Chapter 10

Adaptation of Local Grapevine Germplasm: Exploitation of Natural Defence Mechanisms to Biotic Stresses

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Additional information is available at the end of the chapter

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1. Introduction

The history of human civilization is closely intertwined with the development of viticulture, considering the consumption of grapevine fresh fruits and their use for wine-making since Neolithic Age. Evidences are given by the archeobotanical discovers of ancient seeds belonging to *Vitis vinifera* subsp. *sativa* (domestic grape) and *Vitis vinifera* subsp. *sylvestris* (wild grape) and by the discovery of wine jars and primitive stone wine presses, known locally as "pestarole" [1-2]. These evidences suggest that wine production was initially dependent from the collection of wild fruits, and subsequently from the cultivation of plants derived from the domestication of the Eurasian grapevine. Probably grapevine domestication occurred in different areas, from most ancient Anatolian and Caucasian centers to recent west Mediterranean basin and Central-Europe centers. The study of grapevine domestication is complicated by the presence of large para-domestication areas where wild plants were protected for fruit utilization [3]. Grapevine domestication was significant for the development of Mediterranean agriculture, based on cereal-olive-grape cultivations, typical of Greek and Roman civilizations. During the Middle-Age grape cultivation was maintained by monks as wine is bound to Christian liturgy. During late Middle-Age a description of grape varieties used for the production of high value wines was drawn [4] and among listed varieties, some are currently cultivated. The diffusion through the Europe of several grape pathogens during XIX century, such as downy and powdery mildew, caused the end of the ancient vitiviniculture, the erosion of grape genetic variability and the increase of chemicals used for plant disease protection.

During last years climate changes are causing the increase of favorable environmental conditions for the development of grapevine diseases, with the reduction of suitable areas for traditional crops particularly in some Mediterranean regions [5]. At the same time the large use of copper compounds to control grape diseases lead to accumulation of the toxic heavy metal in soil and groundwater [6]. Considering economical losses, the limits to the use of chemicals for plants protection and the wide market requirement of high quality wines able to express terroir characteristics, the interest of researchers and viticulturist for grape local varieties increased. In many countries with viticultural historical tradition the recovery and description of local varieties are undertaken, due both to vines adaptation to local environment and grape ability to determine typical qualitative characteristics of wines. Even thought the short coevolution period with invasive pathogens and the use of agamic propagation reduced the probability to have disease resistant grapevine genotypes, *V. vinifera* local germplasm can includes minor varieties which characteristics are often unknown with respect to genetic profile, viticultural and oenological potential [7-9] and to the degree of resistance to pathogen, that was already observed during XIX century [10-12]. Different responses to biotic stresses were described among grapevine varieties, particularly concerning the widespread pathogens *Plasmopara viticola, Erysiphe necator* and *Botrytis cinerea,* the causal agents of downy mildew, powdery mildew and of gray mold respectively [13-19]. It is well-known that grapevine genetic resources for pathogen resistance are mainly found in American and Asian wild *Vitis* species [20-23], but at present breeding programs involving *V. vinifera* and aimed to obtain resistant genotypes, released disease resistant interspecifics hybrids able to produce wines suitable only for local markets. For these reasons the study of natural defence mechanisms to biotic stresses of *V. vinifera* varieties has a scientific and applicative interest to improve both the management of grape genetic resources than wine quality.

The aim of the present paper is to review most studied constitutive and inducible defence mechanisms in *V. vinifera*. Among constitutive defences, anatomical and morphological features of leaf, bunch and berry were described. Furthermore induced defence mechanisms, including callose synthesis, stilbenes production and pathogenesis related (PR) proteins induction were discussed. The analysis of different *V. vinifera* varieties indicate that in many cases grapevine varieties have or activate defence responses to biotic stresses.

2. Plant defence mechanisms

The relationships between plant and pathogen start with the initial contact phase between infective propagules and the plant tissue surfaces. As response plants are able to activate defence mechanisms that may be referred to constitutive or inducible defences.

2.1. Constitutive defences

Constitutive defences are active in the plant before pathogen challenge. They are considered able to limit the entry phase of parasite in host tissues through direct penetration or pre-existing tissues opening and to contrast the infection during the first phases. Constitutive defences are generally referred to morpho-anatomical characteristics of leaf, bunch and berry,

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developed independently from fungal attack [13] or include constitutive compounds that can have antimicrobial activity. The synthesis of some antimicrobial constitutive compounds may be also enhanced as plant response to stresses [24-25].

