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Grapevine Biotechnology: Molecular Approaches Underlying Abiotic and Biotic Stress Responses

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Additional information is available at the end of the chapter

Abstract

Grapevine is one of the most abundant crops worldwide, with varieties destined for fresh and dry consumption, as well as wine production. Unfortunately, grapevine plants are affected by both biotic and abiotic stresses, generating significant economic losses. These conditions can negatively impact grape cultivation at different stages: plant and berry development during pre- and post-harvest, production, fresh fruit processing and export, along with wine quality. Most of the grapevine varieties are susceptible to several pathogens and within this chapter, particular attention is given to fungi (*Botrytis cinerea* and *Erysiphe necator)* and viruses, since they are a worldwide concern. Within the latter, special focus is given to the grapevine leafroll disease, a complex and destructive infection. On the other hand, abiotic stress is also relevant in grapevine, and in this chapter it will be exemplified by UV-B radiation and its impact on growth and fruit development, plant adaptive responses and its relationship with the quality of grape berries for winemaking. The main biotic and abiotic grapevine stress factors are reviewed in this chapter, considering a special focus on biotechnological approaches carried out in order to address them and minimize their detrimental consequences.

Keywords: grapevine fungal diseases, *Erysiphe necator*, *Botrytis cinerea*, grapevine viruses, UV-B radiation, grapevine biotechnology

1. Introduction

Grapevine (*Vitis vinifera L.*) is one of the most important crops worldwide. Within the Vitaceae family, the *Vitis* genus has a major agronomic importance. Among them, *V. vinifera* is the only species extensively used in the global industry, dominating the market with only a few cultivars, generally classified according to their final production: wine grapes, table grapes and raisins [1]. This low variability is directly related to grapevine's high susceptibility to biotic and abiotic stresses, which is associated with significant economic losses.

Most of the grapevine varieties are susceptible to several biotic agents, such as phytoplasma, bacteria, fungi, oomycetes, viruses and nematodes, which dramatically reduce plant yield and fruit quality, and negatively impact plant development. In vineyards, the most important diseases are caused by microorganisms such as fungi, oomycetes and viruses. Within pathogenic fungi, *Botrytis cinerea* and *Erysiphe necator* are the most important ones, producing the grey mould and powdery mildew diseases, respectively [2]. *V. vinifera* is classified as a susceptible species, where low or no resistant phenotypes have been described in economically significant cultivars until now.

On the other hand, nearly 60 viruses can infect grapevine plants, a much higher number than the ones affecting other perennial crops. Under natural conditions, grapevine viruses are transmitted by insect or nematode vectors. However, since grapevine is usually propagated by grafting, viruses can also disseminate within plants through these cuttings [3]. It is noteworthy to mention that unlike to other pathogens, grapevine plants present no virus resistance, meaning that they can establish compatible interactions where viral pathogens can spread throughout all tissues, generating a global cellular stress and developmental defects [4–6]. Regarding these infections, the leafroll disease is one of the most complex viral diseases known and is considered one of the most destructive in grapevines. In addition to their economic detriment to grapevine cultures, all viruses are relevant when the sanitary status of the vineyard is considered.

Abiotic stress factors, particularly water availability, temperature and light are also relevant in grapevine. Among them, ultraviolet (UV)-B radiation impacts on grapevine plants growth and normal fruit development. *V. vinifera* is often cultivated in Mediterranean climates with varied UV-B radiation dosages [7]. Grapevines are considered as well adapted to solar radiation due to a variety of physiological adaptive responses mainly based on antioxidant enzyme activities and secondary metabolites [8], which besides their role in defence against abiotic stress are relevant for colour, taste and aroma of grapes. These responses are triggered by UV-B perception and signalling pathway, which was recently identified and characterized in grapes [9, 10]. The increase of flavonols in response to UV-B has been reported in grapevine berry skins [9]. As a consequence, the quality of grape berries for winemaking is correlated with the accumulation of UV-B-induced phenolic compounds. Hence, wines with the highest concentrations of phenolic compounds are generally considered of excellence [11]. Therefore, understanding the mechanisms of perception, signalling and response of the grapevine to UV-B and using this knowledge to improve both productivity and fruit quality by genetic modification are attractive targets for the wine industry.

Biotechnological approaches aimed to solve grapevine stress affections, in areas regarding grapevine physiology and genetics, are a main requirement for optimizing and improving quality of this species through biotechnological tools.

2. Grapevine biotic stress

As mentioned above, biotic stress is related to infection caused by phytopathogenic organisms such as bacteria, nematodes, fungi, oomycetes and viruses, among others. These pathogens get the necessary elements for growth and reproduction from its hosts. According to their infection strategies, plant pathogens can be classified as necrotrophics, biotrophics and hemibiotrophics. Necrotrophic pathogens on one hand, extract nutrients from dead cells during colonization, secreting lytic enzymes and phytotoxins in order to promote necrosis in the host plant. Biotrophic pathogens, on the other hand, feed on living tissue maintaining the viability of the host in order to obtain metabolism products. Finally, hemibiotrophic pathogens start with a biotrophic infection phase, followed by a late necrotrophic one [12].

2.1. Fungal diseases in grapevine: a biotrophic and necrotrophic model

Nowadays, most of the wine, table grape and dried fruit cultivars have the Eurasian grape species *V. vinifera* as a common ancestor, mostly due to its distinctive flavour and aroma. However, another similar trait is their limited genetic resistance against fungal pathogens, making these cultivars highly dependent on the use of fungicides [13].

The most common fungal grape diseases are the powdery mildew and grey mould caused by the biotrophic pathogen *E. necator* and the necrotrophic *B. cinerea*, respectively [2].
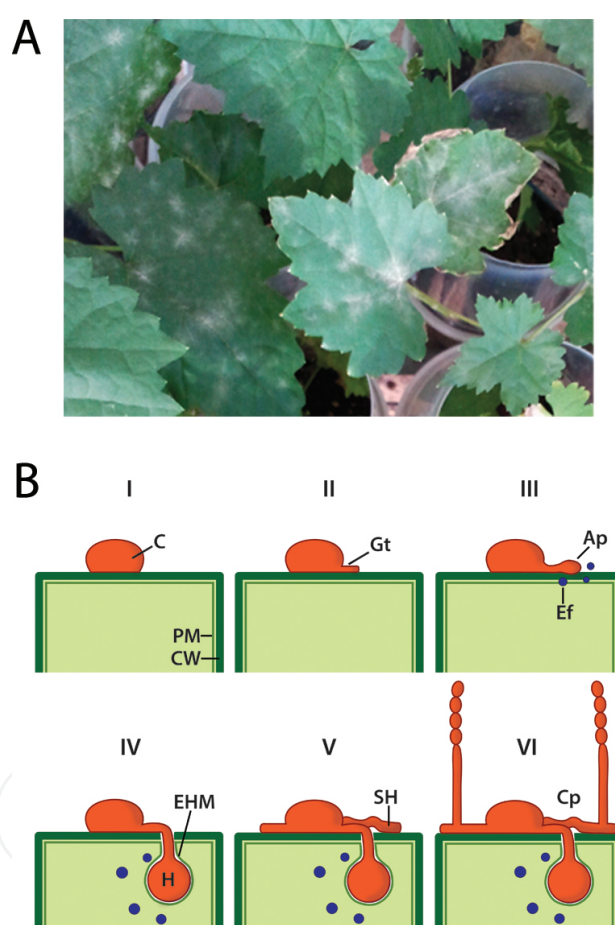
2.1.1. Powdery mildew: E. necator

E. necator Schwein (synonyms: *Uncinula necator* Burr., *E. tuckeri* Berk., *U. americana* Howe and *U. spiralis* Berk. and Curt.; anamorph: *Oidium tuckeri* Berk.) is a biotrophic and filamentous fungus that belongs to the Erisiphaceae family. *E. necator* is the etiologic agent of the powdery mildew disease in species of the *Vitis*, *Cissus*, *Parthenocissus* and *Ampelopsis* genera, being *V. vinifera* one of its most economically important hosts [14].

The powdery mildew disease is associated with large production losses as it reduces yield and fruit quality, mainly affecting the sugar content and acidity of the berries, although it can also infect other green tissues. This pathogen can be found in all grape-growing regions, especially in dry and warm weathers [15].

