frontiers in Environmental Science

REVIEW
published: 19 March 2015
doi: 10.3389/fenvs.2015.00020

Oxidative stress homeostasis in grapevine (*Vitis vinifera* L.)

Luísa C. Carvalho, Patrícia Vidigal and Sara Amâncio *

LEAF Research Centre, Departamento de Recursos Naturais, Ambiente e Território (DRAT), Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

Plants can maintain growth and reproductive success by sensing changes in the environment and reacting through mechanisms at molecular, cellular, physiological, and developmental levels. Each stress condition prompts a unique response although some overlap between the reactions to abiotic stress (drought, heat, cold, salt or high light) and to biotic stress (pathogens) does occur. A common feature in the response to all stresses is the onset of oxidative stress, through the production of reactive oxygen species (ROS). As hydrogen peroxide and superoxide are involved in stress signaling, a tight control in ROS homeostasis requires a delicate balance of systems involved in their generation and degradation. If the plant lacks the capacity to generate scavenging potential, this can ultimately lead to death. In grapevine, antioxidant homeostasis can be considered at whole plant levels and during the development cycle. The most striking example lies in berries and their derivatives, such as wine, with nutraceutical properties associated with their antioxidant capacity. Antioxidant homeostasis is tightly regulated in leaves, assuring a positive balance between photosynthesis and respiration, explaining the tolerance of many grapevine varieties to extreme environments. In this review we will focus on antioxidant metabolites, antioxidant enzymes, transcriptional regulation and cross-talk with hormones prompted by abiotic stress conditions. We will also discuss three situations that require specific homeostasis balance: biotic stress, the oxidative burst in berries at *veraison* and *in vitro* systems. The genetic plasticity of the antioxidant homeostasis response put in evidence by the different levels of tolerance to stress presented by grapevine varieties will be addressed. The gathered information is relevant to foster varietal adaptation to impending climate changes, to assist breeders in choosing the more adapted varieties and suitable viticulture practices.

Keywords: ROS, ascorbate-glutathione cycle, oxidative burst, peroxiredoxins, hormone stress signals, *in vitro* stress, biotic stress

OPEN ACCESS

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***Correspondence:**
Sara Amâncio, LEAF Research Centre, Departamento de Recursos Naturais, Ambiente e Território (DRAT), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Calçada da Tapada, 1349-017 Lisboa, Portugal
samport@isa.ulisboa.pt

Specialty section:
This article was submitted to Environmental Toxicology, a section of the journal Frontiers in Environmental Science

Received: *28 November 2014*
Accepted: *02 March 2015*
Published: *19 March 2015*

Citation:
Carvalho LC, Vidigal P and Amâncio S (2015) Oxidative stress homeostasis in grapevine (Vitis vinifera L.). Front. Environ. Sci. 3:20. doi: 10.3389/fenvs.2015.00020

Introduction

Plants are able to maintain growth and reproductive success by sensing changes in the surrounding environment and reacting through mechanisms at the molecular, cellular, physiological, and developmental levels. These response mechanisms enable plants to react rapidly, within hours or days, to extreme environmental conditions that could otherwise be injuring or lethal. Understanding stress responses is one of the most important issues in plant research nowadays. Both biotic and abiotic stresses can promote the onset of oxidative stress through the accumulation of reactive oxygen species (ROS). Worldwide, extensive agricultural

losses are attributed to drought, often in combination with heat (Mittler, 2006). The available scenarios for climate change suggest increases in aridity in Mediterranean climate regions (Jones et al., 2005), where grapevine traditionally grows. This species is an extremely important crop worldwide, at the economic and cultural levels. In Southern Europe, post flowering phases of the growth cycle usually occur under high temperatures, excessive light and drought conditions at soil and/or atmospheric level. In such situations plants are affected by a combination of abiotic and biotic stresses, triggering synergistic or antagonistic physiological, metabolic or transcriptomic responses unique to each stress combination. Oxidative stress also arises in *in vitro* propagation commonly applied to ornamental species, also used to rapidly propagate grapevine scions for grafting (Carvalho and Amâncio, 2002).

The ultimate "price" to pay for photosynthetic O_2 release and plant aerobic metabolism is the production of ROS. ROS production can also be increased by stress conditions (Apel and Hirt, 2004). When photosynthesis is inhibited, absorption of light energy can be in excess to what can be used by the photosynthetic processes, resulting in ROS production and accumulation. The same is true when stress induced slowdown of other ROS processing metabolic mechanisms results in ROS accumulation. Climate change forecasts indicate a high probability of extreme temperature episodes, both high and low, a decrease in water availability as well as increases in carbon oxide and ozone in the atmosphere. All these factors impact plant growth and development by negatively affecting antioxidant homeostasis, hampering the adaptation to environmental stressors (Munné-Bosch et al., 2013).

A molecule is classified as "antioxidant" when it is able to quench ROS without itself undergoing conversion into a destructive radical, thus interrupting the cascades of uncontrolled oxidation. In that category are included, among others, the metabolites ascorbic acid (AsA, also termed vitamin C), glutathione (GSH), and carotenoid pigments. The ROS signaling or degradation pathways depend on antioxidant redox buffering enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxiredoxins (Prx), ascorbate peroxidase (APX), and glutathione reductase (GOR) (Carvalho et al., 2006; Vidigal et al., 2013).

Upon abiotic stress gene expression profiles are altered and usually genes assigned to the functional categories "protein metabolism and modification," "signaling" and "antioxidative response" undergo significant changes (Carvalho et al., 2011; Rocheta et al., 2014), thus enhancing the common attributes of abiotic stress defense pathways.

The nutraceutical properties of the grape berry and its derivatives, namely wine, are commonly associated with the antioxidant properties of the phenolic compounds they contain (Tenore et al., 2011; De Nisco et al., 2013), from simple flavonoids like anthocyanins to condensed proanthocyanidins (PAs, tannins), which can be solubilized into the vacuole or linked to cell wall polysaccharides, so, it is of great interest to understand their antioxidant mechanisms *in planta*.

Metabolites Involved in Antioxidant Homeostasis: ROS, AsA, GSH, Pigments, and Proline

The different levels of tolerance to stress presented by grapevine varieties relates directly to the genetic plasticity of the antioxidant homeostasis. Some varieties keep low basal levels of antioxidant metabolites thus having to synthesize them at the onset of stress. Such varieties have a slower response than those with higher basal levels of antioxidant metabolites (Carvalho et al., 2014). This is put in evidence by the different pattern of antioxidant metabolites in normal growth conditions, as shown in **Table 1**.

ROS

The first players in antioxidant homeostasis, which in normal conditions induce the need for detoxification/scavenging, are ROS themselves. In chloroplasts O_2^- produced in the Mehler reaction occurs in normal conditions, commonly increasing upon stress. It is now believed that O_2^- formation is the first step in a chain reaction leading to the control and regulation of several cellular processes (Apel and Hirt, 2004), with ROS integrated into signaling pathways (Mullineaux et al., 2006), often in crosstalk with hormonal regulation (Fujita et al., 2006). A mechanism of acclimation of *Nicotiana benthamiana* to high light, driven by the hormone abscisic acid (ABA) and by the accumulation of H_2O_2, also involving Prxs was recently described in Vidigal et al. (2014).

When the energy from triplet excited chlorophylls is transferred to molecular oxygen, $^1O_2$ is formed. This ROS is a strong electrophile agent that can react with lipids, proteins, and DNA (Triantaphylidès and Havaux, 2009). However, $^1O_2$ also reacts with target mediator molecules which trigger signaling cascades that lead either to programmed cell death or to acclimation (Ramel et al., 2012). In grapevine leaves, $^1O_2$ and also H_2O_2 are generated in trace-element stress, such as that caused by boron in excess (Gunes et al., 2006).

