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REVIEW

Recent advances in biotechnological studies on wild grapevines as valuable resistance sources for smart viticulture

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Abstract

Cultivated grapevines, *Vitis vinifera* subsp. *sativa,* are thought to have been domesticated from wild populations *of Vitis vinifera* subsp. *sylvestris* in Central Asia. *V. vinifera* subsp. *sativa* is one of the most economically important fruit crops worldwide. Since cultivated grapevines are susceptible to multiple biotic and abiotic soil factors, they also need to be grafted on resistant rootstocks that are mostly developed though hybridization between American wild grapevine species (*V. berlandieri, V. riparia*, and *V. rupestris*). Therefore, wild grapevine species are essential genetic materials for viticulture to face biotic and abiotic stresses in both cultivar and rootstock parts. Actually, viticulture faces several environmental constraints that are further intensified by climate change. Recently, several reports on biotic and abiotic stresses-response in wild grapevines revealed accessions tolerant to different constraints. The emergence of advanced techniques such as omics technologies, marker-assisted selection (MAS), and functional analysis tools allowed a more detailed characterization of resistance mechanisms in these wild grapevines and suggest a number of species (*V. rotundifolia*, *V. rupestris*, *V. riparia*, *V. berlandieri* and *V. amurensis*) have untapped potential for new resistance traits including disease resistance loci and key tolerance genes. The present review reports on the importance of different biotechnological tools in exploring and examining wild grapevines tolerance mechanisms that can be employed to promote elite cultivated grapevines under climate change conditions.

Keywords Wild grapevines · Smart viticulture · MAS · Omics · Genetic engineering · Stress tolerance · *Vitis*

Introduction

The genus *Vitis* L. encompasses about 70 woody lianas distributed mostly in the temperate regions of the Northern Hemisphere [1]. They include 34 American and 37 Asian species as well as the European–Middle Asian wild grapevine (*V. vinifera* subsp. *sylvestris*), believed to be the ancestor of modern cultivars [1]. To date, *V. vinifera* L. exists as the cultivated form of *V. vinifera* subsp. *sativa* (or *vinifera*) and the wild-form of *V. vinifera* subsp. *sylvestris* [1]. *V. vinifera* subsp. *sativa* is one of the most important crops worldwide with respect to its distribution area, cultural significance and economic value [2].

Grapevine cultivars (*V. vinifera* subsp. *sativa)* are susceptible to various biotic and abiotic factors that are further amplified by climate change [3, 4]. In the Mediterranean region in particular, climate models predict an increase in winter temperatures combined with fluctuations in annual rainfall amount and distribution [5]. In addition, increased salinity from salt-water intrusion may pose the greatest threat to viticulture particularly in the countries with vulnerable coastal regions [6].

Regarding these threats, researchers attempted to find novel resistance sources that can be valuable in grape breeding programs. In this context, several wild and naturally occurring Asian and American *Vitis* species are now recognized as valuable sources of resistant genes against diseases and abiotic factors. In fact, wild grapevines including American species like *V. rotundifolia*, *V. rupestris*, *V. riparia*, and *V. berlandieri*, as well as Asian species with a high degree of resistance to diseases, mainly *V. amurensis*, have been used

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in breeding programs to develop resistant cultivars [2, 6]. Also, wild grapevines from the Mediterranean basin were found to counteract several viral diseases [7, 8].

The undertaken breeding attempts in grapevine have been facilitated by marker-assisted selection (MAS) technology that is based on the use of molecular markers flanking genomic regions that confer resistance to diseases. Molecular markers associated with these regions have been employed in MAS for pyramiding these resistance loci for downy mildew and powdery mildew (PM) in different wild grapevines around the world [2]. Therefore the availability of the vast grapevine genome [9, 10] would efficiently contribute to these breeding programs.

Other advanced high throughput tools involving OMICs and functional studies have been also employed in studying grapevine transcriptomics [11, 12] proteomics [13, 14] and metabolomic aspects [15, 16] in attempt to decipher mechanisms underlying wild grapevines tolerance to biotic and abiotic factors. On the other hand, a failure of some conventional rootstocks in promoting grapevine sustainability under stress conditions has been reported [17]. Researchers thus attempted to search for a new generation of rootstocks with higher adaptation capacities. In this context, the use of advanced biotechnological tools would greatly contribute to a better understanding of the molecular mechanisms deployed by tolerant genotypes to generate reliable molecular markers to select and to rapidly identify new rootstocks and/or cultivars with advantageous loci.

This review focuses on the current understanding of defense mechanisms in wild grapevines and the genetic sources of tolerance to biotic and abiotic stresses, as revealed by recent advanced genomic and transcriptomic studies. We further highlight the priorities that should be addressed to establish an efficient and smart breeding program for grapevine adaptation to environmental constraints.

The efficiency of marker-assisted selection in exploring and pyramiding resistance genes in wild grapes

Grapevines are susceptible to many fungal diseases including powdery mildew caused by *Erysiphe necator* Schw. (syn. *Uncinula necator* Schw.), anthracnose caused by *Elsinoe ampelina* (de Bary) Shear, gray mold caused by *Botrytis cinerea*, and downy mildew caused by *Plasmopara viticola* [18]. These pathogenic fungi affect both grapevine plant growth and fruit quality and their control under field conditions requires a massive usage of fungicides [19]. There is an increasing public pressure to reduce pesticide use due to environmental and human health concerns [20] as well as the cost and risk associated with the development of resistant strains of these pathogens under current control strategies. Thus, the search for environmentally-safe alternatives for the control of these pathogens is of great benefit.

Breeding programs based on the exploitation and the exploration of the potential of different wild grapevine species as a source of disease resistance traits could be a suitable approach. Conventional breeding has been attempted by grape breeders to develop pathogen resistant cultivars with high quality fruit. Nevertheless, the successful introgression of resistance into susceptible grapevine cultivars through conventional breeding is time consuming. Molecular genetic tools like MAS can help breeders in the identification of genomic regions that carry resistance genes to grapevine pathogens from different genetic backgrounds. MAS is useful in pyramiding resistance genes from wild grapevines and their introgression into elite cultivated grapevines.

Numerous North American *Vitis* species are known for their resistance to *P. viticola and U. necator* pathogens [21]. In addition, several Chinese *Vitis* species were also found to be resistant to both pathogens despite that these latter had never been detected in Asia [22]. Using MAS, various resistance genes to these pathogens have been mapped in several genetic backgrounds and hybrids [23, 24].

In fact, a powdery mildew resistance locus, Ren1, has been identified in 'Dzhandzhal kara' and 'Kishmish vatkana' central Asian cultivars, and this discovery significantly contributed to identify new grapevine lines resistant to powdery mildew. Ren1-mediated resistance implicated the inhibition of the pathogen mycelial growth, reduced conidiophore formation and a delay in plant cell death (PCD) establishment at the infection sites [24].

Therefore, simple sequences repeat (SSR) markers have been designed for this locus and allowed the detection of other homologs containing resistance genes to *U. necator* in additional germplasm [25, 26]. Based on MAS, wild Chinese *Vitis* species were found to contain major dominant *R*-genes for *U. necator* resistance. In fact, *Ren4*, isolated from the Chinese species *V. romanetii*, has been successfully introduced into cultivated grapevines and found to segregate as a single dominant R-locus [25]. It is important to note that the majority of the Chinese *Vitis* species may be more advantageous to use for breeding purposes compared to North American *Vitis* ones because of their more neutral fruit flavors while still being fully inter-fertile with *V. vinifera*.

In addition, 392 grapevine accessions from Afghanistan, Pakistan and Central Asia, the Middle East, Turkmenistan, besides to Native American and Chinese *Vitis* species have been screened to identify synonyms and homonyms using 6 microsatellite markers [27]. Thereafter, a set of 266 unique accessions were subjected to a second test using ten additional markers sourced primarily from chromosome 12, 13 and 18. When the allelic diversity of marker VMCNg4e10.1 and UDV124 that flank the Ren1 locus was

assessed, thirty-one accessions were found to contain the marker UDV124 and 30 accessions were found to carry the marker VMCNg4e10.1, both markers were linked to Ren1. However, 6 accessions ('Sochal', Karadzhandal', 'Baidh-ul-Haman', 'Late Vavilov', 'Kishmish vatkana' and 'Husseine') were revealed to contain resistant alleles for both markers. All of the accessions either with one or both alleles associated to resistance have been tested under field conditions to powdery mildew resistance. The results from this field test revealed four additional homologs of Ren1 with significant resistance to powdery mildew [27].

Additionally, based on MAS analysis, a set of 380 unique genotypes including 40 accessions of *V. vinifera* subsp. *sylvestris,* 306 grapevine cultivars, and 34 accessions of *Vitis* species originating from China, northern Pakistan and Afghanistan were evaluated with data generated from 34 SSR markers. Based on the presence of four SSR alleles previously reported to be related to the *U. necator* resistance locus, Ren1, 10 new genotypes were detected, as resistant to powdery mildew disease including 2 V. *vinifera* subsp. *sylvestris* accessions and 8 V. *vinifera* cultivars among which three genotypes were found to have a relationship with 'Karadzhandal' and 'Kishmish vatkana' cultivars [26].