Being an obligated biotrophic pathogen, *E. necator* depends on its host for growth and development, as photosynthesis-active tissues are necessary to complete its life cycle. The infective process begins with the attachment of the asexual spore (conidia) on plant tissues, followed by the formation of a primary germ tube which differentiates in a specialized infective structure called appressorium. The latter then generates a mechanical pressure in order to penetrate and invade the host cell (**Figure 1**). Germination involves the secretion of fungal lytic

enzymes which leads to the release of compounds that enhance fungal germination and development [13]. The successful invasion results in cell membrane invagination and the haustorium formation, which is a specialized hypha that facilitates the dynamic exchange of molecules derived from both fungal and host cells. The fungus retrieves nutrients from the host cells, while at the same time it secretes proteins to suppress host defences. After this establishment, secondary hyphae proliferate in order to colonize a larger area of tissue across the surface, producing more appressoria and haustoria at regular intervals. The overall process culminates with conidiation, which involves the formation and release of asexual spores to distal tissues [16]. The main symptom of *E. necator* colonization is the appearance of a white powder in the infected host tissue, corresponding to mycelial proliferation and conidiophores development [14].

Figure 1. Grapevine powdery mildew and *E. necator* asexual life cycle. (A) Grape leaves infected with *E. necator* exhibit a white powder on the infected tissue surface. (B) Asexual life cycle stages. I: Conidium (C) attachment; II: Conidium germination and germ tube (Gt) formation; III: Appresorium differentiation (Ap); IV: Development of haustorium (H), extra-haustorial membrane (EHM) and secretion of virulence factors or effectors (Ef); V: Colonization and secondary hyphae (SH) formation; VI: Production of asexual reproductive organs or conidiophores (Cp).

When environmental or nutritional conditions become unfavourable, *E. necator* develops a structure of sexual reproduction that contains ascospores, called cleistothecia. Within this structure ascospores can remain dormant for months until favourable conditions allow

germination and, like asexual spores, appressorium formation in order to begin a new infective process [14].

2.1.2. Grey mould: B. cinerea

The necrotrophic fungus *B. cinerea* (Persoon: Fries; teleomorph *Botryotinia fuckeliana*) is widely distributed in nature and it lacks a specific host. This fungus is capable to infect vegetables, fruits and ornamental plants, among others, making it a great problem for many plant species [17]. However, its importance relies on its ability to infect crops of commercial interest, such as grapevine. It can cause soft rotting of all aerial plant tissues, and rotting of post-harvest fruits; production of grey conidiophores and (macro)conidia are typical signs of the disease [18].
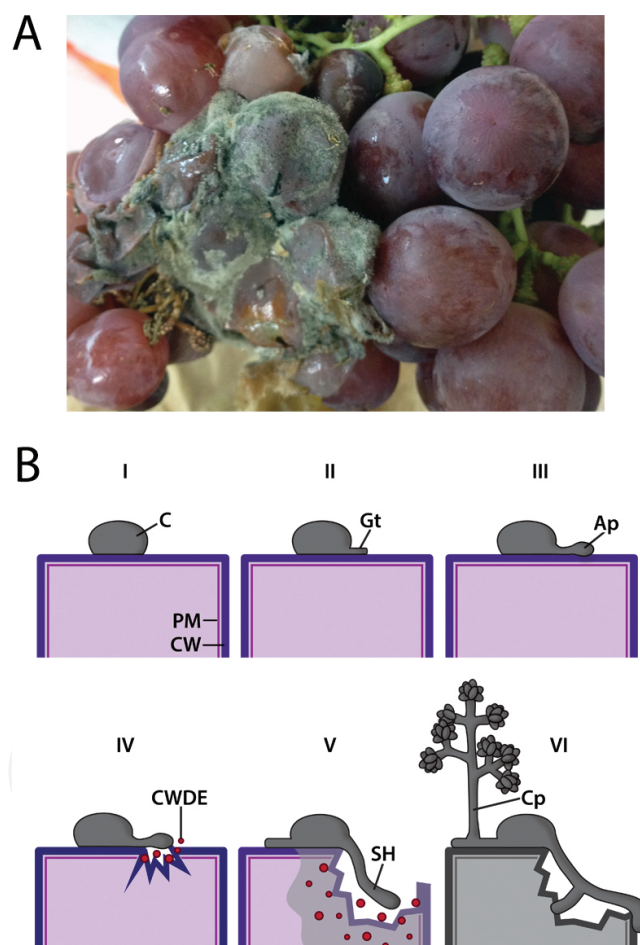
Figure 2. Grey mould and *Botrytis cinerea* asexual life cycle. (A) Grape berry cluster severely infected with *B. cinerea*. (B) Asexual life cycle stages. I: Conidium (C) attachment; II: Conidium germination and germ tube (Gt) formation; III: Appresorium (Ap) differentiation; IV: Secretion of cell wall degrading enzymes (CWDE); V: Colonization and secondary hyphae (SH) formation; VI: Development of asexual reproductive organs or conidiophores (Cp).

Grey mould disease causes heavy yield losses in table and wine grapes all around the world. As a consequence of the increase of the international trade of cold-stored products, this fungus

has gained great importance because it can grow effectively over long periods of time at just above freezing temperatures [18]. In the field, it can spread to other grapes by insects which can carry viable conidia and generate mechanical damage [19]. Although, *B. cinerea* shows a remarkable flexibility to germinate in different host environments, several factors influence the germination of a conidia, such as temperature, surface water, relative humidity, among others [20].

Once the conidium attaches to its host, it can germinate and develop to an infective structure called appressorium, which is able to breach the cuticle by means of a penetration peg (**Figure 2**). The underlying cells are killed by the fungus, and the primary lesion is established. After the skin barrier is damaged, *B. cinerea* causes decomposition of plant tissues in order to consume the plant biomass. At this point it secretes cell wall degrading enzymes (CWDE), toxins and oxalic acid. Subsequently, the hyphal growth is induced in order to begin the sporulation cycle and infection of adjacent cells [17].

In some tissues, *B. cinerea* causes long-lasting quiescent infections, in which no symptoms are discernible at first. It can also penetrate floral tissue of grapes (petals, stigmas, styles or stamens) and remain dormant, often for several weeks, until it resumes activity and invades the fruit later in the season or during ripening. It has been postulated that high levels of phytoalexins in immature fruits contribute to quiescence, acting as fungitoxic or fungistatic compounds; and that post-harvest host physiological and biochemical responses might activate the pathogen [21].

2.2. Grapevine responses to fungal diseases

Plants are considered to have two types of immunity: a general one against a broad spectrum of microorganisms, and other specific one against a particular pathogen. Both responses are characterized by their ability to recognize pathogen components, transduce the stress signal and induce a defence response. However, the main difference between the both is considered to be the robustness and duration of the response [22].

The first type of immunity is known as PTI (pathogen-associated molecular patterns (PAMP) triggered immunity) and is activated by PAMP recognition receptors (PRR) that detect structural pathogen components and transduce the signal for the induction of a basal response. This type of immunity is mainly related to the prevention of pathogen entry into plant cells [23]; however, it is not completely effective against biotrophic and necrotrophic fungi. On the latter case, the response is activated by damage-associated molecular patterns (DAMP) recognition mainly derived from the host cell wall fragments generated by CWDE [24].

The second line of defence is known as ETI or effector triggered immunity, capable of directly or indirectly recognizing specific pathogen effectors through the expression of resistance proteins (R proteins). This recognition induces a more robust and efficient response, mainly against biotrophic pathogens, by preventing them to complete their life cycle in the host, interrupting nutrient uptake and eliminating the infected cells along with the pathogen [23]. Since this response against biotrophic pathogens (and hemibiotrophic too) generally ends with programmed cell death (PCD) of infected tissue, some necrotrophic pathogens induce this

mechanism during infection in order to bypass plant defences and rapidly kill tissue for nutritional benefits [24].

Plant defence mechanisms are finely regulated by plant hormones, mainly jasmonic acid (JA), ethylene (Et) and salicylic acid (SA), which communicate synergistically or antagonistically depending on the type of pathogen. Generally speaking, the defence against necrotrophic pathogens are considered to be mediated by JA and Et, while the defence against biotrophic pathogens by SA [12]. However, *V. vinifera* cultivars are very susceptible to fungal pathogens, likely due to insufficient defence responses to contain these pathogens.

2.2.1. Grapevine defences against E. necator

E. necator corresponds to the only powdery mildew species adapted to *V. vinifera*. Nevertheless, several species from the Vitaceae family have been identified as resistant. In the latter, plants are able to restrict *E. necator* invasion and growth by means of two strategies: penetration resistance and programmed cell death (PCD)-mediated resistance (observed as a hypersensitive response). The first blocks the breach of the cell wall and membrane and thus prevents the formation of the haustorium. On the other hand, the PCD-mediated resistance is exerted once the epidermal cell is penetrated and induces the death of it, thereby interrupting the supply of nutrients required by the biotrophic fungus for further growth and development [13]. This type of resistance is related to the detection of pathogen effectors by the plant due to specific resistance genes (R genes) [25, 26]. Different loci have been found in several species of the Vitaceae family which confer resistance to *E. necator*; carrying resistance gene analogues (RGA) and in some cases associated to complete resistance to powdery mildew mostly related with PCD induction [27]. However, very few candidate R-genes have been identified to date and molecular defence mechanisms triggered by these resistance loci are being studied. A number of genes have been implicated in resistance in certain wild *Vitis* species showing increased transcription during infection or differential expression levels between resistant and susceptible plants [13], but the identification of key components in PTI and ETI responses against *E. necator* in grapevines is still pending.