Peroxisomes are probably the major sites of intracellular H_2O_2 production, although O_2^- and nitric oxide radicals ($NO^{\cdot}$) are also produced in peroxisomes. The photoinhibition that is verified in grapevine leaves upon drought and salinity stress is accompanied of an increase in transcription of genes coding for peroxisome glycolate oxidase, catalase and several photorespiratory enzymes (Cramer et al., 2007).

AsA and GSH

L-ascorbic acid (AsA) is an abundant metabolite playing important roles in plant stress physiology as well as in growth and development. AsA is a key antioxidant (Conklin and Loewus, 2001), able to directly eliminate several different ROS (Potters et al., 2002). Both the chloroplastic lipophilic antioxidant α-tocopherol (vitamin E) and carotenoid pigments (carotenes and xanthophylls) depend on AsA for regeneration from oxidized radicals (Potters et al., 2002). AsA is also the most important H_2O_2 reducing substrate, acting together with glutathione (GSH, γ-L-Glu-L-Cys-Gly) in the ascorbate-glutathione cycle (see Section The Ascorbate-Glutathione Cycle) (Noctor and Foyer, 1998). GSH is a multifunctional metabolite in plants, being

TABLE 1 | Concentration of antioxidative metabolites and activities of antioxidative enzymes quantified in several grapevine varieties and in different tissues.

Metabolite	Kalecic Kerasi	Sultana	Trebbiano	Ökûzgözû	Touriga Nacional	Trincadeira	Thompson Seedless	Tinto Cão	Tinta Roriz	Malbec
Tissue	**Leaves**	***Cells /Leaf disks leaves**	**Leaves**	**Leaves**	**Leaves**	**Leaves**	**Buds**	**Leaves**	**Leaves**	**Leaves**
AsA [μmolg^{-1}FW]			11		0.8*	0.67				
DA sA [μmolg^{-1}FW]			3.3		0.23*	0.08				
GSH[μmolg^{-1}FW]			0.12		19*	25				
GSSG[μmolg^{-1}FW]			0.04		10*	7				
Chlorophyll [mg cm^{-2}LA]					12*	4		208	409	26576
Carotenoids [mg cm^{-2}LA]					5.6*	2.1		58	113	5297
H_2O_2 [μmol g^{-1}FW]	22.74	0.05*,a	35	20	4.3*	2.9	0.023			
Proline [μmol g^{-1}FW]	0.71	0,01*		0.01						
O_2^- [μmol g^{-1}FW]		0.1*,a	0.6							
MDA [μmol g^{-1}FW]				12						
Phenols [mmol g^{-1}FW]			30							
ABA [pmol mg^{-1}DW]		50			6.1*	4.3				1[e]
ENZYME ACTIVITY										
Superoxide dismutase [Units (mg prot min)$^{-1}$]	32.10		4	6.3	1.1					
Peroxidase [μmol GDHP (mg min)$^{-1}$]				250						664
Ascorbate peroxidase [μmol AsA (mg min)$^{-1}$]	237		23[b]	360	200					217.3
Monodehydroascorbate reductase [μmol NA DH (mg min)$^{-1}$]					1.5					
Dehydroascorbate reductase [μmol AsA (mg min)$^{-1}$]			4.5[c]		0.4					
Glutathione reductase [μmol NADPH (mg min)$^{-1}$]			0.08[c]		0.16					
Catalase [Units (mg prot min)$^{-1}$]	5.66			7[d]	3					4.4
Total peroxiredoxin [nmol H_2O_2 (μg prot min)$^{-1}$]					56.4°					
NA DH-GDH [mUnits (g min)$^{-1}$]		150*								
LOX [mmol g^{-1}]	2.42									
References	Gunes et al. (2006)	*Skopelitis et al. (2006), Stoll et al. (2000)	Sgherri et al. (2013)	Ozden et al. (2009)	*Carvalho et al. (2014), Vidigal et al. (2013), °Carvalho et al. (2006)	Carvalho et al. (2014)	Vergara et al. (2012)	Moutinho-Pereira et al. (2007)		Berli et al. (2010)

Units are indicated in each row except the following.
[a]nmol mg prot^{-1}; [b]mU g^{-1} FW; [c]U g^{-1}FW; [d]μmol H_2 O_2mg^{-1} min^{-1}; [e]μg g^{-1}FW; FW, fresh weight; LA, leaf area; DW, dry weight.
*Values followed by * or ° in each column were obtained from the reference labeled accordingly in the Reference line.*

a major reservoir of non-protein reduced sulfur, and a crucial element in cellular defense and protection, preventing the denaturation of proteins caused by oxidation of thiol groups during stress, reacting chemically with a wide range of ROS (Noctor et al., 2002). In grapevine, AsA and GSH pools and their adjustments upon stress seem to be variety dependent and under tight control (Carvalho et al., 2014). In cv. "Touriga Nacional" facing oxidative stress the existing AsA and GSH pools assure the cell buffering capacity while in cv. "Trincadeira" AsA and GSH need to be synthesized *de novo*, leading to a slower response that may be insufficient to maintain the redox pool at working levels (**Table 1**).

Carotenoids

Carotenoids protect the photosynthetic apparatus against photooxidative damage not only by quenching the triplet states of chlorophyll molecules (Koyama, 1991) but also by scavenging ROS, protecting pigments and unsaturated fatty acids from oxidative damage (Edge et al., 1997). In the grapevine variety "Trincadeira" subjected to heat stress, carotenoids play an important role in leaf ROS scavenging, in tandem with ascorbate and glutathione, the usual first line of antioxidative defense in plants (Carvalho et al., 2014, **Table 1**).

Proline

The metabolism of proline, including proline oxidation, is extremely important in the response to stress, as it is one of the most widespread osmoprotectants (Kiyosue et al., 1996), increasing in conditions of water deficit, as shown in the grapevine cv. Riesling (Bertamini et al., 2006). Also in grapevine, ROS generated by salinity stress signal the expression of GDH α-subunit, GDH acting as an anti-stress enzyme not only by detoxifying ammonia but also by producing glutamate which is channeled to proline synthesis (Skopelitis et al., 2006). Artificially increased proline levels also lead to the decrease of H_2O_2 and malondialdehyde. It was thus suggested that the crosstalk between proline and H_2O_2 could play an important role in the response to oxidative stress in grapevine leaves (Ozden et al., 2009, **Table 1**). Proline accumulation increased by two-fold upon salinity stress and by three-fold after water stress, and was accompanied by an increase in transcript abundance of plasma membrane proline transporters and of pyrroline-5-carboxylate synthetase (P5CS), the enzyme that catalyzes the first two steps in proline biosynthesis (Cramer et al., 2007). In parallel, there was an increased transcript abundance of proline dehydrogenase, presumably to enable the removal of excess proline, which can be toxic if allowed to over accumulate (Cramer et al., 2007). Different types of stress can reduce proline levels as it happens in excess boron, leading to an increased lipid peroxidation and APX depletion (Gunes et al., 2006).

Key Enzymes for Antioxidant Homeostasis

Redox homeostasis comprises the interaction of ROS with antioxidant molecules forming an interface for metabolic and environmental signals, thus modulating the induction of appropriate acclimation processes or cell death programs. In the chloroplasts, a decrease of CO_2 fixation together with an over-reduction of the ETC is the foremost source of ROS production during stress; in mitochondria over-reduction of the respective ETC is also a chief mechanism of ROS generation (Yoshida et al., 2007) and in peroxisomes, H_2O_2 is produced when glycolate is oxidized to glyoxylic acid during photorespiration (Mittler et al., 2004).