Another important study, based on SSR markers, conducted on 277 genotypes of an F1 mapping population resulting from a cross of *V. vinifera* selection F2-35 (which is susceptible to powdery mildew disease) and *V. piasezkii* DVIT2027 (a resistant accession to powdery mildew disease) followed by a quantitative trait locus (QTLs) analyses allowed the identification of two major PM resistance loci, Ren6 and Ren7, localized, respectively, on chromosomes 9 and 19.

Further analysis by microsatellites of numerous seedlings of the F1 population besides to others developed from four pseudo-backcross populations allowed the identification of regions of 22 and 330 kb in the *V. vinifera* PN40024 (12X) genome that correspond to Ren6 and Ren7, respectively [28]. Both R loci were found to be implicated in inducing PCD [28].

Apart from the QTL defined for PM disease resistance, others were defined for *U. necator* and *P. viticola* grapevine pathogens using MAS from North American, Chinese, and Asian *Vitis* species. These QTLs were mapped to different chromosomes (4, 7, 9, 12, 13, 15, and 18) that enclose the majority of the *RGA* (repressor of gibberellin) -like genes in grapevines [29]. Among which, a locus for resistance to *U. necator* (Run1) was co-located with another one related to resistance to *P. viticola* (Rpv1) on the chromosome 12 [30]. These loci were found to encode several Toll/interleukin-1 receptor (*TIR*)-*NB-LRR*-type *R* genes. After that, infection assays revealed that only one (*MrRGA10*) of these *TIR-NB-LRR* genes was able to confer resistance to *U. necator* when overexpressed in susceptible grapevines [30]. More recently, Agurto et al. [2] created Run1Ren1 resistant genotypes to *E. necator* and identified them by MAS technology through conventional breeding by cross-pollinating the resistant P09-105/34 accession with the susceptible 'Crimson Seedless' cultivar.

Collectively, these findings confirmed that MAS technology was successful in exploring and examining various genetic resources available in wild grapevines to detect new resistance loci to be employed in developing new cultivated grapevines with better resistance to pathogens.

Overall aspects of OMIC research on wild grapevine species

Wild grapevines have retained high genetic diversity relative to cultivars [31]. Thus, they represent an important and valuable genetic reservoir for biotic and abiotic stress tolerance loci [32]. The quest for genes conferring tolerance to environmental stresses has intensified during the two latest decades. Recently, high throughput sequencing technologies have been used in different studies on wild grapevines to identify novel abiotic [33] and biotic [34, 35] stress tolerance genes and even miRNA regulators of target genes [36, 37]. Based on these findings, several candidate genes from wild grape genotypes have been used to improve biotic [38] and abiotic tolerance [39] in elite-cultivated grapevines (Table 1). However, very few studies examining wild grapevines metabolomics and proteomics related to biotic and/or abiotic stress are available [40]. George and Haynes [41] reported that only a few protein entries from wild species were found in Uniprot database, and these proteins are uncharacterised and unreviewed. Data from multidisciplinary approaches (transcriptomics, proteomics, and metabolomics) should be incorporated into a systems view to gain a clearer image on the tolerance mechanisms in wild grapevine species [42]. In this review, we have uncovered that most molecular studies on wild grapevine species were restricted to Asian and North American species (Table 1). The subspecies *V. vinifera* ssp. *sylvestris* which is mainly Mediterranean, has not yet been the subject of omics studies and is still unexplored. This species is considered to be the progenitor of cultivated grapevines due to their close genetic and phenotypic relationships and recurrent gene flow [43]. Most importantly, this species has been shown to possess important potential for increased abiotic stress tolerance [8, 44], but an understanding of the genetic architecture of these traits is unknown. This is likely due to the very limited wild species-ESTs present in the genome databases and gene annotation. Until very recently there has only been one grape genome sequence currently available, *V. vinifera,* Pinot Noir PN40024. Most studies on cultivated and wild species so far have used this genome sequence

Table 1 Stress related genes from wild grapevine species

Gene	Accession number	Vitis species	Putative function	Inducing stress	References
VyDHN1	JF900497		Dehydrins: protect plant cells from dessication and pathogen attacks	Cold/heat/embryogenesis/ABA	
				SA/MeJA/*E.necator*	
VyDHN2	JQ408442	*V. yeshanensis*		Cold/heat/embryogenesis/ ABA	[68]
VyDHN3	JQ408443			Seed development	
VyDHN4	JQ408444			Late embryogenesis	
VfDHN1a	AB676854	*V. flexuosa*		warer stress	[67]
VrDHN1a	AY70987	*V. riparia*		Cold/water stress/ABA	
VrDHN1b	AY706988				[66]
VaCPK1	KC488321			High salt/high mannitol	
VaCPK2	KF042354			High salt/high mannitol/low and high temperature	[59]
VaCPK3	KF042355			High salt/high mannitol/low and high temperature	
VaCPK9	KC488319	*V. amurensis*	Calcium-dependant protein kinases:	High salinity/mannitol/ high temperature	
VaCPK13	KC488320		sensors of Ca^{2+} flux in plants,	Low temperature	
VaCPK16	KF042356			High salinity/mannitol	
VaCPK21	KC488318			High salt/high mannitol/low and high temperature	
VaCPK26	KC488323			Low and high temperature	
VaCPK29	KC488317			High salt/high mannitol/low and high temperature	
VaCPK20	KC488322	*V. amurensis*	Regulatory transcripton factor	High tolerance to freezing and water stress	[60]
VpERF1	GU393310		Ethylene response factor (ERF)	Water stress /heat	
VpERF2	GU393311	*V. pseudoreticulata*		Water stress /cold/heat	[32]
VpERF3	GU393312			SA/MeJA	
VpWRKY3	JF500755	*V.pseudoreticulata*	Transcription factor	High salinity/ABA	[105]
VpRFP1	FJ356672	*V.pseudoreticulata*	C4C4-type RING finger: transcriptional activator	*U. necator*	[77]
VpPR-10.1	DQ336289	*V. pseudoreticulata*	Pathogenesis-related (PR) proteins	*E.necator*	[33]
VpHsf1	GU393313	*V. pseudoreticulata*	Heat shock related transcription factors	Heat/water stress/ *E. necator*	[76]
VabHLH1	JQ911779	*V. amurensis*	Basic helix-loop-helix (bHLH)-type transcription factor	Cold	[70]
VaICE1	KC815984	*V. amurensis*	Inducer of CBF expression 1/transcription factors	Cold	[69]
VaICE2	KC815985		Inducer of CBF expression 2/transcription factors	ND	
VrCBF4	AY706986	*V. riparia*	CRT-binding transcription factor	Cold	[72]
VaCBF4	HM231149	*V. amurensis*	CRT-binding transcription factor	Cold/salinity/ABA/SA	[73]
VaCOR	HM231146	*V. amurensis*	Cold-stress related transcription factor	Low temperature/salinity/ABA/SA	[74]
VpAPX	DQ150258	*V. pseudoreticulata*	Ascorbate peroxidase	*U. necator*	[84]
EIRP1	JF502034	*V. pseudoreticulata*	E3 ubiquitin ligase *E. necator*-induced RING finger protein	*U. necator*	[99]
VpALDH2B4	DQ150256	*V. pseudoreticulata*	Aldehyde dehydrogenases	*U. necator*	[96]
VpSTS	DQ445490	*V. pseudoreticulata*	Stilbene synthase	*U. necator* and *A. alternata*	[90] [39]
VpGLOX	DQ201181	*V. pseudoreticulata*	Glyoxal oxidase	*E. necator*	[38]
VpNAC1	GR883063	*V. pseudoreticulata*	Transcription factor	*E. necator*/SA/MeJA/ET	[103]
VaNAC26	GSVIVT01019952001	*V. amurensis*	Transcription factor	Cold, water stress and salinity	[101]
VaNCED1	VV78X205727.5	*V. amurensis*	9-cis-epoxycarotenoid dioxygenase	Water stress	[62]

SA salicylic acid, *JA* jasmonic acid, *ABA* abscissic acid, *MeJA* methyl-jasmonate, *ET* ethylene, *ND* not defined; *E necator*: *Erysiphe necator*, *U. necator*: *Uncinula necator*

as a reference genome. Despite this valuable resource, it is widely believed that significant numbers of gene transcripts and proteins are either species specific or so divergent that they are underestimated when compared with the Pinot Noir genome. Recently, based on deep sequencing assays, it was demonstrated that the reliance of studies on a single reference genome might significantly affect the estimation of the variation degree among different genotypes [45]. For example, reconstructing the transcriptomes of different grapevine genotypes has revealed differences in transcripts associated with their phenotypes [46, 47]. Similarly, using RNA-seq experiments, Xin et al. [48] observed a greater number of unidentified sequences from *V. amurensis* compared to the reference genome. In the same context, Jiao et al. [34] performed a deep sequencing of two Chinese wild *Vitis* species (*V. pseudoreticulata* and *V. quinquangularis*) in order to explore genetic diversity. When comparing the wild sequenced genomes to the reference grape (PN40024), high genetic variation was observed as SNPs, small indels, and cis-natural antisense transcript (cis-NAT) pairs. This variation was observed in a broad range of novel regulatory changes in genes related to secondary metabolism and abiotic stress responses.

