

Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update

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Abstract. This review deals with grapevine responses to water stress by examining perturbations to physiological and molecular processes at the root, shoot, leaf and berry levels. Long-distance signalling among organs is also considered. Isohydric or anisohydric *Vitis* genotypes are described in relation to their response to drought, which is linked to stomatal behaviour. Stomatal regulation of grapevine under abscisic acid and hydraulic control (the latter being linked to embolism formation and recovery in water pathways upstream the stomata) is reviewed and linked to impairments of photosynthetic assimilation. We define three stages of photosynthesis regulation in grapevines that are subjected to progressive water stress on the basis of the main causes of assimilation decline. Early and late contributions of aquaporins, which play a fundamental role in water stress control, are discussed. Metabolic mechanisms of dehydration tolerance are reviewed, and variation linked to differences in transcript abundance of genes involved in osmoregulation, photosynthesis, photorespiration, detoxification of free radicals and coping with photoinhibition. Results of these defence strategies accumulated in berries are reviewed, together with perturbations of their molecular pathways. Features observed in different organs show that grapevine fits well as a complex model plant for molecular and physiological studies on plant drought avoidance/tolerance.

Additional keywords: abscisic acid, anisohydric, anthocyanins, aquaporin, genome, isohydric, polyphenols, proteomics, stomatal conductance, transcriptomics, water use efficiency.

Introduction

Advances in the understanding of plant responses to water deficits require information from the molecular level to that of whole plant (Chaves *et al.* 2003). Grapevine has been used as model plant to study ecophysiological responses to water stress since the 1970s. Molecular studies began in the 1990s, and, more recently, applications of ‘-omics’ technologies, such as transcriptomics and proteomics, together with the availability of the grapevine genome sequence (Jaillón *et al.* 2007; Zharkikh *et al.* 2008) have provided a powerful molecular counterpart to physiological analyses (Troggio *et al.* 2008). This review uses a physiological platform to incorporate recent molecular developments to provide an understanding of how grapevine responds to and recovers from drought stress.

Grapevine is grown widely throughout the world, with its production making the top agriculture lists in many countries (Bisson *et al.* 2002). According to FAOSTAT time-series and cross-sectional data (<http://faostat.fao.org>, accessed September 2009) there were 66 271 676 tonnes of grapes produced on 7 501 872 ha in 2007.

Vitis vinifera L. cultivation is traditionally non-irrigated (especially in Europe) and spread widely across dry and semi-dry ecosystems. Yield and berry quality depend strongly on the vine adaptability to drought. Water stress does not imply exclusively negative effects, but a regulated water stress, which is the base of various agronomic practices (e.g. rootstock use, controlled cover crops, tillage, rescue irrigation techniques such as regulated deficit irrigation or partial root-zone drying) has been largely used to balance vine vegetative and reproductive growth with the aim of controlling berry quality. Understanding and manipulating plant–water relations and water-stress tolerance by means of physiology and molecular biology can significantly improve plant productivity and environmental quality, hence, is clearly of wide economic importance in viticulture.

In this review we examine root, shoot, leaf and berry, linking physiological and molecular knowledge to applied research. We focus on the role of aquaporins in water movement and how water constraints modify organ functioning and generate signals directed to other plant organs. Drought signalling among grapevine organs has a dual component: a hydraulic signal

controlled by xylem physiology coexists with chemical signals (involving hormones), transported via xylem, phloem and parenchyma pathways (Loveys 1984b; Lovisolo *et al.* 2002a). Grapevine is a model species to study the different drought signalling mechanisms, as many cultivars and genotypes are available, including the different species of the genus *Vitis*.

Root and shoot sensing of water stress

When roots sense a soil water deficit, root cells respond in terms of their growth and differentiation, and also transmit a signal that is then perceived in the shoot. The response to drought involves first the acidification of the apoplast by protons pumped from the cytoplasm. This acidification stimulates the action of expansins, important in cell wall synthesis. Of the 10 expansin genes in grapevine, *Vlexp2* isolated from *Vitis labruscana* (*Vitis labrusca* L. × *V. vinifera* L.) cv. Kyoho berries, showed high root expression level (Ishimaru *et al.* 2007). From *V. vinifera* cv. Arka Neelamani, Thomas *et al.* (2003) isolated *VvPRP1* and *VvPRP2*, two genes encoding a distinct type of proline-rich proteins (PRPs), a major category of cell wall proteins in plants, involved in different developmental and environmental responses to abiotic stresses. Grape PRP genes are expressed in root tissues and not detected in shoot tip or leaf tissues; they play a role in the initiation of new roots on grape stem cuttings, probably by altering the cell wall mechanical properties to enable root emergence.

Although a reduction in leaf and shoot growth is one of the signs of grapevine water deficit (Stevens *et al.* 1995), the decrease in root growth is less than shoot growth (Dry *et al.* 2000a, 2000b). This results in a higher root : shoot ratio, which ensures adequate water and nutrient transport to the shoots. The tolerance of grapevines to drought has often been attributed to their ability to produce new roots selectively where soil water is available (Morlat and Jacquet 1993; Dry *et al.* 2000b). Droughted roots continue to grow into deeper, wetter soil layers, whereas the roots of irrigated plants proliferate mostly in the topsoil (Bauerle *et al.* 2008). As deeper roots procure soil-mobile nutrients, like nitrate (NO_3^-) (Keller 2005), this mechanism also enhances plant nitrogen uptake.

Shoot growth inhibition comprises inhibition of internode extension, leaf expansion and elongation of tendrils (Schultz and Matthews 1988; Hardie and Martin 2000) and has been used as a sensitive indicator of grapevine water status (Pellegrino *et al.* 2005; Lebon *et al.* 2006). In addition, comparably low water stress levels induce a decrease in the average diameter of grapevine vessels and a decrease of xylem hydraulic conductivity (Lovisolo and Schubert 1998; Lovisolo *et al.* 2002b). Although shoot growth inhibition limits transpiration, reduced vessel size prevents excessive loss of water by reducing xylem conductivity and may help to prevent embolisation, as smaller vessels are less susceptible to cavitation (Salleo *et al.* 1985). Extreme drought consequences at the shoot level involve shedding of leaves and cessation of secondary growth, as reviewed by Keller (2005).

