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38 **Abiotic stress effects on grapevine (*Vitis vinifera* L.): focus on abscisic acid-mediated**

39 **consequences on secondary metabolism and berry quality.**

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49 **Abstract**

50 This review deals with grapevine abscisic-acid (ABA) mediated responses to abiotic stress by

51 addressing stress consequences mainly on berry quality. Accumulation of secondary metabolites

52 as a plant defense strategy to abiotic stress is reviewed, together with perturbations of metabolite

53 molecular pathways. The role of ABA is highlighted as a link between berry ripening process and

54 grapevine response to stress. Abiotic stress (especially water scarcity, light and temperature)

55 modifies growth and development of all plant organs. The response to abiotic stress at the berry

56 level drives the accumulation in berry pulps, seeds and skins of secondary metabolites as a line of

57 defense against cell damages. Viticultural practices can be managed to control stress plant

58 response in order to influence berry secondary metabolite concentrations and profiles, reflecting

59 on an enhancement of table grape and must quality and on their nutraceutical and health

60 benefits, as grape berry secondary metabolites contribute to berry and wine taste and aroma, to

61 the potential antioxidant capacity of fruit and wines, to wine stabilization and protection during

62 ageing. Being stress response mainly ABA mediated, feeding of exogenous ABA to grapevine

63 organs is reviewed as a tool to enhance grape quality and to control abiotic stress. Consequences

64 in viticultural practices are discussed in relation to different abiotic stresses and global warming

65 effects.

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1. Introduction

According to the Food and Agriculture Organization of the United nations time-series and cross sectional data FAOSTAT (http://faostat3.fao.org) 69,654,925.50 tonnes of grapes on 7,086,021.81 ha were produced in the world in 2011. About 29 millions of wine tonnes were produced in 2011, two thirds of them in the Mediterranean basin; considering that the yield of the transformation of grape into wine is averagely 80 %, about 36 millions of tonnes are destined to the winemaking, the remaining ones are fresh or dry consumed.

Regions with Mediterranean climate, where viticulture flourished since 1000 BC, are characterized by long growing seasons, with moderate to warm temperatures. Throughout the year there is little seasonal change in temperatures, and winters are generally warmer than those of maritime and continental climates. During the grapevine growing season, there is very little rain fall (most precipitation occurring in the winter months), which increases the risk of drought hazard in viticulture (Robinson, 2006), especially in sandy and gravelly soils that do not retain water (Tramontini et al., 2013).

Mediterranean climate ensures to grapevine long warm periods during the crucial phenological stages of flowering, fruit set and ripening. The physiological processes of grapevines begin when temperatures are around 10 °C. Below this temperature, vines are usually dormant. Above 35 °C, on the contrary, plant adaptation to heat stress is activated. In addition to temperature, the amount of rainfall and the need for supplemental irrigation are crucial characteristics for the definition of viticultural areas (Keller, 2010). On average, a grapevine needs around 6 to 7 hundreds mm of water for sustenance during the growing season, not all of which may be provided by natural rainfall. In the Mediterranean region, climate may be quite dry during the grapevine growing season and vines may require additional irrigation to limit water deficit stress (Chaves et al., 2007, 2010).

Berry quality, particularly that of winegrape varieties, is largely dependent on secondary metabolites, i.e. on the accumulation of polyphenols and volatiles.

Plant secondary metabolism provides a line of defense in cellular response to abiotic stress (Cramer et al., 2011) and induces an enhancement of grape quality, as secondary metabolites contribute to colour, taste and aroma of fresh and dried grapes and they are involved in wine stabilization and ageing processes. In the last decades this concept has largely been addressed, by linking technological aspects (both agricultural and enological ones) with biological and molecular aspects in viticulture, resulting in an increase of the related scientific literature (Figure 1a). Abscisic

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acid has been proposed as the main mediator of grapevine biological response to abiotic stress, especially drought. If, on one hand, the number of papers published on grape secondary metabolism has linearly increased during the last fifteen years, due to the progress in science applied to viticulture, on the other, it has exponentially increased when linked to abiotic stress and especially to the ABA-mediated response to stress (Figure 1b).

In this review we address grapevine responses to the main abiotic stresses, highlighting the ABA role in controlling environmental stress effects on secondary metabolism especially in berries, but also in vegetative organs.

2. Grape ripening: an ABA-induced process crossing with abiotic stress and secondary metabolite accumulation

In *Vitis vinifera* berries, the onset of ripening (véraison, i.e. the onset of anthocyanin accumulation in colored-skin varieties) is proved to be tied to sugar accumulation (Gambetta et al., 2010) and it is accompanied by a marked increase in ABA concentration (Deluc et al., 2009; Gambetta et al., 2010; Owen et al., 2009).

Pirie and Mullins (1976) were the first to show a synergic effect of ABA and sucrose in grape leaf anthocyanin accumulation. The hypothesis that ABA may drive ripening in grape berries has been reinforced by the demonstration that exogenous ABA treatments at véraison enhance several processes involved in berry ripening, such as soluble solid and anthocyanin accumulation and organic acid concentration decrease (Coombe and Hale, 1973; Pirie and Mullins, 1976; Palejwala et al., 1985; Wheeler et al., 2009; Deis et al., 2011; Sandhu et al., 2011; Xi et al., 2013; Ruiz-Garcia et al., 2013).