Resistance to powdery mildew in the Vitaceae family is closely related to its evolutionary history. *V. vinifera* is native to Eurasia and developed evolutionarily isolated from *E. necator*, a pathogenic fungus from North America, until the 1840s. This is the reason why nearly all *V. vinifera* cultivars lack the genetic protection mechanisms against the fungus and are highly susceptible to infection [14, 26, 28]. Even though *V. vinifera* susceptible plants are able to initiate a basal defence response, they are unable to restrict fungal growth and arrest the disease [29].

Ontogenic, or age-related resistance, also has a role in the defence against *E. necator*. It may operate at a whole plant level or at specific organs or tissues. Grape berries develop ontogenic resistance to *powdery mildew* within 4 weeks after fruit set. However, adhesion of conidia, germination and appressorium formation were not impeded on older berries [30]. Ontogenically resistant berries respond rapidly to infection by synthesis of a germin-like protein that has been previously shown to play a role in the host defence against barley powdery mildew. This type of defence, which conditions ontogenic resistance, operates in the earliest stages of the infection process prior to the formation of a functional haustorium [30].

2.2.2. Grapevine defences against B. cinerea

Low or no resistant phenotypes to grey mould have been described in most common table grape *V. vinifera* cultivars, whereas high level of resistance has only been found in the *Muscadinia rotundifolia* (*Vitis rotundifolia*), *Vitis labrusca* and other grape hybrids species. This resistance appears to be related to mechanical barriers, such as cuticle and wax contents [31]. Pre-existing or basal defences seem to be an important part of the machinery against *B. cinerea*, along with the activation of inducible defence mechanisms mediated by SA or JA/Et pathways, which in turn depend on the developmental stage, and an appropriate kinetics between ROS production and the generation of antioxidant compounds [32, 33].

Structural barriers are related to the fungal primary infection process (i.e. appressoria formation and plant tissue penetration), while inducible responses are associated with subsequent infection ones [34]. In this case, PTI is mainly activated by DAMPs, host cell wall fragments generated by fungal CWDE and PAMPs such as chitin fragments of fungal cell walls, among others. These are identified by specific PRR receptors, such as cell-wall-associated kinases, which in turn activate the defence signalling cascade, culminating in hormones and transcription factors biosynthesis [12, 24]. This response induces protease inhibitors generation and secondary metabolite biosynthesis (i.e. anthocyanins and phytoalexins). The flavonoid phytoalexin plays an important role in the defence response of grapes. The rapid production of resveratrol, major compound of the stilbene family, and its transformation into Viniferins enhance resistance to fungal pathogens in grapevine cultivars [35]. Resveratrol and pterostilbene (two grapevine phytoalexins) produce malformation or growth inhibition of germ tubes, cytoplasmic granulation of the cellular content and the disruption of the plasma membrane in *B. cinerea* conidia [36].

2.3. Biotechnological strategies for fungal control in grapevine

Regarding control of fungal pathogens, major improvement efforts have been directed towards enhancing fungal-disease resistance in table and wine grape cultivars. Development and optimization of alternative strategies to reduce the use of classic chemical inputs for protection against diseases in vineyard is becoming a necessity. Nowadays, fungal-related diseases are controlled through fungicide applications of organic and inorganic composition. The most used compounds are sulphurs, petroleum-based oils, inorganic salts, benzimidazoles and ergosterol biosynthesis inhibitors, among others [14]. However, these management practices usually generate negative impacts on the environment and have elevated health and safety hazards. Various sources have speculated that sulphur, the most heavily used agricultural chemical, can cause respiratory illnesses and other adverse health effects [37]. In soil, sulphur is slowly converted by bacteria to sulphate, which generally does not cause harm. Other synthetic compounds used for treatment and prevention, such as sterol inhibitors have not been reported as having negative environmental or human health effects.

2.3.1. Genetic improvement

Genetic improvement is an agronomic practice widely used to confer interest features to a crop through hybridization between different cultivars or even species, in order to obtain new varieties. In *V. vinifera*, most interesting features vary depending on the use the fruit will be given. Nevertheless, in all cases, the importance of introducing fungal disease resistance is a priority, in order to reduce pathogen management and to minimize environmental impact [38].

Many North American *Vitis* species show various levels of resistance to *E. necator* but lack productive and commercial qualities; however, they represent a valuable germplasm to be used as natural sources of resistance in grapevine breeding programs. Among the resistant North American species identified to date we can find *V. aestivalis*, *V. cinerea*, *V. riparia*, *V. berlandieri*, *V. labrusca* and also *Muscadinia rotundifolia* [14, 26, 39]. However, the powdery mildew resistance character is not restricted to North America. The Central Asian *V. vinifera* cvs. 'Kishmish vatkana' and 'Dzhandzhal kara' have also been identified as resistant genotypes [26, 40–42].

Genetic knowledge of the resistance trait is crucial to achieve a significant improvement of grapevine through breeding. Several powdery mildew resistance loci have been identified and mapped to date. The Run1 locus was described in *M. rotundifolia* and has been successfully introgressed into *V. vinifera*. According to the closest SSR markers VMC4f3.1 and VMC8g9, this locus was mapped to a region in chromosome 12 and co-segregates with the *Plasmopara viticola* resistance locus Rpv1 [14, 26, 43, 44]. The *MrRUN1* and *MrRPV1* genes, which code for TIR-NBS-LRR proteins (a class of R proteins), are the first cloned and functionally characterized resistance genes from grapevine [27]. The Ren1 locus, on the other hand, belongs to the *V. vinifera* cvs. 'Kishmish vatkana' and 'Dzhandzhal kara' from Central Asia. It has been mapped in linkage group 13 with the closest linked SSR markers VMC9H4-2, VMCNG4E10-1 and UDV-020. To date, this gene has not been fully identified, although near the SRR markers, an NBS-LRR and a CAD gene have been recognized, being both probably part of the hypersensitive response [40, 42]. Other identified powdery mildew resistance loci are Run2.1, Run2.2, Ren5 and Ren.4 from *M. rotundifolia* cvs. 'Magnolia', 'Trayshed', 'Regale' and *V. romanetii*, respectively. All of these loci have been mapped in chromosome 18, which have a significant higher density of NBS-LRR genes compared to the other linkage groups, except for Ren5 mapped in linkage group 14. The resistance mechanism mediated by Ren4 may differ from the other loci since extremely low penetration and secondary hyphae development rates with no cell death have been observed at the infection site [45–47].

One of the main concerns about using pathogen resistance genes in plant breeding is the potential appearance of new pathogen strains that could breakdown the resistance. To overcome this latent problem, actual breeding efforts are focusing on stacking or pyramiding two or more resistance genes within a single cultivar to increase the durability of the resistance in the field. In this scenario, pathogen reproduction will be restricted even if infection by a new pathogen strain with a modified or lost effector molecule occurs. Thus, biotechnological tools have become essential for the development of new resistant cultivars. Marker-assisted gene pyramiding is one of the main applications of DNA markers in plant breeding. The use of molecular marker-assisted selection allows the identification of segregants that may exhibit

the same phenotype but carry multiple resistant genes [26]. A grapevine progeny with individuals carrying both Run1 and Ren1 loci was developed in 2010, where Run1 was introgressed from a *M. rotundifolia* × *V. vinifera* hybrid plant derived from a pseudo-backcrossing breeding scheme, while Ren1 was introgressed from the resistant *V. vinifera* cv 'Kishmish vatkana' [48].

Unlike to what happens with *E. necator*, no genetic resistance components against *B. cinerea* have been identified until now, being this the main reason why no breeding programs against this fungus have been reported. All efforts in this area have been developed within the transgenic field, which will be described below.

2.3.2. Genetic manipulation

The development of highly reproducible genetic engineering protocols for grapevine cultivars and rootstocks now allows the identification, screening and/or introduction of grapevine-derived genes related to desirable traits, such as disease resistance.

Pathogenesis-related (PR) proteins were screened for their response to fungal pathogen infection. Genetically modified (GM) grapevines constitutively expressing rice chitinase genes exhibited enhanced resistance to powdery mildew [49, 50]; however, no resistance was observed when plants expressed barley chitinase genes [51]. Other non-grapevine-derived genes, such as the polygalacturonase inhibiting protein (PGIP) and other lytic peptides, were demonstrated to improve fungal disease resistance [49].