V. vinifera Grenache and Chardonnay plants show a higher amount of suberin deposited in cell walls of droughted roots than in well watered roots in response to water stress, both at endodermal and exodermal levels. In Grenache, all cells of the

endodermis appear to become suberised, whereas in Chardonnay some passage cells are still evident. These cellular adaptations assist in modulating stress responses in these two grape cultivars (Vandeleur *et al.* 2009).

In addition to root growth and apoplastic barrier formation, plants can modulate responses to soil water stress by compartmentalising the water transport pathways which are under metabolic control. This implies that roots can either modulate water-driving cell osmotic forces and/or regulate expression and activity of aquaporins (Galmés *et al.* 2007; Lovisolo *et al.* 2008b).

Aquaporins are thought to control the radial movement of water through roots (Maurel *et al.* 2008) and through living tissues adjacent to root and shoot xylem cells (Kaldenhoff *et al.* 2008). Some aquaporins are constitutively expressed (Johansson *et al.* 2000) but the expression of others is regulated by different stimuli, such as adverse environmental conditions (Vera-Estrella *et al.* 2004). Indeed, expression of different aquaporin genes may be stimulated, decreased or unchanged under abiotic stress (Kaldenhoff *et al.* 2008). Grapevine aquaporins have been cloned by Baiges *et al.* (2001), Picaud *et al.* (2003), Perrone *et al.* (2006), Reid *et al.* (2006), Galmés *et al.* (2007), Fouquet *et al.* (2008), Glissant *et al.* (2008), Schlosser *et al.* (2008), Shelden *et al.* (2009) and Vandeleur *et al.* (2009), and reported to be expressed either in roots or shoots.

The role of aquaporins in root water transport was investigated by comparing grapevine cultivars that differed in water use strategies, as described by Vandeleur *et al.* (2009). These workers studied gene expression of *VvPIP1;1* and *VvPIP2;2*, the two PIP aquaporins with higher expression in roots of both Chardonnay and Grenache plants. *VvPIP2;2* showed a constitutive expression in root regardless of cultivar or soil water condition, whereas *VvPIP1;1* transcript expression increased during water stress in Chardonnay, but not in Grenache. Functional assays showed a gain of water permeability (P_{os}) when *VvPIP2;2* and *VvPIP1;1* cRNA were injected together in the oocytes but not when *VvPIP2;2* was injected alone. Hence, it was proposed that Chardonnay plants show a minor reduction of root hydraulic conductance caused by water stress through increased transcellular component of radial water transport. This provides water while transpiration continues; thus, an anisohydric behaviour (see below) is reported for this cultivar. In this way, Chardonnay plants are able to maintain lower water potential differences between soil and xylem, possibly due to lower vulnerability to embolism formation compared with Grenache, as described by Alsina *et al.* (2007).

Grenache plants demonstrate a different strategy: the finding of no variation in transcript level of most important root PIP aquaporins and suberisation implies a lower hydraulic conductance in water deficit conditions. This supports the hypothesis of tight control on stomatal regulation that is typical of isohydric (see below for explanation) cultivars like Grenache, which aims to avoid excessively negative xylematic water potential and, therefore, cavitation (Schultz 2003a; Soar *et al.* 2006; Vandeleur *et al.* 2009). It is hypothesised that in the isohydric Grenache, the drought-induced root abscisic acid (ABA) biosynthesis increases apoplastic concentration because of a concomitance of events: an increase of suberisation of

apoplastic barriers causes a reduction of water conductivity which is not compensated by aquaporin-mediated water transport.

Literature reviewed in this section shows a lack of experiments dealing with root hydraulics; grapevine root aquaporins are, in some cases, performed on *Vitis* spp. rootstocks grafting *V. vinifera* scions (e.g. Schultz 2003a; Alsina *et al.* 2007), whereas others are performed on own-rooted varieties of *V. vinifera* (e.g. Soar *et al.* 2006; Vandeleur *et al.* 2009). However, the iso- or anisohydric response is always detected at the scion level, showing that a strict causal linkage of the scion response with root aquaporins occurs only on own-rooted plants.

Embolism formation and removal: a phenomenon affecting hydraulics and transpiration of grapevines

Embolism of xylem vessels as a result of water stress has been extensively studied in grapevines. Under high levels of tension, gas-filled xylem vessels may become disrupted by breakage of water columns, which causes embolism formation and drastically reduces hydraulic conductance (Schultz and Matthews 1988; Lovisolo and Schubert 1998). In different studies comparing root and shoot embolisms, roots have been shown to be more vulnerable to xylem cavitation than shoots (Lovisolo and Schubert 2006; Lovisolo *et al.* 2008a). This protects the stem from extreme xylem tensions during severe drought (Sperry *et al.* 2006). Froux *et al.* (2005) demonstrated that lateral roots seem to be more prone to embolism than the main root, suggesting a behaviour similar to 'hydraulic segmentation' in petioles. In plants with an anisohydric type response to drought, like *Populus euphratica* (Hukin *et al.* 2005), a lower cavitation vulnerability of the roots compared with shoots was found. In *P. euphratica* stomatal closure occurred relatively late, well after shoot hydraulic conductance was significantly affected by embolisms, showing that in the anisohydric response to drought a lower vulnerability of roots to cavitation may be part of the survival strategy.

Although grapevine petioles and roots are more vulnerable to embolism than shoots (Lovisolo *et al.* 2008a), when plants are rewatered, either root or shoot and petioles recover ~35–40% of hydraulic conductivity within 24 h, suggesting that a common and coordinated mechanism of recovery among all plant organs occurs. The proposed mechanism is based on three points: (i) embolism repair occurs progressively in shoot and then in root and in petioles, following an almost full recovery of leaf water potential; (ii) hydraulic conductance recovery in all plant organs also occurs during diurnal transpiring hours when formation and repair of embolisms occurs in all plant organs; and (iii) a non-hydraulic stress-derived ABA residual signal in rehydrated leaves hinders stomatal opening even when leaf water potential and the overall plant hydraulics are recovered, suggesting that an ABA-induced transpiration control promotes gradual embolism repair in rehydrated grapevines (Lovisolo *et al.* 2008a).

