Cross section in infected trunks shows a central soft, white necrosis (touchwood), surrounded by a brownish hard zone. Esca develops slowly in the grapevine until the plant exhibits a sudden apoplectic decline, eventually killing the vine within a few days. No chemical is currently available to control esca.

3. GRAPEVINE RESISTANCE GENES ANALOGS

The European grape *V. vinifera*, which accounts for about 90% of worldwide grape production for wine making, shows very low disease resistance. On the other hand, wild American species, such as *Muscadinia rotundifolia*, are resistant to various pathogens, including *U. necator* (powdery mildew), *P. viticola* (downy mildew) or *Xylella fastidiosa* (Pierce's disease; Olmo 1986). Enhancing the resistance of cultivated grapevine to diseases therefore constitutes a major goal for breeders (Bisson et al. 2002).

3.1. R-genes and the plant immune system

In the past decade, our understanding of the molecular basis of plant disease resistance has increased steadily. Plants lack the adaptive immune system, which is the privilege of the vertebrates. To detect and successfully ward off pathogens, plants rely solely on innate immunity of each cell and on systemic signals emanating from infected sites (Dangl and Jones 2001). It is now widely admitted that the plant immune system uses two distinct defense systems (Chisholm et al. 2006). A first line of defense uses transmembrane pattern recognition receptors to recognize slowly evolving pathogen-associated molecular patterns (PAMPs), such as bacterial flagellin, lipopolysaccharide or fungal/oomycete cellulose binding elicitor proteins to activate basal defenses (Ausubel 2005). Pathogens, however, have evolved to acquire mechanisms that help them to by-pass PAMP-triggered immunity. Plants struck back by setting up a second line of defense that relies on proteins which recognize pathogen effectors or modifications of their cellular targets, a mechanism called effector-triggered immunity. Both PAMP- and effector-triggered immunity rely on the so-called resistance genes (*R*-genes).

R-genes can be grouped into 5 classes (Ellis et al. 2000, Dangl and Jones 2001) encoding for: (i) cytoplasmic serine/threonine kinases such as the *Pto* gene in tomato (Fig. 1A); (ii) extracellular leucine-rich re-peats (LRRs) proteins anchored to a transmembrane domain, as exemplified by the tomato *Cf-9* gene

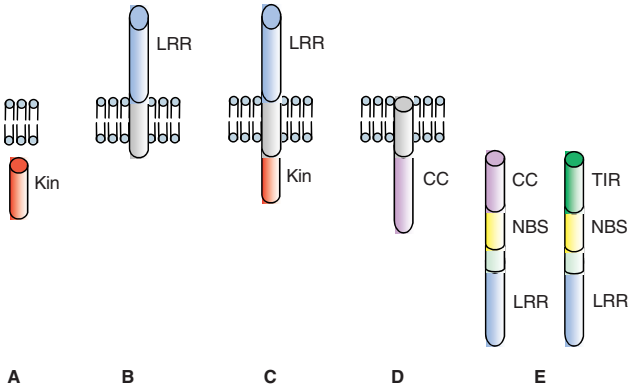

Fig. 1. Schematic representation of the *R*-genes products. (A) Intracellular Pto-like serine/threonine kinases (Kin). (B) Cf-9-like trans-membrane-anchored Leucine-Rich repeats (LRR). (C) Xa21-like proteins with an intracellular serine/threonine kinase domain and an extracellular LRR domain. (D) RPW8 protein with a transmembrane-anchored coiled-coil (CC) domain. (E) Intracellular NBS-LRR proteins with an LRR domain in their C-terminus, a nucleotide binding site (NBS) and either a toll/interleucine-1 receptor (TIR) or a CC domain in their N-terminus. Adapted from Dangl and Jones (2001), with permission.

(Fig. 1B); (iii) receptor-like kinases (RLKs) with an extracellular LRR and an intracellular serine/threonine kinase (i.e. the rice gene *Xa21*, Fig. 1C), (iv) proteins with an N-terminal transmembrane anchor and a cytoplasmic coiled-coil (CC) domain encoded by the *Arabidopsis RPW8* (Resistance to Powdery Mildew 8, Fig. 1D) genes; and (v) proteins with a nucleotide binding site (NBS), and a LRR domain in their C-terminus (NBS-LRR proteins, Fig. 1E). That later class of *R*-genes can be sub-divided in two sub-classes based on their N-terminal domain (Bai et al. 2002), which can either be a toll/interleukine-1 receptor (TIR-NBS-LRR, specific to dicotyledonous species) or a coiled-coil domain (CC-NBS-LRR, present in all angiosperms).

3.2. R-genes in grapevine

NBS-LRR genes are the most largely represented *R*-genes in plant genomes, as exemplified by the 149 genes found in the *Arabidopsis* genome (Meyers et al. 2003), or the 480 in the rice genome (Zhou et al. 2004). Grapevine is no exception and in a survey of the grape cv Pinot Noir draft genome Velasco et al. (2007) detected 233 genes encoding for proteins containing both NBS and LRR domains (InterPro IPR001611 and IPR002182, respectively). Among them, 84 genes belong to the CC-NBS-LRR subfamily while the TIR-NBS-LRR subfamily includes 37 genes. Additionally, 112 truncated NBS-LRR encoding genes are also present in the grape genome.

A complete inventory of defense-related RLKs genes is not easy to make, because these proteins are also implicated in a wide range of developmentally-related signalling pathways (Shiu and Bleecker 2001). Nevertheless, 53 genes encoding putative RLKs have been identified in grapevine (Di Gaspero and Cipriani 2003), eight of them being closely related to the Pto cytosolic protein from tomato and 3 to the products of the *R*-genes *Xa21* from rice and its *Arabidopsis* homolog *FLS2*.

To date, no true homolog of the *Arabidopsis RPW8* resistance genes have been identified in grapevine. Two genes, however, *VRP1-1* and *VRP1-2* (for *Vitis* Resistance to *Plasmopara* 1-1 and 1-2) encode CC-NBS-LRR with a RPW8 domain in their N-termini (Kortekamp et al. 2008). Such chimeric resistance proteins could link the pathogen effector-triggered (gene-for-gene) responses attributed to NBS-LRR proteins with the basal general resistance responses credited to RPW8 proteins (Xiao et al. 2005, Wang et al. 2007). Interestingly, *VRP1-1* and *VRP1-2* sequences show nucleotide polymorphism that led to amino acid substitutions at several positions when compared in the downy mildew resistant *Vitis* accession Regent and the susceptible Pinot noir (Kortekamp et al. 2008), making them potentially interesting to breed resistance.

3.3. Cluster of R-genes map to chromosomal region of grapevine genetic disease resistance

R-genes, particularly the NBS-LRR class, are arranged in clusters in plant genomes, a physical disposition that generates sequence variation and gene family expansion at a high rate, a point that is crucial to generate new resistance specificities (Bergelson et al. 2001, Meyers et al. 2003, Zhou et al. 2004). In grapevine, *TIR-NBS-LRR* gene clusters are preferentially located on linkage group (LG) 18, *CC-NBS-LRR* gene clusters on LG 9 and 14 and truncated *NBS-LRR* on LG 12 and 13 (Velasco et al. 2007, Moroldo et al. 2008). *RLK*s are more evenly dispersed in the grape genome, with LG 14 scoring the highest number of RLK coding genes (Moroldo et al. 2008).