Two endochitinase (*ECH42* and *ECH33*) genes and a *N*-acetyl-β-D-hexosaminidase (*NAG70*) gene related to *Trichoderma* spp. were used to develop a set of genetically modified 'Thompson Seedless' lines in order to evaluate fungal tolerance against *B. cinerea*. The highest resistant plants were the ones expressing the *ECH42–NAG70* double gene construct and the *ECH33* gene [52].

Genetic manipulation of phytoalexins has been done in order to increase disease resistance of plants. Use of modern molecular biology tools for elucidating the control mechanisms of phytoalexin synthesis and for engineering disease-resistant plants is based on the expression of stress- or disease-related genes. Few reports attempting the manipulation of phytoalexins biosynthesis by genetic engineering have been published, with most of them related to resveratrol, the major phytoalexin from Vitaceae. STS, the key enzyme in resveratrol synthesis, uses as substrates precursor molecules that are present throughout the plant kingdom. Therefore, the introduction of a single gene is sufficient to synthesize resveratrol in heterologous plant species [53].

The grapevine rootstock 41-B, overexpressing the grapevine *VST1* stilbene synthase gene under the control of the fungus inducible promoter PR 10.1, produced high stilbene levels and exhibited *in vitro* resistance to *B. cinerea* [54]. Stilbene phytoalexin resveratrol levels in grapes have been directly correlated with grey mould resistance [55].

All the aforementioned results demonstrate that improved fungal tolerance can be accomplished through transgene expression. In addition, they support the use of iterative molecular

and physiological phenotyping in order to select tolerant individuals from GM grapevine populations.

2.3.3. Biological control

Biological control of fungal pathogens is based on the use of microorganisms to prevent or reduce the damage produced during infection. Among the best studied biocontrol agents we can find are the filamentous fungi of the *Trichoderma* genus [56], bacteria of the *Bacillus*, *Pseudomonas* and *Serratia* genera [57] and yeasts of the *Pichia* and *Candida* genera [58]. Within the proposed mechanisms of how biological control of plant pathogenic fungi work, we can describe the competition for ecological niches, especially for nutrient utilization and elements obtaining such as nitrogen and carbon and/or the secretion of toxic molecules for the fungus [59, 60].

Another biocontrol mechanism is the activation of the induced systemic resistance (ISR) in plants; this mechanism can be induced by elicitors released by the biocontrol agent (ranging a wide variety of molecules), and it has been attributed to non-pathogenic microorganisms associated to plants, such as saprophytes [61]. Generally speaking, microorganisms exhibit a combination of the mentioned mechanisms, thus reducing the risk of pathogen resistance [62].

Among the bacteria able to synthesize and secrete anti-fungal molecules, those belonging to the genus *Bacillus* are the most important. These bacteria are characterized by their ability to secrete a wide range of bioactive molecules, including anti-fungal, anti-microbial, insecticides, plant growth promoters and ISR-inducing ones [63]. In addition, these molecules have a low toxicity to animals and humans, and are highly biodegradable, so they do not represent a hazard to the environment unlike chemical fungicides [64]. The best characterized molecules secreted by bacteria of the genus *Bacillus* with anti-fungal activity, are the cyclic lipopeptides. These molecules are mainly classified into three families: iturins, fengicines and surfactins. They are formed by a non-ribosomal peptide synthesis ring, which is attached to a fatty acid chain [65]. An important feature of these molecules is that within each family there are different counterparts, differing in the amino acid composition of the polypeptide in the ring and the chain length of the fatty acid [65].

2.3.4. Elicitors

Another control strategy consists in the stimulation and/or potentiation of the grapevine defence responses by the means of elicitors [66]. Elicitors are defined as a more specific class of purified molecules originated from microorganisms or plants which are able to stimulate an innate immune response in plants [67].

Elicitor perception also increases the level of plant resistance against future pathogen attack [12]. Induced resistance is often related to the 'priming' or potentiation phenomenon, and some molecules perceived by plants have also been shown to induce these effects [66, 68]. The definition of priming is related to the physiological state of the plant after an initial biotic or abiotic stimulus. This priming allows the plant to respond in a faster and/or stronger way to following biotic and/or abiotic challenges, often resulting in an improved tolerance in com-

parison to non-primed plants [68]. The mechanism of this phenomenon remains relatively unknown to date, but recent hypotheses suggest that accumulation of dormant MAPKs, chromatin modifications and alterations of primary metabolism could be involved in the process [66, 68].

Bacterial elicitors were recently shown to stimulate innate immunity in grapevine cultivars through cytoskeleton re-organization, early signalling event activation and defence gene induction [69]. Fungal elicitors have also been proved to be very efficient in stimulating innate immunity in grapevines. The deacetylated derivative of chitin (chitosan) elicitor triggered defence responses and protection against *B. cinerea* [70]. Also, ergosterol has been found to trigger defence gene expression in grapevine plants [71]. However, there are few references that show positive and effective results against pathogens under vineyard conditions [66].

Few of these products have shown acceptable effectiveness against biotrophic pathogens. Therefore, until now, there is not an elicitor-based product that can be used instead of conventional agrochemicals in order to successfully fight *B. cinerea*. Additional research needs to be pursued in order to fully understand the defence mechanisms under vineyard conditions.

2.4. Viral infections in grapevine: an example of compatible host-pathogen interaction

Viral diseases in grapevine are highly complex. This complexity is due to the large amount of different viruses that can infect grapevine plants, occurring most of the time as multiple infections, and because of the nature of the compatible pathogen-host interactions that is
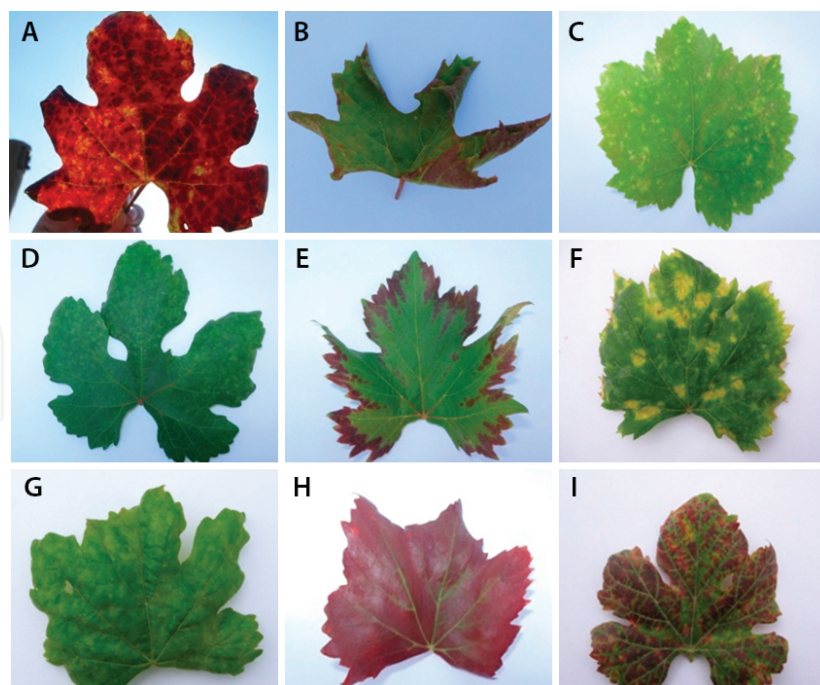
Figure 3. Characteristic symptomatology of the main viruses affecting grapevine. Leaves showing different virus-triggered symptomatology, including reddish areas (A, B, E, H and I), leaf thickness and downward rolling (B), ringspots (D and G), chlorosis (C and F) and yellow veins (H).

established. Viral infections in grapevine plants affect vegetative organs inducing foliar deformations, alterations in leaf colour and, in some cases, graft rejection (**Figure 3**) [72].

Severe infections also reduce berry setting and cause irregular and delayed ripening [72, 73]. Currently, more than 60 viruses have been described in grapevine [73], which together with viroids, phytoplasmas and insect-transmitted xylematic bacteria, correspond to the highest number of intracellular pathogen described for a single crop. Grapevine infecting viruses are classified according to several parameters, including size particle, genome structure, replication strategies, transmission vector and serological information [73]. In general, grapevine infecting viruses exhibit single-stranded RNA (ssRNA) genomes, and the most relevant belong to the Nepovirus, Ampelovirus, Closterovirus and Vitivirus genera.