To reintegrate vessel functionality, plants have developed different repair mechanisms, which, in some cases, are associated with positive root pressure, and in other cases involve active and energy-consuming processes in shoot conductive tissues (Salleo *et al.* 2004). In grapevine,

experiments conducted with the metabolic and water transport inhibitor mercuric chloride point to the presence of an active mechanism involving the contribution of living cells (Lovisolo and Schubert 2006), and also possibly involving the contribution of aquaporins. A role of aquaporins in embolism repair was previously hypothesised based on asymmetrical aquaporin distribution, which appeared enriched at the interface between xylem vessels and associated living cells (Kaldenhoff *et al.* 2008). Aquaporin distribution in grapevine cells has not yet been described, although in water-stressed grapevine shoots the expression of an aquaporin (PIP1) was found to be downregulated (Cramer *et al.* 2007). However, aquaporins can be activated by post-translational modifications, e.g. by phosphorylation, thus, their role in embolism refilling cannot be ruled out on the basis of expression studies.

Aquaporins may be involved in embolism repair. In walnut (*Juglans regia* L.) PIP2 aquaporins, localised in xylem vessel parenchyma cells, were activated during spring embolism recovery (Sakr *et al.* 2003). PIP1 and PIP2 antisense *Arabidopsis* plants were slower than controls in the conductance recovery after rewatering (Martre *et al.* 2002). Tobacco (*Nicotiana tabacum* L.) RNAi plants showing an impaired expression of PIP1 or PIP2 genes led to a direct evidence of aquaporin involvement in embolism recovery. PIP1-RNAi and control plants repaired embolism in a few hours after rehydration, but PIP2-RNAi plants showed a delayed kinetics of recovery (Kaldenhoff *et al.* 2008).

Results of recent work suggest ABA/aquaporin interaction in embolism repair and in aquaporin activation during water stress (Kaldenhoff *et al.* 2008). ABA may be involved in gating mechanisms of water channels by facilitating their structural restoration, possibly acting from the cytoplasmic side of aquaporins (Wan *et al.* 2004). Although downregulation of aquaporins after ABA treatment has been reported (Mariaux *et al.* 1998; Suga *et al.* 2002), more often aquaporins, if responsive, were upregulated by this hormone (Jang *et al.* 2004). Parent *et al.* (2009) obtained sense and antisense transgenic maize (*Zea mays* L.) plant for 9-cis-epoxycarotenoid dioxygenase *NCED/VP14* gene that catalyses the first specific step in ABA biosynthesis. Expression levels of most root PIP isoforms were significantly higher in sense plants than in antisense plants, and analogous results were obtained for the influence on protein content. Moreover, a long-lasting effect was observed. This transgenic approach allows us to distinguish between ABA and non-ABA effects on aquaporins.

Stomatal and non-stomatal limitations to photosynthesis

Under conditions of high irradiance and vapor pressure deficit (e.g. midday of clear summer days), water flow into grapevine leaves, as in many other species, is insufficient to compensate water losses through evapotranspiration, resulting in a midday to afternoon depression of leaf water potential (Schultz 2003a; Chaves *et al.* 2007). As a consequence, midday to afternoon depression of stomatal conductance (g_s) and net photosynthesis (A_N) has been reported in many cultivars, even under sufficient soil water availability (Gómez-del-Campo *et al.* 2004; Moutinho-Pereira *et al.* 2004). Differences among cultivars in stomatal

responsiveness to midday conditions have been described (Winkel and Rambal 1990).

Midday stomatal closure is often associated with accumulation of ABA in the petiole xylem and leaves (Loveys 1984a; Rodrigues *et al.* 2008), although increased xylem pH (Rodrigues *et al.* 2008) and decreased plant hydraulic conductance (Salleo and Lo Gullo 1989; Vandeleur *et al.* 2009) could also be involved. However, stomatal closure is not the only cause of decreased photosynthesis during the midday depression. The depression involves both stomatal and non-stomatal factors, as reflected by lower photosynthesis during the afternoon than in the morning at any given stomatal conductance (Cuevas *et al.* 2006) or substomatal CO₂ concentration (Downton *et al.* 1987; Quereix *et al.* 2001). In addition, g_s in the afternoon is less sensitive to ABA and more sensitive to CO₂ than during the morning, also supporting the idea that reduced photosynthesis is limiting g_s in the afternoon and not *vice versa* (Düring 1991; Correia *et al.* 1995).

Non-stomatal limitations may also be partly responsible for the midday depression. Photoinhibition, feedback inhibition through source–sink interactions, and decreased mesophyll conductance to CO₂ have been suggested as important limiting factors, but none of these processes has been demonstrated to predominate (Flexas *et al.* 2008). For instance, Escalona *et al.* (2003) showed that, in irrigated plants, a midday depression occurs in the most exposed leaves of the canopy, but not in shaded leaves. Leaves exposed to constant high light, temperature and vapor pressure deficit had a maximum stomatal conductance 1 h after illumination, which declined thereafter (Correia *et al.* 1990; Lu *et al.* 2003). Also, using remote sensing of chlorophyll fluorescence, Flexas *et al.* (2000) showed that the quantum efficiency of PSII was lower during the afternoon than during the morning at any given light intensity. All these observations suggest a possible involvement of photoinhibition. Indeed, the maximum quantum efficiency of PSII (F_v/F_m) declines slightly at midday, although typically less than 20% (Quick *et al.* 1992; Bertamini and Nedunchezian 2004). Bertamini and Nedunchezian (2004) reported that this decline was initially (2 h after high light exposure) associated with a decrease in the concentration of the D1 protein (the core protein of PSII components), and later (4 h after high light exposure) with a decline of the 33KDa protein (the water-splitting complex) while D1 was recovered, suggesting that both acceptor side and donor side photoinhibition are involved in midday depression of photosynthesis. However, in other cases F_v/F_m declines somewhat, it correlates better with the accumulation of de-epoxidated xanthophylls than with degradation of D1 protein (Chaumont *et al.* 1995). The mechanism of repair of D1 is very effective in grapevines; in its presence, the fraction of functional PSII is kept higher than 50% (and F_v/F_m between 0.6 and 0.8) even exposing leaves to photon exposures higher than those of a normal sunny day. Conversely, when the D1 repair is blocked with lincomycin the fraction of functional PSII and F_v/F_m rapidly decline to zero with photon exposures similar to those received by a grapevine at midday (Flexas *et al.* 2001). Moreover, in other experiments, F_v/F_m remained constant during the day (Iacono and Sommer 1996; de Souza *et al.* 2003), and there was no degradation of chlorophyll (Medrano *et al.* 2002a), suggesting that effective photoprotection is associated with midday