In the absence of reference genomes, RNA-seq data have been used to construct transcriptional profiling in new species by de novo assembly [49, 50]. To manage these large datasets, several Vitis databases were built, such as the VitisExpDB [51] which might provide grapevine genomic resources for the identification of genes and their functional analysis. For example, the VitisNet database (www.sdstate.edu/ps/research/vitis/pathways.cfm) focused on the molecular networks observed in grapevine and can be used for expression analyses at transcriptome, proteome and metabolome levels [52, 53]. Naithani et al. [54] developed a metabolic pathway knowledge base for grapevine and enriched for plant-specific pathways and grape-specific metabolites and responses. Recently, Pulvirenti et al. [55] created a novel platform BIOWINE to manage and analyze genomic data of *V. vinifera* which is an open web-based resource allowing users to browse and query *V. vinifera* RNA-seq data properly integrated with knowledge from, transcripts, proteins, microRNAs, pathways and Gene Ontology (GO) associations. Grapevine network models with a new gene co-expression database, VTCdb (https://vtcdb.adelaide.edu.au/Home.aspx) [56] and VESPUCCI [57] have been successfully used to study complex transcription factor regulatory networks, helpful for targeted functional studies. Furthermore, metabolite networks were developed to search for secondary metabolic pathway regulators in grapevine [58]. Thus, whole genome sequencing for wild *V. vinifera ssp. sylvestris* would promote omics studies for an efficient and reliable characterization of novel tolerance genes by building on this foundation of tools build around *V. vinifera*.

Outputs of Omic research on abiotic stress responses in wild grapevines

High throughput sequencing methods are needed to identify novel genes and to characterize the potential genetic diversity among wild grapevines. In this respect, RNA-seq studies have been achieved to identify novel micro RNA (mi-RNA) with regulatory roles in grape growth, development, and stress response [37]. Several novel genes have been identified and some of which have been functionally characterized (Table 1). Dubrovina et al. [59], discovered 12 novel calcium-dependent protein kinases (CDPKs) genes actively expressed under osmotic and temperature stresses in the highly resistant *V. amurensis*. The authors suggested that a *VaCPK20* gene might act as a regulatory factor during response to cold and water stress [60]. CDPKs have been reported to be important sensors of Ca^{2+} flux in plants, and to play an essential role in plant development and external factors signaling [61]. More recently, Zhu et al. [32] identified three new ethylene response factor (ERF) genes, *VpERF1*, *VpERF2*, and *VpERF3* from the Chinese wild *V. pseudoreticulata*, involved in the regulation of abiotic stress responsive pathways. These genes could be good candidates for improving tolerance toward abiotic stress in plants knowing that ERF transcription factors are considered as important players that interact with different signaling pathways to regulate responses to abiotic stress.

Recently a *VaNCED1* gene encoding a 9-cis-epoxycarotenoid dioxygenase (*NCED*), an enzyme involved in abscisic acid (ABA) biosynthesis, was found to be induced under osmotic stress (PEG) in the drought-tolerant wild grapevine *V. amurensis* [62]. The upregulation of *VaNCED1* gene modulated the expression of the ABA-responsive element 1 (*ABRE1*), ABRE binding factors2 (*ABF2*), plasma membrane intrinsic proteins 2 (*PIP2*), C-repeat/DRE-Binding Factor 4(*VvCBF4*) and ABA-insensitive 5 (*ABI5*) [62] leading to an improved drought tolerance in plants. However, under freezing stress, the downregulation of ABA synthesis resulted to a loss of cold hardiness in both wild *V. amurensis* and cultivated grapevines [63]. These findings indicate that ABA is a key element in abiotic stress signaling in *V. amurensis* [64]. In addition, when this cold-tolerant wild grapevine was subjected to low temperature stress, a higher accumulation of sugar-related transcripts and metabolites (raffinose, fructose and mannose) was noted compared to the grapevine cultivars [65]. These metabolites were found to act as compatible solutes to ensure cellular membrane stability under low-temperature stress [65].

Dehydrins (DHN1a and DHN1b), known to play a major role in protecting plants from desiccation, were found to be differentially induced among tolerant wild grapevine (*V. riparia)* and susceptible cultivated grapevine (*V. vinifera* Cv. Chardonnay) in response to cold stress [66]. Furthermore,

only DHN1a appears to be involved in cold tolerance as in silico analysis revealed that DHN1b lacks the amino acid string PGVLNR and includes an important proline residue. This mutation likely imposes a bend in its secondary structure, and this folding might impair dehydrin activity and/or cold acclimation [66]. DHN1a/b genes were found to be induced earlier and faster in *V. flexuosa* under water stress and lasted longer compared to the cultivated grapevines. DHN1a and DHN1b are alternative spliced transcripts with high sequence homology (having the 18-bp deleted-region distinguishing them, [67]. Moreover, Yang et al. [68] identified four DHN genes (DHN 1, 2, 3 and 4) from *V. yeshanensis* that are upregulated by water stress. Studies by Xu et al. [69] identified a novel transcription factor *VabHLH1* from cold-tolerant *V. amurensis* and this gene functions as a positive regulator of stress-related gene expression, particularly targeting CBF3 and resulting in an increased accumulation in RD29A. Promoter sequence homology analysis of *VabHLH1* and *VvbHLH1* (from cold-sensitive *Vitis* genotype) revealed differences in the position and types of stress-related cis-regulatory elements by having (MYBGAHV and MYB2AT) or lacking abiotic stress related elements (MYBGAHV and MYB2AT, [69]). Furthermore, differences in bHLH expression in cold-tolerant *V. amurensis* and sensitive *V. vinifera* species are the result of a 12-bp deletion in the VabHLH1 promoter region [70]. Transcripts of the regulatory element CBF4 were found to be induced rapidly following cold treatment in *V. riparia* and their expression lasts for a longer duration compared to those in cultivated grapevines [71].

Agro-infiltration experiments in tobacco leaves revealed that *VrCBF4* activates the expression of reporter genes driven by a CRT (C repeat element) -containing promoter [72]. However, no detectible differences in the expression pattern of *VvCBF4* among the tolerant *V. riparia* and the sensitive *V. vinifera* under cold stress was observed. Indeed, cold tolerance of *V. riparia*, might be attributed to subtle differences in the *VrCBF4* and *VvCBF4* proteins, with a glutamic acid (E) to aspartic acid (D) substitution at position 164. Similarly, Dong et al. [73] characterized new *VaCBF4* genes involved in signaling events during high salinity and hormone adaptation in *V. amurensis*. High-throughput sequencing experiments (RNAseq) allowed the identification of several candidate genes known to be involved in metabolism, transport, signal transduction and transcription which are also upregulated in response to cold stress in the tolerant *V. amurensis* [48]. Recently, Xu et al. [69] identified two ICE-orthologs (Inducer of CBF expression) from *V. amurensis*. *VaICE1* and *VaICE2* encode MYC-Type bHLH transcription activators and are implicated in freezing tolerance through acting as key regulators at the early phase of the transcriptional reprogramming underlying freezing tolerance and modulation of the expression of various cold related-genes that are involved in the C-repeat binding factor (CBF) pathway. According to Xu et al. [70], these genes confer cold tolerance in transgenic *A. thaliana* when overexpressed.

Finally, a cold-regulated (COR) gene in the freezing-tolerant wild grape *V. amurensis* (*VaCOR*) has been reported to be up-regulated under cold stress but not in a freezing sensitive *V. vinifera* Cv [74]. The *VaCOR* gene was found to be also involved in various stress responses. Such findings provided new information useful to better understand the entire network regulating stress signaling and tolerance in grapevine species highlighting the great potential of the various genetic resources available in wild grapevine species.

Outputs of Omic research on biotic stress responses in wild grapevines

Numerous studies have reported that Chinese wild grapevines (approximately 38 species) possess resistance genes as well as novel resistance mechanisms against grapevine pathogens [75]. For example, several candidate genes underlying resistance to PM involved in various hormonal signaling pathways in wild grapes *V. pseudoreticulata* have been identified [35]. Hormone modulation in response to pathogens includes an enhancement of jasmonic acid (JA) biosynthesis, which regulates the accumulation of phytoalexins, especially stilbenes. RNA-seq experiments performed on Chinese wild grapevines after infection by *Erysiphe necator* (the causal agent of powdery mildew), revealed that 70% of the transcription factors belonged to the ethylene-responsive factor (ERF) family, which has been previously reported to be implicated in the control of both primary and secondary metabolism as well as responses to environmental constraints [34].

Transcriptomic studies revealed an up-regulation of DHN1 gene in disease-resistant *V. yeshanensis* compared to a disease-susceptible *V. vinifera* cv. after inoculation with *Erysiphe necator*, highlighting the possible role of DHN1 in plant defense mechanisms [68]. Moreover, for the first time, a novel member of the Hsf class B2 family of heat shock transcription factors was isolated from Chinese wild *V. pseudoreticulata* (*VpHsf1*), and was found to act as a trans-activation repressor of defense responses [76]. Indeed, *VpHsf1* over-expression in tobacco enhanced its susceptibility to *Phytophthora parasitica* var. nicotianae Tucker.