ROS signaling pathways depend upon a strict homeostatic regulation accomplished through antioxidant redox buffering. Antioxidants determine the lifetime and the specificity of the ROS signal or processing products. In this process, enzymes such as SOD, CAT, Prx, APX, and GOR are the key players in antioxidant homeostasis (Carvalho et al., 2006; Vidigal et al., 2013). See **Table 1** for reference values of enzyme activity in different grapevine varieties. A thorough search of the genes coding for these enzymes in grapevine was performed in NCBI (http://www.ncbi.nlm.nih.gov/) and 297 sequences were retrieved, including 109 peroxidases (**Supplementary Table 1**). Functional annotation is still incomplete and many of those sequences are redundant.

Phylogenetic dendrograms of grapevine non-redundant sequences of *APX*, *SOD*, *CAT*, *GOR,* and *Prx* gene families were generated based on the members annotated so far of *Vitis vinifera*, *Arabidopsis thaliana, Populus trichocarpa,* and *Oryza sativa* (var. japonica) retrieved from NCBI (**Supplementary Figure 1**). From the analysis of those dendrograms it was observed that *V. vinifera* has as many isoforms as *A. thaliana* and also has the highest sequence homology with this species. However, further annotation is still necessary such as in the case of GOR, for which no records of peroxisome and/or mitochondria isoforms are available (**Figure 1**).

Enzymes of the Water-Water Cycles

Under normal conditions, electrons obtained from the splitting of water molecules at PS II are channeled through the photosynthetic apparatus and transferred to molecular oxygen by PS I. Under stress conditions that decrease CO_2 availability due to stomata closure or increase exposure to continuous excessive light there is an excess of electron transfer toward molecular oxygen, generating O_2^- ions in PS I, through the Mehler reaction (Asada, 2006). In this situation, redox homeostasis can be guaranteed in two consecutive steps, the membrane attached copper/zinc superoxide dismutase (CZSOD) which converts O_2^- into H_2O_2 that is redirected to the ascorbate-glutathione cycle, where it is converted to water. This whole process is referred to as the water–water cycle (Rizhsky et al., 2003) as depicted in **Figure 1**. In "Trincadeira" grapevines submitted to heat stress both *CZSOD* and *FeSOD* are induced to scavenge plastidial O_2^- and the resulting H_2O_2 is scavenged by the *MDHAR-GOR* branch of the ascorbate-glutathione cycle (Carvalho et al., 2014).

Catalase

Peroxisomes are subcellular organelles with a single membrane that exist in almost all eukaryotic cells, containing as basic enzymatic constituents CAT and H_2O_2-producing flavin oxidases (Corpas et al., 2001; **Figure 1**). Despite their simplicity, they perform essential functions (Del Río et al., 2006); namely in the

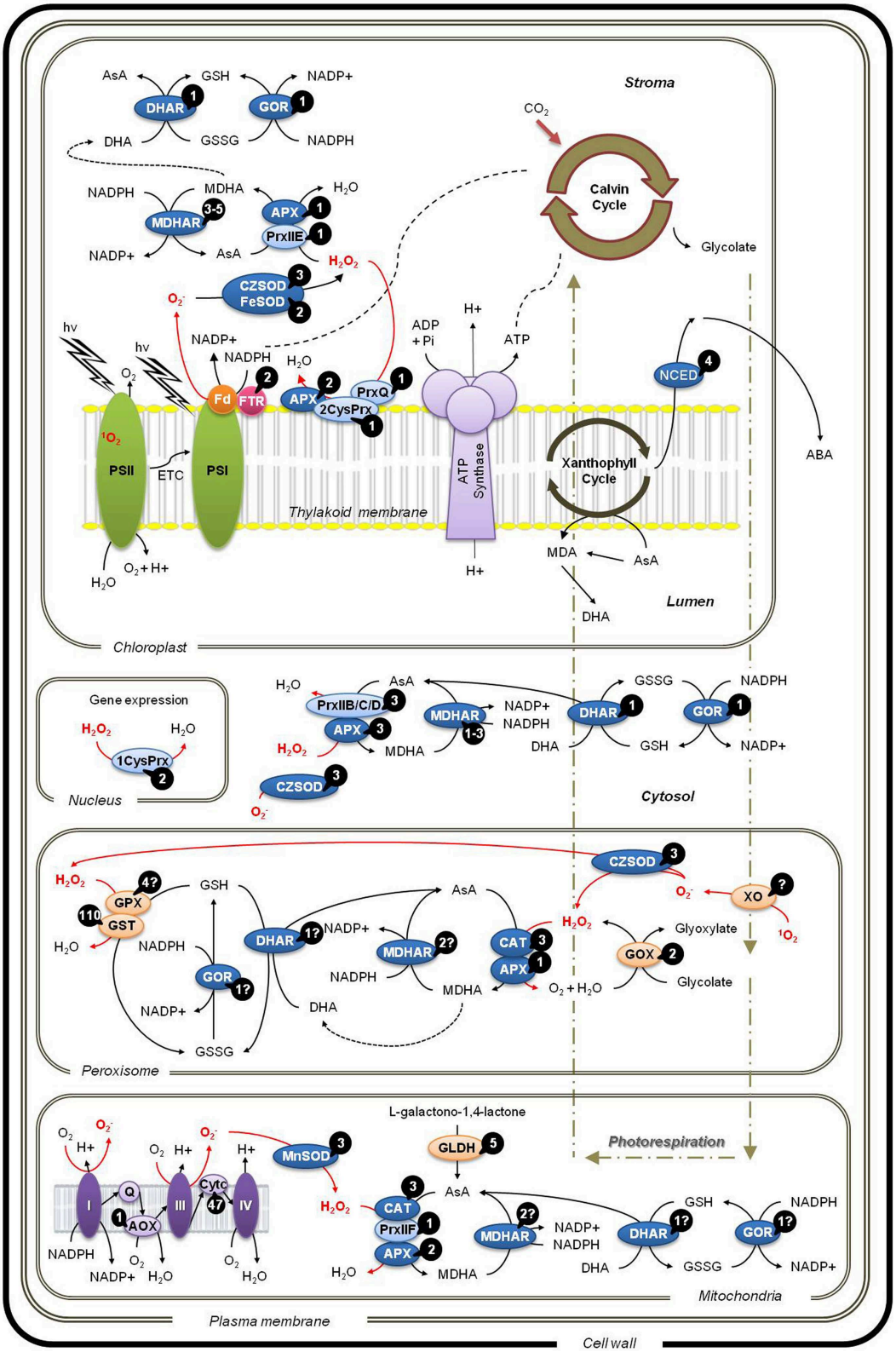

FIGURE 1 | Localization of reactive oxygen species (ROS) scavenging pathways in different cellular compartments. ROS: $^1O_2$, hydroxyl radical; H_2O_2, hydrogen peroxide; O_2^-, superoxide. Water-water cycle and ascorbate-glutathione cycle: APX, ascorbate peroxide; GOR, glutathione reductase; SOD, superoxide dismutase; CAT, catalase; DHAR, dehydroascorbate reductase; MDHAR, monodehydroascorbate reductase. Peroxiredoxin-mediated alternative water–water cycle: 1CysPrx,

(Continued)

FIGURE 1 | Continued
1-cysteine peroxiredoxin; 2CysPrx, 2-cysteine peroxiredoxin; PrxII; Type II-peroxiredoxin; PrxQ, peroxiredoxin Q. ABA, abscisic acid; AOX, alternative oxidase; AsA, ascorbate; Cytc, cytochrome c; DHA, dehydroascorbate; ETC; electron transport chain; Fd, ferredoxin; FTR, ferredoxin-thioredoxin reductase; GLDH, L-galactono-1,4-lactone dehydrogenase; GOX, glycolate oxidase; GPX, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; MDA, monodehydroascorbate; NCED, 9-cis-epoxycarotenoid dioxygenase; PSI and II, photosysthem I and II; Q, Coenzyme Q; XO, xanthine oxidase. Numbers in black circles indicate known isoforms in *V. vinifera*. The question marks indicate uncertainty on the actual number of isoforms, based in NCBI records; the given number is the most likely value.

antioxidative metabolism. They have an essentially oxidative type of metabolism, and great metabolic plasticity, as their enzymatic content varies with the organism, cell/tissue-type and environmental conditions (Baker and Graham, 2002). Upon photo-oxidative stress the peroxisomal CAT is the most responsive of catalases in grapevine (Carvalho et al., 2011; Vidigal et al., 2013) and it was recently shown that peroxisomal CAT influences Prx activity in the cytosol of *N. benthamiana* (Vidigal et al., 2014).