depression of photosynthesis. Chlorophyll fluorescence data corroborate this view, showing midday to afternoon decline in the steady-state fluorescence emission and a constant or slightly declined in the effective quantum efficiency of PSII (Φ_{PSII}) and rate of electron transport (ETR) (Correia *et al.* 1990; Flexas *et al.* 2000). Meanwhile, non-photochemical quenching parameters (q_P , NPQ) largely increase and the photochemical reflectance index (PRI) decreases (Evain *et al.* 2004). These changes are associated with increased trans-thylakoid ΔpH and de-epoxidation of xanthophylls, reflecting safe thermal energy dissipation (Düring 1999; Medrano *et al.* 2002a; Evain *et al.* 2004). The rate of photorespiration increases or is kept constant in the afternoon in irrigated plants (Iacono and Sommer 1996; Flexas *et al.* 1999a, 2000), while the foliar pools of ascorbate and glutathione slightly increase, and their oxidised forms increase over the reduced forms (Chaumont *et al.* 1995). Together these mechanisms confer an effective photoprotection to grapevines leaves during the midday depression of photosynthesis.

Alternatively, a phloem-based feedback signal could be involved in the regulation of the balance between source and sinks activities, leading to an afternoon decline of photosynthesis (Quereix *et al.* 2001). This would be supported by the observed accumulation of sucrose and starch in leaves during the course of the day (Chaumont *et al.* 1994), although in other experiments they rather remain constant or even decrease (Quick *et al.* 1992). Alternatively, a decrease in mesophyll conductance to CO₂ (g_m) concomitant to decreased g_s , as observed by Moutinho-Pereira *et al.* (2004) in three different grapevine cultivars, may result in a further decrease in chloroplast CO₂ availability, explaining the non-stomatal component of photosynthesis reductions.

Regulation of leaf water potential: isohydric and anisohydric cultivars

In the next section we provide a generalised view of grapevine leaf responses to water stress.

When soil water stress combines with high atmospheric water demand, reductions in leaf water potential and gas exchange become larger and longer-lasting (Liu *et al.* 1978; Flexas *et al.* 1998; Schultz 2003a; Pou *et al.* 2008). Based on their water potential behaviour in response to water stress, grapevine cultivars have been classified as isohydric or anisohydric (Schultz 2003a; Vandeleur *et al.* 2009). Isohydric cultivars are those that keep their leaf water potential above a certain threshold regardless of soil water availability or atmospheric water demand. Anisohydric cultivars are those in which leaf water potential drops with decreasing soil water availability or increasing atmospheric water demand (Fig. 1a). In isohydric grapevines, leaf water potential rarely drops below -1.5 MPa (Fig. 1a). This is close to the threshold for severe cavitation in this species (Salleo and Lo Gullo 1989; Lovisolo *et al.* 2008a), although some cavitation occurs at lower water potentials in petioles, shoot nodes and internodes and roots (Schultz and Matthews 1988; Salleo and Lo Gullo 1989; Schultz 2003a; Lovisolo *et al.* 2008a). According to observations in other isohydric species as laurels (*Laurus nobilis* L.) (Salleo *et al.* 2000), it may be argued that these cultivars present a fine co-regulation of g_s and leaf hydraulic conductivity, allowing them to avoid cavitation. Grapevines typically reported as isohydric include the hybrid species