In agreement with the role of *R*-genes in plant innate immunity, several clusters of *NBS* genes map to chromosomal regions where genetic resistance to bacterial, fungal or oomycete-induced diseases were previously assigned (Di Gaspero et al. 2007, Velasco et al. 2007). The *Run1* locus (Resistant to *Uncinula necator* 1), originating from *M. rotundifolia* (Pauquet et al. 2001), which confers resistance to powdery mildew, has a counterpart in the *Vitis* genome physically located on LG 12, in a region which contains several copies of *R*-genes (Barker et al. 2005). Additional loci for powdery mildew resistance have been reported on LG 15 and 14 in *Vitis* hybrids (Dalbo et al. 2001, Fischer et al. 2004). In the same region of LG 14, a primary locus for resistance to Pierce's disease causal agent, *Xylella fastidiosa*, was identified in the wild grape *Vitis arizonicai* (Krivanek et al. 2006). Quantitative trait loci for downy mildew resistance have been mapped with SSR markers to the distal part of LG 18 (Fischer et al. 2004), and in the middle of LG 7 (Grando et al. 2003) in *Vitis* resistant accessions, nearby regions where *NBS-LRR* genes are clustered. Another major determinant responsible for resistance to *P. viticola* has been identified on LG 12 (Merdinoglu et al. 2003). In conjunction with the knowledge of the grape genome sequence, the availability of linkage maps based on transferable molecular markers (reviewed by Doligez et al. 2006) will constitute valuable tools for pathogen resistance breeding in premium *Vitis* cultivars.

4. ELICITORS ACTIVE ON GRAPEVINE

Several molecules coming from microorganisms, plants or algae have been characterized as elicitors. These molecules, which encompass lipids, oligosaccharides and proteins, trigger defense responses in plants, such as the hypersensitive response (HR), the localized acquired resistance (LAR) or the systemic acquired resistance (SAR). Besides, some molecules coming from non-

pathogenic microorganisms potentiate ISR (Induced Systemic Resistance) in plants, leading to tolerance against many pathogens. These signal molecules, often recognized by a receptor (see *R*-genes, § 3), offer several possible applications as natural inducers of defense and tolerance in plants.

4.1. Oligosaccharide elicitors

Several elicitors such as β-1,3-glucans or α-1,4-oligogalacturonides are known to be active in many plant species. In grapevine some oligosaccharides appear to be efficient, like β-1,4-cellodextrins (Aziz et al. 2007), cyclodextrins (Morales et al. 1998, Bru et al. 2006), laminarin extracted from algae (Aziz et al. 2003) and induce tolerance against *B. cinerea* or *P. viticola*. Sulfated glucans like β-1,3-glucan sulfate enhance tolerance to *P. viticola* (Trouvelot et al. 2008). In addition, two novel oligosaccharidic elicitors were purified from *B. cinerea*. These molecules, obtained from crude mycelium cell wall extracts and culture filtrate preparations, were named Botrycin and Cinerein, respectively (Repka et al. 2001a, Repka 2002, 2006). In all cases, treatment with these elicitors triggered the classical PR (Pathogenesis Related) proteins accumulation, reactive oxygen species (ROS) production, as well as Ca^{2+}, jasmonic acid (JA) and salicylic acid (SA) signalling pathways.

4.2. Lipid elicitors

If several lipid molecules are known to act as elicitors in plants, in grapevine the main lipidic elicitor described up to now is the ergosterol molecule. This sterol, which is typical of fungi, was described as an inducer of a specific set of defense-related genes in tobacco and associated signal transduction pathways (Kasparovsky et al. 2004, Lochman and Mikes 2006, Rossard et al. 2006). Some PR-proteins (PR-14) and enzymes of the stilbene biosynthesis pathway are highly induced in grapevine by ergosterol treatment, most probably through the activation of WRKY trans-activator factors (Gomès et al. 2003, Laquitaine et al. 2006, Marchive et al. 2007). The putative specific receptors of ergosterol remain to be identified.

4.3. Proteinaceous elicitors

In terms of proteinaceous elicitors, two major lines of work have emerged these last years. Poinssot et al. (2003) described an endo-polygalacturonase secreted in the culture medium of *B. cinerea*, which is able to trigger full-scale early defense reactions in grapevine cell suspension cultures. Apparently, elici-

tor effect is not due to the enzyme activity. More recently, it was demonstrated that oligandrin, an elicitin of *Pithium oligandrum*, enhances *Vitis* tolerance towards *B. cinerea* (Mohamed et al. 2006). These results are of interest because elicitins were previously described to be active on tobacco but not on other plants (Ponchet et al. 1999). The fact that an elicitin could induce protection against fungal pathogens without HR response, but with modifications of the redox status of the cells, is indeed a very innovative concept.

5. EARLY CELLULAR EVENTS IN DEFENSE REACTIONS

Numerous studies of early plant defense reactions have been made in the last ten years. In Fig. 2 the principal steps, keys of the knowledge about this signal transduction pathway, are summarized. In model plants such as tobacco or *Arabidopsis* all these steps including perception, calcium flux activation, ROS synthesis, MAPKs (mitogen-activated protein kinases) or phosphatases activation are well characterized. In grapevine, the amount of information available, regarding early events of defense reactions, was less developed until recently (Busam et al. 1997, Jacobs et al. 1999). In the past few years, several publications aimed to decipher defense-related early signalling events in grape-

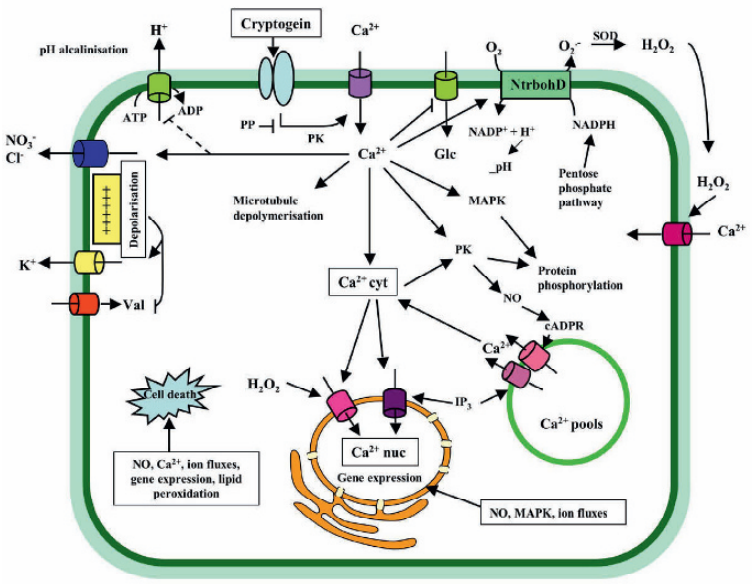

Fig. 2. Early cellular events triggered by pathogen recognition, as exemplified by the case of the signalling cascades induced on tobacco cells by cryptogein, an elicitin from *Phytophthora cryptogea*. Reprinted from Garcia-Brugger et al. (2006), with permission.

vine using model cell suspension cultures or entire plants (Repka 2006, Vandelle et al. 2006).

As described in paragraph 4, many elicitors have been characterized and some putative receptors identified in the Pinot Noir genome sequence. After the classical step of pathogen (or elicitor) perception, most of the crucial signalling events have been identified in grapevine. It seems that Ca^{2+} influx is the first event occurring after elicitation, by modulation of plasmamembrane Ca^{2+}channels. Later on, $NO^{\bullet}$ is synthesized and mobilizes internal stores of Ca^{2+}. Then come ROS ($O_2^{\bullet-}$, H_2O_2) production, MAPK and phosphatase activities that have also been evidenced in elicited plants.