Peroxidases

Peroxidases are a large family of ubiquitous enzymes that have numerous roles in plant metabolism (For review, Passardi et al., 2005), including that of removing the H_2O_2 formed as a consequence of stress. In the grapevine genome 109 peroxidases were found (**Supplementary Table 1**). They use different electron donors, such as AsA, in the case of APX in the ascorbate-glutathione cycle; glutathione peroxidase (GPX) uses GSH as its reductant and the generically termed "peroxidases" use phenolic compounds (able to use guaiacol as substrate sometimes they are called "guaiacol-peroxidases"). In grapevine, GPX has been implicated in stress responses against *Elsinoe ampelina* and *Rhizobium vitis* and *GPX* is up-regulated in response to abiotic stresses such as drought and salinity (Cramer et al., 2007).

The main role of APX is the scavenging of H_2O_2 in the ascorbate-glutathione cycle, keeping its levels tightly controlled in order to maintain redox homeostasis, a similar role as that of GPX (Asada, 2006). Conversely, peroxidases oxidize a large variety of organic substrates and the resulting products are involved in important biosynthetic processes, such as lignification of the cell wall, degradation of IAA, biosynthesis of ethylene, wound healing, and defense against pathogens (Kvaratskhelia et al., 1997).

The Ascorbate-Glutathione Cycle

The reduction of H_2O_2 undertaken by AsA is only possible due to the activity of APX, an enzyme that uses two molecules of AsA to reduce H_2O_2 to water. AsA itself is oxidized to monodehydroascorbate (MDHA), an unstable compound that suffers spontaneous disproportionation to AsA and dehydroascorbate (DHA) (Potters et al., 2002). Monodehydroascorbate reductase (MDHAR) regenerates AsA from MDHA, using NADPH as a reducing agent and DHA is reduced to AsA through the action of dehydroascorbate reductase (DHAR), using GSH as the reducing agent. In this reaction GSH is oxidized to GSSG that, in turn is reduced back to GSH using NADPH as reducing potential. This is the ascorbate-glutathione cycle, also called Foyer-Halliwell-Asada cycle (**Figure 1**), where AsA and GSH act together to detoxify H_2O_2 in a cycle of oxidation-reduction, without being consumed and using electrons derived from NAD(P)H (Noctor and Foyer, 1998).

In grapevine, function of the ascorbate-glutathione cycle and its contribution to the detoxification process depend upon several factors, namely, the type, intensity and duration of the stress and the genotype under study. This became quite evident in a study comparing two greenhouse-grown genotypes ("Trincadeira" and "Touriga Nacional") subjected to heat stress with previous acclimation to moderate heat stress (Carvalho et al., 2014). The levels of expression of the plastidial SOD genes (*CZSOD* and *FeSOD*) were induced, mostly in "Trincadeira," suggesting the scavenge of chloroplast O_2^- as a consequence of over-reduction of the ETC, while in "Touriga Nacional" only an increase of O_2^- scavenging in the mitochondria (through *MnSOD*) was observed. H_2O_2 accumulation induced the expression of *CAT, APX1*, and *APX3*, together with the *MDHAR-GOR* branch of the ascorbate-glutathione cycle in both genotypes. This, together with similar results obtained for micropropagated grapevine subjected to high light (Carvalho et al., 2006) and of plants under viral infection (Sgherri et al., 2013) shows that, upon a severe stress, MDHAR alone is unable of maintaining the AsA pool in the reduced form, thus calling for the function of the whole ascorbate-glutathione cycle. After 3 days of acclimation to high light (Carvalho et al., 2006) or 24 h after the end of heat stress (Carvalho et al., 2014), the MDHAR branch of the cycle can keep the ascorbate pool reduced. However, the activation of the ascorbate-glutathione cycle is not, on its own, a trustworthy indicator of oxidative stress, as its activity can be triggered by differentiation of emerging structures in developing plants (Carvalho and Amâncio, 2002; Carvalho et al., 2006).

Glutathione S-Transferases

Glutathione S-transferases (GSTs) are enzymes that detoxify cytotoxic compounds by conjugation of GSH to several hydrophobic, electrophilic substrates (Marrs, 1996). Plant GSTs have been intensively studied because of their ability to detoxify herbicides, and several GSTs conferring herbicide tolerance have been characterized in many major crop species. Another plant GST subclass is implicated in stress responses, including those arising from pathogen attack, oxidative stress, and heavy-metal toxicity. In grapevine, the induction of GSTs upon pathogen attack occurs in parallel with that of phenylalanine ammonia-lyase (PAL) and stilbene synthase (STS) (Aziz et al., 2004). GSTs also play a role in the cellular response to auxins and during the normal metabolism of plant secondary products like anthocyanins and cinnamic acid. In grapevine, 107 GST isoforms were found (**Supplementary Table 1)** and seven genes belonging to the *phi* class and 57 belonging to the *tau* class, the two most important classes of plant-specific GSTs (Edwards et al., 2000),

are implicated in anthocyanin metabolism during berry development (Zenoni et al., 2010), *GST1* and *GST4* being implicated in anthocyanin transport to the vacuole (Conn et al., 2008). Also in grapevine, the expression of several GSTs genes was affected by defoliation (Pastore et al., 2013).

Peroxiredoxins

Prxs catalyze the reduction of H_2O_2, alkylhydroperoxides, and peroxynitrite to water, alcohols, or nitrite, respectively (for review, Dietz, 2011; **Figure 1**). Prxs are redox sensitive proteins that can undergo reversible oxidation–reduction and as a result, switch "on" and "off" depending on the redox state of the cell. In *V.vinifera,* under conditions of light and heat stress, Prx activity decreased as a result of the decrease in H_2O_2 levels while, under water stress, Prx activity increased, mirroring the increase in H_2O_2 (Vidigal et al., 2013).

2CysPrx is the most abundant stromal protein, protecting the photosynthetic apparatus against oxidative stress (König et al., 2003). When in deficiency leads to inhibition of photosynthesis, decrease in chlorophyll and impaired grapevine development (Vidigal et al., 2013). In light of its function in photosynthesis, *Vv2CysPrx01* (Vidigal et al., 2013), and *Vv2CysPrxB* were up-regulated in grapevine under light stress while *Vv2CysPrxA* was down-regulated under similar light stress conditions (Carvalho et al., 2011). These results were different from those obtained in *A. thaliana,* where an increase in light intensity resulted in little consequence to the expression of *2CysPrxA* and *2CysPrxB* (Horling et al., 2003). Transcription of *2CysPrxA* is induced by H_2O_2 and repressed by ABA (Baier et al., 2004). In grapevine several ABA-responsive-genes were consistently up-regulated (Carvalho et al., 2011), which could be an explanation for the discrepancy in *Prx* expression between these studies. The other possibility could be connected to the dual function of Prx, both in antioxidant defense and in signaling (Dietz, 2003). *Vv2CysPrx01* was up-regulated in grapevine under heat and water stress (Vidigal et al., 2013) a result that could be related with the chaperone function of 2CysPrx (Kim et al., 2010) and its role in drought tolerance (Rey et al., 2005).