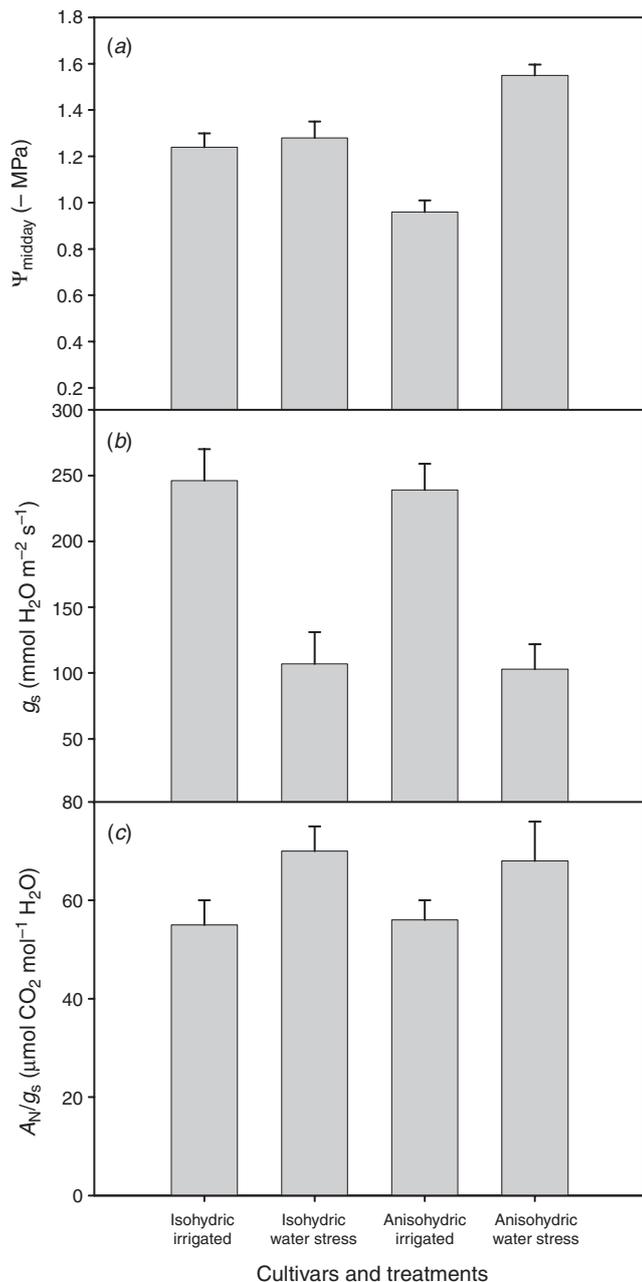


Fig. 1. Average midday (a) leaf water potential, (b) stomatal conductance and (c) intrinsic water use efficiency in isohydric and anisohydric grapevine species or cultivars fully irrigated or subjected to some degree of water stress. Data + s.e. Data from a literature survey including the following references: Baigorri *et al.* (2001), Bota *et al.* (2001), Chaves *et al.* (2007), Correia *et al.* (1990, 1995), Dobrowski *et al.* (2005), Downton *et al.* (1987), Escalona *et al.* (2003), Flexas *et al.* (1999b, 2009), Gamon and Pearcy (1990), Gómez-del-Campo *et al.* (2004), Liu *et al.* (1978), Maroco *et al.* (2002), Moutinho-Pereira *et al.* (2004), Naor and Wample (1994), Naor *et al.* (1994), Padgett-Johnson *et al.* (2000), Patakas *et al.* (2005), Poni *et al.* (1993), Pou *et al.* (2008), Quick *et al.* (1992), Rodrigues *et al.* (1993, 2008), Satisha *et al.* (2006), Schultz (2003a), Sivilotti *et al.* (2005), de Souza *et al.* (2005a, 2005b), Winkel and Rambal (1993), Zufferey *et al.* (2000).

V. labruscana (*Vitis labrusca* × *V. vinifera*) and the rootstock Richter-110 (*Vitis berlandieri* Planch. × *Vitis rupestris* Scheele), widely used *V. vinifera* cultivars such as Grenache, Trincadeira Preta and Tempranillo, as well as some cultivars native to dry viticultural areas. Anisohydric cultivars, by contrast, drop leaf water potential through the day as a function of soil water deficit (Fig. 1a), which is often achieved by means of osmotic adjustment (Downton 1983; Düring 1984; Schultz and Matthews 1993; Patakas and Noitsakis 1997), although may also be through changes in cell wall elasticity (Patakas and Noitsakis 1997). Grapevines typically reported as anisohydric include the species *V. labrusca* and *Vitis californica* Benth., as well as many *V. vinifera* cultivars, including Chardonnay, Cabernet Franc, Cabernet Sauvignon, Syrah, Riesling, Carignan, Muscat, Thomson seedless, Touriga Nacional, as it appears from a literature survey (references in the legend of Fig. 1).

Although it has been suggested that differences between iso- and anisohydric cultivars may include different stomatal responses and water use (Schultz 2003a), on average, they show similar decreases in g_s (Fig. 1b) and A_N (data not shown) in response to water stress. As a consequence, both iso- and anisohydric cultivars present similar values of leaf intrinsic water-use-efficiency (A_N/g_s) both under irrigation and under water stress (Fig. 1c). Indeed, differences among grapevine cultivars in water use efficiency (WUE) have been reported, based on instantaneous gas-exchange data (Gómez-del-Campo *et al.* 2004), isotopic composition ($^{13}\text{C}/^{12}\text{C}$) of leaf and/or fruit dry matter (Gibberd *et al.* 2001; Gaudillère *et al.* 2002) or biomass accumulation per unit of water used (Gibberd *et al.* 2001). However, no clear pattern of correlation is observed in these studies between WUE and the iso- or anisohydric character of the studied cultivars.

Moreover, the same cultivar can behave as iso- or anisohydric, depending on the conditions. For instance, *V. labruscana* was reported to be anisohydric by Liu *et al.* (1978), but as isohydric by Naor and Wample (1994). Pinot Noir behaves as anisohydric when water stress is applied *pre-véraison* and as isohydric when it is applied *post-véraison* (Poni *et al.* 1993). In contrast, during most of the growing season (June–July), Tempranillo and Manto Negro are often reported as isohydric (Flexas *et al.* 1998; Medrano *et al.* 2003), but later in the season (August), however, they behave as iso- or anisohydric, depending on the year (J. Flexas and H. Medrano, unpubl. data). However, the cavitation threshold of -1.5 MPa described for grapevines (Salleo and Lo Gullo 1989) is rarely and barely reached even during the ‘anisohydric years’, suggesting that grapevines are effective in avoiding catastrophic cavitation.

Soil water stress and mechanisms of stomatal closure

Stomatal closure is one of the first processes that occurs in the leaves in response to soil water stress. Many signals are involved in sensing environmental variations during soil drought-induced stomatal closure. They are related more to ABA metabolism, hydraulic signals (cavitation), regulation of expression and activity of aquaporins, and/or electric signals, than changes in leaf water status, measured as leaf water potential. Indeed, with some exceptions, stem water potential has been described as a more preferable indicator of grapevine water status than leaf

water potential (Choné *et al.* 2001; Intrigliolo and Castel 2006; Williams and Baeza 2007), and g_s often correlated better with leaf water potential determined at pre-dawn ($\Psi_{\text{pre-dawn}}$) than Ψ_{midday} (Winkel and Rambal 1990; Schultz 2003b; Intrigliolo and Castel 2006). This is also observed in the survey of iso- and anisohydric cultivars used in Fig. 1 (Fig. 2). Although a significant correlation is found when plotting midday g_s against $\Psi_{\text{pre-dawn}}$, pooling all the data together (Fig. 2a), no clear relationship is observed between g_s and Ψ_{midday} (Fig. 2b), even if only anisohydric cultivars (triangles) are considered. These observations suggest that g_s in grapevines decreases under water stress in response to some root- or stem-based signal(s). Indeed, this is the basis of the partial root drying technique used to reduce vegetative growth and water use and improve WUE in grapevines (Dry and Loveys 1998; Chaves *et al.* 2007).