A second class of signalling compounds (JA, SA and ethylene) are produced as endogenous signalling molecules and elicit pathogen protection process. Finally, a pool of defense genes gets activated, including PR proteins encoding genes, as well as phenylpropanoid and stilbene biosynthesis genes (Repka 2006, Vandelle et al. 2006). A particular class of PR-proteins is induced, the lipid transfer proteins (LTP, PR-14). These secreted proteins are able to bind JA, at least *in vitro* and trigger protection against *B. cinerea* (Girault et al. 2008). LTP-JA complexes could be one element of SAR signalling, leading to global protection of the plant against pathogens (Grant and Lamb 2006).

In conclusion, it seems that grapevine species possess all the signalling elements to respond to a pathogen attack. Nevertheless, it is clear that there are some differences between wild grapevine species and the European *V. vinifera*. For example, some *R* genes are absent from *V. vinifera* (*Run1*) and could explain the sensitivity of premium cultivars to pathogenic fungi (Barker et al. 2005).

6. GRAPEVINE PR-PROTEIN GENES

As already mentioned, one of the major steps in plant defense reactions is the synthesis of a particular class of proteins classically termed as ‘PR’ proteins. These proteins were distributed in 1999 in 11 classes. Recently, Van Loon et al. (2006) published a broader classification with 17 classes. The PR-proteins are defined as proteins that are not expressed in plants without pathogen interaction or largely induced during infection. For a protein to be classified as a PR-protein, it is necessary that its induction is described for two different plant/pathogen systems in two different laboratories. Not all the PR-proteins classes have been described in grapevine so far.

A putative sequence of a *V. vinifera* PR-1 protein was identified and cloned by Bertsch et al. (2003). The expression kinetics of a *PR-1* defense-related gene is strongly dependent on the nature of elicitor used (Repka 2001b). Comparison of *PR-1* expression in grapevine cell cultures after inoculation with

a host and a non-host pathogen revealed a high *PR-1* expression rate 3 weeks post-inoculation in *V. vinifera* cv Riesling and *Vitis riparia* cv Gloire de Montpellier, even if pathogens development was not blocked. Thus, the role of *PR-1* expression in impeding the downy mildew pathogen remains equivocal. It seems that expression of *PR-1* genes is a general stress response in some grapevine culture systems (Wielgoss and Kortekamp 2006). Three PR-1-like proteins were found to accumulate in grapevine leaves after infection by *U. necator*. Expression of these proteins was also induced by elicitor treatments in grapevine cell suspension culture (Repka et al. 2000).

The PR-2, -3, -4 and -5 classes are better documented in grapevine. In a susceptible *V. vinifera* cv, such as Riesling, inoculated with *Pseudoperonospora cubensis* (downy mildew of cucumber), a non-host pathogen in grapevine, ß-1,3-glucanases (PR-2) and chitinases (PR-3 and -4) are largely accumulated in comparison with a host situation (*P. viticola*). Following treatment with *P. cubensis*, sporulation intensity was significantly reduced in Riesling after subsequent inoculation with *P. viticol*a (Kortekamp 2006). Several PR-proteins are expressed in berries at maturity and liquid chromatography-mass spectrometry analysis of grape juice revealed the presence of several PR-3 and PR-5 (thaumatin-like) isoforms with different molecular masses, as a function of the varieties (Hayasaka et al. 2001).

U. necator, the causal agent of grapevine powdery mildew, induces expression of chitinases and ß-1,3-glucanases in leaves and berries in various grapevine cultivars, including susceptible ones. Indeed, Jacobs et al. (1999) showed that the hydrolytic activity was directly related to the severity of infection at the pathogen location. PR-2, -3 and -5 were also observed in infected berries at pre-véraison stage and were highly induced by ethephon treatment. These results demonstrate a paradox: even if these classes of PR-proteins are expressed during pathogen invasion this does not offer complete protection against *U. necator*. Probably, some other more specific proteins are necessary. Alternatively, the key of the protection might be more determined by the presence or the absence of *R*-genes (resistance genes).

Furthermore, the diversity of PR proteins expressed decreases during grape maturation (Monteiro et al. 2007) and could explain the enhanced susceptibility of the berries during the last stages of ripening. Accordingly, it was demonstrated that constitutive expression in transgenic *V. vinifera* of thaumatin-like protein protects grapevine plants against anthracnose (Jaysankar et al. 2003). These plants, however, were not protected against other fungi. In addition, induction of chitinase genes in *V. vinifera* depends on the infecting pathogen, but also the type of chitinase is different in a compatible or incompatible interaction (Robert et al. 2002). The selective expression of specific chitinases might be a reliable indicator of the SAR response in *V. vinifera* (Busam et al. 1997).

A grapevine class 10 PR-protein was cloned from *V. vinifera* leaves infiltrated with the incompatible bacterial pathogen *Pseudomonas syringae* pv Pisi (Robert et al. 2001). To our knowledge, it is the only example of *PR-10* gene characterized in grapevine. The accumulation of the corresponding mRNA was observed from 3 to 96 h post-inoculation and was followed by the accumulation, between 24 and, at least, 96 h after inoculation, of the encoded polypeptide, detected by immunoblotting.

The story of the PR-14 family is more complicated. These proteins (Lipid Transfer Proteins) were shown to be involved in several physiological processes. It is now quite clear that some isoforms are clearly involved in defense reaction signalling process (Maldonado et al. 2002, Blein et al. 2002, Grant and Lamb 2006). Several isoforms of LTP have been described in grapevine (Coutos-Thévenot et al. 1993). Some of them were induced by fungal elicitor treatments (Gomès et al. 2003). Ergosterol-induced protection of grape against *B. cinerea* relies on the expression of a type I lipid transfer protein, which is mediated by a WRKY trans-activating protein (Laquitaine et al. 2006). In addition, Girault et al. (2008) demonstrated that some grapevine LTP were able to bind JA, and that exogenous application of a LTP-JA complex induces protection of grapevine towards infection by *B. cinerea*. All LTPs, however, are not defense-related. Other isoforms seem to be involved in other physiological process, like somatic embryo development and epidermal layer formation. Accordingly, over-expression of the *VvLTP1* gene interferes with somatic embryo development in grapevine and abolishes the bilateral symmetry of embryos (François et al. 2008).

The last classes of PR-proteins that have been described in grapevine are the germin and germin-like proteins (PR-15 and -16). Recently, 7 members of the grapevine germin-like multigenic family were cloned in *V. vinifera* (Godfrey et al. 2007). Among them, one gene, *VvGLP3* (*V. vinifera* germin-like 3), have no basal expression level and is strongly induced by powdery mildew infection. Another member of the family, *VvGLP7*, responds to both *P. viticola* and *B. cinerea* infection. Some germin-like proteins exhibit oxalate oxidase or superoxide dismutase activities, but their exact role in plant defense reactions is far from being elucidated.

According to the literature, PR-6, -7, -8, -9, -11, -12, and -13, have not been described in grapevine yet. Nevertheless, using the sequences of the first member of each class to be published (Van Loon et al. 2006), a BLAST analysis indicate the presence of putative homolog genes in the Pinot noir genome (http://www.genoscope.cns.fr/externe/GenomeBrowser /Vitis/) for all these PR-protein classes, with the noticeable exception of PR-13 (thionins). Table 1 summarizes the BLAST results, by presenting for each PR-protein class the first

Table 1. Putative homolog genes for PR-6, 7, 8, 9, 11, 12 and 13 in the Pinot noir genome. For each PR-protein class, the grapevine genome database was probed with the sequence a typical member, as described by Van loon et al. (2006).