PrxQ has a specific function in protecting photosynthesis, different from that of 2CysPrx (Lamkemeyer et al., 2006). In grapevine under abiotic stresses, *VvPrxQ* was either repressed or unresponsive (Vidigal et al., 2013), the same result as after 24 h of high light in *in vitro* propagated plants (Carvalho et al., 2011). However, in the same work, after 48 h, *PrxQ* transcripts increased significantly, pointing to a delayed transcription response. PrxQ is responsive to H_2O_2 and ABA (Guo et al., 2004), and in grapevine, neither H_2O_2 nor ABA increased upon light stress, another explanation for the verified discrepancies.

1CysPrx is a seed specific Prx that is targeted to the cytosol (Dietz, 2011). In grapevine leaves *Vv1CysPrx03* is located in the cytosol and is connected to drought and heat tolerance (Vidigal et al., 2013).

PrxIIC was very responsive to light stress in grapevine, with the tendency to increase with time (Carvalho et al., 2011). In *A. thaliana, PrxIIE* expression was highly induced by light stress (Horling et al., 2003), while in grapevine it was down-regulated (Carvalho et al., 2011; Vidigal et al., 2013). However, the up-regulation of *PrxIIE* in grapevine under water stress, correlating well with the increase in H_2O_2, suggests a role in drought tolerance (Vidigal et al., 2013). The expression of the mitochondrial isoform, *VvPrxIIF,* was unaltered upon high light (Carvalho et al., 2011; Vidigal et al., 2013), in agreement with results obtained in poplar under photo-oxidative conditions or heavy-metal treatments (Gama et al., 2007). Conversely, it was up-regulated in grapevine under heat and water stress, with strong correlation with H_2O_2 and ABA (Vidigal et al., 2013) suggesting a role for *VvPrxIIF* under light independent stress conditions.

Two new possible chloroplast *Prx* genes were identified in grapevine, *VvPrxII-1* and *VvPrxII-2* (Vidigal et al., 2013). Transcript levels of *VvPrxII-2* showed a strong response to heat stress and an analysis of its promoter region revealed the presence of the ABA-responsive element ABRE. Furthermore, the up-regulation of *VvPrxII-2* was positively correlated with ABA concentration, suggesting that this *Prx* gene may play a role in ABA-mediated heat tolerance (Vidigal et al., 2013) while *VvPrxII-1* transcripts were down-regulated under light stress and significantly up-regulated under water stress.

Crosstalk with Hormone Signals

Phytohormones are essential for the ability of plants to adapt to stresses by mediating a wide range of adaptive responses often by the regulation of gene expression mediated by the ubiquitin–proteasome degradation of transcriptional regulators (Santner and Estelle, 2009). One of the key players in the response of plants to abiotic stress, especially when involving water shortage, is ABA. However, other hormones such as cytokinins (CK), salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) play significant roles in keeping cell homeostasis under oxidative stress, and their interactions are schematized in **Figure 2**.

Abscisic Acid

Drought and high salinity result in strong increases of plant ABA levels, accompanied by major changes in gene expression and in

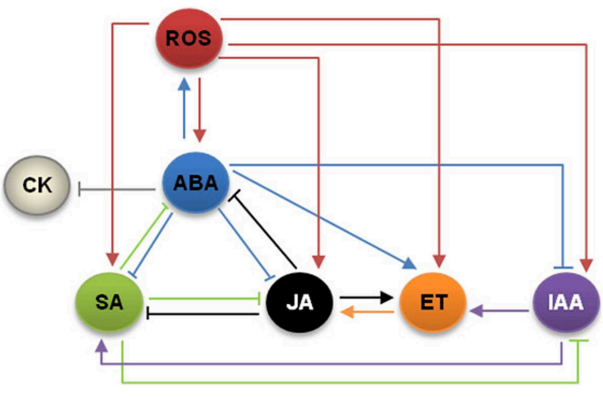

FIGURE 2 | Crosstalk with hormone signals. ROS, reactive oxygen species; ABA, abscisic acid; CK, cytokinins; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; IAA, auxins.

adaptive physiological responses (Seki et al., 2002; Rabbani et al., 2003). The expression of ABA-inducible genes that leads to stomatal closure, thus reducing water loss through transpiration and eventually restricting cellular growth, occurs promptly upon the sensing of stress (Peleg and Blumwald, 2011). Several loss of function mutants for genes related to *de novo* ABA synthesis, to ABA receptors and to downstream signaling are now available in several species (Fang et al., 2008; Cutler et al., 2010). In grapevine however, such tools are not available, thus ABA research has been taking place in a more classical approach.

To improve plant water status and decrease leaf temperature of grapevine plants different irrigation regimes are applied in Mediterranean vineyards (Costa et al., 2012). One method of applying regulated irrigation is by partial root-zone drying (PRD). The main rationale to use PRD in grapevine is the action of ABA in modeling stomatal conductance and the demonstration that, by keeping some root areas dry and others wet, the necessary hormonal signals to regulate stomatal conductance are provided by the dry root-zones and the water needed to prevent severe water deficit is delivered by the wet root-zones (Stoll et al., 2000). ABA accumulation depends both on an accelerated ABA biosynthesis under water deficit, and on the rate of ABA catabolism and conjugation, which is quite fast, and is the main factor controlling the disappearance of ABA signal (Jia and Zhang, 1997). Thus, the accumulation of this so-called stress ABA is controlled by a dynamic equilibrium between ABA biosynthesis at the level of NCED (9-cis-epoxycarotenoid dioxygenase) transcription and its catabolism and conjugation.

In berries, the onset of ripening (*veraison*) when anthocyanin accumulation begins in red varieties, is accompanied by a marked increase in ABA concentration (Deluc et al., 2009; Gambetta et al., 2010) and correlates well with sugar accumulation (Gambetta et al., 2010). ABA has been shown to activate anthocyanin biosynthetic genes and the anthocyanin-synthesis related *VvmybA1* transcription factor (Jeong et al., 2004), and to induce the delay of expression of proanthocyanidin biosynthetic genes (*VvANR* and *VvLAR2*) (Lacampagne et al., 2009).

Cytokinins

CK exert an opposite function as ABA, and CK levels decrease upon water shortage (Peleg and Blumwald, 2011). In grapevine the effect of ABA on root growth may be augmented by a reduction in CK concentration in the roots that leads to the enhanced ABA to CK ratios obtained in PRD irrigation cycles (Stoll et al., 2000), since it is known that root growth is inhibited by increased endogenous CK (Werner and Schmülling, 2009). In fact, it is possible to reverse the effects of PRD in stomatal conductance by exogenous application of benzyladenine, that also leads to lateral shoot development (Stoll et al., 2000). Also, when comparing fully irrigated and PRD vines a significant decrease in zeatin and zeatin riboside concentration in shoot tips and axillary buds is observed (Dry and Loveys, 1999).

Salicylic Acid

SA is a phenolic plant growth regulator, with several physiological and biochemical functions (Raskin, 1992) under normal conditions and, especially, in the response to abiotic stresses, namely heat stress. SA is known to counteract the effects of heat stress by up-regulating the antioxidative system. The treatment of grapevine leaves with SA before, during and after heat stress maintained photosynthesis rates high, chiefly by keeping high levels of Rubisco activation state, and also accelerated the recovery of photosynthesis through effects on PS II function. These effects may be partially related to the presence of a heat shock protein, HSP21, during the recovery period in SA-treated leaves (Wang et al., 2010). Treatment with SA also protects mesophyll cells against cold and heat stress in leaves of young grape plants, affecting Ca^{2+} homeostasis, and enzymatic and non-enzymatic components of the ascorbate-glutathione cycle (Wang and Li, 2006). SA treatment also induced the expression of *PAL* and the synthesis of new PAL protein and increased its activity in grape berries (Wen et al., 2005). PAL is a crucial enzyme of the phenylpropanoid metabolism, catalyzing the formation of trans-cinnamic acid. Its induction results in the accumulation of phenolic acids and flavonoids, thus enhancing the quality of berries.