Grapevines were among the first plant species in which a direct role of ABA in stomatal closure was demonstrated (Loveys and Kriedemann 1974; Liu *et al.* 1978; Loveys

1984a, 1984b). In different grapevine genotypes during the gradual imposition of soil water stress (non-irrigation) or partial root drying, tight negative correlations are often observed between g_s and either xylem (Pou *et al.* 2008; Rodrigues *et al.* 2008) or leaf tissue (Loveys and Kriedemann 1974; Liu *et al.* 1978; Lovisolo *et al.* 2002a) ABA contents. 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 mediates most of the stomatal response in grapevines, although a reverse transport, i.e. from leaves to roots has also been shown (Loveys 1984b). However, gradients in both xylem and leaf ABA along shoots of grapevines, from higher concentrations close to the apex to lower concentrations downwards have been observed (Soar *et al.* 2004). These gradients, which resulted in a gradient of g_s among leaves with different positions along the shoot, are inconsistent with the concept of root-derived ABA. Analysing the patterns of expression along the shoots of two genes involved in ABA synthesis, Soar *et al.* (2004) concluded that

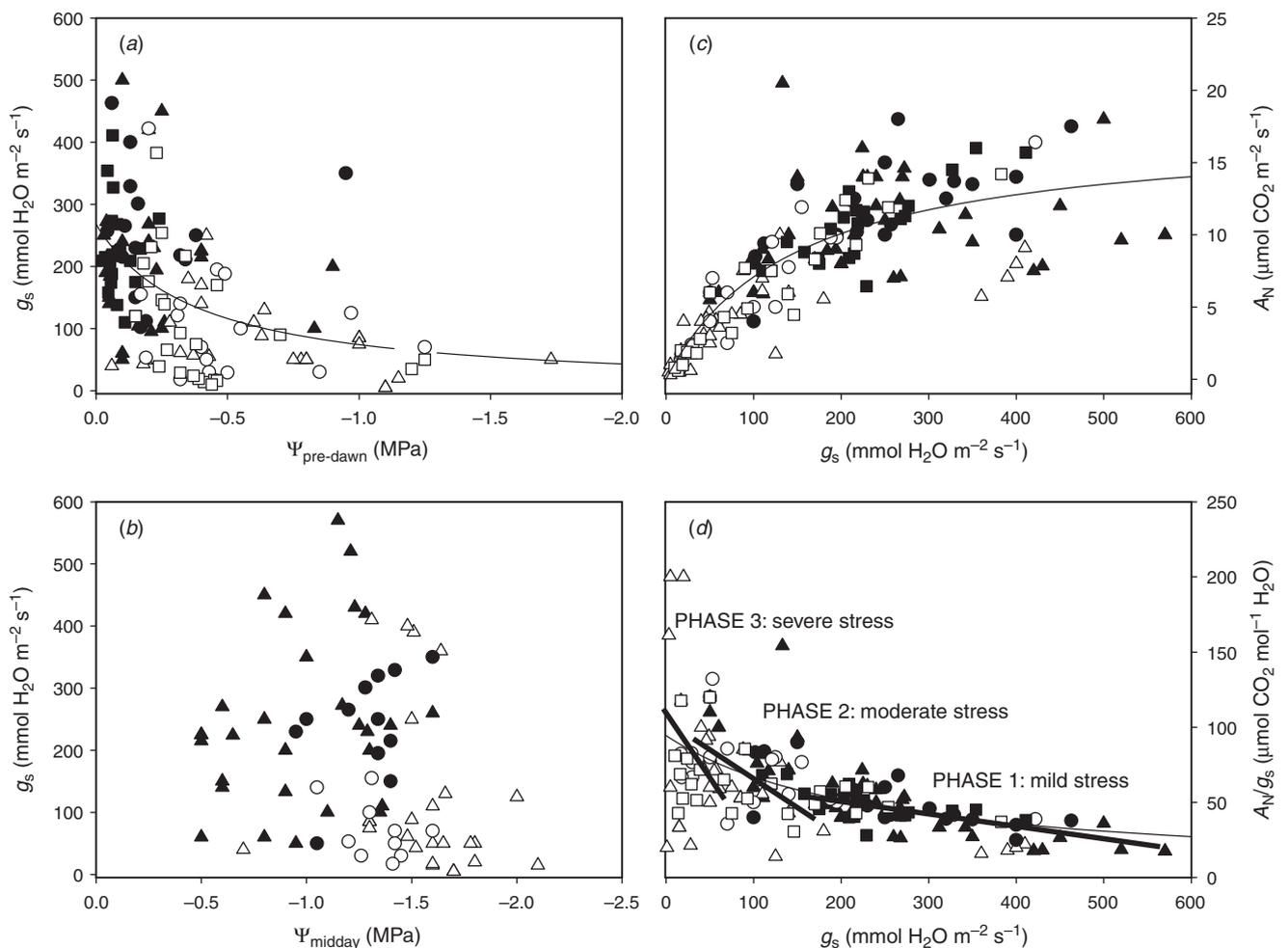


Fig. 2. Some relationships among leaf parameters in isohydric (circles) and anisohydric (triangles) grapevine species or cultivars under irrigation (closed symbols) or subjected to some degree of water stress (open symbols). The shown relationships are between stomatal conductance and (a) pre-dawn and (b) midday leaf water potential, (c) net photosynthesis and (d) intrinsic water use efficiency. Data from same references as Fig. 1.

the observed differences in ABA were due to differences in the *in situ* synthesis of ABA in shoots and leaves. Soar *et al.* (2006) further confirmed that regulation of gene expression in response to increased vapour pressure deficit in leaf tissue and not in roots was associated with the higher xylem ABA concentrations found in Grenache than in Syrah. In *Arabidopsis*, it has been clearly demonstrated that g_s response to soil water stress is due to increased ABA synthesis in the shoots, not in the roots (Christmann *et al.* 2007). The observations of Soar *et al.* (2004, 2006), although not conducted on water stressed plants, suggest that this may also be the case in grapevines.