PR-protein class	Typical member	Properties	Putative grape homolog genes*	Linkage group
6	Tomato inhibitor I	Proteinase inhibitors	GSVIVT00020160001	5
			GSVIVT00020161001	5
			GSVIVT00029370001	13
7	Tomato P_{69}	Endoproteases	GSVIVT00001054001	2
			GSVIVT00001055001	2
			GSVIVT00001051001	2
			GSVIVT00001053001	2
			GSVIVT00001034001	2
			GSVIVT00001056001	2
8	Cucumber chitinase	Type III Chitinases	GSVIVT00006464001	Unknown
			GSVIVT00026961001	15
			GSVIVT00026949001	15
			GSVIVT00026950001	15
			GSVIVT00006463001	Unknown
			GSVIVT00020672001	14
9	Tobacco lignin-forming peroxidase	Peroxidase	GSVIVT00024722001	6
			GSVIVT00025396001	8
			GSVIVT00024724001	6
			GSVIVT00024717001	6
			GSVIVT00037460001	8
			GSVIVP00018771001	12
11	Parsley PR-1	Ribonuclease-like	GSVIVP00012304001	Unknown
			GSVIVP00012300001	Unknown
			GSVIVP00012296001	Unknown
			GSVIVP00005601001	Unknown
			GSVIVP00005604001	Unknown
			GSVIVP00005606001	Unknown
12	Radish Rs-AFP3	Defensins	GSVIVT00035146001	1
			GSVIVT00014577001	18
			GSVIVT00002075001	19
13	*Arabidopsis* THI2.1	Thionins	None detected	

* Putative grape PR-proteins transcripts are designated by their Genoscope annotation number (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/).

6 transcripts detected in the database (only 3 where detected for PR-12, defensins). Such an analysis is of course far from being exhaustive and is just intended to point the need of additional studies in the future to better characterize PR-protein families in grapevine.

7. GRAPEVINE PHYTOALEXINS BIOSYNTHESIS AND METABOLISM

7.1. Stilbene synthase genes

The knowledge of grapevine phytoalexin metabolism (Fig. 3) has increased vastly in the past decade. In woody plants, the classical phenylpropanoid pathway is split at the coumaroyl-CoA step by the stilbene synthase activity, which synthesizes resveratrol by condensation of 3-malonyl-CoA with one molecule of coumaroyl-CoA (Langcake and Pryce 1976, Langcake and Pryce 1977, Pont and Pezet 1990, Pezet and Pont 1995). This enzyme activity belongs to a multigenic family and has been already characterized by Wiese et al. (1994).

The first genes coding a *Vitis* stilbene synthase (*VST*) was cloned by Melchior and Kindl (1990), and afterwards several other genes were characterized (Richter et al. 2005). The recent publication of the grapevine Pinot Noir genome revealed the presence of 21 putative stilbene synthase genes, essentially on LG 10 and 16 (Velasco et al. 2007). This number fits nicely with a recent stilbene synthase gene expression analysis in infected grapes leaves (Richter et al. 2005). Stilbene synthases exhibit a high degree of homology with chalcone synthase and are known to be induced by several stimuli, including UV light, which is the major abiotic inducer (Adrian et al. 2000, Bonomelli et al. 2004).

Stilbene synthase genes are down-regulated during grape berry ripening, due to a competition between stilbene and chalcone synthase activities; that latter enzyme being activated after véraison for anthocyan and flavonoid accumulation in pellicula. A large group of biotic inducers are able to promote stilbene synthase expression and microorganisms were shown to have a direct effect on resveratrol accumulation. *B. cinerea* is the most common fungus able to act on phenylpropanoid pathway (Liswidowati et al. 1991, Bais et al. 2000, Keller et al. 2003). The level of the response, however, depends largely on the cultivar (Gabler et al. 2003), and it is possible to class the level of tolerance of cultivars in the field in regard to the capacity to accumulate resveratrol after UV light induction, as summarized in the Table 2 (Coutos-Thevenot et al. 2001).

Several other fungi were found to induce resveratrol production. *Aspergillus* induces expression of *VST* genes (Jean-Denis et al. 2006) and more problematic pathogens like three of the fungi associated with Esca, promote stilbene production (Bruno and Sparapano 2006a, 2006b). Bacteria-like *Pseudomonas*

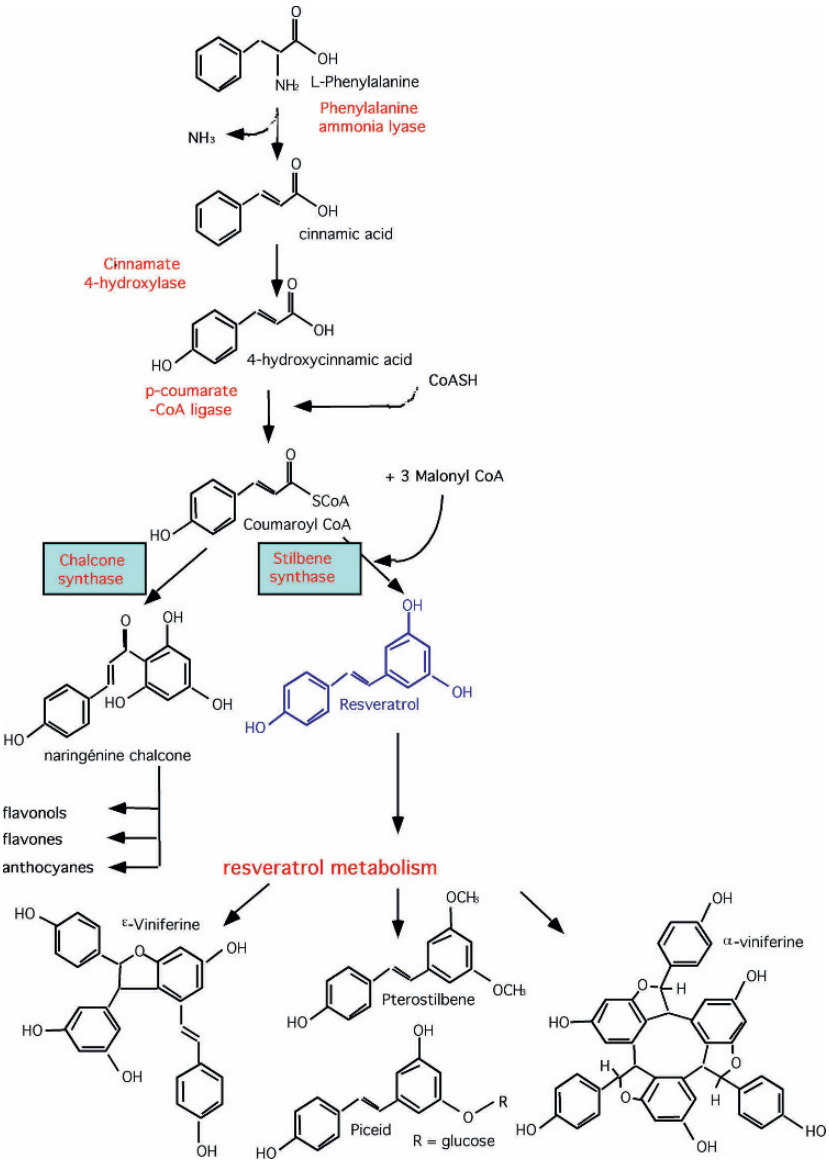

Fig 3. Biosynthesis pathway of grapevine stilbenes. Reprinted from Coutos-Thevenot et al. (2001) with permission.

syringae pv Pisi seem also to act as inducers during HR of grapevine (Robert et al. 2001). Various studies have demonstrated the ability of purified elicitors to induce *VST* gene activation or resveratrol synthesis. Ergosterol, a specific sterol of fungi and a non-specific elicitor, is highly efficient (Laquitaine et al.