- Leaf Hairs

Grapevine leaf hairs (trichomes and bristles) are morphological characters with ampelographic value. The density of leaf prostrate and erect hairs is included in the OIV descriptors list for grape varieties and *Vitis* species [26]. The number and length of leaf hairs differ according to *Vitis* species and varieties and may be influenced by environmental conditions (Figure 1). The hairs of abaxial leaf surface can constitute a hydrophobic barrier able to reduce the contact area among water droplets and leaf lamina, with the reduction of wettability of epidermal tissues. The presence of very dense leaf hairs leads to a reduction of water retention capacity of the leaf surface [27-28], that are decisive during the infection process [29-31]. The density of abaxial leaf hairs has been related to the different degree of tolerance of *Vitis* species to pathogens [32-33]. In *V. doaniana* and *V. davidii*, downy mildew resistant species, the reduction of leaf wettability prevent zoospores emission from zoosporangia and the pathogen is hampered to reach host tissues. In these species the use of wetters to reduce water surface tension increases the infection and lead to the regular sporulation of the pathogen. A similar behavior was demonstrated in *V. cinerea* e *V. labrusca*, which downy mildew infections were enhanced by wetter use, but the subsequently pathogen growth was blocked, supporting the hypothesis of the presence of further defence mechanisms in these two species. In *V. vinifera,* even though any significative correlation was demonstrated between the hair density of abaxial leaf surface and plant resistance to downy mildew [34-35], furthers investigations might be useful, according to the great variability of the character among varieties and clones.

- Stomata

Stomata are plant natural openings bordered by two guard cells, that exert a control over plant water and carbon cycles by variation in both size and number. In grapevine leaf they occupy a small percentage of the surface and are mainly located in the abaxial side. In *V. vinifera* cultivars, stomatal leaf density (number of openings per leaf surface unit) varies according to environmental conditions, including CO_2 concentration, light intensity, air temperature, photoperiod [36] and genotype [37-39] (Figure 2). Stomata are one of the most important way for pathogen entry [40-42]. The penetration of the grapevine obligate biotrophic parasite *P. viticola* occurred exclusively through stomata, while sporulation can rarely occurs also through other tissue openings [43]. During sporulation stomatal density can affect *P. viticola* secondary infections [43]. The mobility of pathogen zoospores to health stomata was related to a chemotaxis process that is regulated by chemical compounds as aminoacids, isoflavons, pectins and cell wall fragments, which production might be influenced by stomata opening [44]. Other hypothesis have been evaluated to explain a functional relationship between stomata and zoospore, including the presence of electrical fields produced by stomata [45-46]. Infected stomata are preferential sites of attraction for zoospores. This process, known as adelphotaxis, is the cause the accumulation of more than one zoospore on the same stoma [47-48]. Studies carried out on *V. vinifera* varieties showed a no clear relation between leaf stomatal density and susceptibility to downy mildew [35], even though the lower percentage of infected stomata occurred in *V. vinifera* varieties with the lower number of stomata per surface unit (Paolocci and Muganu, unpublished data) (Figure 3). Functional stomata found on the berry surface are possible entries for pathogens [49]. After berry set and under the influence of climate, stomata are quickly covered by wax layers and originates lenticels, that are often surrounded by cuticle tears [50-51]. These morphological transformation was correlated to the acquisition of berry ontogenetic resistance to downy mildew, even though the occurrence of berry infection during this phase remains still possible through berry pedicel [49]. Starting from veraison lenticels and peristomatic tissues represent the main entry sites for *B. cinerea* infection, but a significative correlation between the number of lenticels and the degree of berry susceptibility to gray mold was not demonstrated [52, 13]. *V. vinifera* stomatal opening/closure is influenced also by plant health, considering that in downy mildew infected leaves stomata are open in darkness and during water stress, leading to an increase of transpiration. This functional relationships is not systemic, being restricted to the infected area, and could be related to non-systemic compounds affecting stomatal activity and produced by pathogen or by the infected plant [53].

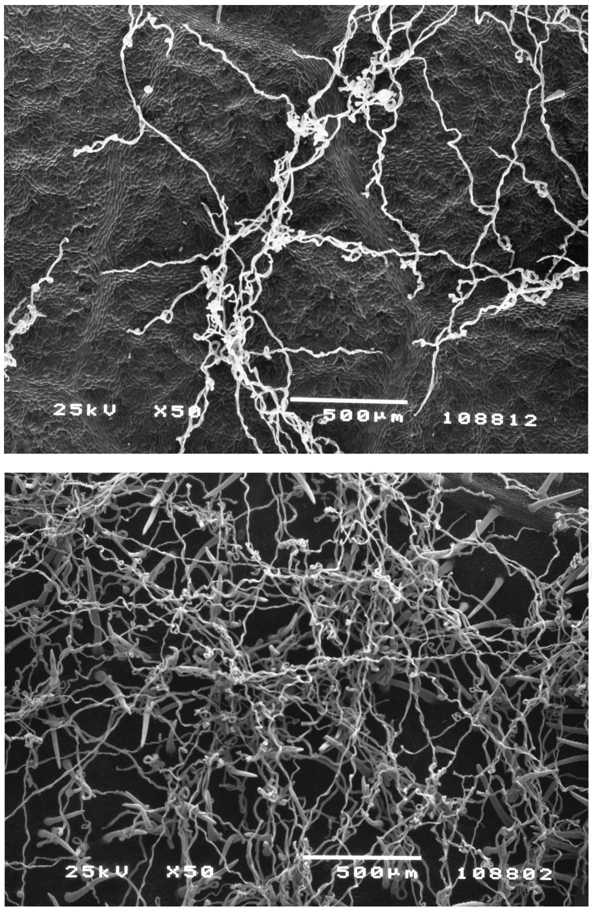

Figure 1. Scanning electron micrographs of hair density assessed on abaxial leaf surface on the two V. vinifera local varieties Romanesco (above) and Trebbiano giallo (below) (pictures by Muganu and Paolocci)