In addition, a novel C4C4-type RING finger protein from mildew-resistant *V. pseudoreticulata* (*VpRFP1*) has been shown to be involved in powdery mildew resistance based on transcriptomic studies. After inoculation with *U. necator*, *VpRFP1* was rapidly and strongly induced in mildew-resistant *V. pseudoreticulata* plants while down-regulated in mildew-sensitive *V. vinifera* ones. Furthermore, *VpRFP1* over-expression enhanced the resistance of

transgenic Arabidopsis lines to both Arabidopsis powdery mildew caused by *Golovinomyces cichoracearum* and bacterial disease caused by *Pseudomonas syringae* pv. tomato DC3000. Likewise, functional studies demonstrated that the C-terminal RING finger protein region had a significant role in the regulation of transcriptional activity [77].

Under environmental stresses or after pathogen attacks, plants produce different types of defense proteins such as lytic enzymes, proteinase inhibitors or low molecular weight proteins, defined as pathogenesis-related (PR) proteins [19]. For instance, PR-10 isoforms were found to contribute in plant defense mechanisms. Novel VpPR10.1 proteins have been identified from the Chinese wild *V. pseudoreticulata* by Xu et al. [79] and this protein exhibited not only RNase and antifungal activities but also DNase activity [80]. In addition, Xu et al. [33] confirmed that *VpPR10.1* is related to hypersensitive reaction (HR) establishment in tobacco BY-2 suspension cells and provided resistance to *E. necator* infection.

In silico analysis revealed that while *VpPR10.4* and *VpPR10.7* contain characteristic amino acid domains with nuclease activity, this was not the case for *VpPR10.9* and *VpPR10.6*. Hence, it could be suggested that VpPR10.4 and VpPR10.7 proteins may not have the same function as VpPR10.6 and VpPR10.9 proteins [81]. Such differences in wild grape species genomic structure was also observed by comparative analysis of the novel stilbene synthase gene and promoter region in PM-resistant *V. pseudoreticulata* and PM-susceptible *V. vinifera*. The differential regulation pattern between PM-resistant wild grapevine *V. pseudoreticulata* and PM-susceptible *V. vinifera* was attributed to differential regulatory mechanisms governed by cis-regulatory elements in the promoter region [39].

In silico analysis of structure and promoter activity revealed marked differences in the stilbene synthase promoter of the Chinese wild *V. pseudoreticulata* compared to those of the cultivated *V. vinifera* cv.[82, 83]. This suggests that *V. pseudoreticulata* might contain a new promoter system inducible by pathogen attacks and, thereby, useful for generating engineered grapevine lines with enhanced disease-resistance levels. Lin et al. [84] identified a novel *VpAPX*, the ascorbate peroxidase-related gene from wild *V. pseudoreticulata,* by mRNA differential display approach. APX gene is the key enzyme that scavenges H_2O_2 in plant cells, [85]. *VpAPX* was strongly induced by *U. necator* inoculation and seems to be implicated in disease resistance in *V. pseudoreticulata.* Solexa-based sequencing technology analysis identified several novel candidate genes and pathways that may contribute to downy mildew resistance in *V. amurensis* [86].

Having available the gene pool and genome of a Mediterranean representative of the subspecies *V. vinifera* ssp. *sylvestris* might increase our appraisal of the natural diversity of *V. vinifera* to meet the challenges of future biotic stresses.

Functional studies on selected genes from wild-Vitis species

Functional analyses aim to decipher the role of genes via either over-expression or silencing tools using heterologous and/or homologous expression systems. Wild grapes, subjected to extensive natural selection over evolutionary scales, generally have some historically important traits that provide tolerance to different environmental constraints. Thus, functional studies on stress-related genes from wild grapevines can not only promote our understanding of stress tolerance mechanisms in grape but also provide an important resource for genetic engineering of cultivated *V. vinifera* cultivars.

Incorporating tolerance genes from wild *Vitis* species into *V. vinifera* has been proven successful (Tab. 1) leading to an increased tolerance to multiple environmental factors [87]. Genetic transformation was developed for wild *Vitis* species [88, 89], but almost of the functional studies were conducted on cultivated genotypes because of their susceptibility to various stresses. It was shown that the constitutive overexpression of the *VaNCED1* gene from the wild grapevine *V. amurensis* increased ABA content and conferred drought tolerance to the sensitive cultivated grapevine 'Thompson Seedless' [62]. Recently, a calcium-dependent protein kinase gene from *V. amurensis* (*VaCPK20*) was the subject of functional studies in cultured suspension cells of transgenic *V. amurensis* plants and found to act as a regulatory factor mediating both cold and water stress tolerance [60]. Unlike most *V. vinifera* cultivars, the Chinese *V. pseudoreticulata* showed natural resistance to *E. necator* and *P. viticola*. The over-expression of a stilbene synthase (*VpSTS*) gene from *V. pseudoreticulata* increased resveratrol concentrations in transgenic Thompson Seedless plants resulting in an enhanced resistance to both *U. necator* and *Alternaria alternata* [90]. Similarly, over-expression of the stilbene synthase gene *VaSTS19* from the Chinese wild grape, *V. amurensis* enhanced resistance to both powdery mildew and gray mold diseases in transgenic Arabidopsis plants [91]. The *VaSTS19* over-expression increased the expression of JA- and/or salicylic acid (SA) -dependent defense reactions.

Another set of defense genes belonging to PR gene family, known to be involved in basal resistance mechanisms in *V. pseudoreticulata*, were also the subject of functional studies. Enhanced resistance to both *E. necator* and *P. viticola* was observed in transgenic grapevine cultivar over-expressing *VpPR10.1* and *VpPR4-1* [33, 92]. Moreover, Guan et al. [38] reported that the over-expression of a *VpGLOX* gene, which encodes a glyoxal oxidase enzyme in wild *V. pseudoreticulata* led to a complete suppression of powdery mildew disease in transgenic cultivated grapevine plants. Zhao et al.

[93] suggested that *VpGLOX* might have a role in maintaining H_2O_2 homoeostasis, a hypothesis supported by many lines of evidence for a signaling role for H_2O_2 produced by the glyoxal oxidase enzyme as a secondary messenger responsible for the induction of PR genes.

Diverse signalling events are required for appropriate modulation of plant defense responses to stress factors. The Mitogen-activated protein kinases (MAPKs) are one of the most important proteins involved in defense signaling cascades responsible for target gene activation. The MAPKKKs is the first enzyme of the MAPK cascades known for the transduction of signals by sequential phosphorylation. A candidate gene *VqMAPKKK38* was firstly identified and functionally studied in a Chinese wild grapevine species *V. quinquangularis*. Transient over-expression and gene silencing experiments in *V. quinquangularis* leaves revealed that the *VqMAPKKK38* is involved in a stilbene-type phytoalexin biosynthesis by mediating the transcription of stilbene synthase (STS genes) through activation of the MYB14 transcription factor [94]. In addition, both H_2O_2 and calcium influx were found to modulate *VqMAPKKK38* expression and stilbene biosynthesis, suggesting that *VqMAPKKK38* might be a part of a signaling network involving both calcium and ROS components [95].

In addition, the over-expression of *VpALDH2B4* from *V. pseudoreticulata* encoding an aldehyde dehydrogenase promoted resistance to mildews through the SA-signaling pathway [96] and alleviated salt stress via upregulation of the stress-responsive superoxide dismutase activity for cellular reactive oxygen species (ROS) detoxification in Arabidopsis plants [96]. Zhou et al. [97] described that over-expressing of a Plant U-Box (PUB) family of E3 Ligases gene *VpPUB23* from a Chinese wild *V. pseudoreticulata* decreased resistance to E. necator in the cultivated Thompson Seedless grapevine Cv. Using transgenic Arabidopsis plants, the involvement of the *VpRFP1* and *VpEIRP1* (Erysiphe necator-induced RING finger protein 1 harboring E3 ligase activity) from *V. pseudoreticulata* in mediating resistance to *E. necator*, *G. cichoracearum* and *P. syringae* pv. Tomato has been evidenced. The *VpRFP1* transcription factor is a transcriptional activator of defense-related genes in grapevines and its accumulation seems to be associated with increased mRNA transcripts of PR genes in transgenic Arabidopsis [98, 99]. Another RING-H2-type named *VpRH2* that was found to interact with *VpGRP2A* (a glycine-rich RNA-binding protein) in yeast two-hybrid assay and when over-expressed in transgenic grapevines cv. Thompson Seedless, offered significant resistance to *E. necator* [100].

Fang et al. [101] described that the heterologous expression of *VaNAC26* induced tolerance to water and salinity stresses in Arabidopsis plants. In addition, the over-expression of *V. amurensis VaICE1*, *VaICE2* and *VabHLH1* transcription factors offered freezing tolerance in Arabidopsis [69, 102]. The C-repeat binding factors *VrCBF* isolated from *V. riparia* were characterized and also found to confer either drought tolerance (*VrCBF1*) or freezing tolerance (*VrCBF4*) by inducing cold responsive genes (*AtCOR15a*, *AtRD29A*, *AtCOR6.6*, and *AtCOR47*) in transgenic *A. thaliana* [46].