Gene-specific studies on ABA molecular effects in grapevine berries have shown an activation of anthocyanin biosynthetic genes and of the anthocyanin-synthesis related *VvmybA1* transcription factor (Ban et al., 2003; Jeong et al., 2004), as well as in cucumber *R2R3Mybs* (Li et al., 2012), and a delayed expression of condensed tannin biosynthetic genes (VvANR and VvLAR2) (Lacampagne et al., 2010).

In berries ABA mostly acts through the regulation of the same proteins involved in the ripening process, and several of these changes share common elements with the ABA-induced responses in vegetative tissues (Giribaldi et al., 2010). Whether the increase of ABA concentration at véraison is due to an *in loco* biosynthesis of ABA, consequently to the demonstrated activation of NCED genes (Wheeler et al., 2009) or to a translocation of ABA from vegetative organs, is still unclear

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(Castellarin et al., 2011). These authors studied the spatio-temporal berry ripening in a colored-flesh berry variety ('Alicante Bouschet') pointing out that ripening begins in the stylar end flesh, afterwards it moves to the pedicel end flesh and to skins; they showed a progressive and correspondent up-regulation of VvNCED genes in the different tissues hand in hand colour accumulates. However, as NCED genes are up-regulated in response to ABA itself (Koyama et al., 2010; Sun et al., 2010; Wheeler et al., 2009), it is still unclear what causes the initial ABA accumulation. ABA signaling during grape ripening interacts with plant responses to exogenous stress, by regulating the process of plant adaptation through two interacting steps. First, ABA acts via differential signal transduction pathways on cells which are the least and most affected by the imposed stress. Second, ABA may regulate some genes and gene products, which control the expression of stress or adaptative-specific genes. Some genes are up-regulated and others are down regulated, resulting in overall synthesis of genomic products which may play a role in plant survival under different environmental conditions (Swamy and Smith, 1999). Abscisic acid also plays a role in plant response to biotic stress (Cao et al., 2011; Hayes et al., 2010). Moreover, the interactions of biotic and abiotic stress signal transduction are known and reviewed (Kim, 2012; Yang et al., 2012). In *Vitis vinifera* the ABA mediated responses to abiotic stress (especially water stress) have been investigated since the 70s, whereas reports about ABA mediated response to biotic stress are more recent. Many key genes (*VvMybA1* and *VvUFGT*) of the flavonoid biosynthetic pathway were proved to be up-regulated during ripening so one can speculate that stress conditions, leading to an ABA accumulation and inducing the activation of key genes of the flavonoid biosynthesis, resolve in a berry quality increase, i.e. the accumulation of secondary metabolites, polyphenols in particular. Demonstrations of this aspect are generally focused on the detection of total concentrations of different polyphenolic groups so whether this increase is always related to the accumulation of molecules deemed positive for the technological transformation of grapes into wines or as nutraceuticals in table grape varieties, is still little known; as a matter of fact few papers investigate how stress conditions can modify the profile of specific polyphenols.

3. Water stress

Grapevine performances and berry quality depend on the vine adaptability to drought (Lovisolo et al., 2010). Water deficit does not exclusively cause negative effects, but a regulated water deficit,

which is the base of various agronomic practices, has largely been used to balance grapevine vegetative and reproductive growth with the aim of controlling berry quality (Chaves et al., 2010). In grapevines a direct role of ABA in stomatal closure upon water stress was demonstrated since the 70s (Liu et al., 1978; Loveys, 1984; Loveys and Kriedemann, 1974). In different grapevine genotypes, during the gradual imposition of soil water deficit, negative correlations between stomatal conductance and either xylem (Pou et al., 2008; Rodrigues et al., 2008; Rogiers et al., 2012) or leaf tissue (Liu et al., 1978; Lovisolo et al., 2002; Perrone et al., 2012a and 2012b) ABA contents were often observed. On the basis of these observations, it is often assumed that root ABA synthesis in response to water stress and transport through the xylem into leaves, or stress induced ABA mobilization in leaf, mediate most of the stomatal response in grapevines. As a determinant factor of stomatal closure, ABA controls both transpiration and assimilation, enhancing expansive cell growth by saving leaf water and reducing xylem tension, likely altering structural growth by limiting CO_2 entry (Pantin et al., 2012). Aquaporins are another target for ABA to regulate both water and carbon fluxes. ABA affects aquaporin regulation in response to abiotic stresses (Kaldenhoff et al., 2008) by modulating their gene expression and protein abundance or activity, affecting, in turn, cellular water relations and cell metabolism in response to water stress. By negative control of stomatal conductance, ABA drives drought avoidance mechanisms. In addition, by controlling secondary lines of plant defense, ABA is an active mediator of plant tolerance to water stress.

Adaptation of plants to water deficit is a wide and complex biological process that implies global changes in gene expression and primary and secondary metabolite composition. NCED genes were shown to be up-regulated under water stress conditions in *Arabidopsis thaliana* where ABA exerted a role as transcriptional regulator of the biosynthesis of the branched-chain amino acids, saccharopine, proline and polyamine (Urano et al., 2009). The grapevine response to water stress, lowering cell turgor, above all if applied in pre-véraison, increased berry sugar influx and ABA concentration (Davies et al., 2006). The expression of genes such as F3H, F305'H, LDOX and DFR, notably involved in the biosynthesis of anthocyanins, proanthocyanidins and flavonols, increased in water-deficit conditions (Castellarin et al., 2007a; Castellarin et al., 2007b). Moreover, during grape berry ripening the expression of genes such as UFGT and GST, strictly associated with the anthocyanin accumulation the former and the anthocyanin transport into the vacuole, the latter (Ageorges et al., 2006; Deluc et al., 2007), were strongly up-regulated under water stress conditions. The effect of water stress on polyphenol accumulation in grapevine berries has widely