Jasmonates

JA and their derivatives are known to play important roles in activating genes coding for proteins involved in the defense against abiotic (drought, salt, and ozone) and biotic (insects and microbial pathogens) stresses. The activity of JA responses can be regulated by antagonistic cross-talk with SA signaling (for review, Balbi and Devoto, 2008). In fact, SA can suppress the JA-dependent response to wounding and pathogen or insect attack (Leon-Reyes et al., 2010).

In grapevine, salt stress and biotic defense signaling share common pathways, e.g., the activity of a gadolinium-sensitive calcium influx channel and transient induction of JAZ/TIFY transcripts. Exogenous JA application can rescue growth in salt-sensitive *Vitis riparia* (Ismail et al., 2012). In line of these data, the authors proposed a model where the default pathway is salt stress signaling that is modulated by a parallel signal chain triggered by biotic factors downstream of JA signaling.

JAs are also described as promoting the synthesis and accumulation of the stilbene compound resveratrol in grapevine berries (Tassoni et al., 2005). A transcriptional study of the different berry tissues during development revealed that JA signaling genes are preferentially expressed in the pericarp while JA-biosynthesis genes have differential expressions, lipoxigenase-related genes in the pericarp while the conversion of linoleic acid to jasmonic acid appears to be seed-exclusive (Grimplet et al., 2007). Recently, high levels of expression of both JA and ethylene signaling related genes was reported in berries before *veraison* (Fasoli et al., 2012).

Ethylene

ET biosynthesis is induced in response to abiotic stresses and this hormone affects membrane permeability, osmotic potential (sugar and proline accumulation) and the control of cell water potential. Together with H_2O_2, ET acts as a signaling molecule in the response of grapevine buds to hypoxia, leading to the activation of antioxidative stress genes (Vergara et al., 2012). The oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC) to ET is catalyzed by the membrane associated ACC oxidase. ACC

oxidase has been reported to increase in response to cold in grapevine (Tattersall, 2006).

Auxins

Auxins play an important role in fruit development, and the grape berry is no exception. Indole-3-acetic acid (IAA) content is high from anthesis to *veraison*, then declines to very low levels at maturation (Conde et al., 2007). Amidase (AMI1), responsible for the synthesis of IAA from indole-3-acetamide decreases its levels of gene expression during ripening (Pilati et al., 2007), in tandem with the decline of IAA levels.

Auxins have been implicated in the response to UV-acclimation in grapevine, genes belonging to auxin responsive SAUR and Aux/IAA family, auxin response factors and auxin transporter-like proteins are down-regulated in grapevine leaves exposed to low UV-B, supporting evidence for a role in the response to low UV-B fluence light (Pontin et al., 2010).

Oxidative Stress in *In Vitro* Systems

In vitro systems offer a practical and easily manipulated method for studying oxidative stress. *In vitro* cultures are usually grown in contained environments with low photon flux density and high relative humidity. Culture media contain high quantities of an organic carbon source and growth regulators, contributing to the development of characteristic features such as abnormal leaf anatomy, poor development of grana (Wetztein and Sommer, 1982) and low photosynthesis rates (Chaves, 1994). Oxidative stress due to photoinhibition is prone to occur upon transplantation to *in vivo* conditions. The extent in which photoinhibition affects the survival of the plant depends on its physiological status dictated by the prevailing environmental conditions, the efficiency of protective mechanisms against excess energy and the repair processes to restore normal photosynthesis (Krause and Weis, 1991). Nitro-oxidative abiotic stress can also cause damage to *in vitro* cultures and, in grapevine, procyanidins were shown to have a protective action against the damage caused by peroxisome peroxynitrite thus formed (Aldini et al., 2003).

Immediately after transplantation to *ex vitro*, *in vitro* propagated grapevine plants showed severely affected photosynthetic capacity, that recovered after 1 week (Carvalho et al., 2001) as clearly seen in the heat map of **Figure 3**. ROS concentration is maximal on the first 2 days after transfer while chlorophyll fluorescence indicators show symptoms of photoinhibition and the ROS scavenging machinery is activated. Photoinhibition symptoms are less severe when the first stages of *in vivo* growth are conducted at CO_2 concentrations double the normal atmospheric values and light intensities six-fold higher than *in vitro* (Carvalho and Amâncio, 2002).

In the early stages of *ex vitro* growth a stabilization of Rubisco is observed (Carvalho et al., 2005), allowing photosynthesis to regain normal levels. In parallel, the activation of the ascorbate-glutathione cycle after 24 h of *ex vitro* growth helps to maintain cell redox homeostasis and regulates the antioxidative response (Carvalho et al., 2006). When the transcriptome of grapevine plants after transplantation was scanned an activation of signaling pathways up to 48 h was reported together with the up-regulation of the protein rescuing mechanism that involves the cooperation of HSP100 and HSP70, two ATP-dependent chaperone systems that remove non-functional and potentially harmful polypeptides deriving from misfolding, denaturation, or aggregation caused by stress (Carvalho et al., 2011). This was an unusually late and time-prolonged reaction when compared with "typical" abiotic stress responses (Mittler, 2006; Cramer, 2010). During this short period, H_2O_2 is accumulating and is used as a second messenger to trigger the pathways that are essential for plant survival at this delicate developmental phase (Vilela et al., 2008), as confirmed by the activation of genes related to stress defense pathways, hormones and protein metabolism in the same timeframe (Carvalho et al., 2011).

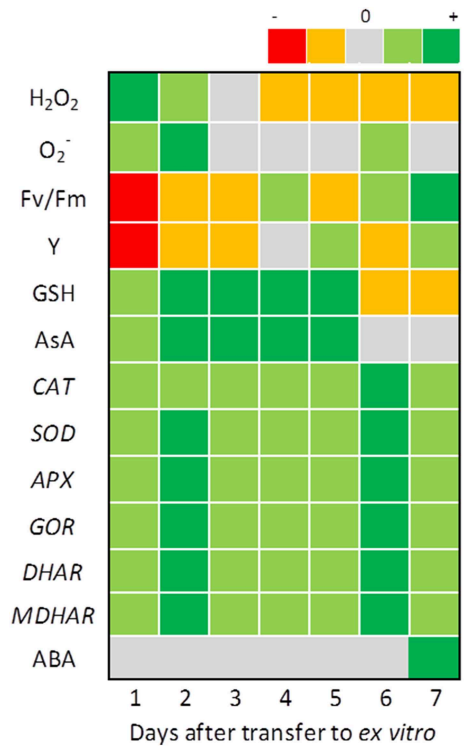

FIGURE 3 | Monitoring changes in antioxidant homeostasis of *in vitro* propagated grapevine plants during the first 7 days of growth in *ex vitro* conditions. H_2O_2 was quantified in μmol g^{-1} FW; O_2^- was visualized through nitroblue tetrazolium staining, Fv/Fm (maximum efficiency of PSII photochemistry in dark-adapted leaves), and Y (maximum quantum efficiency of PSII in light adapted leaves) were both measured using a Fluorimager chlorophyll fluorescence imaging system (Technologica Lda. Colchester, UK) and the Fluorchart software to isolate the individual leaves and calculate the values of the parameters, GSH and AsA were both quantified in μmol g^{-1} FW; *CAT, SOD, APX, GOR, DHAR,* and *MDHAR* expression was quantified through RT qPCR and ABA was quantified in nmol g^{-1} DW. The heat map represents the differences between the values measured at the moment of transfer to *ex vitro* (control) and those monitored for 7 days of *ex vitro* growth: dark green, very significant increase from the control; light green, significant increase from the control; gray, no significant differences to the control; orange, significant decrease from the control; red, very significant decrease from the control. Values were retrieved from Carvalho et al. (2006) and Vilela et al. (2007).