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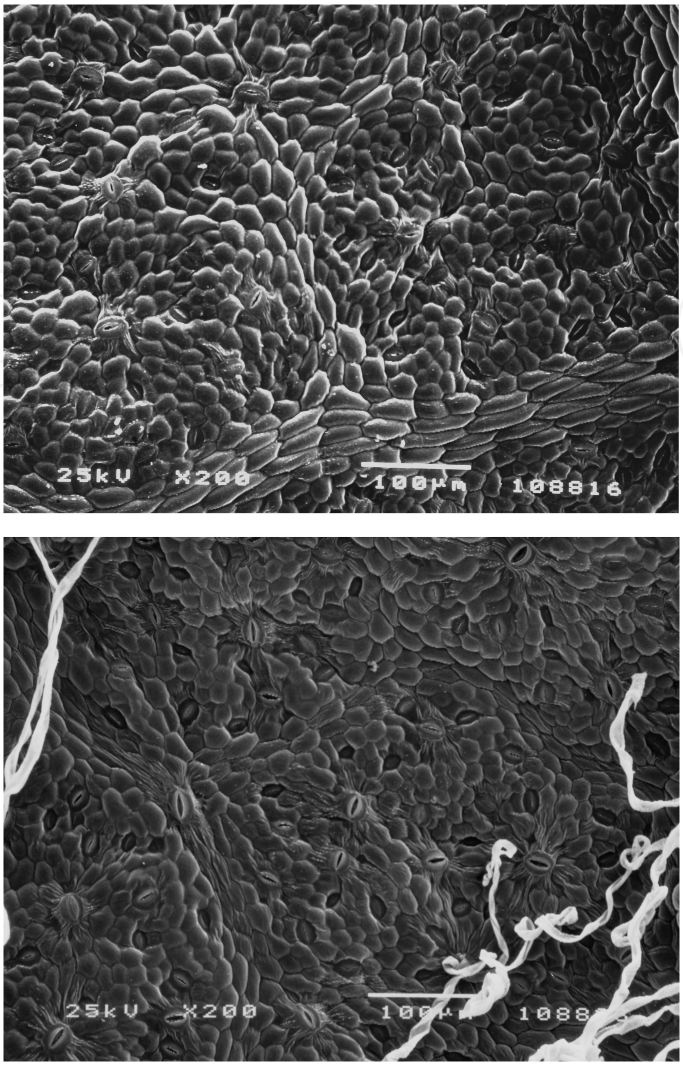

Figure 2. Scanning electron micrographs of stomata density assessed on abaxial leaf surface on the two V. vinifera local varieties Romanesco (above) and Trebbiano giallo (below) (pictures by Muganu and Paolocci)

• Cuticular membrane

The cuticle is a protective membrane of aerial plant tissues able to maintain a stable tissue form, to reduce water loss and to control gas exchanges [54-55, 51]. The cuticle is formed by an insoluble cutin layer and a soluble epicuticular wax layer. Quantitative differences in cuticle content among varieties have a genetic control even though wax amount and plate-like structure are influenced by environmental factors [13, 56-57]. The cuticle membrane is the first defence barrier that many plant pathogens must overcome to infect plant tissues and its variation in thickness, structure and composition have been analyzed to study its protective role against several grapevine diseases. The thickness of leaf cuticle of different grape varieties was positively correlated to their susceptibility to *E. necator* [58-59]. Nevertheless the increase of cuticle thickness during berry growth was not related to the acquisition of ontogenetic resistance to *E. necator* of mature berries [60].