Viruses belonging to the Nepovirus genus are widely disseminated and are responsible for the degeneration disease. The most representative are the GFLV (Grapevine Fanleaf Virus), ArMV (Arabis Mosaic Virus), SLRSV (Strawberry Latent Ringspot Virus), ToRSV (Tomato Ringspot Virus) and TRSV (Tobacco Ringspot Virus) [72–75]. Most viruses of these groups are not serologically related but share physical and biological attributes [75]. Regarding their infection vectors, Nepoviruses can be transmitted by one or more nematodes species [76, 77]. Moreover, it has been established that GFLV is transmitted by *Xiphinema index*, ArMV by *Xiphinema diversicaudatum* and SLRSV by *Paralongidorus maximus*. Nepoviruses induce grapevine degeneration and leaf decline and produce serious yield losses [74]. However, leaf, steam and berries symptoms vary according to the graft and scion combination, virus strains and environmental conditions. These symptoms include delay in bud break, irregular bud growth, leaf deformity and reduced berry size. Together with the virus-induced decay, a reduction in vegetative growth takes place and even plant death can occur [78, 79]. Decay disease is a major threat for the grape industry since vigour reduction triggered by GFLV infection can reduce yields in about 80% or more [73, 80].

Grapevine leafroll disease (GLD) is one of the most important viral diseases affecting grapevines worldwide [3, 81–83]. It is generally accepted that this disease is caused by 11 viral agents, named GLRaV-1 to GLRaV-11 [3], and according to specific genome sequences, their taxonomic classification includes members of the Ampelovirus genus (GLRaV-1, -3, -4, -5, -6 and -9), the Closterovirus genus (GLRaV-2) and the Velarivirus genus (GLRaV-7). Besides the diversity of viral agents associated with GLD, it is widely assumed that GLRaV-3 is the main etiological factor contributing to the disease. Viral agents responsible for GLD are flexuous filaments, 1800 × 12 nm in size with the unique Closterovirus architecture [3]. These particles are responsible for the characteristic GLD symptom, expressed as red colour leaves with green vein pattern, often curled downwards and brittle [83]. In red cultivars, GLD symptomatology is much more evident in comparison to white cultivars, where the disease can be asymptomatic [84]; nevertheless, white cultivars can show inter-veinal yellowing of leaves and leaf rolling [83].

Grapevine virus A and B (GVA and GVB), which belong to the Vitivirus genus, are also relevant [85]. GVA is related with the Kober stem grooving symptom, where severe grooving on the grafted stems occurs [86, 87], while GVB is associated with the corky bark syndrome consisting of soft, rubbery and abnormal swelling of the basal internodes of the canes, longitudinal cracks and cork forming, typical of the rugose wood complex [88]. Vitivirus genus

has other species less ubiquitous, named GVD, GVE, and the most recently discovered GVF [89], causing similar symptoms to the corky rugose wood but its role is still unclear [90].

Interestingly, in many cases viruses are present in grapevine as multiple infections [91, 92], where the symptomatology can be a combination of those triggered by individual viral agents. This situation is exacerbated by the fact that grapevine is propagated through cuttings. Asexual propagation is the predominant method to generate clones which are genetically identical to the parental plants, allowing worldwide distribution since centuries, together with the dissemination of infectious agents across the grapevine-growing regions, spreading their detrimental consequences to grape production [3].

It is noteworthy to mention that, unlike to other pathogens, grapevine plants show no resistance to viruses, meaning that plants and viruses establish compatible interactions where pathogens can spread throughout all tissues without any active resistance response, generating a global cellular stress and developmental defects. It is well known that susceptible hosts are not completely passive against a pathogen, and can set up a defence response that could be less intense and not strong enough to stop viral replication and dissemination [4, 6]. Within the latter, the emergence of visible plant symptoms is none other than the sum of different molecular, cellular and physiological variations of the plant defence processes in response to viral infections. Moreover, as seen in compatible interactions, several changes in gene expression occur which determine the disease symptom development and the viral levels in the infected tissues [93]. The dynamics of compatible interactions can be even more complex, considering that the infections could be chronic, and that there are variables to take into account, such as cultivars, species and environmental clues, among others [94]. All of these aspects modify the manner the infection is phenotypically expressed.

2.5. Molecular and physiological changes in grapevine in response to viral diseases

Current understanding of host-virus interactions derives mostly from studies in leaves of red-berry *V. vinifera* cultivars, and few studies have been carried out in this area up until now. Considering that GLRaV-3 is one of most significant grapevine-infecting viruses, special attention has been given to the physiological changes and molecular responses against this virus in leaves [5, 95, 96] and berries [84, 97] (**Figure 4**).

Transcript profiles of leaves from the red cultivars Cabernet Sauvignon and Carménère naturally infected with GLRaV-3, were characterized using the *Vitis vinifera* GeneChip® microarray that contains 14,000 and 1700 transcripts from *V. vinifera* and other Vitis species, respectively [5]. This work showed that viral infection induces changes in grapevine transcript profiling in a wide spectrum of biological functions, with significant induction of stress- and defence-related proteins, including lipid transfer proteins (*LTP*), stress-responsive proteins such as the patatin-like protein, the agenet domain containing protein and MAP kinase phosphatase (*MKP1*), aging genes like tropinone reductase and harpin-induced family protein (*HIN-1*) and the detoxifying gene gluthathione S-transferase (*GST*). Viral response also includes changes in hormones transporters (auxins and cytokinins), lipids, sugars and oligopeptides, cell wall remodelling proteins, such as extensin and hydroxyproline-rich proteins which are anchored to the cell membrane. On the other hand, among the most

significant down-regulated genes, we found genes coding for photosynthetic proteins, as well as photosystems constituents and chlorophyll biosynthetic enzymes.

LEAVES		BERRIES		
YOUNG	OLD	FRUIT SET	VÉRAISON	HARVEST
ASYMPTOMATIC	SYMPTOMS	ASYMPTOMATIC	SYMPTOMS	
- no symptoms development	- decrease in photosynthetic rate - senescence - dwarfism - yellowing (chlorosis) - leaf roll - ring spots - leaf necrosis - malformations	- no symptoms development	- altered sugar metabolism - reduction in anthocyanin accumulation - delayed ripening - yield loss	

Figure 4. Different symptomatology triggered by grapevine viruses in leaves and berries. Certain developmental stages such as young leaves and berries at fruit set show no symptoms of viral diseases. However, as development continues mature leaves and berries at véraison and harvest can exhibit the characteristic symptomatology, depending on the varieties and viral agent combination.

It has been proposed that some overlap exists between leaf-senescence and pathogen-defence programs, with transcript profiling in red cultivars further supporting this concept [5, 95]. Several marker genes of the leaf senescence process are expressed during natural viral infection in grapevines. Genes induced during viral disease in grapevine plants are also induced during leaf senescence triggered by natural factors, showing a clear correspondence between the senescence program and plant responses during viral compatible disease. The generation of ROS could be responsible for the partial activation of the senescence program during viral diseases, since ROS are necessary for the expression of defence-related genes and also act as promoters of senescence [98]. This relationship may represent a strategy used by plants in order to adapt to viral pathogens, recycle nutrients from infected leaves and mobilize them to distant tissues, and allow a plant-pathogen relationship to be established, even for long periods of time [95].

A different study characterized the expression of flavonoid biosynthetic pathway genes in GLRaV-3 infected symptomatic leaves in a red-fruited wine grape cultivar (cv. Merlot) [96]. Based on the accumulation of specific flavonoids in GLRaV-3 infected plants, these authors suggest that the expression of the flavonoid biosynthetic pathway is activated during the infection, and is responsible for the characteristic changes in leaf colour. These molecules could confer protection from oxidative stress and opportunistic pathogens during the infection.

Even though berries are the most valuable part of grapevine plants, little attention has been given to the effect of viruses during fruit development and ripening. Evidence suggest that autotrophic leaves located near berry clusters serve as the main source of photoassimilates to ripening berries [3]. Photoassimilates are normally transported via phloem, as well as viruses

such as GLRaV-3. Therefore, it is reasonable to think that the infection may alter the molecules flow towards the berries, and that this effect may vary according to the asymptomatic or symptomatic phases of the infection and grapevine phenological stages [3, 83].

The effects of a chronical infection with GLRaV-3 during berry ripening in grapevine have been studied in the red cultivar Cabernet Sauvignon [97]. Interestingly, this virus affects the normal fruit ripening process, resulting in incomplete berry ripening in terms of gene expression patterns. Genes associated with anthocyanin biosynthesis and sugar metabolism are down-regulated in berries from infected plants, consistent with a decrease in up to 40% in total anthocyanin content. These changes are observed specifically at ripening, where the infection has a greater impact in comparison with other stages of berries development. These authors also suggest the presence of viral particles in berries, probably colonizing the organ through the vasculature during fruit development.

Lately, the effect of GLRaV-3 on the chemical properties of fruit, juice and wine from *V. vinifera* L. cv. Sauvignon blanc was assessed, allowing comparisons between recent and established infections [84]. Authors propose that the duration of the infection is significant to this comparison, and that established infections modify berry development at later stages. The pathogen causes a delay in grape ripening, with a concomitant delay in harvest date. However, when berries from uninfected and infected plants reached similar ripeness, minimal effects on juice and wine chemistry were observed.