The over-expression of *VpSBP16*, a Squamosa promoter binding protein (SBP)-box transcription factor, enhanced tolerance to salt and water stress in *A. thaliana* germinating seeds, seedlings and mature plants by regulating SOS (salt overly sensitive signaling) and ROS signaling cascades [103]. Also, overexpression of *VaPAT1*, a GRAS transcription factor from *V. amurensis* encoding a phytochrome A signal transduction module, conferred tolerance to different abiotic stresses in Arabidopsis. In fact, *VaPAT1* was found to modulate different sets of stress-related genes [78] via interactions with GAI (gibberellin acid insensitive), RGA, and SCR (scarecrow).

The over-expression of *V. pseudoreticulata VpNAC1*, that is known to be a positive transcription activator of disease resistance that act through regulating PR gene expression, enhanced the resistance of transgenic tobacco plants to both *Phytophtora parasitica* and *Erysiphe cichoracearum* [104].

Over-expression of *VpERF1*, *VpERF*2, and *VpERF3* induced resistance in tobacco plants against both *P. parasitica var. nicotianae* and *Ralstonia solanacearum* [32]. However, the *VpERF1*-overexpressing Arabidopsis plants were more susceptible to these latest [32]. Finally, the transcription factors, *VpWRKY1* and 2 were found to be rapidly induced in *V. pseudoreticulata* after both SA treatment and *E. necator* infection [105]. The ectopic expression of VpWRKY1 promoted resistance to *E. cichoracearum* and alleviated both cold and salt stresses in Arabidopsis plants and the over-expression of *VpWRKY3* induced resistance to *R. solanacearum* [106] in tobacco plants, thus, providing an important basis for understanding defense mechanisms that are regulated by WRKY transcription factors in China wild grapevines.

Hypothetical model of the pathways involved in biotic and abiotic stress adaptation in wild grapevines

Wild grapevines may be more adapted to changing climate compared to the cultivated ones due to their history of local adaptation and freedom from selection during domestication. The molecular process and gene interactions related to environmental stress adaptation are becoming clearer, at least for Asian *Vitis* species (*V. pseudoreticulata* and *V. amurensis*) (Fig. 1). Nearly all of the genes mentioned in our proposed model (Fig. 1), were functionally validated via transgenesis experiments in different model plants where they offered an enhanced tolerance state to abiotic (salt, water, cold) and biotic factors (downy mildew, powdery mildew, etc.).

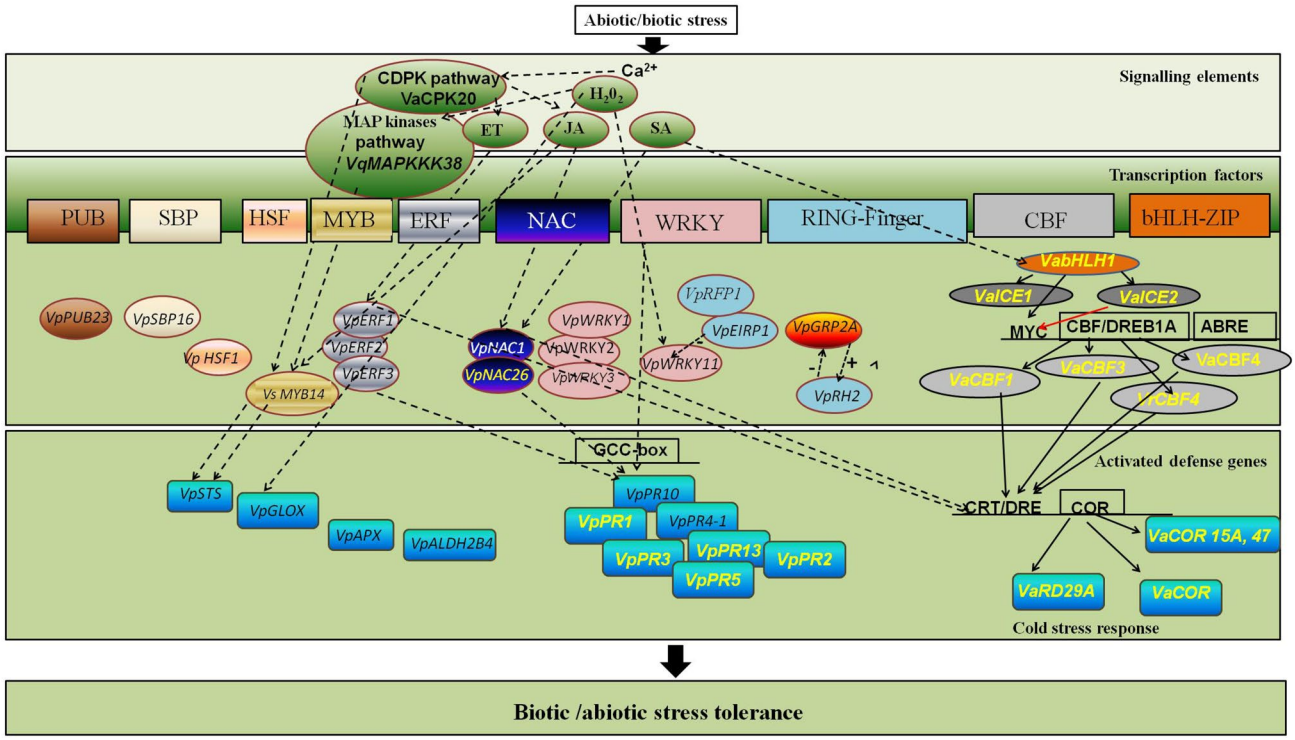

Fig. 1 A representative scheme of defense mechanisms in wild grapevine species to environmental constraints. *ET* ethylene, *JA* Jasmonic acid and *SA* salycilic acid, *MAPK* mitogen-activated protein kinase, *CDPK* calcium-dependent protein kinases, *NAC* (*NAM* no apical meristem, *ATAF* Arabidopsis transcription activation factor, *CUC* cup-shaped cotyledon), *HSF* heat stress transcription factor, *ERF* ethylene response factor, *RING-Finger* (Really Interesting New Gene) finger domain, *PUB* plant U-Box, *SPB-Box* Squamosa promoter binding protein (SBP)-box, *CBF* C-Repeat-Binding Factor, *bHLH-ZIP* basic helix-loop-helix leucine zipper transcription factors. *PR* pathogenesis related protein, *STS* stilbene synthase, *GLOX* glyoxal oxidase, *APX* ascorbate peroxidase, *ALDH* aldehyde dehydrogenases. *ICE* inducer of CBF expression, *DREB* dehydration-responsive element B, *ABRE* ACGT-containing abscisic acid response element, *CRT* C repeat element, *COR* cold-regulated, *RD29A* response to dehydration 29A

The mechanisms of stress tolerance in wild grapevines seem to be controlled by an efficient signal perception process and transduction modules that are responsible for transmitting the stress signal. After stress signal perception, a generation of secondary messengers such as Ca^{2+} and calcium-dependent protein kinases (CDPKs) initiates protein phosphorylation cascades targeting proteins directly involved in cellular protection or transcription factor activation. CDPKs are serine/threonine protein kinases with a C-terminal calmodulin-like domain and can directly bind Ca^{2+}. In wild grapevines, the CDPKs have been reported to have an important role during plant development and in response to external factors [61] and seem to be more connected to cold and water stress. Furthermore, the *V. amurensis VaCPK20* gene was found to regulate the biosynthesis of stilbenes in grape [61]. Another important signaling pathway identified in wild grapevines is the MAP kinase pathway which is activated by the calcium influx and by the change of the redox state due to the production and accumulation of H_2O_2. The activated MAPK then migrates to the nucleus to activate the transcription factor directly, or activate additional signal components to regulate gene expression. For example, in the wild grapevine *V. quinquangularis*, *VqMAPKKK38* was found to mediate the induction of STS genes via activation of the MYB14 transcription factor [95]. Other signaling hormones such as ethylene (ET), JA, and SA have been also reported to play an important role in mediating wild grapevine defense responses mainly through activating a wide range of transcription factors. Both JA and SA were found to regulate NAC transcription factors (*VpNAC1*/*VpNAC26*) known to regulate the expression of target genes containing the cis acting elements GCC-box in their promoter region including PR genes (*VpPR1*, *VpPR3*, *VpPR5*, *VpPR10*, *VpPR4-1*, *VpPR13*, *VpPR2*). The PR gene (*VpPR10*) was reported to be also regulated by the ET signaling pathway via the induction of VpERF3 transcription factor. Additionally, JA was found to activate MYB14 transcription factor [94], while, SA was reported to activate the bHLH transcription factors *VaICE1* and *VaICE2* and modulate the expression of cold related-transcription factors involved in the C-repeat binding factor (CBF) pathway (*VaCBF1*,

VaCBF3, *VaCBF4*, *VrCBF4*), and which also target downstream COR genes (*VaRD29A*, *VaCOR*).