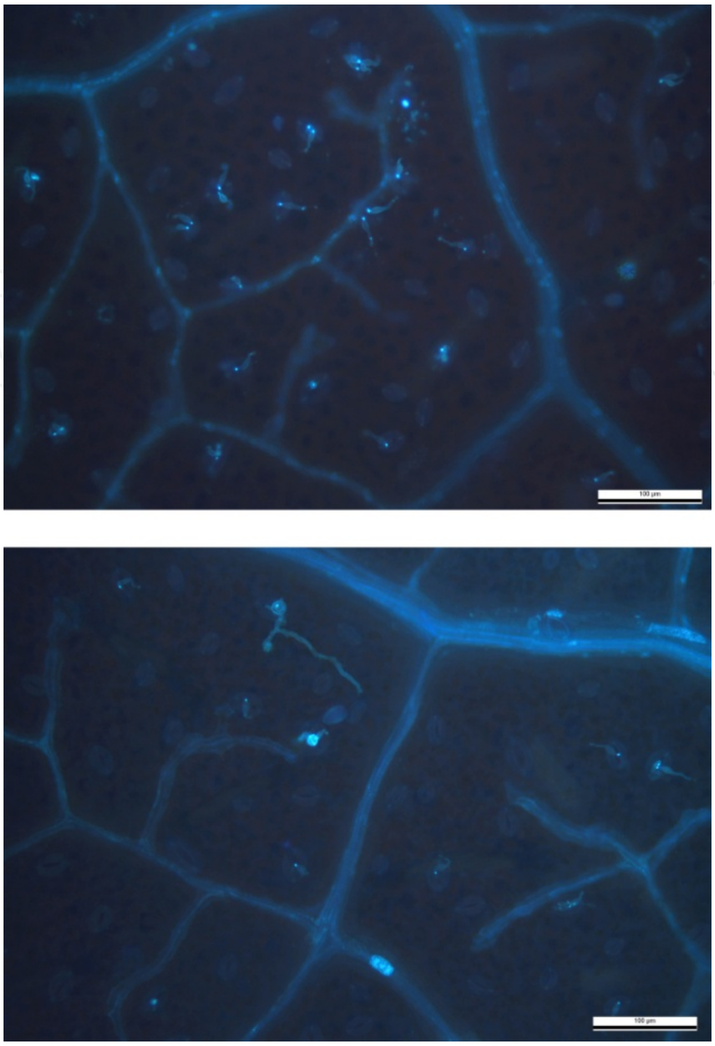

Figure 3. Epifluorescence micrographs of leaf stomata infected by P. viticola assessed at 24 hours post inoculation on the two V. vinifera varieties Aleatico (above) and Trebbiano toscano (below) (pictures by Muganu and Paolocci)

The amount of berry epicuticular wax positively affected the level of resistance to *B. cinerea* of different *V. vinifera* varieties as the influence of wax content on berry skin hydrophobicity and reduction of pathogen adhesion [13]. The removal of berry epicuticular wax increases the susceptibility to *B. cinerea*, indicating a role of wax layer on the infection phase [61]. The significant decrease of cutin content per surface unit of berry skin from berry set to veraison influenced the susceptibility to gray mold of three different clones of Pinot noir [62].

Anyway mere dimensional or quantitative variations of the cuticle membrane seem not explain the changes of grapevine degree of resistance to pathogen during annual vine cycle [63]. For this reason the presence of morphological and/or chemical differences occurred at cuticle level during berry growth and able to influence the development of infection, must be considered. Several studies analyzed the chemical composition of berry epicuticular wax from berry set to harvest. Variations of lipidic and alchoolic composition of the cuticle were shown in the transition from bunch closure to veraison phase and the presence of compounds with an inhibitory effect on the germination of *B. cinerea* conidia were detected [64-65].

- Bunch and berry features

Morphological characteristics of bunch and berry anatomy can affect grape resistance to pathogens. The evaluation of bunch density allows us to distinguish loose bunches, with movable berries, and dense or very dense bunches with not movable and sometimes deformed berries, as consequence of the contact with each other. Tight bunches determine the presence of micro-environmental conditions in the fruit zone, such as the increase of air temperature, low ventilation and relative humidity, that could promote pathogen growth, as showed for *B. cinerea,* which occurrence could be enhanced [62]. A part the frequency of berry skin cracks, that cause the release of free water required in the germination of *B. cinerea* conidia, the increase of physical contact among berries during growth leads to the development of flattened areas on berry contact surfaces and affects the structure of epicuticular wax. The contact surfaces show the larger areas of amorphous and thinner wax and the higher number of gray mold infections compared to non-contact berry skin surfaces [61, 54]. Recent studies about ampelographic characters described a negative correlation between bunch density and berry degree of resistance to *E. necator*, whereas any relation was obtained among powdery mildew infection and bunch length, width and shape [18].

Some morphological and anatomical characteristics of the berry were related to the susceptibility to *B. cinerea.* A positive correlation was found among berry resistance to gray mold and berry skin thickness and number of epidermal cell layers [13]. The intravarietal evaluation of Spanish Albariño variety showed that clones with small berries and short pedicels were low susceptible to gray mold [66].

- Constitutive compounds

Constitutive compounds with antimicrobial activity are preformed in plant tissues before host-parasite interaction. Many of these compounds may be related to the group of phytoanticipins according to the following definition “phytoanticipins are low molecular weight antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents” [67]. These metabolites are complementary to phytoalexins, antimicrobial metabolites which synthesis occurs after plant-parasite contact [68]. Preformed phenolic compounds were demonstrated to have antimicrobial activity [69-70] such as constitutive pterostilbene that showed antifungal properties against *B. cinerea.* Pterostilbene was detected in low concentration in gray mold resistant young berries, but its toxic activity against pathogen was enhanced by the high content of glycolic acid during berry set [71]. Other constitutive phenols, such as cathechin, epicatechin-3-*O*-gallate, caftaric acid and cutaric acid are able to inhibit fungal stilbene oxidase activity between flowering and veraison, and a high content of catechin was detectectd in *B. cinerea* resistant grape varieties after veraison [72]. The non-specific inhibition of *B. cinerea* lytic enzymes was related to the detection of proantocyanidins, polymeric flavonoids which are considered inhibitors of the oxidative fungal enzyme laccase, responsible of pterostilbene detoxification [73]. These results suggest that the resistance of young berries to gray mold depends on both catechins and proantocyanidins contents which contribute in maintaining the pathogen in a quiescent state [72, 74]. Considering that the decrease of proantocyanidins content during berry ripening lead to the increase of gray mold susceptibility, proantocyanidins are considered as markers of grapevine *B. cinerea* resistance [75].