2.6. Diagnostic and control methods for grapevine viruses

2.6.1. Diagnostic methods

Since grapevine viruses can show detrimental effects on plant physiology, it is necessary to have appropriate and reliable diagnosis methods to achieve an efficient control of pathogens propagation. So far, several techniques have been applied to identify infected plant material, including biological indexing, serology and molecular assays [3, 83, 99].

Biological indexing, mostly performed as part of certification programs, refers to grafting of candidate vine on woody indicators of the Vitis genus. Later on, the indicator plant is observed for the development of virus disease symptoms. However, this approach is time-consuming, labour intensive and dependent on virus titer, the success of the viral inoculation, strain variations and skilled personnel [83]. Serological methods are based on the recognition of viral proteins by specific antibodies. Of these, the enzyme-linked immunosorbent assay (ELISA) is the most widely applied [83, 99], and commercial kits are available. Serological approaches are robust and scalable, although less sensitive than nucleic acid-based techniques. Special attention must be given to sampling, considering differences in virus accumulation through plant tissues and seasonal variations in virus titer, and it has been described that genetic variants can affect the robustness of these methods [3, 83]. Nucleic acid-based methods, on the other hand, detect the genomic components of the viruses. These methods are commonly used due to their high sensitivity, in comparison with other diagnostic approaches. They can detect the presence of viral genomes even at low viral titer, are rapid, allow the scaling and the

simultaneous analysis of a high number of samples or several viruses at once [100]. Since most of grapevine viruses have RNA genomes, reverse transcription-polymerase chain reaction (RT-PCR) is the selected molecular assay for the detection of these pathogens [83, 101]. Several techniques have been developed based on PCR variants [83, 102], but the use of real-time PCR allows quantification of virus titer [103]. Recently, new generation sequencing (NGS) has been used for rapid identification and sequencing of all putative viruses present in a candidate sample, allowing the identification of new viral agents as well [3, 81, 99, 104]. The use of NGS technologies as diagnostic tool requires no prior knowledge of the pathogens present in the sample, but is still expensive in order to be used as a routine procedure.

2.6.2. Multiplex PCR to detect complex viral infections

As it was mentioned before, viral diseases in grapevine often occurs as multiple infections, where several viral agents are present simultaneously and can contribute to the overall symptoms development. Several papers describe viral detection by molecular approaches, which are reviewed in [83, 99]. However, a simple and efficient commercial method for the
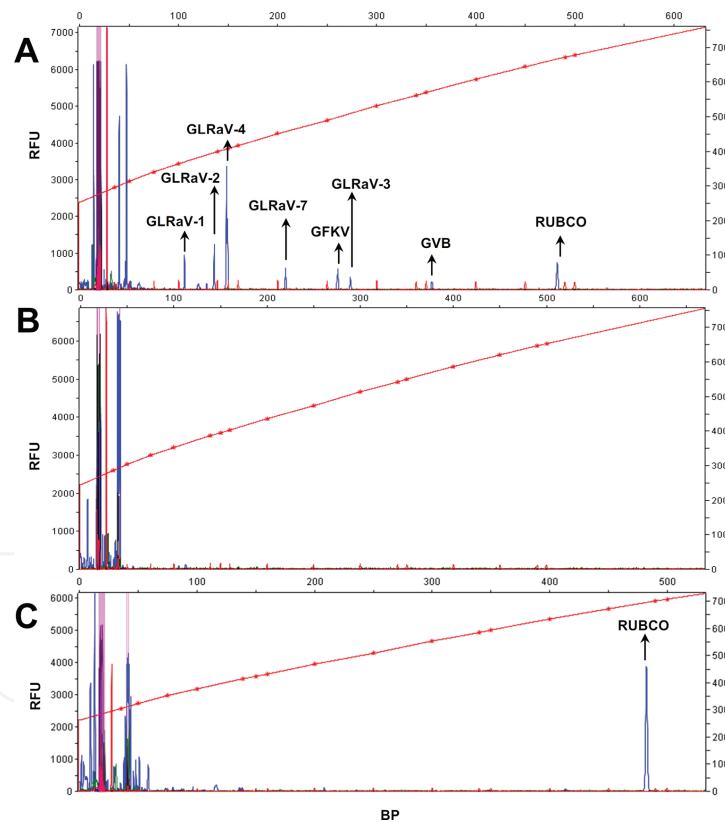
Figure 5. Multiplex PCR detection of grapevine viruses in complex samples. PCR fragments were analysed by capillary electrophoresis in order to detect the different amplicons. (A) Detection of the specific fragments corresponding to GFKV, GVB, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4 and GLRaV-7, in a sample containing a mix of cDNA from plants infected with these viruses. (B) Negative control of the PCR. (C) PCR using a virus-free plant. The ROX500 standard was used to estimate the size of each fragment. Blue peaks correspond to the amplified fragments obtained in each reaction. Red line represents the linear regression obtained with the Peak Scanner Software V.1.0 to estimate fragment size. Base pairs of each fragment (*X*-axis), fluorescence intensity (RFU) of amplified fragments (*Y*-axis, left) or RFU of the standard (*Y*-axis, right) are shown.

detection of several grapevine viruses at once is currently not available. A method for virus detection must fulfil several criteria, such as sensitivity, specificity, accuracy, number of samples that can be tested simultaneously and cost, among others. Therefore, to have reliable diagnosis methods is a permanent challenge for grapevine growers.

In our laboratory (unpublished work), we have designed a system for simultaneous virus detection in vines, consisting in a multiplex PCR that can detect up to seven RNA viral genomes, in addition to the detection of the gene coding for the small sub-unit of grapevine Rubisco enzyme as a plant positive control. Using bioinformatics tools, specific primers against different viruses were designed to generate products of different sizes. Then, primers were labelled at the 5′ end with 6-FAM fluorophore, in order to be detected by capillary electrophoresis. This method allows the specific and simultaneous detection of GFKV, GVB, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4 and GLRaV-7, in a quick, efficient and single PCR (**Figure 5**).

This type of multiplex PCR can be used to generate commercial kits that can serve to detect viral agents present in a vineyard or to test the plant material that will be later used in clonal propagation. With the proper bioinformatics analysis, more viruses can be added to the system, allowing a much more versatile detection kit.

2.6.3. Control methods

There are several control methods that are routinely applied in order to prevent virus dissemination. For instance, sanitary selection and certification of propagation material helps to reduce potential virus dispersion [99]. Since viruses are transmitted by vectors, control of viral diseases can be achieved by the restriction of such vectors with the use of agrochemicals [105]. However, agrochemicals utilization increases production costs, and additionally are associated with detrimental effects to the environment and human health, while most of modern agronomical practices tend to reduce its use. Sanitation techniques, on the other hand, are aimed to treat infected material and eliminate viral titer. Among these techniques, thermotherapy is the most frequently applied although it is not effective for all grapevine viruses [106]. The *in vitro* culture of meristems, somatic embryos and shoot tips allows the regeneration of virus-free plantlets [99], an approach that is probably based on the unequal distribution of viruses along plant tissues. Other sanitation techniques include chemotherapy, very often applied as an alternative to eliminate more recalcitrant viruses and cryotherapy, a highly efficient method which is effective when the treatment of high number of samples is needed, but its implementation is difficult as some genotypes are refractory to it [99].