2006). The endo-polygalacturonase BcPG1 produced by *B. cinerea* induces *VST1* mRNA accumulation 4 hours after treatment of grapevine cell suspension and is more active than oligogalacturonate elicitors (Poinssot et al. 2003). Several other molecules are also effective inducers of resveratrol production in grapevine (Borie et al. 2004, Laura et al. 2007), like oligogalacturonates (Aziz et al. 2004), β-1-3 glucane sulfate (Trouvelot et al. 2008), as well as chemicals like aluminium chloride (Adrian et al. 1996) or benzothiazole (Iriti et al. 2004, see also Chapter 12 in this book). Even if its mechanism of action is not well understood, resveratrol inhibits the growth of several fungi or oomycetes. Microbiologic tests revealed a good effect on *Botrytis* and *Eutypa lata* mycelium growth (Adrian et al. 1997, Coutos-Thevenot et al. 2001), *P. viticola* (Pezet et al. 2004) and *Venturia inaequalis* growth (Schulze et al. 2005). If resveratrol is active, its metabolites like pterostilbene (methylated form) and viniferins (oligomer metabolites) seem to be much more efficient (Pezet et al. 2004). On the other hand, the glycosylated form (piceid) seems to be less active and could be a soluble storage form (Pezet et al. 2004). It is suspected that enzymes, like methyl transferases and oxidases, could be involved in these mechanisms.

Table 2. Effects of *Botrytis* infection or UV treatment on resveratrol accumulation by various grapevine cultivars. Data are means of four independent experiments ± standard error. Nd: not detected. Reprinted from Coutos-Thévenot et al. (2001), with permission.

Variety / cultivars	Control (not induced)	*Botrytis* Resveratrol ($\mu g.g^{-1}$ DW)	UV light Resveratrol ($\mu g.g^{-1}$ DW)
Rupestris	nd	nd	350 (± 115)
41B Rootstock	nd	112 (± 30)	240 (± 120)
Ugni blanc 479	nd	86 (± 45)	210 (± 74)
Pinot Noir 386	nd	103 (± 31)	87 (± 49)
Folle blanche	nd	101 (± 16)	38 (± 11)

7.2. Hormones and signalling

Signalling pathways involved in stilbenes accumulation in grapevine are less clear-cut. It seems that stilbene synthase activation is probably due to a cross- talk between several pathways. The role of JA and methyl-JA are well documented with a very efficient induction (Zhang et al. 2002, Curtin et al. 2003, Larronde et al. 2003, Repka et al. 2004, Tassoni et al. 2005, Vezzulli et al. 2007), but the one of SA is less understood. It seems that SA acts on the phenylpropanoid pathway by inducing phenylalanine ammonia lyase and stilbene synthase genes expression (Chen et al. 2006, Wen et al. 2005, Wen et al.

2008). Ethylene also appears be involved (Grimmig et al. 2002).

7.3. Use in transgenic plants

After the fist evidence that tobacco plants, which do not naturally produce stilbenes, become tolerant to *B. cinerea* when transformed with *p35S-VST1* chimeric gene (Hain et al. 1993), the idea of generating transgenic grapevine that over-express stilbene synthase rapidly gained ground. The 41B rootstock was transformed with the *PR10* promoter, which is highly inducible by *B. cinerea* infection, fused to the *VST1* coding sequence. Some transgenic clones showed a high level of *in vitro* tolerance to *Botrytis* and could be also tolerant to *E. lata* (Coutos-Thevenot et al. 2001). More recently, these results were confirmed by several groups on various plant species, like grapevine (Fan et al. 2008), hop (Schwekendiek et al. 2007), oilseed rape (Husken et al. 2005), papaya (Zhu et al. 2004), poplar (Giorcelli et al. 2004) and apple (Szankowski et al. 2003). These examples prove the importance of stilbenes in plant defense mechanisms and open many future applications. Moreover, treatment of post-harvested fruits with resveratrol improves their resistance for conservation (Urena et al. 2003).

8. CONCLUSIONS

In the last 15 years, our understanding of molecular aspects of grapevine-fungi interactions has increased largely. The recent publication of the Pinot Noir genome will undoubtedly be a valuable tool for future studies. However, a fair deal of work remains to be done, to precisely decipher and finely characterize the different steps of the pathogen detection by the plant and the subsequent activation and establishment of defense reactions.

This applies, for example, to the characterization of the not yet described, but present in the grapevine genome, PR-protein families; or to the comparative studies of the genetic diversity of resistance genes and other defense-related genes, in the various cultivated and wild grapevines. The global outcome of the knowledge of grapevine defense reaction studies at the molecular level has already started to be integrated to breeding experiments, either through genetic transformation or through marker-assisted selection. It is reasonably safe to bet that this tendency will increase in the future.

REFERENCES

Adrian M, Jeandet P, Bessis R, Joubert JM (1996) Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminum chloride ($AlCl_3$). J Agric Food Chem 44:1979-1981

Adrian M, Jeandet P, Veneau J, Weston LA, Bessis R (1997) Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. J Chem Ecol 23:1689-1702

Adrian M, Jeandet P, Douillet-Breuil AC, Tesson L, Bessis R (2000) Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. J Agric Food Chem 48:6103-5

Ausubel FM (2005) Are innate immune signalling pathways in plant, animals concerved? Nat Immunol 6:973-797

Aziz A, Poinssot B, Daire X, Adrian M, Bezier A, Lambert B, Joubert J M, Pugin A (2003) Laminarin elicits defense responses in grapevine, induces protection against *Botrytis cinerea*, *Plasmopara viticola*. Mol Plant Microbe Interact 16:1118-1128

Aziz A, Heyraud A, Lambert B (2004) Oligogalacturonide signal transduction, induction of defense-related responses, protection of grapevine against *Botrytis cinerea*. Planta 218:767-774

Aziz A, Gauthier A, Bezler A, Poinssot B, Joubert JM, Pugin A, Heyraud A, Baillieul F (2007) Elicitor, resistance-inducing activities of beta-1,4 cellodextrins in grapevine, comparison with beta-1,3 glucans, alpha-1,4 oligogalacturonides. J Exp Bot 58:1463-1472

Bai J, Pennill LA, Ning J, Lee SW, Ramalingam J, Webb CA, Zhao B, Sun Q, Nelson JC, Leach JE, Hulbert SH (2002) Diversity in leucine in nucleotide binding site-Leucine-rich repeat genes in cereals. Genome Res 12:1871-1884

Bais AJ, Mutphy P J, Dry IB (2000) The molecular regulation of stilbene phytoalexin biosynthesis in *Vitis vinifera* during grape berry development. Austral J Plant Physiol 27:425-433

Barker CL, Donald T, Pauquet J, Ratnaparkhe MB, Bouquet A, Adam-Blondon AF, Thomas MR, Dry I (2005) Genetic, physical mapping of the grapevine powdery mildew resistance gene *Run*1, using a bacterial artificial chromosome library. Theor Appl Genet 111:370-377

Bergelson J, Kreitman M, Stahl EA, Tian D (2001) Evolutionary dynamics of plant *R*-genes. Science 292:2281-2285

Bertsch C, Joly D, Walter B (2003) Sequence of a putative *Vitis vinifera* PR-1. Vitis 42:103-104

Bisson LF, Waterhouse AL, Ebeler SE, Walker AM, Lapsley JT (2002) The present, the future of the international wine industry. Nature 418:969-699