It is well known that light exposure affect the synthesis of phenolic compounds. For this reason several studies evaluate the relationships between the intensity of tissue sun-light exposure and grapevine susceptibility to pathogens [76-78]. Shaded *P. viticola* infected leaves, besides showing the lower content of flavonoids compared to full light exposed ones, also displayed the highest disease severity [69]. A similar result was obtained with the artificial inoculation of detached berries with *E. necator:* in this case shaded berries showed the highest susceptibility to the pathogen [79].

Insects use of plants as a source of nutrients causes tissue mechanical damages and, in many cases, compromises plant health as consequence of virus or phytoplasma transmissions. Phytophagous find host plant using mainly olfactory signals produced by the host plant itself. These chemical signals, known as volatile organic compounds (VOCs), are plant secondary metabolites including alcohols, aldehydes, terpenoids and aromatic phenols, that showed a different role in plant-insect relationships [80]. Each plant species releases specific bouquets, which blend is influenced by plant phenology and health conditions [81]. Grape berries and leaves release hundred of volatiles compounds among which α-farnesene, (E)-β-farnesene and (Z)-3-hexenyl acetate. These compounds detected in Chardonnay varieties between pre-flowering and green berry developmental phases, significantly elicited female attraction of *Lobesia botrana*, the most important insect of *V. vinifera* in Europe [82]. *Lobesia botrana* feeds on all *V. vinifera* cultivars but a different susceptibility to the insect among grape varieties was shown [83].

In the study of Grapevine Yellows the involvement of VOCs in the ecology of *Scaphoideus titanus* Ball (the causal agent of Flavescence Doreé) and of *Hyalesthes obsoletus* Signoret (the causal agent of Bois noir), are under investigation. Observation on preference of *H. obsoletus* for different plant species were made testing different plant extracts among which *V. vinifera* [84].

2.2. Induced defences

The induced defences are the result of plant reaction to pathogen attack and require the perception of plant-tissues signals resulting from pathogen infection.

Plants have evolved different active defence strategies aimed at the protection against biotic stresses. A first strategy is founded on the recognition, by host extra-cellular receptors, of pathogen associated molecular patterns (PAMPs) which are microbial products, among which chitin [85-87]. This recognition triggers active plant defence mechanisms (PAMP-Triggered Immunity PTI), including the synthesis of pathogenesis related (PR) proteins, and the strengthening of plant tissue cell walls [88]. PTI strategy is considered a plant basal immunity against non-host specific pathogens and can be overcame from host specific pathogens, which developed the ability to produce effectors, molecules able to suppress PTI resistance. As consequence plants evolved effector-triggered immunity (ETI) defence mechanism, which enable plant recognition of the PTI-suppressing effectors [86-87]. This strategy, which involves the activation of specific resistance (*R*) genes, lead to a hypersensitive response (HR) which is one of the most efficient mechanism used by plants to arrest biotrophic pathogen infections. HR involves the massive production and accumulation of reactive oxygen species (ROS), among which hydrogen peroxide (H_2O_2), that can modulate localized plant cell death (PCD) of infected tissues which prevents pathogen nutrition and growth. It must be considered that H_2O_2 can act also as diffusible signals for the induction of different plant defence reactions, among which the production of phytoalexins, of PR proteins and of cell wall polymers. HR mechanism was described in the american species *V. rotundifolia*, resistant to *E. necator* and in some of their hybrids with *V. vinifera* [89]. Recently PCD activity was also proved for the two grape varieties Kishmish vatkana e Dzhandzhal kara, belonging to *V. vinifera* subsp. *sativa* proles *orientalis* subproles *antasiatica*, native of Uzbekistan [90]. It may be useful to highlight that effector-triggered immunity is dependent on the activation of single, dominant genes and can be overcome by the deletion or mutation of a single effector [86-87, 91].

Besides to tissue-localized defence activities, plant pathogen recognition also induce plant systemic reactions, known as systemic acquired resistance (SAR). SAR enhances defence responses against a wide range of biotrophic pathogens in plant organs remotely located from the initial site of infection [92-94]. It has long been thought that salicylic acid (SA) is a key signaling molecule in plant defence resistance against biotrophic pathogens and it is required for activation of SAR [95- 96]. Endogenous salicylic acid level was higher in powdery mildew resistant *V. aestivalis* than in susceptible *V. vinifera* varieties, which salicylic acid content increased only at 120 hours after infection, being inadequate to limit disease progression [97]. Evidence of the involvement of salicylic acid in SAR is showed by the exogenous application of SA that increases the synthesis of stilbenes [98] and of PR proteins [99-100].