been investigated over the past years (Kennedy et al., 2002; Koundouras et al., 2006; Matthews and Anderson, 1988; Ojeda et al., 2002; Poni et al., 2007). Many studies dealt with the accumulation of some classes of polyphenols such as anthocyanins and tannins in grapevine varieties characterized by different behavior as to water relation. *Vitis* genotypes in fact, show either an isohydric or anisohydric response to water stress. In isohydric cultivars, an ABA control of stomatal conductance reduces transpiration avoiding decrease in water potential and delaying the onset of stress tolerance mechanisms. On the contrary, a weak ABA control of stomatal closure does not avoid midday drop in water potential in anisohydric grapevines (Lovisolo et al., 2010). Upon water stress, polyphenolic concentration increases in berries both in isohydric varieties such as 'Grenache' (Coipel et al., 2006), 'Tempranillo' (Santesteban et al., 2011), 'Manto negro' (Medrano et al., 2003), and in anhysohydric varieties such as 'Cabernet Sauvignon' (Bindon et al., 2008; Kennedy et al., 2002), 'Cabernet Franc' (Matthews and Anderson, 1988), 'Muscat of Alexandria' (dos Santos et al., 2007), with different temporal dynamics linkable to putative ABA induction. As to the anthocyanin profile, Castellarin and co-workers (2007b) showed that water stress conditions favored the accumulation of more hydroxylated and more methylated anthocyanins (peonidin 3-O-glucoside and malvidin 3-O-glucoside); Deluc and co-workers (2009) reported for 'Cabernet Sauvignon' a 4-fold higher accumulation of peonidin 3-O-glucoside in water stressed plants respect to well watered ones. As to the profile of polyphenols other than anthocyanins, few information are currently available in grapevine. No effects were exerted by water stress on berry skin proanthocyanidin concentration in 'Cabernet Sauvignon' (Castellarin et al., 2007a) as well as in concentration and profile in 'Syrah' (Olle et al., 2011). However, in green leaf tea it was assessed in vivo that water deficit could increase the concentration of proanthocyanidins and their profile changed greatly due to the transformation of –(-)epicatechin and –(-) epigallocatechin gallate into their correspondent quinones (Hernandez et al., 2006). Quinones are involved in the modulation of lipoxygenase activity both as pure molecules and as soybean extract (Chedea et al., 2010); this aspect could have important consequences at the berry level both in terms of nutraceutical implications (in table grapes) and of technological transformation in winemaking (Cheynier et al., 1997).

The flavonol synthase (FLS), a pivotal enzyme of flavonol biosynthesis, was up-regulated in water stress conditions (Deluc et al., 2009) and flavonol concentration increased in 'Chardonnay' berries but not in 'Cabernet Sauvignon' ones. At present, to the best of our knowledge, the possible

involvement of water stress on flavonol profile is not known as well as no studies dealt with the effect of water deficit on cinnamic and benzoic acids and their ester derivatives.

The response of *Vitis vinifera* to water stress conditions as to carotenoid accumulation was not univocal but it was shown to be dependent on the soil characteristics: in a high water retention capacity soil no differences in the accumulation of carotenoids were detected among vines undergoing different water regimes, whereas in a low water retention capacity soil, berry carotenoid accumulation was higher in plants suffering from a severe water stress (Oliveira et al., 2003). One can speculate that, being the water intakes similar, soils or portions of soils in the same vineyard, able to induce water stress earlier, besides influencing the anthocyanin concentration, as well documented, are able to influence the accumulation of carotenoids in berries. Water deficit increased the transcript abundance of isoprenoids and carotenoids in 'Chardonnay' at maturity (Deluc et al., 2009), together with the increase of flavonol synthase; in the same work however authors assessed that the transcript abundance of CCDs and terpene synthases was not up-regulated in 'Cabernet Sauvignon' and no increase in flavonol concentration was measured in colored berries of 'Cabernet Sauvignon', as well; they speculated that the increased concentration of flavonols in water-stressed 'Chardonnay' berries, together with the higher transcript abundance of enzymes of terpenoid and carotenoid biosynthesis in the white grape variety, indicates a greater need for photoprotection, probably tied to the constitutive lack of anthocyanins. However, deficit irrigation in 'Cabernet Sauvignon' (Bindon et al., 2007) increased the accumulation of C-13 norisoprenoids, one of the carotenoid cleavage products. Some important differences among white and red wines, such as the higher floral and fruity notes of the former respect to the latter could rely on the fact that white varieties, lacking of anthocyanins, have adapted to abiotic stress through the enhancement of carotenoid (norisoprenoid precursors) and terpenoid byosynthesis. Further transcriptomic and metabolomic insights would be necessary to clarify the hypothesis that genotypes, on one hand, and conditions (cultural practices, environment, etc…), on the other, able to favour the accumulation of polyphenols, reduce the accumulation of volatiles and vice-versa; longtime ago Di Stefano and co-workers through the exclusive recourse to analytical measurements pointed out that varieties with a high anthocyanin concentrations were less rich in flavors, terpenols in particular, respect to low-concentration anthocyanin varieties (Cravero et al., 1994).