Conclusions and perspectives

Climate change is expected to have a significant negative impact on grapevine growth and yield. As covered in this review, it is necessary for the development of omics methods in cultivated grapevine to elevate grapevine genetics to a level where we can now target specific strategies to preserve and sustain vineyard productivity. In this respect, wild *Vitis* species are a highly valuable source of tolerance traits. Significant advances in high throughput analysis techniques have been made and have resulted in better resolution of the traits related to biotic and abiotic stress tolerance in wild *Vitis* species. The future examination of wild grapevine genomic resources coupled with the development of *Vitis* omics databases is of prime interest to develop innovative strategies based on molecular breeding programs for a modern and durable viticulture.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. This P, Lacombe T, Thomas MR (2006) Historical origins and genetic diversity of wine grapevines. Trends Genet 22:511–519
2. Agurto M, Schlechter RO, Armijo G, Solano E, Serrano C, Contreras RA, Zúñiga GE, Arce-Johnson P (2017) RUN1 and REN1 Pyramiding in Grapevine (*Vitis vinifera* cv. Crimson Seedless) displays an improved defense response leading to enhanced resistance to powdery Mildew (*Erysiphe necator*). Front Plant Sci 8:758
3. Carvalho LC, Amâncio S (2019) Cutting the gordian knot of abiotic stress in grapevine: from the test tube to climate change adaptation. Physiol Plant 165:330–342
4. Fraga H, Malheiro AC, Moutinho-Pereira J, Santos JA (2014) Climate factors driving wine production in the Portuguese Minho region. Agric For Meteorol 185:26–36
5. Ashour EK, Al-Najar H (2012) The impact of climate change and soil salinity in irrigation water demand in the Gaza Strip. J Earth Sci Clim Chang 3(2):120
6. Reisch BI, Owens CL, Cousins PS (2012) Grape. In: Badenes ML, Byrne DH (eds) Fruit breeding. Springer, New York, pp 225–262
7. Arnold C, Gillet F, Gobat JM (1998) Situation de la vigne sauvage *Vitis vinifera* subsp. *Silvestris* en Europe. Vitis 37:159–170
8. Askri H, Daldoul S, Ben Amar A, Rejeb S, Rejeb MN, Mliki A, Ghorbel A (2012) Molecular and physiological characterization of salt stress in wild grapevines (*Vitis vinifera* ssp. S*ylvestris*). Acta Physiol Plant 34:957–968
9. Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyere C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lecharny A, Scarpelli C, Artiguenave F, Pe ME, Valle G, Morgante M, Caboche M, Adam-Blondon AF, Weissenbach J, Quetier F, Wincker P (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–467
10. Costantini L, Battilana J, Lamaj F, Fanizza G, Grando MS (2008) Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. BMC Plant Biol 8:38
11. Daldoul S, Guillaumie S, Reustle GM, Krczal G, Ghorbel A, Delrot S, Mliki A, Höfer MU (2010) Isolation and expression analysis of salt induced genes from contrasting grapevine (*Vitis vinifera* L.) cultivars. Plant Sci 179:489–498
12. Cramer GR, Ergül A, Grimplet J, Tillett RL, Tattersall EAR, Bohlman MC, Vincent D, Sonderegger J, Evans J, Osborne C, Quilici D, Schlauch KA, Schooley DA, Cushman JC (2007) Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Funct Integr Genomics 7:111–134
13. Cramer GR, Van Sluyter SC, Hopper DW, Pascovici D, Keighley T, Haynes PA (2013) Proteomic analysis indicates massive changes in metabolism prior to the inhibition of growth and photosynthesis of grapevine (*Vitis vinifera* L.) in response to water deficit. BMC Plant Biol 13:49
14. George IS, Haynes PA (2014) Current perspectives in proteomic analysis of abiotic stress in Grapevines. Front Plant Sci 5:686. https://doi.org/10.3389/fpls.2014.00686
15. Grimplet J, Wheatley MD, Jouira HB, Deluc LG, Cramer GR, Cushman JC (2009) Proteomic and selected metabolite analysis of grape berry tissues under well-watered and water-deficit stress conditions. Proteomics 9:2503–2528
16. Conde A, Regalado A, Rodrigues D, Costa JM, Blumwald E, Chaves MM, Gerós H (2015) Polyols in grape berry: transport and metabolic adjustments as a physiological strategy for water-deficit stress tolerance in grapevine. J Exp Bot 66(3):889–906
17. Serra I, Strever A, Myburgh PA, Deloire A (2013) Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine. Aust J Grape Wine Res 20:1–14. https://doi.org/10.1111/ajgw.12054
18. Pavlousek P (2007) Evaluation of resistance to powdery mildew in grapevine genetic resources. J Cent Eur Agric 8:C105–C114
19. Boubakri H (2017) The role of ascorbic acid in plant–pathogen interactions. In: Hossain M, Munné-Bosch S, Burritt D, Diaz-Vivancos P, Fujita M, Lorence A (eds) Ascorbic acid in plant growth, development and stress tolerance. Springer, Cham
20. Chen W-J, Delmotte F, Cervera SR, Douence L, Greif C, Corio-Costet M-F (2007) At least lwo Origins of fungicide resistance in grapevine downy mildew populations. Appl Environ Microbiol 73(16):5162–5172
21. Staudt G (1997) Evaluation of grapevine powdery mildew (*Uncinula necator*, anamorph Oidium tuckeri) in accessions of *Vitis* species. Bachelhurst. Merzhausen Germany. Vitis 36(3):151–154
22. Wan Y, Schwaniniger H, He P, Wang Y (2007) Comparison of resistance to powdery mildew and downy mildew in Chinese wild grapes. Vitis 46:132–136