Blein J-P, Coutos-Thévenot P, Marion D, Ponchet M (2002) From elicitins to lipid-transfer proteins:a new insight in cell signalling involved in plant defense mechanisms. Trends Plant Sci 7:293-296

Bonomelli A, Mercier L, Franchel J, Baillieul F, Benizri E, Mauro MC (2004) Response of grapevine defenses to UV-C exposure. Am J Enol Vitic 55:51-59

Borie B, Jeandet P, Parize A, Bessis R, Adrian M (2004) Resveratrol, stilbene synthase mRNA production in grapevine leaves treated with biotic, abiotic phytoalexin elicitors. Am J Enol Vitic 55:60-64

Bru R, Selles S, Casado-Vela J, Belchi-Navarro S, Pedreno MA (2006) Modified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. J Agric Food Chem 54:65-71

Bruno G, Sparapano L (2006a) Effects of three esca-associated fungi on *Vitis vinifera* L.:II. Characterization of biomolecules in xylem sap, leaves of healthy, diseased vines. Physiol Mol Plant Pathol 69:195-208

Bruno G, Sparapano L (2006b) Effects of three esca-associated fungi on *Vitis vinifera* L.:III. Enzymes produced by the pathogens, their role in fungus-to-plant or in fungus-to-fungus interactions. Physiol Mol Plant Pathol 69:182-194

Busam G, Kassemeyer HH, Matern U (1997) Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators or fungal challenge. Plant Physiol 115:1029-38

Calonnec A, Cartolaro P, Poupot C, Dubourdieu D, Darriet P (2004) effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) wine. Plant Pathol 53:434-445

Chen JY, Wen PF, Kong WF, Pan QH, Zhan JC, Li JM, Wan SB, Huang WD (2006) Effect of

salicylic acid on phenylpropanoids, phenylalanine ammonia-lyase in harvested grape berries. Postharvest Biol Technol 40:64-72

Chen W-J, Delmotte F, Richard-Cervera S, Douence L, Greif C, Corio-Costet M-F (2007) At least two origins of fungicide resistance in grapevine downy mildew populations. Appl Ennviron Microbiol 73:5162-5172

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune system. Cell 124:803-814

Coutos-Thévenot P, Jouenne T, Maes O, Guerbette F, Grosbois M, Le Caer JP, Boulay M, Deloire A, Kader J, Guern J (1993) Four 9-kD proteins excreted by somatic embryos of grapevine are isoforms of lipid-transfer proteins. Eur J Biochem 217:885-889

Coutos-Thevenot P, Poinssot B, Yean H, Buffard D, Esnault R, Hain R, Boulay M (2001) Tolerance of grapevine 41B rootstock in *in vitro* transgenic plants expressing the stilbene synthase *vst1* gene under the control of a fungi inducible PR 10 promoter. J Exp Bot 358:949-959

Curtin C, Zhang W, Franco C, Curtin C, Kikuchi M (2003) Manipulating anthocyanin composition in *Vitis vinifera* suspension cultures by elicitation with jasmonic acid, light irradiation. Biotechnol Letters 25:1131-1135

Dalbo MA, Ye GN, Weeden NF, Wilcox WF, Reisch BI (2001) Marker-assisted selection for powdery mildew resistance in grapes. J Am Soc Horticult 126:83-89

Dangl JL, Jones JDG (2001) Plant pathogens, integrated defense responses to infection. Nature 411:826-833

Délye C, Laigret F, Corio-Costet M-F (1997) A mutation in the 14α-demethylase gene of *Uncinula necator* that correlates with resistance to a sterol biosynthesis inhibitor. Appl Environ Microbiol 63:2996–2970

Di Gaspero G, Cipriani G (2003) Nucleotide biding site/leucine-rich repeats, Pto-like, repceptor-like kinases related to disease resistance in grapevine. Mol Gen Genomics 269:612-623

Di Gaspero G, Cipriani G, Adam-Blondon A-F, Testolin R (2007) Linkage maps of grapevine displaying the chromosomal location of 420 microsatellite markers, 82 markers for *R*-gene candidates. Theor Appl Genet 114:1249-1263

Doligez A, Adam-Blondon A-F, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith CP, Riaz S (2006) An integrated SSR map of grapevine based on five mapping populations. Theor Appl Genet 113:369-382

Ellis J, Dodds P, Pryor T (2000) Structure, function, evolution of plant disease resistance genes. Curr Opin Plant Biol 3:278-284

Fan CH, Pu N, Wang XP, Wang YJ, Fang L, Xu WR, Zhang JX (2008) Agrobacterium-mediated genetic transformation of grapevine (*Vitis vinifera* L.) with a novel stilbene synthase gene from Chinese wild *Vitis pseudoreticulata*. Plant Cell Tissue Organ Cult 92:197-206

Fischer BM, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Töpfer R, Zyprian EM (2004) Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor Appl Genet 108:501-515

François J, Lallemand M, Fleurat-Lessard P, Laquitaine L, Delrot S, Coutos-Thévenot P, Gomès E (2008) Overexpression of the *VvLTP1* gene interferes with somatic embryo development in grapevine. Function Plant Biol 35:394-402

Gabler FM, Smilanick JL, Mansour M, Ramming DW, Mackey BE (2003) Correlations of morphological, anatomical,, chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology 93:1263-1273

Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A (2006) Early signalling events induced by elicitors of plant defenses. Mol Plant Microbe Interact 19:714-724

Giorcelli A, Sparvoli F, Fulvio MY, Tava A, Balestrazzi A, Vrhovsek U, Calligari P, Bollini R, Confalonieri M (2004) Expression of the stilbene synthase (*StSy*) gene from grapevine in transgenic white poplar results in high accumulation of the antioxidant resveratrol glucosides.