Water stress significantly affected the transcript abundance of four lipoxygenases (LOxs) in berries

of 'Cabernet Sauvignon' and 'Chardonnay' (Deluc et al., 2009) even though two of them showed a reduced transcript abundance in 'Cabernet Sauvignon' after véraison; authors argue that the interpretation of data is difficult, however evidences of the influences exerted by water stress on this metabolism was demonstrated in *Arabidopsis thaliana* (Melan et al., 1993) and it is likely also in *Vitis vinifera*. LOXs are a wide gene family ubiquitous in plants and tissues (Liavonchanka and Feussner, 2006); they catalyze the membrane fatty acid lipoxygenation determining the production of C6 volatile aldehydes, the most abundant volatiles in different plant organs, involved in plant defense signaling (Kishimoto et al., 2006), in storage lipid mobilization and in jasmonic acid biosynthesis. These compounds, with herbaceous notes, can be transformed into the correspondent alcohol by alcohol-dehydrogenases (ADHs) and they can react with organic acid to produce esters, giving evident relapses on wine quality. These compounds have recently been indicated as the "master-switch" in plant development and stress adaptation (Liavonchanka and Feussner, 2006).

The known water deficit effects on grapevine physiology have allowed an improvement of viticulture through the enhancement of berry quality: rescue irrigation techniques such as regulated deficit irrigation or partial root-zone drying, the use of rootstocks tolerant to water scarcity, the use of controlled cover crops, soil tillage and green pruning have been proposed to improve grape berry development and quality, as reviewed by Dai et al. (2010; 2011) and Keller (Keller, 2005). In this sense, cultural practices can be managed to control the effects of a stress-impaired plant metabolism on the accumulation of sugars and secondary metabolites in berry, including responses to severe osmotic stress in berry cells, and the consequences at the berry level of the chemically-mediated long distance stress signaling between root and shoot.

4. Light stress

The light stress implies photosynthetic activity variations, photoinhibition, and photoxidation. In leaves light deficiency and excess induce stomatal and metabolic responses such as the accumulation of antioxidants, namely ascorbate, glutathione, flavonoids, carotenoids and of enzymes controlling their oxido-reductive state (Fini et al., 2011); in *Arabidopsis thaliana* leaves (Page et al., 2012) light intensity was shown to be able to influence the accumulation of anthocyanins confirming the role of these molecules, together with ascorbate, as photoprotectants. On the contrary, the accumulation of the flavonol kaempferol 3-O-glucoside was unaffected and, in ascorbate deficient mutants, there was no significant accumulation of ABA,

suggesting that in this model plant ABA could not be involved in the mediation of the response to light stress. However, in potatoes an up-regulation of anthocyanidin glucosyltransferase (GT) was demonstrated in response to UV, cold, light, salt and ABA, resulting in 5.5, 6.0, 5.0, 5.0 and 8.0 fold increase in enzyme activity, respectively. Moreover, a synergistic effect of ABA+cold and cold+light was detected, as well (Korobczak et al., 2005). In *Vitis vinifera* leaves the effect of light on the increase of photoprotective compound concentration is known and documented (Kolb et al., 2001).

Recently, Berli and co-workers (2010, 2011) have suggested that the leaf antioxidant defense system is activated by UV-B irradiation and ABA acts downstream in the signaling pathway for most enzymes of the photoprotection response. Tossi et al. (2009) suggested that UV-B perception triggers an increase in ABA concentration, which activates NADPH oxidase and hydrogen peroxide generation; this, through a nitric oxide (NO) synthase-like-dependent mechanism, increases NO production to maintain cell homeostasis and attenuate UV-B-derived cell damage. Interestingly, the same authors recently proposed that the induction of common signaling components, such as ABA and NO in either plant or animal cells occurs in response to high doses of UV-B, showing that the evolution of a general mechanism activated by UV-B is conserved in divergent multicellular organisms, and it could be interpreted as a challenge to a changing common environment (Tossi et al., 2012).

Some responses, such as the accumulation of quercetin and kaempferol in grape tissues were activated both by UV-B and ABA applications whereas some others, such as caffeic and ferulic acid concentration increase, were activated by ABA alone. The possible implications of these aspects at the level of berry quality is well evident, together with the fact that also leaf carotenoids were enhanced by UV-B treatment: in this case, the level of endogenous ABA increased, as well, being carotenoids its precursors. The pigment analysis of 'Sauvignon blanc' berries allowed the identification of the xanthophyll, lutein 5,6 epoxide (Young et al., 2012); this molecule occurs in the alfa-carotene branch of the carotenoid biosynthetic pathway and in *Cucumis sativus* (Esteban et al., 2009) its accumulation has been shown to be a plant early response to shade conditions. Grapevine synthesizes carotenoids both from the violanthin cycle and the lutein epoxide cycle (Young et al., 2012): possible implications on berry quality could also rely on the formation of lutein 5,6 epoxide catabolites, impacting on berry quality. Moreover, even though in *Vitis vinifera* at present there are no information about a possible transcriptional regulation of carotenoid biosynthetic genes, it can be speculated that environmental conditions and vineyard management

techniques can drive the profile of carotenoid accumulation in berries, besides influencing their total final concentration. In addition, possible further implications on fruit quality are evident being carotenoids the precursors of C-13 norisoprenoids, a class of volatiles whose sensorial impact in many fruits, grapes and wines is known.