2.6.4. Inducing virus resistance in grapevine by transgenesis

Biotechnology arises as an alternative to allow the generation of virus-resistant grapevine plants by transgenesis, mainly involving the expression of viral components and exploiting the naturally occurring gene silencing [107–117]. This strategy requires plant transformation with a short sequence of the pathogen genome in a way that a double-strand RNA structure is formed during transcription, initiating gene silencing in the host. In our lab, induction of virus silencing was accomplished in grapevine rootstocks in order to be used for grafting [118].
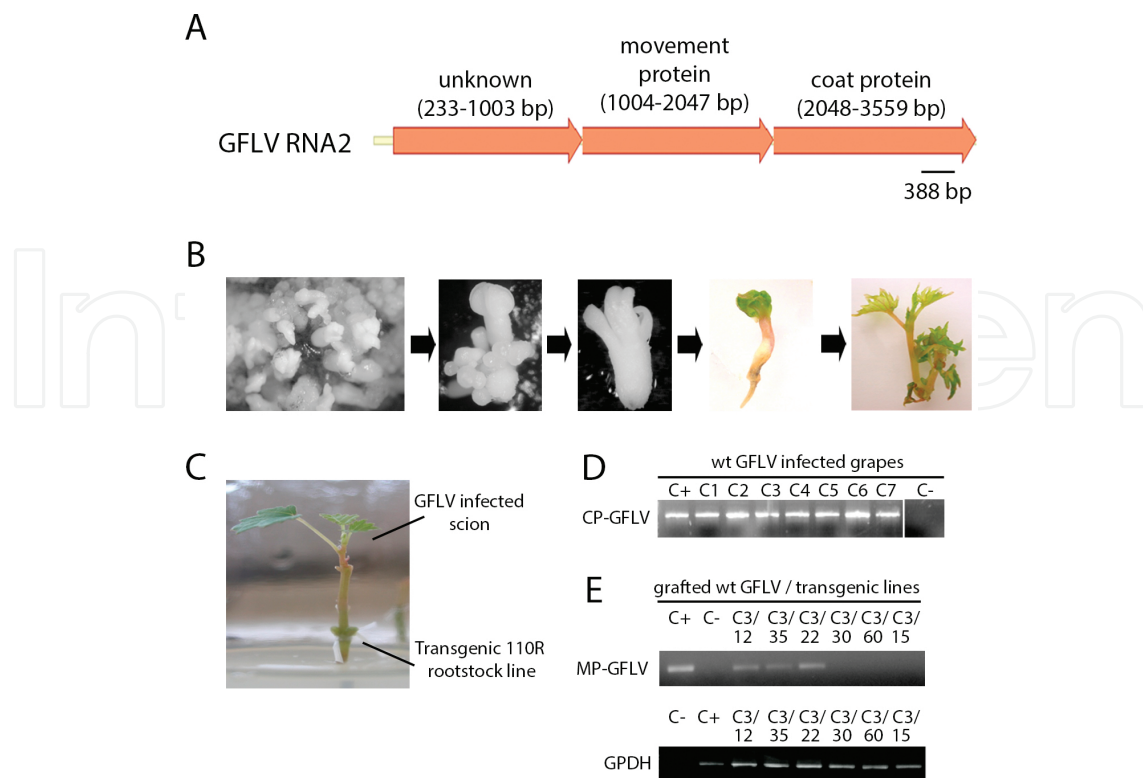
Figure 6. GFLV silencing induced by grafting strategy. (A) RNA 2 of GFLV genome showing the unknown, movement and coat protein corresponding ORFs; the 388 bp region of coat protein used to produce the inverted duplicate that triggers PTGS is denoted. (B) To obtain transgenic rootstock, globular embryos were used for transformation. (C) *In vitro* grafting of GFLV infected plants on transgenic rootstock expressing the *cp-gflv* transgene. (D) Presence of GFLV in infected wild-type (wt) plants (C1–C7) showed by amplification using CP-GFLV primers. (E) Evaluation of GFLV presence in upper leaves of the grafts with MP-GFLV primers, 30 days after grafting. MP-GFLV was undetected in C3 plants grafted on transgenic lines 30, 60 and 15. Expression of the GPDH gene was used as a housekeeping control.

It is expected that the mobile signal-inducing virus silencing in the rootstock will also be able to reach the scion, and as a consequence, trigger virus silencing in the non-transgenic scion. This approach is very versatile, since the resistance against a specific virus can be obtained in all the varieties used as scion with a particular virus-resistant transgenic rootstock. We have transformed rootstocks plants (110 Richter and Harmony) by co-culture of embryogenic and organogenic tissues with *Rhizobium radiobacter* carrying a vector containing a silencing sequence of the coat protein of the grapevine fanleaf virus (GFLV) cloned as an inverted duplicate in a way that triggers post-transcriptional gene silencing (PGTS) at transcription. Twenty-six transgenic plants of the 110 Richter rootstock have been recovered, analysed by RT-PCR against the GFLV sequence, and lines properly expressing the construction were propagated to obtain several plants of each line. The transgenic rootstocks have been grafted with GFLV-infected plants that were positive for virus presence by RT-PCR analysis. Once the grafts were set, the GFLV detection was made in the scion using primers for the viral movement protein. After 1 month of grafting, the detection of the virus has been abolished in the scion, in three of the six analysed rootstocks lines (**Figure 6**).

Therefore, a viral infection of a non-transgenic scion could be silenced if it is grafted on a transgenic rootstock carrying sequences that triggers PTGS. This strategy is an interesting

alternative to considerate in virus-free breeding programs because the infection in non-transgenic grapevines from any cultivar could be abolished using a transgenic rootstock, keeping cultivars and, more important, the fruit produced non-transgenic.

3. Grapevine abiotic stress

Grapevine crops are often exposed to sub-optimal growing conditions which cause several abiotic stresses, as they are constantly exposed to different water regimes, nutrient deficiency or excess, extreme heat or low temperatures and deficit or excess of light [8]. All plants, including grapevine, need Sun energy in order to produce organic compounds through photosynthesis, but sunlight is a sum of different wavelengths. Among them, the ultraviolet radiation (UVR) plays an important role, however, the main problem with UV light is that as the wavelength declines, its energy content increases, mainly as UV-B radiation, and therefore its potential to cause photo-biological damage increases. UV-B is not only potentially harmful, but it also serves as an environmental information source, though information about it is still scarce. As general abiotic stresses have been extensively reviewed [119–125], we will have special focus on UV-B-mediated perception and signalling responses of grapevine and photo-biotechnological approaches to improve fruit quality for winemaking.

3.1. Solar ultraviolet B levels, ozone layer depletion and increase of UV-B radiation

Solar energy is the primary source of energy for all surface phenomena, especially autotrophic organisms. Among them, plants use solar radiation not only as an energy source, but also as a key signal containing vital information about the environment in which they live [126, 127]. Solar radiation not only includes the visible spectrum (400–700 nm) necessary for photosynthesis, but also other types of radiation. Near 7% of the electromagnetic radiation emitted by the Sun is within the ultraviolet radiation (UVR) spectrum (200–400 nm) [128–130]. UVR has been divided into three different bands: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm) [130, 131]. As it passes through the atmosphere, the total transmitted radiation flux is considerably reduced, and the composition of UVR is modified. Shortwave UV-C is completely absorbed by atmospheric gases, while UV-B is partially absorbed by the strato-spheric ozone (O_3), leaving only a small fraction (<0.5% of total sunlight energy) transmitted to the Earth surface. UV-A, on the other hand, is not absorbed by ozone [130, 132]. Over the last 50 years, the ozone concentration has diminished by 5%, mainly due to the release of anthropogenic pollutants, such as chlorofluorocarbons (CFCs) and other halogenated ozone-depleting substances [126, 133]. As a direct consequence of the ozone reduction, an increase in the flux of UV-B radiation has been registered during the last years [126, 128, 132]. Although, UV-B radiation is only a minor component of solar radiation, due to its high energy, its potential for causing biological damage is exceptionally high [133]. Besides the regulation of solar UV-B by the ozone layer, there are several factors influencing UV-B radiation levels, such as latitude, altitude, season, time of the day, weather conditions, surface reflection, atmospheric pollution and shading by plant canopies [126, 133]. From a wine producer's point of view, the

establishment, planning and vineyard management are additional factors to take into account that can influence UV-B levels on plants. These factors including climate, presence and slope aspect, site elevation, trellis and training system and vine vigour, among others, could be directly influencing both intercepted light in canopies and fruit zone [8].

3.2. Effects of UV-B in plants

Due to the sessile lifestyle, plants are forced to adapt to changes in environmental conditions while achieving an equilibrium between optimal photosynthetically active radiation (PAR) capture and UV-B protection [131, 132]. The UV-B radiation has several detrimental effects but it also serves as a key regulator of plant morphology and physiological, biochemical and genetic mechanisms [127, 129, 133, 134]. Plants actively respond to irradiation with high or low UV-B doses, either by the activation of repair mechanisms or by stimulation of photomorphogenic processes [128, 129]. In general, low UV-B doses reduces growth and expansion of leaves, produces leaf thickness, increases epicuticular waxes, trichomes number and axillary branching, reduces stem elongation, suppresses both hypocotyl extension and root growth and enhances flavonoid biosynthesis, mostly flavonol [127–129, 132, 134]. Plants under UV-B radiation present a compact architecture, although different phenotypes have been reported. This may relate to UV-induced morphological changes being underpinned by different mechanisms at high and low UV-B doses [127].

In grapevine, the high UV-B doses reduce shoot length and leaf area, increase both leaf thickness [135] and accumulation of terpenes with antioxidant properties [136]. On the other hand, flavonol biosynthesis is dramatically activated under both high and low UV-B exposures in the berry skin [9, 10]. Also, membrane-related terpenes are increased in low fluence of UV-B in grapevine leaves [136].