Transgenic Res 13:203-214
Girault TJF, Rogniaux H, Pascal S, Delrot S, Coutos-Thevenot P, Gomès E (2008) Exogenous application of a lipid transfer protein-jasmonic acid complex induces protection of grapevine towards infection by *Botrytis cinerea*. Plant Physiol Biochem 46:140-149
Godfrey D, Able AJ, Dry IB (2007) Induction of a grapevine germin-like protein (*VvGLP3*) gen is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? Mol Plant Microbe Interact 20:1112-1115
Gomès E, Sagot E, Gaillard C, Laquitaine L, Poinsot B, Sanejouand H-Y, Delrot S, Coutos-Thévenot, P (2003) Non specific lipid-transfer protein genes expression in grape (*Vitis* sp.) cells in response to fungal elicitor treatments. Mol Plant Microbe Interact 16:456-464
Grando MS, Bellin D, Edwards KJ, Pozzi C, Stefanini M, Velasco R (2003) Molecular linkage maps of *Vitis vinifera* L., *Vitis riparia* Mchx. Theor Appl Genet 106:1213-1224
Grant, M Lamb C (2006) Systemic immunity. Curr Opin Plant Biol 9:414-420
Grimmig B, Gonzalez-Perez MN, Welzl G, Penuelas J, Schubert R, Hain R, Heidenreich B, Betz C, Langebartels C, Ernst D, Sandermann H (2002) Ethylene-, ozone-induced regulation of a grapevine resveratrol synthase gene:different responsive promoter regions. Plant Physiol Biochem 40:865-870
Hain, R Reif HJ, Krause E, Langebartels R, Kindl H, Vornam B, Wiese W, Schmelzer E, Schreier PH, Stocker RH et al. (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361:153-6
Hayasaka Y, Adams KS, Pocock KF, Baldock GA, Waters EJ, Hoj PB (2001) Use of electrospray mass spectrometry for mass determination of grape (*Vitis vinifera*) juice pathogenesis-related proteins:a potential tool for varietal differentiation. J Agric Food Chem 49:1830-9
Husken A, Baumert A, Milkowski C, Becker HC, Strack D, Mollers C (2005) Resveratrol glucoside (piceid) synthesis in seeds of transgenic oilseed rape (*Brassica napus* L.). Theor Appl Genet 111:1553-1562
Iriti M, Rossoni M, Borgo M, Faoro F (2004) Benzothiadiazole enhances resveratrol, anthocyanin biosynthesis in grapevine, meanwhile improving resistance to *Botrytis cinerea*. J Agric Food Chem 52:4406-4413
Jacobs AK, Dry IB, Robinson SP (1999) Induction of different pathogenesis-related cDNAs in grapevine infected by powdery mildew, treated with etephon. Plant Pathol 48:325-336
Jaysankar S, Zhijin LI, Gray DJ (2003) Constitutive expression of *Vitis vinifera* thaumatin-like protein after in vitro selection, its role in anthracnose resistance. Function Plant Biol 30:1105-1115
Jean-Denis JB, Pezet R, Tabacchi R (2006) Rapid analysis of stilbenes, derivatives from downy mildew-infected grapevine leaves by liquid chromatography-atmospheric pressure photoionisation mass spectrometry. J Chromatogr 1112:263-268
Kasparovsky T, Blein JP, Mikes V (2004) Ergosterol elicits oxidative burst in tobacco cells *via* phospholipase A2, protein kinase C signal pathway. Plant Physiol Biochem 42:429-35
Keller M, Viret O, Cole FM (2003) *Botrytis cinerea* infection in grape flowers:defense reaction, latency, disease expression. Phytopathology 93:316-322
Kortekamp A (2006) Expression analysis of defense-related genes in grapevine leaves after inoculation with a host, a non-host pathogen. Plant Physiol Biochem 44:58-67
Kortekamp A, Welter L, Vogt S, Knoll A, Schwander F, Töpfer R, Zyprian E (2008) Identification, isolation, characterization of a CC-NBS-LRR candidate disease resitance gene family in grapevine. Mol Breeding:doi 10.1007/s11032-008-9186-2.
Krivanek AF, Riaz S, Walker MA (2006) Identification, molecular mapping of *PdR*1, a primary resistance gene to Pierce's disease in *Vitis*. Theor Appl Genet 112:1125-1131
La Guerche S, Dauphin B, Pons M, Blancard D, Darriet P (2006) Characterization of some mushroom, earthy off-odors microbially induced by the development of rot on grapes. J Agric Food Chem 54:9193-9200

Langcake P, Pryce RJ (1976) The production of resveratrol by *Vitis vinifera,* other members of the *Vitaceae* as o response to infection or injury. Physiol Plant Pathol 9:77-86

Langcake P, Pryce RJ (1977) A new class of phytoalexins from grapevines. Experientia 33:151-2

Laquitaine L, Gomès E, François J, Marchive C, Pascal S, Hamdi S, Atanassova R, Delrot S, Coutos-Thevenot P (2006) Molecular basis of ergosterol-induced protection of grape against *Botrytis cinerea*: induction of type I Lipid Transfer Protein promoter activity, WRKY, stilbene synthase gene expression. Mol Plant Microbe Interact 19:1103-1112

Larignon P, Dubos B (1997) Fungi associated with esca disease in grapevine. Eur J Plant Pathol 103:147-157

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

Laura R, Franceschetti M, Ferri M, Tassoni A, Bagni N (2007) Resveratrol production in *Vitis vinifera* cell suspensions treated with several elicitors. Caryologia 60:169-171

Leroux P, Chapeland F, Arnold A, Gredt M (1998) Résistance de *Botrytis cinerea* aux fongicides. Phytoma, la défense des végétaux 504:62-67

Liswidowati F, Melchior F, Holmann F, Schwer B, Kindl H (1991) Induction of stilbene synthase by *Botrytis cinerea* in cultured grapevine cells. Planta 183:307-314

Lochman J, Mikes V (2006) Ergosterol treatment leads to the expression of a specific set of defense-related genes in tobacco. Plant Mol Biol 62:43-51

Maldonado AM, Doerner P, Dixon RA, Lamb CJ, Cameron RK (2002) A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. Nature 419:399-403

Marchive C, Mzid R, Deluc L, Barrieu F, Pirrello J, Gauthier A, Corio-Costet MF, Regad F, Cailleteau B, Hamdi S, Lauvergeat V (2007) Isolation, characterization of a *Vitis vinifera* transcription factor, VvWRKY1,, its effect on responses to fungal pathogens in transgenic tobacco plants. J Exp Bot 58:1999-2010

Melchior F, Kindl H (1990) Grapevine stilbene synthase cDNA only slightly differing from chalcone synthase cDNA is expressed in *Escherichia coli* into a catalytically active enzyme. FEBS Lett 268:17-20

Merdinoglu D, Wiedemann-Merdinoglu S, Coste P, Dumas V, Haetty S, Butterlin G, Greif C (2003) Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. Acta Horticult 603:451-456

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of the NBS-LRR-encoding genes in *Arabidopsis*. Plant Cell 15:809-834

Mohamed N, Lherminier J, Farmer MJ, Fromentin J, Beno N, Houot V, Milat ML, Blein J-P (2006) Defense responses in grapevine leaves against *Botrytis cinerea* induced by application of a *Pythium oligandrum* strain or its elicitin, oligandrin, to roots. Phytopathology 97:611-620

Moller WJ, Kasimatis AN (1978) Dieback of grapevine caused by *Eutypa armeniacae*. Plant Disease Rep 62:254-258

Monteiro S, Carra-Pereira MA, Loureiro VB, Teixeira AR, Ferreira RB (2007) The diversity of pathogenesis-related proteins decreases during grape maturation. Phytochemistry 68:416-425

Morales M, Bru R, Garcia-Carmona F, Ros-Barcelo A, Pedreno MA (1998) Effect of dimethyl-ß-cyclodextrins on resveratrol metabolism in Gamay grapevine cell cultures before, after inoculation with *Xylophilus ampelinus*. Plant Cell Tissue Organ Cult 53:179-187

Moroldo M, Paillard S, Marconi R, Legeai F, et al. (2008) A physical map of the heterozygous grapevine Carbernet Sauvignon allows mapping candidate genes for disease resistance. BMC Plant Biol 6:66

Mullins MG, Bouquet A, Williams LE (1992) Biology of the Grapevine. Cambridge University Press, Cambridge, UK

Munkvold GP, Marois JJ (1994) *Eutypa* dieback of sweet cherry, occurence of *Eutypa lata* perithecia in the central valley of california. Plant Disease 78:200-207

Olmo H P (1986) The potential role of (*vinifera* x *Rotundifolia*) hybrids in grape variety improvement. Experientia 42:921-926

Pascoe I (1999) Grapevine trunk disease - black goo decline, esca, *Eutypa* dieback, others. Aust Grape Wine 429:27-28

Pauquet J, Bouquet,A, This P, Adam-Blondon AF (2001) Establishment of a local map of AFLP markers around the powdery mildew resistance gene *Run*1 in grapevine, assessment of their usefulness for marker assisted selection. Theor Appl Genet 103:1201-1210

Pezet R, Pont V (1995) Mode of toxic action of Vitaceae stilbenes on fungal cells. In: Daniel M, Purkayastha RP (eds) Handbook of Phytoalexin Metabolism. Action, Marcel Dekker Inc