In berries of *Vitis vinifera* the influence exerted by light on the accumulation of some polyphenols is quite documented but at present there are no demonstrations of a possible talk-cross with ABA in this response. Downey et al., (2004) did not find, in 2 out of 3 years, significant difference between shade and light exposure treatments in 'Syrah' berry anthocyanin accumulation confirming previous results by Price et al., (1995) in 'Pinot noir'. Other authors, on the contrary, pointed out that shading considerably (32 %) even though not significantly, reduced the anthocyanin content at harvest in 'Pinot noir' berries (Cortell and Kennedy, 2006). Chorti et al., (2010), in 'Nebbiolo' grapes, a peonidin 3-O-glucoside prevalent grapevine variety, assessed that shading reduced the anthocyanin total content and enhanced the level of acylation with p-coumaric acid, i.e. favored the formation of p-coumaroyl derivatives which are the prevalent anthocyanin derivatives in most grapevine genotypes. The effects of light on grapevine physiology have been exploited to address grape berry quality: different training systems, influencing the vine light use efficiency (Cavallo et al., 2001; Louarn et al., 2008; Orlandini et al., 2008; Petrie et al., 2009) exert pivotal consequences on berry quality. Trellis system able to increase shading within the vine canopy resulted in berries with reduced anthocyanin and phenol concentrations respect to controls in 'Aglianico' (Cavallo et al., 2001); on the contrary, no influences were detected in 'Syrah' grapevines trained to five different trellis system on polyphenolic concentration over two years (Wolf et al., 2003).

If the effect of light exposure on berry anthocyanin concentration is not univocal, no doubts about the light effect on flavonol accumulation exist: berry skin flavonol concentration is known to be very sensitive to light exposure, resulting in a higher concentration in exposed berries respect to shaded ones (Cortell and Kennedy, 2006; Kolb et al., 2003; Matus et al., 2009). At present there are no information about the effect of light exposure on the flavonol profile in berries; however in berry skins of different varieties (Ferrandino et al., 2012b) and of different clones (Ferrandino and Guidoni, 2010) over two years, some flavonol profile characteristics were not dependent on vintage (i.e. different light dynamics over the season), so we can speculate that the light effect on the accumulation of some specific flavonols (kaempferol 3-O-glucoside, in particular) is probably negligible, in line with studies on *Arabidopsis thaliana* where increasing light intensities did not

influence the accumulation of this compound in leaves (Page et al., 2012). In *in vitro* cultured plants of *Vitis vinifera* exposed to two different intensities of 'field-like' UV-B light it was assessed that low UV-B intensities increased the levels of triterpenes sitosterol, stigmasterol and lupeol, in young leaves, whereas in mature leaves the accumulation of antioxidants such as diterpenes, tocopherols, phytol, E-nerolidol and of monoterpenes such as carene, alfa-pinene and terpinolene was maximum under high UV-B (Gil et al., 2012). Also ABA concentration increased after high UV-B irradiation; one can wonder if the ABA concentration increase was a direct response to light stress or a consequence to the increase of carotenoid concentration, precursors of ABA itself. Except for the documented effect of artificial shading on the reduction of monoterpenols and norisoprenoids in 'Muscat of Frontignan' grapes (Bureau et al., 2000) and in 'Sauvignon blanc' (unpublished data), no specific information are at present available about the effect of light on volatiles in berries even though, being most of these molecules, present in tissues other then berries where their concentration generally increases following stress conditions (Gil et al., 2012), studies on the influence of light intensity and profile on berry volatile accumulation deserve future deepening.

5. Temperature stress

Temperature plays an important role in the ripening of many fruits, including grape berries. Temperature trends are so determinant for grape phenology, that historical series of grape ripening have been used to reconstruct temperatures to provide insight into regional-scale climate variations (Chuine et al., 2004). In the last decade much attention has been focused on the global warming effects in many fields of agriculture (Wolfe et al., 2008) and in viticulture, as well (Hannah et al., 2013; Jones et al., 2005). Effects of temperature increase in viticulture have been discussed in the light of political, social, ecological implications in the Mediterranean area, following a predicted average temperature increase of 2 °C in the next 50 years (Hannah et al., 2013; Jones et al., 2005). Accordingly, regions producing at present high quality and valuable wines at the margins of *Vitis vinifera* climatic limits, in conditions of further climate change, will be in consistent difficulties to maintain high quality levels of production with the traditionally cultivated varieties. On the contrary, the warmer conditions of locations which are at the moment out of the favored areas for quality grape production, will probably turn into areas more favorable to grapevine growing and fine wine production (Goode, 2012). Although the main meteorological parameter implicated in global climate change is temperature, few are information, particularly as

to field grown plants, on temperature effects on ABA-mediated phenolic and volatile compound accumulation.