23. Hoffmann S, Di Gaspero G, Kovacs L, Howard S, Kiss E, Galbacs Z, Testolin R, Kozma P (2008) Resistance to *Erysiphe necator* in the grapevine 'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. Theor Appl Genet 116:427–438
24. Coleman C, Copetti D, Cipriani G, Hoffmann S, Kozma P, Kovacs L, Morgante M, Testolin R, Di Gaspero G (2009) The powdery mildew resistance gene REN1 cosegregates with an NBS-LRR gene cluster in two central Asian grape varieties. BMC Genet 10:89
25. Ramming DW, Gabler F, Smilanick J, Cadle-Davidson M, Barba P, Mahanil S, Cadle-Davidson L (2010) A single dominant locus, Ren4, confers rapid non-race-specific resistance to grapevine powdery mildew. Phytopathology 101:502–508
26. Riaz S, Boursiquot J-M, Dangl G, Lacombe T, Laucou V, Tenscher A, Walker AM (2013) Identification of mildew resistance in wild and cultivated central Asian grape germplasm. BMC Plant Biol 13:149
27. Riaz S, Tenscher AC, Ramming DW, Walker MA (2011) Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use in marker-assisted breeding. Theor Appl Genet 122:1059–1073
28. Pap D, Riaz S, Dry IB, Jermakow A, Tenscher AC, Cantu D, Oláh R, Walker MA (2016) Identification of two novel powdery mildew resistance loci, Ren6 and Ren7, from the wild Chinese grape species *Vitis piasezkii*. BMC Plant Biol 16:170
29. Yang S, Zhang X, Yue J-X, Tian D, Chen J-Q (2008) Recent duplications dominate NBS-encoding gene expansion in two woody species. Mol Genet Genomics 280:187–198
30. Feechan A, Anderson C, Torregrosa L, Jermakow A, Mestre P, Wiedemann- Merdinoglu S, Merdinoglu D, Walker AR, Cadle-Davidson L, Reisch B, Aubourg S, Bentahar N, Shrestha B, Bouquet A, Adam-Blondon AF, Thomas MR, Dry IB (2013) Genetic dissection of a TIR-NB-LRR locus from the wild North American grapevine species *Muscadinia rotundifolia* identifies paralogous genes conferring resistance to major fungal and oomycete pathogens in cultivated grapevine. Plant J 76:661–674
31. Sean M, Boyko AR, Owens CL, Brown PJ, Grassi F, Aradhya MK, Prins B, Andy R, Chia J, Wareh D, Bustamante CD, Bucklera ES (2011) Genetic structure and domestication history of the grape. PNAS 3530–3535:108
32. Zhu Z, Shi J, Xu W, Li H, He M, Xu Y, Xu T, Yang Y, Cao J, Wang Y (2013) Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways. J Plant Physiol 170(10):923–933
33. Xu TF, Zhao XC, Jiao YT, Wei JY, Wang L, Xu Y (2014) A pathogenesis related protein, *VpPR-10.1*, from *Vitis pseudoreticulata*: an insight of its mode of antifungal activity. PLoS ONE 9:e95102
34. Jiao C, Gao M, Wang X, Fei Z (2015) Transcriptome characterization of three wild chinese *Vitis* uncovers a large number of distinct disease related genes. BMC Genomics 16:223
35. Weng K, Li Z-Q, Liu R-Q, Wang L, Wang Y-J, Xu Y (2014) Transcriptome of Erysiphe necator-infected *Vitis pseudoreticulata* leaves provides insight into grapevine resistance to powdery mildew. Hortic Res 1:14049
36. Jiu S, Zhu X, Wang J, Zhang C, Mu Q, Wang C, Fang J (2015) Genome-Wide mapping and analysis of grapevine microRNAs and their potential target genes. BMC Genomics 16:223
37. Wang C, Han J, Liu C, Kibet KN, Kayesh E, Shangguan L, Li X, Fang J (2012) Identification of microR-NAs from Amur grape (*Vitis amurensis* Rupr.) by deep sequencing and analysis of microRNA variations with bioinformatics. BMC Genomics 13:122
38. Guan X, Zhao H, Xu Y, Wang Y (2011) Transient expression of glyoxal oxidase from the Chinese wild grape *Vitis pseudoreticulata* can suppress powdery mildew in a susceptible genotype. Protoplasma 248:415–423
39. Xu W, Yu Y, Ding J, Hua Z, Wang Y (2010) Characterization of a novel stilbene synthase promoter involved in pathogen-and stress-inducible expression from Chinese wild *Vitis pseudoreticulata*. Planta 231:475–487
40. Victor KJ, Fennell AY, Grimplet J (2010) Proteomic analysis of shoot tissue during photoperiod induced growth cessation in *V. riparia* Michx. Grapevines. Proteome Sci 8:44
41. George IS, Haynes PA (2014) Current perspectives inproteomic analysis of abiotic stress in Grapevines. Front Plant Sci 5:686
42. Daldoul S, Ben Amar A, Guillaumie S, Mliki A (2014) Integration of omics and system biology approaches to study grapevine (*Vitis vinifera* L.) response to salt stress: a perspective for functional genomics—a review. J Int Sci Vigne Vin 48:189–200
43. Heywood V, Zohary D (1991) A catalogue of the wild relatives of cultivated plants native to Europe. Flora Mediter 5:375–415
44. Kok D (2007) Responses of *V. vinifera* subsp. *sylvestris* (C.C.Gmlin) ecotypes originated from two different geographical region of Turkey to salinity stress at seed germination and plantlet stages. Pak J Biol Sci 10(16):2631–2638
45. Venturini L, Ferrarini A, Zenoni S, Tornielli GB, Fasoli M, Dal Santo S, Minio A, Buson G, Tononi P, Zago ED, Zamperin G, Bellin D, Pezzotti M, Delledonne M (2013) De novo transcriptome characterization of *Vitis viniferacv*. *Corvina unveils varietal diversity*. BMC Genomics 14:41
46. Siddiqua M, Nassuth A (2011) Vitis *CBF1* and Vitis *CBF4* differ in their effect on Arabidopsis abiotic stress tolerance, development and gene expression. Plant Cell Environ 34:1345–1359
47. Da Silva C, Zamperin G, Ferrarini A, Minio A, Dal Molin A, Venturini L, Buson G, Tononi P, Avanzato C, Zago E, Boido E, Dellacassa E, Gaggero C, Pezzotti M, Carrau F, Delledonne M (2013) The high polyphenol content of grapevine cultivar tannat berries is conferred primarily by genes that are not shared with the reference genome. Plant Cell 25:4777–4788
48. Xin H, Zhu W, Wang L, Xiang Y, Fang L, Li J, Sun X, Wang N, Londo JP, Li S (2013) Genome wide transcriptional profile analysis of *Vitis amurensis* and *Vitis vinifera* in response to cold stress. PLoS ONE 8(3):e58740
49. Fu CH, Chen YW, Hsiao YY, Pan ZJ, Liu ZJ, Huang YM, Tsai WC, Chen HH (2011) Orchid Base: a collection of sequences of the transcriptome derived from orchids. Plant Cell Physiol 52:238–243
50. Su CL, Chao YT, Alex Chang YC, Chen WC, Chen CY, Lee AY, Hwa KT, Shih MC (2011) De novo assembly of expressed transcripts and global analysis of the *Phalaenopsis aphrodite* transcriptome. Plant Cell Physiol 52:1501–1514
51. Doddapaneni H, Lin H, Walker MA, Yao J, Civerolo EL (2008) Vitis ExpDB: a database resource for grape functional genomics. BMC Plant Biol 8:23. https://doi.org/10.1186/1471-2229-8-23
52. Grimplet J, Cramer GR, Dickerson JA, Mathiason K, Van Hemert J, Fennell AY (2009) VitisNet :"Omics" integration through grapevine molecular networks. PLoS ONE 4:e8365
53. Grimplet J, Van Hemert J, Carbonell-Bejerano P, Díaz- Riquelme J, Dickerson J, Fennell A, Pezzotti M, Martínez-Zapater JM (2012) Comparative analysis of grapevine whole-genome gene predictions, functional annotation, categorization and integration of the predicted gene sequences. BMC Res Notes 5:213
54. Naithani S, Raja R, Waddell EN, Elser J, Gouthu S, Deluc LG, Jaiswal P (2014) VitisCyc: a metabolic pathway knowledgebase for grapevine (*Vitis vinifera*). Front Plant Sci 5:644
55. Pulvirenti A, Giugno R, Distefano R, Pigola G, Mongiovi M, Giudice G, Vendramin V, Lombardo A, Cattonaro F, Ferro A

(2015) knowledge base for *Vitis vinifera* functional analysis. BMC Syst Biol 9(Suppl 3):S5
56. Wong DC, Sweetman C, Drew DP, Ford CM (2013) VTCdb: a gene co-expression database for the crop species *Vitis vinifera* (grapevine). BMC Genomics 14:882. https://doi.org/10.1186/1471-2164-14-882
57. Moretto M, Sonego P, Pilati S, Malacarne G, Costantini L, Grzeskowiak L, Bagagli G, Grando Maria S, Moser C, Engelen K (2016) VESPUCCI: exploring patterns of gene expression in grapevine. Front Plant Sci 7:633
58. Wong DCJ, Matus JT (2017) Constructing integrated networks for identifying new secondary metabolic pathway regulators in grapevine: Recent applications and future opportunities. Front Plant Sci 8:505
59. Dubrovina AS, Kiseleva KV, Khristenkoa VS (2013) Expression of calcium-dependent protein kinase (CDPK) genes under abiotic stress conditions in wild-growing grapevine *Vitis amurensis*. J Plant Physiol 170:1491–1500
60. Dubrovina AS, Kiseleva KV, Khristenko VS, Aleynova OA (2015) *VaCPK20*, a calcium-dependent protein kinase gene of wild grapevine *Vitis amurensis* Rupr., mediates cold and drought stress tolerance. J Plant Physiol 185:1–12
61. Kiselev KV, Dubrovina AS, Shumakova OA, Karetin YA, Manyakhin AY (2013) Structure and expression profiling of a novel calcium-dependent protein kinase gene, CDPK3a, in leaves, stems, grapes, and cell cultures of wild-growing grapevine *Vitis amurensis* Rupr. Plant Cell Rep 32:431–442
62. He R, Zhuang Y, Cai Y, Agüero CB, Liu S, Wu J, Deng S, Walker MA, Lu J, Zhang Y (2018) Overexpression of 9-cis-epoxycarotenoid dioxygenase cisgene in grapevine increases drought tolerance and results in pleiotropic effects. Front Plant Sci 9:970
63. Kovaleski AP, Londo JP (2019) Tempo of gene regulation in wild and cultivated *Vitis* species shows coordination between cold deacclimation and budbreak. Plant Sci 287:110781
64. Sah SK, Reddy KR, Li J (2016) Abscisic acid and abiotic stress tolerance in crop plants. Front Plant Sci 7:571
65. Chai F, Liu W, Xiang Y, Meng X, Sun X, Cheng C, Liu G, Duan L, Xin H, Li S (2019) Comparative metabolic profiling of *Vitis amurensis* and *Vitis vinifera* during cold acclimation. Hortic Res 6:8
66. Xiao H, Nassuth A (2007) Stress and development-induced expression of spliced and unspliced transcripts from two highly similar dehydrin 1 genes in *V. riparia* and *V. vinifera*. Plant Cell Rep 25:968–977
67. Choi Y-J, Hur YY, Jung S-M, Se-H K, Jung-Ho N, Park S-J, Park K-S, Yun H-K (2013) Transcriptional analysis of dehydrin1 genes responsive to dehydrating stress in grapevines. Hortic Environ Biotechnol 54(3):272–279
68. Yazhou Y, He M, Zhu Z, Li S, Xu Y, Zhang C, Singer SD, Wang Y (2012) Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. BMC Plant Biol 12:140
69. Xu W, Jiao Y, Li R, Zhang N, Xiao D, Ding X, Wang Z (2014) Chinese wild-growing *Vitis amurensis ICE1* and *ICE2* Encode MYC-Type bHLH Transcription Activators that regulate cold tolerance in Arabidopsis. PLoS ONE 9(7):e102303
70. Xu WR, Zhang NB, Jiao YT, Li RM, Xiao DM, Wang ZP (2014) The grapevine basic helix-loop-helix (bHLH) transcription factor positively modulates CBF-pathway and confers tolerance to cold-stress in Arabidopsis. Mol Biol Rep 41:5329–5342
71. Xiao H, Siddiqua M, Braybrook S, Nassuth A (2006) Three grape *CBF/DREB1* genes respond to low temperature, drought and abscisic acid. Plant Cell Environ 29:1410–1421
72. Xiao H, Tattersall EAR, Siddiqua MK, Cramer G, Nassuth A (2008) CBF4 is a unique member of the *CBF* transcription factor family of *Vitis vinifera* and *Vitis riparia*. Plant Cell Environ 31:1–10
73. Dong C, Zhang Z, Qin Y, Ren J, Huang J, Wang B, Lu H, Cai B, Tao J (2013) *VaCBF1* from *Vitis amurensis* associated with cold acclimation and cold tolerance. Acta Physiol Plant 35:2975–2984
74. Dong C, Qin Y, Wang B, Lu H, Xiao L, Yang R, Wang Y, Chen L, Feng Y (2014) COR-like gene is involved in induced-expression response to multiple abiotic stresses in grapevine (*Vitis amurensis*) tissues. Agric Sci 5:604–610
75. Li F, Fan X-C, Liu C-H, Zhang Y, Jian G-F, Li M (2012) Investigation resistance to white rot in grape germplasm resources. China Fruit 72–74.
76. Peng S, Zhu Z, Zhao K, Shi J, Yang Y, He M, Wang Y (2013) A novel heat shock transcription factor, *VpHsf1*, from Chinese wild *Vitis pseudoreticulata* is involved in biotic and abiotic stresses. Plant Mol Biol Rep 31(1):240–247
77. Yu Y, Xu W, Wang S, Xu Y, Li H, Wang Y, Li S (2011) *VpRFP1*, a novel C4C4-type RING finger protein gene from Chinese wild *Vitis pseudoreticulata*, functions as a transcriptional activator in defence response of grapevine. J Exp Bot 62(15):5671–5682
78. Yuan Y, Fang L, Karungo SK, Zhang L, Gao Y, Li S, Xin H (2016) Over-expression of *VaPAT1*, a GRAS transcription factor from *Vitis amurensis*, confers abiotic stress tolerance in Arabidopsis. Plant Cell Rep 35:655–666
79. Xu Y, Yu H, He M, Yang Y, Wang Y (2010) Isolation and expression analysis of a novel pathogenesis-related protein 10 gene from Chinese wild *Vitis pseudoreticulata* induced by *Uncinula necator*. Biologia 65:653–659
80. He MY, Xu Y, Cao JJ, Zhu ZG, Jiao YT, Wang YJ, Guan X, Yang YZ, Xu WR, Fu ZF (2013) Subcellular localization and functional analyses of a *PR10* protein gene from *Vitis pseudoreticulata* in response to *Plasmopara viticola* infection. Protoplasma 250:129–140
81. Wang L, Wei J, Zou Y, Xu K, Wang Y, Cui L, Xu Y (2014) Molecular characteristics and biochemical functions of *VpPR10s* from *Vitis pseudoreticulata* associated with biotic and abiotic stresses. Int J Mol Sci 15:19162–19182
82. Xu W, Yu Y, Zhou Q, Ding J, Dai L, Xie X, Xu Y, Zhang C, Wang Y (2011) Expression pattern, genomic structure, and promoter analysis of the gene encoding stilbene synthase from Chinese wild *Vitis pseudoreticulata*. J Exp Bot 62:2745–2761
83. Zheng X, Shi J, Yu Y, Shen Y, Tan B, Ye X, Li J, Feng J (2017) Exploration of elite stilbene synthase alleles for resveratrol concentration in wild Chinese *Vitis* spp. and *Vitis* cultivars. Front Plant Sci. 8:487
84. Lin L, Wang X, Wang Y (2006) cDNA clone, fusion expression and purification of the novel gene related to ascorbate peroxidase from Chinese wild *Vitis pseudoreticulata* in *E. coli*. Mol Biol Rep 33:197–206
85. Teixeira FK, Menezes-Benavente L, Margis R, Margis-Pinheiro M (2004) Analysis of the molecular evolutionary history of the ascorbate peroxidase gene family: Inferences from the rice genome. J Mol Evol 59:761–770
86. Wu J, Zhang Y, Zhang H, Huang H, Folta KM, Lu J (2010) Whole genome wide expression profiles of *Vitis amurensis* grape responding to downy mildew by using Solexa sequencing technology. BMC Plant Biol 10:234
87. Jelly NS, Valat L, Walter B, Maillot P (2014) Transient expression assays in grapevine: a step towards genetic improvement. Plant Biotechnol J 12:1231–1245
88. Zhao T, Wang Z, Su L, Sun X, Cheng J, Zhang L, Karungo KS, Han Y, Li S, Xin H (2017) An efficient method for transgenic callus induction from *Vitis amurensis* petiole. PLoS ONE 12(6):e0179730