After overcoming the initial stress of transfer, plants undergo a new cycle of up-regulation of the antioxidative machinery, which reaches a maximum on day 6, and is not accompanied by photo-oxidative stress symptoms or increase in GSH/AsA pools (Carvalho et al., 2006). This coincides with the protrusion of new roots and the expansion of the first *ex vitro* leaf and culminates with a peak of ABA and H_2O_2 concentration on the seventh day after transfer, both produced in the newly-expanded and functional roots (**Figure 3**; Neves et al., 1998; Vilela et al., 2007). At this point, acclimatization to *ex vitro* is not yet complete but photo-oxidative stress is no longer a problem for the growing grapevine plants.

Response to Biotic Stress

One of the first attempts at "cataloging" biotic stress response genes in grapevine was undertaken with the aid of the MapMan onthology, adjusted to encompass a few pathways in detail, such as phenylpropanoid, terpenoid, and carotenoid biosynthesis, very responsive upon biotic stress, with a marked effect on wine production and quality (Rotter et al., 2009). The authors describe an overview of transcriptional changes after the interaction of a susceptible grapevine with *Eutypa lata*, and show that the responsive genes belong to families known to take part in plant biotic stress defense, such as PR-proteins and enzymes of the phenylpropanoid pathway (Rotter et al., 2009).

Several stress response processes are common between biotic and abiotic stresses, exerting either synergistic or antagonistic actions, depending upon the specific stress combination that the plant is facing. One example is the role of dehydrins (DHNs) in the protection of plant cells from drought and also in host resistance to various pathogens. In the genus *Vitis*, the wild *V. yeshanensis* is tolerant to both drought and cold, and moderately resistant to powdery mildew due to the precocious induction of *DHN1*, occurring earlier in drought conditions than in *V. vinifera* and having more than one up-regulation peak during the infection with *Erysiphe necator* as compared to *V. vinifera* (Yang et al., 2012).

ROS signaling seems to have an important role in *Plasmopara viticola* resistance, as resistant varieties display a specific chronological set of events upon infection, that is not observed in susceptible genotypes, beginning with an increase in O_2^-, followed by a hypersensitive response, an increase in peroxidase activity in cells flanking the infection area and finally, an increased accumulation of phenolic compounds (Kortekamp and Zyprian, 2003). Specifically, the peroxidase activity after an infection with *P. viticola* is strongly correlated with resistance *to P. viticola* in field plants (Kortekamp and Zyprian, 2003).

In grapevine, the most ubiquitous reaction to fungal infection is the accumulation of phytoalexins. Since the 1970s that it is known that grapevine synthesizes resveratrol in response to fungal attacks (Peter and Pryce, 1977). Viniferins, products of resveratrol oxidation, are also produced in response to biotic and abiotic stresses, and also classified as phytoalexins. These compounds present biological activity against a wide range of pathogens and are considered as markers for plant disease resistance (Pezet et al., 2004b). Resveratrol is synthesized from coumaroyl CoA and malonyl CoA by STS (**Figure 4**). STS is closely related to chalcone synthase (CHS), the key enzyme in flavonoid-type compound biosynthesis leading to the production of chalcones while STS leads to the production of stilbenes (**Figure 4**). Indeed, under certain conditions, such as oxidative stress, the transcriptional response of *VvSTS* and *VvCHS* genes appears to be diametrically opposed suggesting that under those conditions, the plant refocuses its metabolism on stilbene biosynthesis, taking precedence over flavonol biosynthesis, as schematized in **Figure 4**, the pathway highlighted in red (Vannozzi et al., 2012).

Resistant grape genotypes artificially inoculated with *P. viticola* show very high amounts of stilbenes at the site of infection, that actively inhibit the motility of *P. viticola* zoospores and subsequent disease development (Pezet et al., 2004a). Interestingly, *PAL* seems to be constitutively expressed in resistant and susceptible genotypes, but was totally repressed in tissues after mock inoculation using the non-host pathogen *Pseudoperonospora Cubensis*. *CHS* and *STS*, however, had their expression increased after inoculation with *P. viticola*, indicating an activation of the resistance response, in accordance with the increase of stilbenes (Kortekamp, 2006).

The antioxidative response of the grapevine genotype "Trebbiano" when infected by the grapevine fanleaf virus was thoroughly scrutinized. At the early stages of infection, increases in H_2O_2 were observed and probably due to enhanced dismutation of O_2^- by SOD, whereas, toward the late phase of infection, increases in AsA, GSH, and APX activity might be the reasons for H_2O_2 to regain control levels (Sgherri et al., 2013).

Upon infection by necrotrophic pathogens, which need to kill their host cells to gain access to nutrients, an activation of JA-dependent defense mechanisms takes place (Avanci et al., 2010). Many plant pathogens can either produce auxins themselves or manipulate host auxin biosynthesis to interfere with the host's normal developmental processes. In response, plants have evolved mechanisms to repress auxin signaling during infection as a defense strategy, mediated by the accumulation of SA. In grapevine, auxin responsive genes (including *SAUR*, *Aux/IAA*, auxin importer *AUX1*, auxin exporter *PIN7*) are also significantly repressed in pathogen resistance responses (Wang et al., 2007), supporting the hypothesis that down-regulation of auxin signaling contributes to induce immune responses in plants (Bari and Jonathan, 2009).

Oxidative Burst

Plant species such as pear, tomato, strawberry, and pineapple show a specific oxidative stress response during fruit development, termed oxidative burst. The respective fruits are themselves named "climacteric." As grapevine is not amongst them, it came somewhat as a surprise when Pilati et al. (2007) reported an oxidative burst in cv. "Pinot Noir" that began at *veraison* and was characterized by rapid accumulation of H_2O_2 and by the modulation of many ROS scavenging enzymes, previously thought not to be up-regulated in this species. This work comprised a thorough transcriptomic analysis of the grape berry in the stages close to *veraison* and the quantification of H_2O_2. The