Cold temperatures induce ABA synthesis, reflecting on phenolic accumulation and/or biosynthesis enhancement (Keller, 2010; Xin et al., 2013). However, in Mediterranean cool viticultural areas an increase of temperature is often linked to an enhancement of phenolic accumulation. In such situations a heat increment optimizes primary metabolism, allowing fresh carbon available to flavonoid biosynthesis, when an up-regulation of genes of flavonoid biosynthesis does not occur. A positive effect of ABA on non-structural carbohydrate metabolism (both starch and hexoses, Çakir et al., 2003) becomes crucial in light of hexose-induced activation of flavonoid metabolism. In this sense, a likely threshold effect could occur in grapevine grown in cool climate areas, as Meng et al. (2008) showed that cucumber seedlings treated with 50 and 150 µM ABA under low temperature accumulate substantial amounts of soluble carbohydrates whereas 250 µM ABA treated seedlings show, on the contrary, decreased levels of all soluble carbohydrates, as compared to the control seedlings treated with 0 µM ABA. Not only an ABA-mediated effect of temperature stress can influence leaf and berry secondary metabolites, but in addition, through ABA negative control on stomatal aperture and transpiration rate, an increased concentration of ABA tends to buffer the day-night alternations of metabolite biosynthesis in response to heat and cold. This mechanism, suggested for ABA regulation of leaf growth rate by Tardieu et al. (2010), is amplified in presence of water stress, *per se* causing an ABA increase. The antagonistic effect of ABA to high night temperatures was demonstrated (Mori et al., 2005a): as a matter of fact by feeding 'Pinot noir' vines with ABA, the suppressive effect of high temperatures on total anthocyanin amount was annulled even though no effects were exerted on the anthocyanin profile. In an *in vivo* incubation of 'Cabernet Sauvignon' grape berries with salicylic acid, the increase in total polyphenols detected via the Folin-Ciocalteu method was consequent to the activation of phenyl alanine ammonia-lyase (PAL) induced under high temperature stress conditions (Wen et al., 2008); authors argued that salicylic acid can act as an answer to oxidative stress induced by very high temperatures (40 °C) via the induction of the phenylpropanoid pathway, thus favoring the consequent accumulation of antioxidants as polyphenols.

High temperatures repressed the anthocyanin accumulation in various plants, including grapevines where in an interspecific hybrid, high night temperature conditions depressed the expression level of the key enzymes of the anthocyanin biosynthesis, UFGT, in particular, resulting in a significantly

lower anthocyanin concentration in grape berries (Mori et al., 2005b). The same authors in a work on 'Cabernet Sauvignon' grapes showed that high temperatures reduced the total anthocyanin amount but they did not change the concentration of malvidin glucoside and of its acylated derivatives; in addition, as the anthocyanin biosynthetic genes were not strongly, but only partially, down-regulated they concluded that under high temperatures, the reduction of berry skin anthocyanin concentrations was also imputable to anthocyanin degradation, probably due to both chemical and enzymatic reactions occurring in berries still on the vine. As a matter of fact, high temperatures would probably induce an oxidative stress leading to the formation of H_2O_2 with the subsequent induction of peroxidases and of oxidoreduction enzymes, responsible of anthocyanin degradation (Mori et al., 2007).

Little is known about the impact of temperature on proanthocyanidin accumulation in grape skins: in an experiment hold on field-grown Merlot vines cooled during the day or heated at night by +/− 8 °C, from fruit set to véraison in three years, the total PA content per berry varied only in one year, when PA content was the highest in heated berries and the lowest in cooled berries. Differences in the profile were instead detected in two years over three as cooled berries resulted in a significant increase in the proportion of (−)-epigallocatechin as extension subunit. When the expression level of several genes involved in the flavonoid biosynthetic pathway was assessed, it emerged that berry heating and cooling altered the initial rates of PA accumulation but, as PA accumulation is concentrated prior to véraison, berries were able to compensate the initial effects of temperature on PA biosynthesis resulting in similar concentration of PA at harvest (Cohen et al., 2012). Cooling berries during the day induced a higher flavonol content at véraison but lowered flavonol and PA concentration at harvest (Cohen et al., 2008).

Cool temperatures increased the concentrations of C6 volatile aldehydes in 'Traminette' berries whereas the monoterpenes concentration was higher in hot conditions respect to cool conditions (Ji and Dami, 2008). Some insights exist about the effect of temperatures on the expression of alcohol dehydrogenases (ADHs): ADHs, involved in the volatile biosynthesis thorough the reduction of aldehydes into the correspondent alcohol and providing substrates for the ester formation, have a specific role in the regulation of aroma biosynthesis in melon fruit (Manriquez et al., 2006) and in *Vitis vinifera*, as well (Tesniere et al., 2006): grapevines over-expressing ADH displayed a lower sucrose content, a high degree of proanthocyanidin polymerization and higher concentration of volatiles, namely carotenoids and shikimate derivatives. Few studies report about the effect of temperature on ADHs and they all were conducted in post-harvest conditions where,

different temperatures can lead to the over- or down-expression of ADHs, resulting in a higher or lower ester concentration: however, during grape berry dehydration, the effect of temperature on aldehydes dehydrogenase (ADH) and on carotenoid cleavage dioxygenase (CCD1) was demonstrated: Cirilli et al. (2012) pointed out that in dehydration conditions the expression of ADH2 and CCD1 in Aleatico grapes was the highest in grapes dehydrated at 10 °C respect to the one detected at higher temperatures. To the best of our knowledge there are little information related to the effect of temperature in grape berries still on the vine. This opens to important future perspectives as recent interest as arisen around volatile alcohols and aldehydes due to their involvement in plant response to biotic stress (Matsui, 2006; Shiojiri et al., 2006) and to the fact that they represent the majority of varietal and pre-fermentative volatile in fresh grapes (Ferrandino et al., 2012a; Kalua and Boss, 2010; Yang et al., 2009).

6. Stress-mediated variations of berry and must quality improve the nutraceutical value of grapes

Epidemiological data indicate a beneficial effect of Mediterranean diets on human health, especially associated to the lower incidence of cardiovascular diseases. Heinrich et al. (2005) tested *in vitro* Mediterranean plant extracts through different tests: four antioxidant tests (2,2-diphenyl-1-picrylhydrazyl scavenging –DDPH-, prevention of oxyhaemoglobin bleaching, prevention of lipid peroxidation, and protection from DNA damage), three enzyme inhibition tests (inhibition of xanthine oxidase, inhibition of myeloperoxidase-catalysed guaiacol oxidation, and inhibition of acetylcholine esterase), one test investigating the extract potential cytotoxicity, one assay measuring their anti-proliferation potential, one test assessing the anti-diabetic activity, and one investigating the extract effect on mood disorder-related biochemical parameters. They concluded that grape extracts, together with those derived from *Berberis vulgaris*, *Reichardia picroides*, *Scandix australis*, *Satureja montana*, *Thymus piperella*, and *Lythrum salicaria* show the highest activity in a broad range of assays, suggesting that grapes and grape derivates may contribute to the observed better aging of rural Mediterranean populations.