As above described the different grapevine defence mechanisms trigger the production of physical barrier or the synthesis of anti-microbial compounds that are involved in grapevine pathogen resistance strategies. Among which:

- Callose synthesis

The synthesis and accumulation of callose, a sugar polymer of (1-3)-β-D-glucose, occurs in phloematic tissues, root hairs, epidermal cells and in parenchimatic tissues as a consequence of fungal infections. Callose synthesis is considered a grapevine induced defence response to powdery and downy mildew [101-102]. Callose deposition on stomata as response to *P. viticola* infection is able to block the penetration of zoospores to the substomatal cavity 7 hours post infection (hpi) and at 24 hpi infected stomata are surrounded by necrotic areas, showing a HR-like reactions. The deposit of callose was also detected at 120 hpi in stomata close to infections sites, even though the presence of necrotic areas did not occur. The nature of signals that affect neighboring health stomata are unknown, but their callose deposition is able to prevent secondary infection and could be referred to a systemic acquired resistance process (SAR) against *P.viticola* [43]. The percentage of infected stomata that showed callose deposition at 48 hpi is used as a histological marker to evaluate the degree of resistance to downy mildew of grape varieties [103]. Late callose deposition was detected in the mesophyll both in susceptible than in resistant *Vitis* varieties. At this time the pathogen block, occurred at 3-4 days after inoculation, was observed only in resistant varieties and was related with the presence of further defence mechanisms [102]. The presence of callose deposit was observed as a consequence of the grape leaf infection of *E. necator.* In this case the penetration of the haustorium in epidermal cells was stopped by the formation of a papilla, a structure formed by different layers containing carbohydrates, silica and phenolic compounds and callose deposits could be observed around the haustorial neck and papilla [101].

The role of callose in grape defence mechanisms was validated by the increase of the number of sporangia produced in leaf tissues infected with *P. viticola* treated with 2-deoxy-D-glucose (DDG), an inhibitor of callose synthesis. The increase of sporangia was observed also in *P. viticola* resistant variety Solaris, even thought Solaris treated tissues showed higher resistance compared to the basal resistance of susceptible Chasselas variety, indicating the involvement of further resistance factors, besides callose synthesis, in Solaris [104].

- Stilbenes synthesis

Stilbenes are low molecular weight phenolic compounds found in several plant genera, included many *Vitis* species. Stilbenes show low solubility in water and high solubility in organic solvents. In *V. vinifera* they are costitutive compounds of the berry and of woody tissues [105]. Grapevine stilbenes include several compounds among which resveratrol, with *cis* and *trans* isomers, piceid and resveratroloside, two glucosides of resveratrol [106] and different molecules derived from resveratrol, that include pterostilbene and viniferins [71, 107-108]. Resveratrol was the first described stilbenic compound and its activity is studied since the first half of XX century. Resveratrol content in grape berries is influenced by environmental conditions, vineyard agronomic management and genotype characteristics [105-106,109-110]; it is included among wine components [105-106] and recently its regular presence in human diet was positively correlated with the protection from cancer and other cardiovascular diseases [110-112]. Stilbenes production has been related to plant response to abiotic elicitors among which UV-irradiation, ozone, fosetyl-Al, metyl-jasmonate, benzothiadiazole, chitosan olygomers, ciclodextrins and salicylic acid [113-114]. Stilbenes are induced in non-woody vine tissues, such as flowers, leaves and berries, by different pathogen infections among which *B. cinerea,* one of the first studied elicitors [107], and by *P. viticola, E. necator*, *Phomopsis viticola*, *Rhizopus stolonifer*, *Aspergillus* spp., *Trichoderma viride* [106, 114-115]. Induced stilbenes are considered phytoalexins, compounds with antimicrobial activity and the involvement of stilbenic phytoalexins in grapevine induced defences against *B. cinerea* was observed for a long time [113]. Stilbenes are able to inhibit some fungal ATPases and fungal cells respiration [73, 116], and their effectiveness is related to the rapidity of their synthesis. Stilbene-sinthase is the key enzyme in resveratrol synthesis. The decrease of berry resveratrol content during berry ripening and sugar accumulation goes with the increase of berry susceptibility to *B.cinerea.* Resveratrol reduction in ripe berries was related to the decline of stilbene-sinthase gene expression and to the contemporary increase of chalcone-sinthase enzyme which is bound with flavonoyds synthesis [73, 116]. Among stilbenes, resveratrol did not show an instant antimicrobial activity [117], even though the long term incubation of the pathogen together with resveratrol can inhibit conidia germination and the growth of germ tubes [118]. Also the production of resveratrol in micropropagated grape explants was correlated with the severity of gray mold infection [119]. *B. cinerea* evolved the ability to detoxify grape berry phytoalexins by stilbene oxidase activity [72,74] and the accumulation of resveratrol in leaf tissues of *in vitro* transgenic plants for stilbene synthase gene was related to the reduction of disease severity [120]. Among other stilbenes the role of pterostilbene against gray mold infection remains unclear, considering that its content did not increase after berry inoculation [73].