89. Dhekney SA, Li ZT, Grant TN, Gray DJ (2016) Somatic embryogenesis and genetic modification of *Vitis*. Methods Mol Biol 1359:263–277
90. Fan CH, Pu N, Wang XP, Wang YJ, Fang L, Xu WR et al (2008) Agrobacterium-mediated genetic transformation of grapevine (*Vitis vinifera* L.) with a novel stilbene synthase gene from Chinese wild *Vitis pseudoreticulata*. Plant Cell Tissue Org 92:197–206
91. Wang Y, Wang D, Wang F, Huang L, Tian X, van Nocker S, Gao H, Wang X (2017) Expression of the Grape *VaSTS19* gene in Arabidopsis improves resistance to powdery mildew and botrytis cinerea but increases susceptibility to *Pseudomonas syringe* pv Tomato DC3000. Int J Mol Sci 18:2000
92. Dai L, Wang D, Xie X, Zhang C, Wang X, Xu Y, Wang Y, Zhang J (2016) The Novel Gene *VpPR4-1* from *Vitis pseudoreticulata* increases powdery mildew resistance in transgenic *Vitis vinifera* L. Front Plant Sci 27(7):695
93. Zhao H, Guan X, Xu Y, Wang Y (2013) Characterization of novel gene expression related to glyoxal oxidase by agro-infiltration of the leaves of accession Baihe-35-1 of *Vitis pseudoreticulata* involved in production of H_2O_2 for resistance to *Erysiphe necator*. Protoplasma 250:765–777
94. Duan D, Fischer S, Merz P, Bogs J, Riemann M, Nick P (2016) An ancestral allele of grapevine transcription factor MYB14 promotes plant defence. J Exp Bot 67:1795–1804
95. Jiao Y, Wang D, Wang L, Jiang C, Wang Y (2017) *VqMAPKKK38* is essential for stilbene accumulation in grapevine. Hortic Res 4:17058
96. Wen Y, Wang X, Xiao S, Wang Y (2012) Ectopic expression of *VpALDH2B4*, a novel aldehyde dehydrogenase gene from Chinese wild grapevine (*Vitis pseudoreticulata*), enhances resistance to mildew pathogens and salt stress in Arabidopsis. Planta 236:525–539
97. Zhou Q, Dai L, Cheng S, He J, Wang D, Zhang J, Wang Y (2014) A circulatory system useful both for long-term somatic embryogenesis and genetic transformation in *Vitis vinifera* L. cv. Thompson seedless. Plant Cell Tissue Org 118:157–168
98. Yu Y, Xu W, Wang J, Wang L, Yao W, Xu Y, Ding J, Wang Y (2013) A core functional region of the RFP1 promoter from Chinese wild grapevine is activated by powdery mildew pathogen and heat stress. Planta 237:293–303
99. Yu Y, Xu W, Wang J, Wang L, Yao W, Yang Y, Xu Y, Ma F, Du Y, Wang Y (2013) The Chinese wild grapevine (*Vitis pseudoreticulata*) E3 ubiquitin ligase Erysiphe necator-induced RING finger protein 1 (EIRP1) activates plant defense responses by inducing proteolysis of the *VpWRKY11* transcription factor. New Phytol 200:834–846
100. Wang L, Xie X, We Y, Wang J, Ma F, Wang C, Yang Y, Tong W, Zhang J, Wang YXX, Zhang C, Wang Y (2017) RING-H2-type E3 gene *VpRH2* from *Vitis pseudoreticulata* improves resistance to powdery mildew by interacting with *VpGRP2A*. J Exp Bot 68(7):1669–1687
101. Fang LC, Su LY, Sun XM, Li XB, Sun MX, Karungo SK et al (2016) Expression of *Vitis amurensis NAC26* in Arabidopsis enhances drought tolerance by modulating jasmonic acid synthesis. J Exp Bot 67:2829–2845
102. Xu W, Li R, Zhang N, Ma F, Jiao Y, Wang Z (2014) Transcriptome profiling of *Vitis amurensis*, an extremely cold-tolerant Chinese wild *Vitis* species, reveals candidate genes and events that potentially connected to cold stress. Plant Mol Biol 86:527–541
103. Hongmin H, Hui J, Qin Y, Xiping W (2018) Over-expression of a SBP-Box Gene (*VpSBP16*) from Chinese Wild *Vitis* Species in Arabidopsis Improves Salinity and Drought Stress Tolerance. Int J Mol Sci 19(4):940
104. Zhu Z, Shi J, He M, Cao J, Wang Y (2012) Isolation and functional characterization of a transcription factor *VpNAC1* from Chinese wild *Vitis pseudoreticulata*. Biotech Lett 34:1335–1342
105. Li H, Xu Y, Xiao Y, Zhu Z, Xie X, Zhao H, Wang Y (2010) Expression and functional analysis of two genes encoding transcription factors, *VpWRKY1* and *VpWRKY2*, isolated from Chinese wild *Vitis pseudoreticulata*. Planta 232(6):1325–1337
106. Zhu Z, Shi J, Cao J, He M, Wang Y (2012) VpWRKY3, a biotic and abiotic stress-related transcription factor from the Chinese wild *Vitis pseudoreticulata*. Plant Cell Rep 31(11):2109–2120