Kelsey et al. (2010) reviewed the literature pertaining to various classes of nutraceutical antioxidants and discussed their potential therapeutic value in neurodegenerative diseases, by dividing natural antioxidants into several distinct groups based on their chemical structures. Among them, flavonoid polyphenols like epigallocatechins and non-flavonoid polyphenols such as resveratrol, deriving from grapes, were included either because they directly scavenge free

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radicals or they indirectly increase endogenous cellular antioxidant defenses. In addition, neuroprotective effects of anthocyanins on apoptosis (i.e. programmed cell death) induced by mitochondrial oxidative stress have been reported (Kelsey et al., 2011).

Options of grapes to prevent cancer and chronic diseases (atherosclerosis, cataract, diabetes, neurological diseases, immune-inflammatory disorders) to improve life quality in maturity, following main antioxidant defense mechanisms have been reported (Ferrari and Torres, 2003), as well as antioxidant and antiobesity activities of 'Campbell Early' grape seeds (Oh et al., 2013).

Recently, malvidin 3-0-glucoside effects on the cardiovascular function have been tested by using red grape skin extracts, containing a malvidin 3-O-glucoside amount of about 65 mg/g of fresh skin. On isolated and Langendorff perfused rat hearts, Quintieri et al. (2013) found that increasing doses (1-1000 ng/ml) of the extract induced positive inotropic and negative lusitropic effects associated with coronary dilation. In addition, they found that malvidin 3-O-glucoside acts as a post-conditioning agent, being able to elicit cardioprotection against ischemia/reperfusion damages, and proposed malvidin as a new cardioprotective principle.

In plants, all phenylpropanoids, particularly flavonoids, play primary antioxidant functions in the responses of plants to a wide range of abiotic stresses. Stress-responsive dihydroxy B-ring-substituted flavonoids have great potential to inhibit the generation of reactive oxygen species (ROS) and reduce the levels of ROS once they are formed, performing antioxidant functions (Agati et al., 2012). A strong antioxidant ROS-scavenging activity, determined by both DPPH and FRAP assays, has recently been reported in the berry skin and wine of 'Aglianico' grapes harvested from plants cultivated upon controlled drought and high light levels (De Nisco et al., 2013). Even if the significance of flavonoids as scavengers of reactive oxygen species (ROS) in humans has been questioned, based on the observation that the flavonoid concentration in plasma and most tissues is too low to effectively reduce ROS, flavonoids play key roles as signaling molecules in mammals, through their ability to interact with a wide range of protein kinases, including mitogen-activated protein kinases (MAPK), that control key steps of cell growth and differentiation (Brunetti et al., 2013). Flavonoid function in plants is actually restricted to flavonols, the ancient and widespread class of flavonoids. Upon light stress, flavonols are not as efficient as other secondary metabolites in absorbing wavelengths in the 290-320 nm spectral region, but display the greatest potential to keep stress-induced changes in cellular reactive oxygen species homeostasis under control, and to regulate the development of individual organs and the whole plant (Pollastri and Tattini, 2011).

7. Feeding grapevine organs with exogenous ABA: a tool to enhance grape quality and to control abiotic stress

Exogenous ABA application are moderately used in the production of table grapes, to hasten ripening, to increase berry color and to regulate other key processes such as dormancy (Zhang et al., 2011). Since few years a commercial product containing ABA was authorized in Chile and in California where it is essentially used for the enhancement of color development in table grapes. Research on the application of exogenous ABA is not so wide at present and is focalized on table grape varieties such as 'Flame seedless' (Peppi and Fidelibus, 2008; Peppi et al., 2006), 'Crimson Seedless' (Cantin et al., 2007; Lurie et al., 2009; Peppi et al., 2008), 'Redglobe' (Omran, 2011) and 'Benitaka' (Roberto et al., 2012); evidences about exogenous ABA application in 'Chambourcin' and 'Cabernet franc' grapes were reported in relation to the increase of freeze tolerance (Zhang and Dami, 2012a; Zhang and Dami, 2012b), as well. Xiao et al (2006) reported that the C-repeat (CRT)-binding factor/dehydration-responsive element (DRE) binding protein 1 (CBF/DREB1) transcription factors (CBF1), controlling freezing and drought tolerance in plants, were accumulated in young *Vitis vinifera* leaves in response to exogenous ABA application. Moreover, foliar application of exogenous ABA were applied to increase the phenolic compound content of xylem sap extracted from 'Pinot noir' vines, resulting in an increase in the Pierce's disease curing (Meyer and Kirkpatrick, 2011).