Several studies analyzed stilbene production during downy mildew infection. The toxicity of pterostilbene and of the two resveratrol dimers δ-viniferin and ε-viniferin against *P. viticola* was demonstrated, while piceid, a resveratrol derived compound, did not show antimicrobial activity as its high synthesis and accumulation was showed in infected leaf tissues of the susceptible Chasselas variety [103, 121]. The involvement of stilbenes in induced defence mechanisms against *P. viticola* was shown in *V. rotundifolia,* which infection with the oomycete lead both to the extrusion of pathogen cells from stomata and to the accumulation in infected tissues of one hundred fold of stilbenic molecules compared to the stilbene content detected in infected tissues of resistant hybrids [122]. Pterostilbene is considered the most toxic stilbene compound against downy mildew. Its inhibition of the mobility of *P.viticola* zoospores was shown in laboratory tests, whereas resveratrol and piceide did not influence pathogen propagules activeness [121]. In the resistant grape hybrid IRAC 2091 pterostilbene was one of the most synthetized stilbenes in infected tissues, and its toxic activity caused the reduction of pathogen growth and development [122]. Anyway the average constitutive content of pterostilbene in *V. vinifera* varieties is very low: less than 5 μg/g in leaves and fruit [73] and its concentration still remains very low in infected leaves and berries. As consequence its role in defense mechanisms is difficult to study [121]. Among stilbenes also viniferins showed a toxic activity agaist *P. viticola* zoospores, particularly δ-viniferin that has higher toxicity compared to ε-viniferina. Both compounds were identified as the major stilbenes synthesized in grape leaves infected with *P. viticola*, playing an important role in grapevine resistance to downy mildew [121]. Stilbenes have been proposed as early selection markers for resistance in grapevine breeding programs aimed to obtain downy mildew resistant genotypes. The analysis of contents of viniferins in the leaves of seedlings at 48 hpi can predict the degree of resistance to downy mildew in the selection of resistant hybrids [103].

Plant stilbene synthesis was related to the grapevine disease powdery mildew [123]. The exogenous application of methyl-jasmonate on susceptible Cabernet-Sauvignon variety increased its resistance to *E. necator* and the content of resveratrol, piceide, ε and δ- viniferins and of pterostilbene in the epidermis of leaves, suggesting a role of stilbenes in plant defence mechanisms against powdery mildew [124]. The determination of viniferins content as marker of resistance to powdery mildew has been proposed to carry out genetic selection programs. Considering that *E. necator* infections are restricted to the first layer of epidermis the amount of viniferins must be related to the number of fungal appressoria [119].

- Other phenolic compounds

Plant phenolic compounds are a very heterogeneous group of metabolites which presence in plant tissues is considered an adaptive response to adverse environmental conditions. The role of these metabolites may be physiologically important as a means of storing carbon in presence of plant nutritional deficiencies [126] and the abundance of different phenolic compounds in plant tissues has been explained as an evolutive strategy of protection against plant tissues photodamages [25]. Anyway many evidences suggest that phenolic compounds accumulation may be related to plant defence responses induced by pathogen infection [25]. The analysis of plant responses showed that the accumulation of polyphenols in cell wall of infected tissues and non-infected neighbouring tissues is related to plant HR response induced by pathogen penetration [59]. The accumulation of electrondense deposits referable to phenolic compounds was observed in *V. rotundifolia* spongy mesophyll and palisade as a consequence of *P. viticola* infection [122].

Among phenolic compounds the synthesis of flavonoids besides by light intensity can be influenced by biotic elicitors [25]. Their accumulation in grapevine tissues was related to induced defence mechanisms as shown in different comparative studies on *Vitis* species. In downy mildew resistant *V. rotundifolia*, the rapid plant response to the infection and the inhibition of pathogen growth was associated with the occurrence of small tissue necrotic spots and the detection at 2 days post infection (dpi) of a high content of flavonoids in infected stomata and closer tissues. A similar accumulation of flavonoids was detected in *V. rupestris*, an intermediate resistant species to *P. viticola,* that at 8 dpi showed the presence of peroxidase activity and the occurrence of wide tissue necrosis, resveratrol accumulation and delayed synthesis of lignin (15 dpi). In *V. vinifera* cv Grenache any HR activity were observed after infection and delayed flavonoid accumulation, detected at 8 dpi, was not able to limit high pathogen sporulation. These data suggest a key role of flavonoids during downy mildew infection as their fast synthesis is able to limit pathogen growth [127].

Grapevine berries show a different resistance to *E. necator* during their growth, considering the development of berry ontogenetic resistance [60]. The presence of autofluorescent polyfenolic compounds induced by powdery mildew infection was monitored in *V. vinifera* during berry growth. The accumulation of phenols occurred in infected cells near fungal appressoria and in non infected contiguous cells with higher frequency in susceptible young berries compared to resistant older berries which showed the lowest rate of polyfenolic oxidization [63].

A different regulation of chalcone-flavonone isomerase, a key enzyme involved in the biosynthesis of flavones, a class of flavonoids, was also found in *V.vinifera* Nebbiolo variety as consequence of the Flavescence dorée disease, suggesting the possible involvement of polyphenols in plant response to phytoplasmas [128].

- Pathogenesis-Related Proteins

Pathogenesis-related (PR) proteins may be produced in host plants as response to biotic and abiotic stresses, chemical elicitors, tissue injured by the induction of specific PR genes [100, 129-131]. They are characterized by different structure and biological activity and include 17 families of proteins with low molecular mass, high resistance to proteolysis and soluble in acid buffers [132]. Different PR proteins families have been detected in grapevine: PR-2 proteins (β-1,3-glucanases) and PR-3 and 4 proteins (chitinases) are able to hydrolyse β-1,3-glucans and chitin respectively that are known to be components of cell wall of different higher fungi; PR-5 proteins (thaumatin-like proteins) which antifungal activity is associated with the permeabilization of fungal membrane or to chitinase activity [133]. Recently PR-10 proteins family was also described [134-135].