That ABA synthesis in the shoots and leaves increases in response to soil water stress implies that some other root-based signal may trigger this response. Other hormonal signals have been suggested, such as phaseic acid (Loveys and Kriedemann 1974) or cytokinins (Stoll *et al.* 2000), as well as the influence of xylem sap pH (Rodrigues *et al.* 2008), but no such roles have been confirmed. In contrast, in *Arabidopsis* the signal triggering an increase of shoot ABA synthesis has been shown to be of hydraulic nature (Christmann *et al.* 2007). Furthermore, there is a gradient of hydraulic conductivity in nodes and internodes along grapevine shoots, similar to the gradient described for ABA synthesis (Salleo *et al.* 1982, 1985; Lovisolo and Schubert 1998). In internodes, both xylem hydraulic conductivity (K_h) and leaf specific conductivity (LSC) are lower in the most apical parts, but in lower internodes they are similar (Salleo *et al.* 1982, 1985) or increase slightly from the shoot base to intermediate shoot positions, thereafter declining towards the apex (Lovisolo and Schubert 1998). In the nodes, LSC is often lower than in internodes and continuously declines from the base to the apex (Salleo *et al.* 1982). This distribution of hydraulic conductivity could explain why apical leaves synthesize more ABA and have lower g_s (Soar *et al.* 2004). There is evidence that hydraulic conductivity of roots, shoot nodes and internodes and petioles, as well as whole-plant conductivity decline in grapevines subjected to water stress (Schultz and Matthews 1988; Salleo and Lo Gullo 1989; Winkel and Rambal 1993; Lovisolo and Schubert 1998, 2006; Lovisolo *et al.* 2002a, 2008a, 2008b; Schultz 2003a; Pou *et al.* 2008). This decline can be due to water stress-induced changes in xylem development (Mapfumo *et al.* 1993; Mapfumo and Aspinall 1994), but most often occurs by means of drought-induced cavitation (Schultz and Matthews 1988; Salleo and Lo Gullo 1989; Schultz 2003a). Roots and leaf petioles appear to be more sensitive than shoots to drought-induced cavitation (Schultz 2003a; Lovisolo *et al.* 2008a), although in the shoots, internodes are much more sensitive than nodes (Salleo and Lo Gullo 1989). In addition to cavitation, aquaporins have been suggested to play a role in the regulation of hydraulic conductivity in grapevines. All known aquaporins in grapevines are much more abundant in roots than in any other tissue, although they are still present in shoots and leaves. Experiments of gene expression during drought and recovery in Richter-110 have shown that several PIP and TIP aquaporins are upregulated at early stages of water stress in roots, while they are mostly downregulated in leaves (Galmés *et al.* 2007). In contrast, after re-watering stressed plants, most aquaporins are upregulated in leaves but not in roots (Galmés *et al.* 2007). Using mercury as an inhibitor of the activity of some

aquaporins, it has been suggested that aquaporins are involved in the recovery after water stress of shoot (Lovisolo and Schubert 2006) and root (Lovisolo *et al.* 2008b) hydraulic conductivity. Using this technique it has been shown that in drought-resistant rootstocks, aquaporin-regulated decrease of K_h is more important than cavitation, the opposite being true for drought-sensitive rootstocks. Moreover, it has been suggested that a reduced transpiration induced by ABA after re-watering promotes aquaporin-mediated embolism repair after water stress (Lovisolo and Schubert 2006; Lovisolo *et al.* 2008a).

Regardless of the mechanism for its regulation, whole-plant hydraulic conductivity often correlates well with g_s during drought imposition (Winkel and Rambal 1993; Schultz 2003a; Pou *et al.* 2008), and much better during recovery after water stress (Lovisolo *et al.* 2008a; Pou *et al.* 2008), suggesting that g_s may be regulated by hydraulic signals during water stress. However, in a factorial experiment involving water stress, partial root drying and downwards shoot position, Lovisolo *et al.* (2002a) presented clear evidence that it was leaf ABA and not whole-plant hydraulic conductivity that determines g_s in grapevines. Further work is required to understand the role of hydraulics on stomatal regulation in grapevines.

Alternatively, electrical signalling could be involved in stomatal regulation in grapevines, as suggested in other species (Fromm and Fei 1998; Grams *et al.* 2007). In grapevines, the two types of wound-induced electrical signals, namely variation potentials (VPs) and action potentials (APs), propagate very fast ($\sim 3 \text{ mm s}^{-1}$ and 100 mm s^{-1} for VPs and APs, respectively) in leaf tissues (Mancuso 1999). Preliminary data (J. Fromm and J. Flexas, unpubl. data) showed that re-watering water stressed grapevine plants generates APs that are transmitted very quickly (less than 10 s) from roots to leaves, similar to the observations in *Zea mays*, where the electrical rather than the hydraulic signal was shown to induce stomatal re-aperture (Grams *et al.* 2007).

Gradual downregulation of photosynthesis under soil water stress

As in other species, a tight curvilinear correlation between g_s and A_N has been described in grapevines (Flexas *et al.* 2002a). This relationship is also observed when pooling together all data of the survey described in previous sections (Fig. 2c). The observed dispersion may depend on temperature and atmospheric vapour pressure deficit (Zufferey *et al.* 2000), leaf age (Zufferey *et al.* 2000) and time of the day or season (Gómez-del-Campo *et al.* 2004; Cuevas *et al.* 2006), but primarily due to intrinsic differences among cultivars (Düring 1987; Bota *et al.* 2001).