Koyama et al. (2010) comprehensively examined the effect of exogenous ABA application on the transcriptome and on the phenolic profiles of 'Cabernet Sauvignon' berry skins. As expected, exogenous ABA induced anthocyanin and hexose accumulation in the skins. Moreover, the analysis of transcripts revealed that about half of ABA-induced transcripts in the berries corresponded to ripening-specific genes; these ripening-specific genes were showed to be up- and down-regulated in the berry skins. ABA- and ripening-induced genes included genes associated with stress response, such as beta-1,3-glucanase, chitinases, thaumatins, LEA proteins, as well as genes associated with cell wall modification such as polygalacturonase PG1 and proline-rich cell wall proteins, in addition to anthocyanin biosynthetic genes. Moreover, the ABA- and ripening-induced genes included those associated with photosynthesis such as chlorophyll a/b binding protein, photosystem components and those associated with auxin response. The relapses on the berry skin transcriptional response were shown to be wide and for some of them possible implications on secondary metabolite accumulation were evident; further insights will help to elucidate possible consequences on grape quality (Wheeler et al., 2009).

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8. Conclusions

In grapevine, abiotic stress modifies growth and development of all plant organs. The response to abiotic stress at the berry level drives the accumulation in berry pulps, seeds and skins of secondary metabolites as a line of defense against cell damages. Viticultural practices can be managed to control stress plant response in order to increase secondary metabolite concentrations, reflecting on an enhancement of table grape and must quality, as secondary metabolites of grapes contribute to fruit and wine taste and aroma, to the potential antioxidant capacity of fruit and wines, to wine stabilization and protection during ageing.

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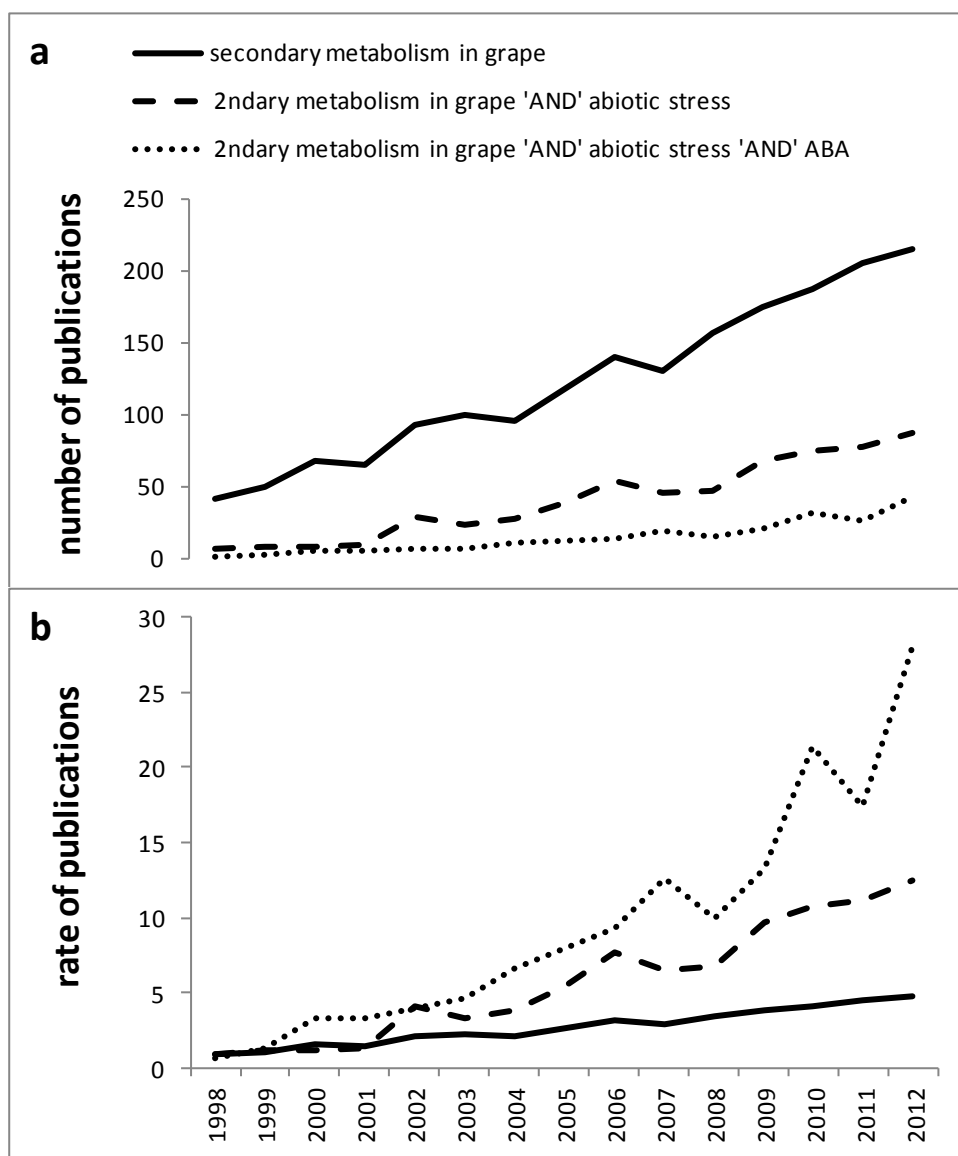
Figure 1. Publications published during the last 15 years on grape secondary metabolism and links to abiotic stress and to the ABA-response to stress: (a) number and (b) rate (i.e. the numbers of papers published each year divided by the average number of papers published in the first three years of the observed period). Key words used in the search of ISI Web of Knowledge[SM] included: *Vitis* OR grape*, secondary metabolism (including sub-terms; e.g. flavon* OR phenol* OR anthoc* OR metabolite*), AND abiotic stress (including stress sub-terms; e.g. drought OR water deficit OR dehydration OR temperature OR cold OR heat OR light stress), AND abscisic acid OR ABA.