Some members of different PR families show antifungal activity strengthening their possible role in plant defence [129, 136]. Isoforms of grape berry chitinases proved to have high toxicity against *B.cinerea* as their *in vitro* reduction of fungal conidia germination and inhibition of hyphal growth [100, 137]. Also thaumatin-like protein derived from mature berries of *B. cinerea* resistant varieties inhibited hyphal growth of grape pathogen *Botrytis cinerea* [137].

Anyway, even though some classes of these PR proteins showed in vitro toxic activity against grape pathogens, their role in plant defence mechanisms must be elucidated. Several studies analyzed the synthesis of PR-like proteins in non infected grape berries during ripening. From veraison to harvest there is a significant increase in total content of berry proteins. During this period most induced soluble proteins are chitinase and a thaumatin-like proteins also considering the decrease of photosynthetic enzymes. The accumulation of antifungal proteins in berries during this period occurr in ripe berries as they acquire resistance to powdery and downy mildew. Experimental results show that the antifungal efficacy of PR-like proteins is enhanced by sugar concentrations, showing the possible role of berry hexoses in the preservation of protein structure [100, 137]. Transcriptional changes in pathogen susceptible and resistant grape varieties were observed after tissue infections and in several studies the largest proportion of common transcripts were related to disease resistance, including several encoding PR proteins such as chitinases and β-1,3-glucanases.

The variation of chitinase and of β-1,3-glucanase activities was analyzed during grape leaves infection with *B.cinerea*. Pathogen infection significantly elicited the biosynthesis of chitinases starting from 48 hpi. A similar trend was observed for glucanase activity which increased from 48 to 72 hpi. Both chitinases and β-1,3-glucanases presence was observed around leaf dead cells, were the accumulation of secondary metabolites, among which phenols, was detected [100]. High levels of chitinases and of β-1,3-glucanases, which showed a lytic activity against germinative tubes of *E. necator*, were detected in infected grape leaves and green berries [138]. Among defense-related proteins that accumulated in Cabernet Sauvignon infected leaves, two members of PR-10 familiy were identified at different times from inoculation as response to powdery mildew infection [139].

Some studies suggest that the different level of resistance to *P. viticola* between resistant and susceptible varieties is induced after infection and is not related to differences in basal gene expression. Transcriptional changes associated with *P. viticola* infection indicate that whereas in *V. riparia* the resistance is a post-infection condition related to the early activation of signal transduction and to the synthesis of defence metabolites, in susceptible *V. vinifera* only a weak and abortive defense response was shown after infection [140]. In downy mildew susceptible Pinot noir variety the induction of PR proteins occurred in the leaves at 48 hpi and the synthesis of most PR-10 defense related proteins increased significantly by 96 hpi, which was too late to produce an effective impact on the infection [141]. Anyway the increase of chitinase transcripts detected after *P.viticola* infection of susceptible young leaves of Pinot noir and the presence of a systemic induction of lytic enzyme activities were correlated with the expression of SAR [99].

The role of salicylic acid as molecular signal in the production of several chitinase isoforms in leaves and berries was showed [100] and recently in a comparative study between *V. riparia* and *V. vinifera* during the infection of the biotrophic pathogen *P. viticola* the significant increase of the basal level of jasmonic acid was detected only in resistant *V. riparia,* while in *V. vinifera* any difference between health and infected plants was observed [140]. A different regulation of thaumatin-like and osmotin-like proteins of the PR-5 family was also found in *V.vinifera* Nebbiolo variety as consequence of the phytoplasma disease Flavescence dorée [128].

It seems useful here to consider that the possibility to increase grapevine resistance to fungal pathogens by biotechnological techniques that can permit the overexpression of PR proteins could lead to the increase of the risks of wine turbidity.

3. Conclusion

In most suitable areas of grapevine cultivation a large number of hazaurdous pests and pathogens are able to compromise plant health and fruit quality. With the aim to protect vines from parasite attacks, viticulturists have developed agronomical strategies that include the use of chemical compounds, most of which have been successively found in mature grapes, causing the reduction of fruits and wine quality. The decrease of grape biodiversity and the present genetic homogeneity of most vineyards due to the wide cultivation of a restricted number of varieties, increase plant disease susceptibility and make difficult the implementation of protection strategies. The use of selective chemical compounds has significantly improved the control of some plant diseases, but different grape pathogens have developed resistant strains that reduced the effectiveness of plant chemical protection. At present the availability of disease resistant grape varieties or selected clones has became a key strategy in many viticultural areas. During last years the conservation of grapevine germplasm increased as the characterization of endangered genotypes can improve the study of grapevine natural defence mechanisms. Plants evolved different level of response against microbial attack and the studies on different disease mechanisms suggest that susceptible grapevine varieties show basal defences similarly to resistant genotypes, but in most cases delayed in time or weak for intensity. The study of morphological characteristics, genetic basis and chemical signals that regulated natural defence mechanisms in grapevine could allow us to develop significant advances in the exploitation of Vitis biological resources and in the use of marker assisted selection aimed to reduce the time to select resistant genotypes for fruit quality improvement and environmental costs reduction.

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