3.3. UVR8-mediated photomorphogenic mechanisms in response to UV-B in plants

In order to maximize its growth and survival, plants detect, respond and adapt to UV-B rays. This type of radiation is a key environmental cue, which initiates diverse pathways affecting metabolism, development and viability. Many of the UV-B radiation effects involve differential regulation of gene expression. This response depends on the exposition nature (high or low UV-B doses), the degree of adaptation and acclimation to the radiation, and the interaction with other environmental factors. UV-B radiation responses are mediated by two signalling pathways in *Arabidopsis thaliana*, (1) the non-specific signalling pathway, which involves DNA damage, accumulation of reactive oxygen species (ROS) and synthesis of defence-related molecules in response to high levels of UV-B radiation; and (2) the specific signalling pathway on the other hand, which is mediated by photomorphogenic responses to low levels of UV-B radiation [128]. It is important to note that photomorphogenic signalling promotes the expression of genes involved in the protection and acclimation against UV-B radiation and, hence, promotes the survival of exposed plants. Photomorphogenic signalling implies the participation of a specific component in *Arabidopsis*, the UV-B photoreceptor UVR8 (UV Resistance Locus 8) with specific tryptophan residues which act as intrinsic chromophores [137]. UVR8 perceives radiation, triggering the dissociation from its non-active homodimer

configuration [137, 138]. Following monomerization, UVR8 accumulates in the nucleus and interacts with the positive regulator Constitutively Photomorphogenic 1 (COP1) [139–142], a WD40/RING-E3 ubiquitin ligase that in non-inductive conditions targets HY5 (Elongated Hypocotyl 5) for proteosome-dependent degradation [143]. HY5 is a key effector of UV-B protection and light photomorphogenic responses [144, 145], and it is transcriptionally activated by UV-B in a UVR8- and COP1-dependent manner [146–148]. Other components of the UVR8 signalling pathway are repressor of UV-B photomorphogenesis 1 (RUP1) and RUP2. Both RUP1 and RUP2 act as feedback inhibitors of UVR8 signalling by facilitating UVR8 redimerization after exposure to UV-B and thus preserve responsiveness to changing levels of the input signal [149, 150].

3.4. Elucidating the grapevine UV-B signalling pathway

Grapevine (*Vitis vinifera* L.) is a woody species often cultivated in Mediterranean climates with varied UV-B radiation dosages, generally ranging between moderate (5 kJ m^{-2} d^{-1}) to high (12 kJ m^{-2} d^{-1}) levels [7]. Grapevines are considered well-adapted plants to solar radiation due to a variety of physiological adaptive responses, mainly based in antioxidant enzyme activities and secondary metabolites production [7]. The most common protective mechanisms against potentially harmful radiation are the synthesis of phenolic compounds that absorb UV-B radiation [128, 132]. Among the versatile range of functions flavonoids possesses, the most important related to UV-B include the ability to attenuate radiation by filtering, an antioxidant activity capable of scavenging free radicals, and the modulation of reactive oxygen signalling cascades involved in growth and development [151]. These secondary metabolites, phenolic compounds, flavonoids and cinnamate esters, among others, accumulate in vacuoles of epidermal cells in response to UV-B radiation and attenuate the further diffusion of solar UV-B in deeper cell layers [128, 132]. In grapevine, it has been reported that in leaf epidermis and fruit berry skins, anthocyanins and flavonols increase in response to UV-B [11, 152–154]. Considered as a relevant model for studying adaptive responses, several approaches have been conducted for understanding the effects of UV-B in grapevine. Analysis of the transcriptomic variations caused by a particular UV-B radiation dose (4.75 kJ m^{-2} d^{-1}) given at high and low

Gene	ID	Expression[a]	Reference
VvUVR1	VIT_07s0031g02560	No change	Carbonell-Bejerano et al. (2014)
VvHY5	VIT_04s0008g05210	Up-regulated	Loyola et al. (2016)
VvHYH	VIT_05s0020g01090	Up-regulated	
VvCOP1-1	VIT_12s0059g01420	No change	
VvCOP1-2	VIT_10s0523g00030	No change	
VvRUP	VIT_16s0050g00020	Up-regulated	

[a]Differential expression in UV-B radiation treatments compared to the control.

Table 1. UV-B perception and signalling grape homologues.

fluence rates demonstrated that DNA repair, synthesis of UV-B sunscreens and general multiple-stress pathways were the main activated processes [155]. Additionally, has been reported the identification and characterization of the grapevine homologues of the Arabidopsis UV-B signalling components (**Table 1**) and that this UV-B radiation-specific signalling cascade is activated in berry skin along with the accumulation of secondary metabolites, mainly flavonols [9, 10].

3.5. Manipulation of UV-B perception and signalling components to improve plant shape and fruit quality in grapevine

It is known that grapevine is a vigorous growing plant; hence, one of the main objectives for viticulture practices is to reduce the size of the canopies and alter the shape of the vine, in order to increase field plant density and improve fruit organoleptic qualities, among others [8, 156]. Moreover, a higher plant density means greater productivity per area unit. To meet these objectives, conventional genetic improvement of most fruit crops, including grapevine, has been extensively done, with several obstacles in the way. Among the latter we can find long juvenility periods, seedlessness, self-incompatibility, high heterozygosity and sterility. Therefore, conventional breeding techniques are difficult, expensive and time consuming [156]. Because of this, genetic improvement through genetic engineering techniques offers an attractive alternative in order to overcome these problems.

Gene name	Role in UV-B signalling[a]	Grape homologue	Experimental approach	Phenotype or trait of interest[a]	Reference
AtUVR8	Photoreceptor	VvUVR1	Over-expression	UV-B tolerance, dwarfing, increased flavonoids levels, enhanced *B. cinerea* resistance.	Rizzini et al. [137]; Demkura and Ballaré [157]
AtCOP1	Positive regutator	VvCOP1-1 VvCOP1-2	Over-expression	UV-B tolerance, dwarfing, increased flavonoids levels.	Oravecz et al. [142]
AtHY5	Positive regulator	VvHY5	Over-expression	UV-B tolerance, dwarfing, increased flavonoids levels.	Ulm et al. [148]
AtHYH	Positive regulator	VvHYH	Over-expression	Moderated UV-B tolerance, increased flavonoids levels.	Brown and Jenkins [158]
AtRUP2	Negative regulator	VvRUP	Silencing or down-regulation	UV-B tolerance, extreme dwarfing, increased flavonoids levels.	Heijde and Ulm [150]

[a]Derived from studies in *Arabidopsis*.

Table 2. UV-B signalling target genes that could be genetically engineered in grapevine.

In vine growing, the production of dwarf and semi-dwarf canopies with short and numerous shoots, in order to increase field vine density, are normally used for both dwarfing rootstocks and spur varieties [8, 156]. However, rootstocks and spur varieties are available for only a few

species and graft compatibility is often a problem. Therefore, an alternative to this is the use of photo-biotechnology techniques which may contribute to the creation of dwarf varieties by genetic engineering, modifying, for example, UV-B perception and/or signalling components (see **Table 2**).

Photo-biotechnology refers to over- or down-expressing genes with photo-biological relevance [159]. Since photoreceptors and/or light signalling cascade components regulate the expression of critical development and plant growth genes, genetic manipulation of these is viewed as a promising strategy to develop fruit crops with improved agronomic traits [159]. Therefore, photo-biotechnology offers a promising approach for studying the influence of UV-B signal transduction components on plant development and may be used to improve crop yield, shade tolerance, growth and fruit ripening, canopies shape, hormone synthesis and biosynthesis of metabolites and pigments. For example, a promising study in tomato showed that down-regulation of *LeHY5* by RNAi-mediated gene repression exhibited defects in light photomorphogenesis response, loss of thylakoid organization and reduced carotenoid accumulation. In contrast, repression of *LeCOP1LIKE* expression resulted in plants with an exaggerated photomorphogenesis response, high levels of chlorophyll and elevated fruit carotenoid levels [160]. These results demonstrate that genes encoding components of UV-B signalling cascade represent a promising genetic tool for manipulation of fruit quality. Additionally, several studies summarized by [159] in various plant species show that modulation of the expression of phytochromes (mainly PhyA and PhyB) can be used to produce high-yielding crops.

The quality of grape berries for winemaking integrates various aspects, but as for red wines, the accumulation of phenolic compounds by UV-B is highly necessary [161]. Wines with the highest phenolic concentrations are generally considered of excellence, therefore, these molecules are said to play a significant role in winemaking since they are key determinants of wine quality [161]. All of the aforementioned evidence suggests that UV-B protective mechanisms may potentially lead to important industrial applications, relevant to the wine industry. UVR8 may prove to be an attractive and suitable target to manipulate plant growth and/or plant tolerance to abiotic stress, generating UV-B-resistant grapevines with enhanced secondary metabolites levels (i.e. phenolic compounds).

In summary, the elucidation of the UV-B signalling pathway and the role of photomorphogenesis, in addition to advances in genetic manipulation of grapes, are unique biotechnological tools that could be used to improve grapevines in order to meet and surpass market expectations.

Acknowledgements

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