Pezet R, Gindro K, Viret O, Richter H (2004) Effects of resveratrol, viniferins, pterostilbene on *Plasmopara viticola* zoospore mobility, disease development. Vitis 43:145-148

Poinssot B, Vandelle E, Bentéjac M, Adrian M, Levis C, Brygoo Y, Garin J, Sicilia F, Coutos-Thevenot P, Pugin A (2003) The endopolygalacturonase 1 from *Botrytis cinerea* activates grapevine defense reactions unrelated to its enzymatic activity. Mol Plant Microbe Interact 16:553-563

Ponchet M, Panabières F, Milat M-L, Mikes V, Montillet J-L, Suty L, Triantaphylides C, Tirilly Y, Blein J-P (1999) Are elicitins cryptograms in plant-*Oomycetes* communication? Cell Mol Life Sci 56:1020-1047

Pont V, Pezet R (1990) Relation between the chemical structure, the biological activity of hydroxystilbenes against *Botrytis cinerea*. J Phytopathol 130:1-8

Repka V, Kubikova J, Fischerova L (2000) Immunodetection of PR-1-like proteins in grapevine leaves infected with *Oidium tuckeri*, in elicited suspension cell cultures. Vitis 39:123-127

Repka V, Fischerova I, Silharova K (2001a) Biological activity of the elicitor released from mycelium of a grapevine isolate of the necrotrophic fungus *Botrytis cinerea*. Vitis 40:205-212

Repka V (2001b) Elicitor-stimulated induction of defense mechanisms, defense gene activation in grapevine cell suspension cultures. Biol Plant 44:555-565

Repka V (2002) A bestatin primes grapevine cells for augmented elicitation of the hypersensitive-like cell death, associated defense responses by methyl jasmonate. Vitis 41:69-76

Repka, V, Fischerova, I, Silharova, K, Repka, V, Fischerova, I (2004) Methyl jasmonate is a potent elicitor of multiple defense responses in grapevine leaves, cell-suspension cultures. Biol Plant 48:273-283

Repka V (2006) Early defense responses induced by two distinct elicitors derived from a *Botrytis cinerea* in grapevine leaves, cell suspensions. Biol Plant 50:94-106

Richter H, Pezet R, Viret O, Gindro K (2005) Characterization of 3 new partial stilbene synthase genes out of over 20 expressed in *Vitis vinifera* during the interaction with *Plasmopara viticola*. Physiol Mol Plant Pathol 67:248-260

Robert N, Ferran J, Breda C, Coutos-Thevenot P, Boulay M, Buffard D, Esnault R (2001) Molecular characterization of the incompatible interaction of *Vitis vinifera* leaves with *Pseudomonas syringae* pv. pisi: expression of genes coding for stilbene synthase, class 10 PR protein. Eur J Plant Pathol 107:249-261

Robert N, Roche K, Lebeau Y, Breda C, Boulay M, Esnault R, Buffard D (2002) Expression of grapevine chitinase genes in berries, leaves infected by fungal, bacterial pathogens. Plant Sci 162:389-400

Rossard S, Luini E, Perault JM, Bonmort J, Roblin G (2006) Early changes in membrane permeability, production of oxidative burst, modification of PAL activity induced by ergosterol in cotyledons of *Mimosa pudica*. J Exp Bot 57:1245-52

Schulze K, Schreiber L, Szankowski I (2005) Inhibiting effects of resveratrol, its glucoside piceid against *Venturia inaequalis*, the causal agent of apple scab. J Agric Food Chem 53:356-362

Schwekendiek A, Spring O, Heyerick A, Pickel B, Pitsch NT, Peschke F, De-Keukeleire D, Weber G (2007) Constitutive expression of a grapevine stilbene synthase gene in transgenic hop (*Humulus lupulus* L.) yields resveratrol, its derivatives in substantial quantities. J Agric Food

Chem 105:195-203
Shiu S-H, Bleecker AB (2001) Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci USA 98:10763-10768
Szankowski I, Briviba K, Fleschhut J, Schonherr J, Jacobsen HJ, Kiesecker H (2003) Transformation of apple (*Malus domestica* Borkh.) with the stilbene synthase gene from grapevine (*Vitis vinifera* L.), a PGIP gene from kiwi (*Actinidia deliciosa*). Plant Cell Rep 22:141-149
Tassoni A, Fornale S, Franceschetti M, Musiani F, Michael AJ, Perry B, Bagni N, Franceschetti M, Ferri M, Tassoni A (2005) Jasmonates, Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. New Phytol 166:895-905
Trouvelot S, Varnier AL, Allegre M, Mercier L, Baillieul F, Arnould C, Gianinazzi-Pearson V, Klarzynski O, Joubert JM, Pugin A, Daire X (2008) A beta-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HR-like cell death. Mol Plant Microbe Interact 21:232-243
Urena AG, Orea JM, Montero C, Jimenez JB, Gonzalez JL, Sanchez A, Dorado M (2003) Improving postharvest resistance in fruits by external application of *trans*-resveratrol. J Agric Food Chem 51:82-89
Van Loon LC, Rep M, Pieterse CM (2006) Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol 44:135-162
Vandelle E, Poinssot B, Wendehenne D, Bentéjac M, Pugin A (2006) Integrated signalling network involving calcium, nitric oxide, active oxygen species but not mitogen-activated protein kinases in Bc-PG1-elicited grapevine defenses. Mol Plant Microbe Interact 19:429-440
Velasco R, Zharkikh A, Troggio M, Cartwright DA, et al. (2007) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE 12:e1326
Vezzulli S, Civardi S, Ferrari F, Bavaresco L (2007) Methyl jasmonate treatment as a trigger of resveratrol synthesis in cultivated grapevine. Am J Enol Vitic 58:530-533
Wang W, Devoto A, Turner JG, Xiao S (2007) Expression of the membrane-associated resistance RPW8 enhances basal defense against biotrophic pathogens. Mol Plant Microbe Interact 20:966-976
Wen PF, Chen JY, Kong WF, Pan QH, Wan SB, Huang WD (2005) Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. Plant Sci 169:928-934
Wen PF, Chen JY, Wan SB, Kong WF, Zhang P, Wang W, Zhan JC, Pan QH, Huang WD (2008) Salicylic acid activates phenylalanine ammonia-lyase in grape berry in response to high temperature stress. Plant Growth Reg 55:1-10
Wielgoss A, Kortekamp A (2006) Comparison of PR1 expression in grapevine cultures after inoculation with a host-, a non-host pathogen. Vitis 45:9-13
Wiese W, Vornam B, Krause E, Kindl H (1994) Structural organization, differential expression of three stilbene synthase genes located on a 13 kb grapevine DNA fragment. Plant Mol Biol 26:667-677
Xiao S, Calis O, Patrick E, Zhang G, Charoenwattana P, Meuskett P, Parker JE, Turner JG (2005) The atypical resistance gene, RPW8, recruits components of basal defense for powdery mildew resistance in *Arabidopsis*. Plant J 42:95-110
Zhang W, Curtin C, Kikuchi M, Franco C (2002) Integration of jasmonic acid, light irradiation for enhancement of anthocyanin biosynthesis in *Vitis vinifera* suspension cultures. Plant Sci 162:459-468
Zhou T, Chen JQ, Araki H, Jing Z, Jiang K, Shen J, Tian D (2004) Genome-wide identification of NBS-LRR genes in *japonica* rice reveals significant expansion of divergent non-TIR NBS-LRR genes. Mol Gen Genet 271:402-415
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