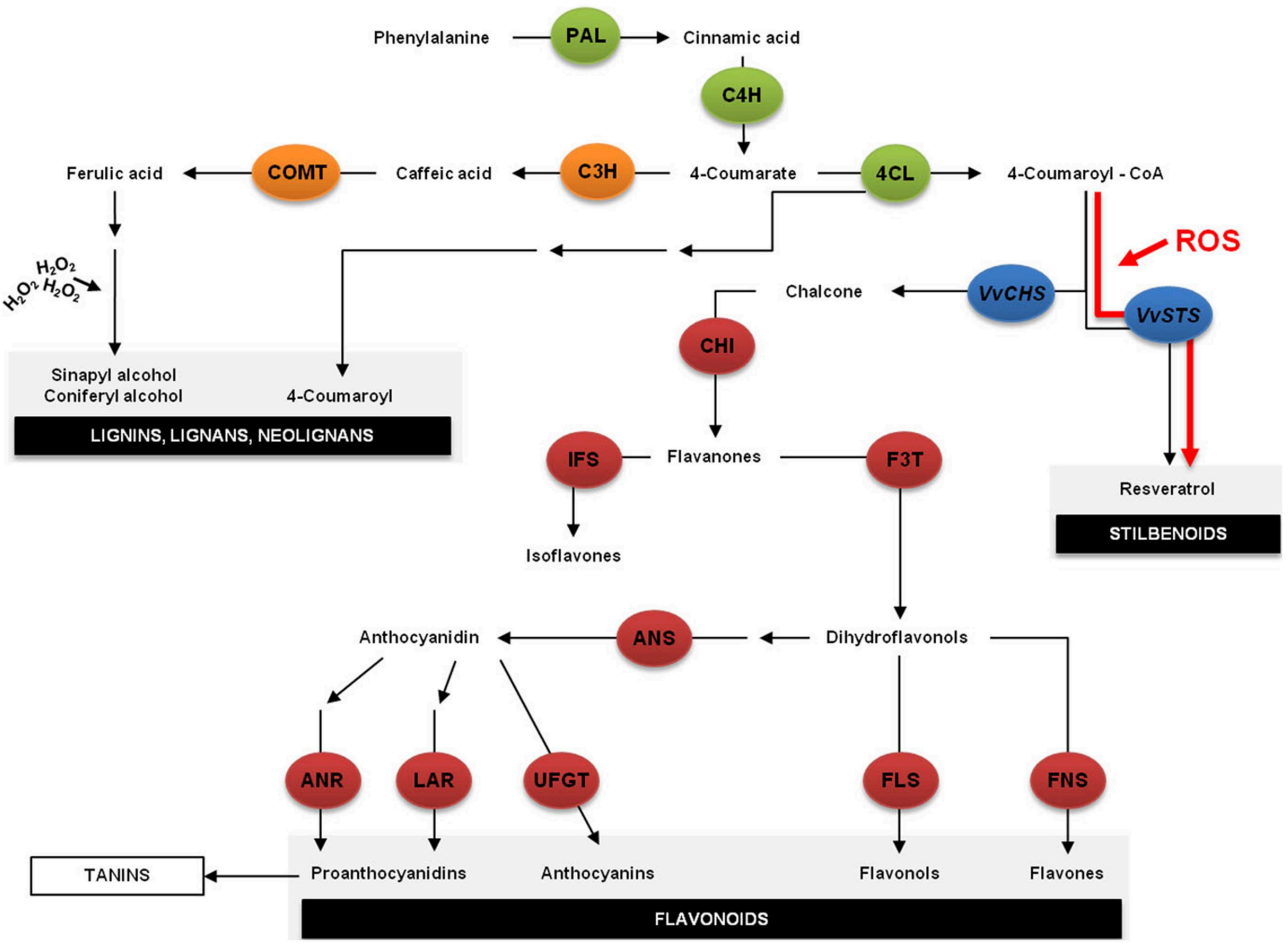

FIGURE 4 | The phenylpropanoid pathway is part of the secondary metabolism and it is responsible for the synthesis of different classes of metabolites, such as lignins, flavonoids and stilbenoids. The first step is the deamination of phenylalanine to cinnamate by the action of phenylalanine ammonia lyase (PAL). Cinnamic acid is then hydroxylated by cinnamate-4-hydroxylase (C4H) to 4-coumarate, which is then activated to 4-coumaroyl-coenzyme A (CoA) by 4-coumaroyl—CoA ligase (4CL). After this reaction the main pathway is divided into two major branches: the flavonoid biosynthesis pathway and the lignin biosynthetic pathway. However, under stress the balance between the transcription rates of *VvSTS* and *VvCHS* appears to shift dramatically suggesting that the plant refocuses its metabolism on stilbene biosynthesis, taking precedence over flavonol biosynthesis. H_2O_2, hydrogen peroxide; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; C3H, coumaroyl 3-hydrolase; CHI, chalcone isomerase; COMT, caffeic acid/5-hydroxyferulic acid 3/5-O-methyltransferase; F3T, flavanone 3-hydroxylase; FLS, Flavonol synthase; FLS, flavonol synthase; FNS, flavone synthase; IFS, isoflavone synthase; LAR, leucoanthocyanidin reductase; ROS, reactive oxygen species; UFGT, UDP-glucose:flavonoid 3-O-glucosyl transferase; *VvCHS*, *Vitis vinifera chalcone synthase*; *VvSTS*, *Vitis vinifera stilbene synthase*.

latter increased at the moment of *veraison*, reaching its maximum 1–2 weeks after, and then decreasing at a slower pace toward ripening. In tandem, transcripts coding APX, GPX, Prxs, Trxs, glutaredoxins, GSTs and metallothioneins were up-regulated, in accordance with the onset of a well-orchestrated antioxidative response. Shortly after, grape's oxidative burst was again reported and associated with high sugar content that impairs photosynthesis in the berries, possibly through ABA signaling (Lijavetzky et al., 2012). It must be referred that high levels of ACC oxidase transcript accumulation have been reported immediately preceding *veraison*, together with a peak in ACC accumulation and ET emission (Chervin et al., 2004). Proteomics studies also reported an increase in ROS scavenging enzymes toward ripening (Giribaldi et al., 2007; Negri et al., 2008). The subject remained wrapped in controversy because other authors did not obtain the same results (Terrier et al., 2005), until Rienth et al. (2014) shed some light onto what might be causing such disparity of results. The authors, in yet another transcriptomic assay of grape berries, found that the oxidative burst occurs markedly during the night, at ripening, following the same trend as sugar transport and phytoalexin synthesis. Together with H_2O_2, $^1O_2$ was also found to increase in chloroplasts together with enzymatic peroxidation of membrane galactolipids (Pilati et al., 2014).

Concluding Remarks

Grapevine can be considered a model for fruit species. Several transcriptomic studies are now complementing the existing

information on abiotic stress responses of many grapevine varieties, previously described at the physiological level. Knowledge of gene expression patterns points to specific varietal responses and to different levels of stress tolerance, confirming the high phenotypic plasticity of this species. The results obtained so far suggest that some varieties keep redox homeostasis without an apparent boost in their antioxidant pool, just adjusting the activities of antioxidant enzymes and/or the accumulation of antioxidant molecules, while others need to synthesize those antioxidant molecules *de novo*. The former demonstrate a well-timed and efficient ROS removal and a broad plasticity in adapting to environmental shifts. At the genomic level, for instance, grapevine Prx isoforms are specifically targeted and highly responsive to major abiotic stresses. Examples are the gene expression of cytosol PrxIIE apparently with a role in grapevine drought tolerance, while in other species it was reported as only responding to light; and the identification of two new possible chloroplast Prx genes, *VvPrxII-1,* and *VvPrxII-2*, the former down-regulated by light stress and up-regulated by water stress, the latter induced by heat stress in tandem with increased ABA concentration and with an ABRE sequence in its promoter.

Specific development processes can shift redox homeostasis. An obvious example is the oxidative burst in berries, a singular feature occurring in this non-climacteric species during *veraison*, mostly during the night. Specifically, this metabolic event is accompanied by sugar transport and resveratrol synthesis. Reports from *in vitro* grapevine systems reveal stress and developmental-related signaling mediated by ROS in growing leaves and roots.

As a whole, the phenotypic plasticity of different grapevine varieties which behave as more tolerant to environmental aggressions that cause oxidative stress can improve crop yield and quality, and thus the species economic value.

Acknowledgments

This work was funded by Fundação para a Ciência e Tecnologia (FCT) through CBAA Funding (PestOE/AGR/UI0240/2011) and the post-doc grants SFRH/BPD/85767/2012 and SFRH/BPD/43898/2008 to LC and PV, respectively. This work also benefited from European project KBBE InnoVine (ref. 311775) and the European COST Action FA1106 "QualityFruit."

Supplementary Material

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fenvs.2015.00020/abstract

Supplementary Table 1 | Complete list of isoforms for the major antioxidative response genes in grapevine: superoxide dismutase, catalase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase, glutathione peroxidase, glutathione S-transferase, peroxidase, peroxiredoxin and thioredoxin. Accessions were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) and the gene name, symbol, grape gene accession numbers and gene ID are given.

Supplementary Figure 1 | Dendrogram analysis of APX, SOD, CAR, GOR, and Prx of *V. vinifera*, *Populus trichocarpa*; *Oryza sativa* L. ssp. Japonica and *Arabidopsis thaliana*, with the respective intracellular locations and confidence that the sequences belong within the respective group.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.