Due to the tightness of this correlation and to the fact that intrinsic WUE (i.e. photosynthesis to g_s ratio) increases as g_s decreases (Fig. 2d), it is often assumed that the drought-induced decrease of photosynthesis is mediated by stomatal closure. Indeed, under mild water stress this may be the case. Using the daily maximum value of g_s as an indicator of water stress that allows comparison of plants with iso- and anisohydric behaviours, Flexas *et al.* (2002b) and Medrano *et al.* (2002b) defined several stages of photosynthesis regulation in grapevines subjected to progressive soil water stress on the

basis of the main causes of A_N decline. These stages are general regardless of the cultivar under study and are as follows:

- (1) Stage 1. Mild water stress: g_s decreases from a maximum (typically 200–500 mmol H₂O m⁻² s⁻¹ in grapevines) to 150 mmol H₂O m⁻² s⁻¹.
- (2) Stage 2. Moderate water stress (or transition stage): g_s ranges between 50 and 150 mmol H₂O m⁻² s⁻¹.
- (3) Stage 3. Severe water stress: g_s drops below 50 mmol H₂O m⁻² s⁻¹.

At Stage 1, the effects of water stress consist in a relatively small decline of A_N , which results in a progressive increase of intrinsic WUE (Fig. 2d) and a slight decline of substomatal CO₂ concentration (C_i). As a consequence of decreased CO₂ availability in the mesophyll, the rate of photorespiration increases somewhat (Flexas *et al.* 1999a, 1999b; de Souza *et al.* 2005b). Under these conditions there is no increase in photoprotection mechanisms or heat energy dissipation (Flexas *et al.* 2002a; Medrano *et al.* 2002a) and there is no effect on parameters reflecting photosynthetic capacity, such as F_v/F_m (Flexas *et al.* 1998; de Souza *et al.* 2003, 2005b), ETR (Düring 1998; de Souza *et al.* 2003, 2005b) or the apparent maximum capacity of carboxylation (V_{c,max,C_i}) or for electron transport (J_{max,C_i}) both derived from A_N - C_i curves (de Souza *et al.* 2003). There is also no inhibition of photosynthetic enzymes such as Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) (Bota *et al.* 2004b), glyceraldehydes-3-phosphate dehydrogenase (G3PDH), ribulose-5-phosphatase kinase (Ru5PK) or fructose-1,6-biphosphate phosphatase (FruBPase) (de Souza *et al.* 2005b). Therefore, at this stage, diffusional limitations are the only cause for decreased photosynthesis. These are mostly due to stomatal closure, although if the stage is prolonged, mesophyll conductance to CO₂ also starts declining, contributing to restricted CO₂ diffusion (Flexas *et al.* 2002a; Moutinho-Pereira *et al.* 2004), particularly under conditions of high irradiance and temperature (Flexas *et al.* 2009).

Stage 2 represents a transition phase between predominant stomatal to non-stomatal limitations, and occurs invariably when g_s ranges between 50 and 150 mmol H₂O m⁻² s⁻¹. During this phase a further reduction in A_N occurs and WUE usually increases to reach maximum levels, and C_i decreases to minimum levels (Flexas *et al.* 2002a; Maroco *et al.* 2002). This indicates that stomatal limitations still dominate, although in some cultivars WUE starts decreasing at this stage, indicating predominant non-stomatal limitations (e.g. Naor *et al.* 1994). For instance, V_{c,max,C_i} decreases sometimes, suggesting impairment of Rubisco, which has been shown to decrease its activity (along with activities of G3PDH, Ru5PK and FruBPase) at this stage in some experiments (Maroco *et al.* 2002). In other experiments, however, Rubisco activity is unaffected (Bota *et al.* 2004b) and decreases of V_{c,max,C_i} are due to decreased g_m , since when it is calculated on a chloroplast CO₂ (C_c) rather than a C_i basis (V_{c,max,C_c}) it remains unaffected by stress (Flexas *et al.* 2002a). F_v/F_m remains unaffected (Flexas *et al.* 1998). There is also no net feedback inhibition of photosynthesis by sugar accumulation (Maroco *et al.* 2002). Instead, ETR characteristically declines during this phase (Flexas *et al.* 1999a, 1999b, 2002b), concomitant with increased NPQ,

decreased PRI and decreased steady-state chlorophyll fluorescence (F_s) under high light (Flexas *et al.* 2000; Medrano *et al.* 2002a; Evain *et al.* 2004). These changes do not represent permanent damage to photosystems, but rather, they are dynamic and reverse immediately upon shading, e.g. during the passage of a cloud (Evain *et al.* 2004). As a consequence of both diffusional (i.e. reduced CO₂ availability) and biochemical (i.e. impaired Rubisco, decreased ETR) limitations, the rate of photorespiration often declines at this stage back to values similar to those of non-stressed plants (Guan *et al.* 2004).

Stage 3, when g_s drops below 50 mmol H₂O m⁻² s⁻¹, results in more generalised and dominant non-stomatal limitations to photosynthesis, particularly under conditions where water stress is accompanied by very high temperature and irradiance. This is demonstrated by the fact that photosynthesis cannot be restored even using very high CO₂ concentrations (Quick *et al.* 1992; Flexas *et al.* 1999b), and there is a drought-induced depression of gross oxygen evolution as seen using O₂ isotope discrimination in a mass spectrometer (Flexas *et al.* 1999b). At this stage, steeper reductions of A_N , ETR and V_{c,max,C_i} occur and NPQ further increases. During this phase A_N/g_s often decreases and C_i increases steeply (Düring 1987; Flexas *et al.* 2002a; Sivilotti *et al.* 2005), indicating that non-stomatal limitations to photosynthesis become dominant. Nevertheless, this is not always observed (see e.g. Fig. 2d). The rate of photorespiration is decreased, but the ratio of photorespiration to photosynthesis still increases, maintaining ETR relatively high with respect to A_N (Flexas *et al.* 1999a, 1999b; de Souza *et al.* 2005b). Under these conditions, changes in the expression of genes and proteins associated with stomatal functioning and photosynthesis occur (Cramer *et al.* 2007; Vincent *et al.* 2007).

Even under these conditions, F_v/F_m is extremely resistant to water stress (Gamon and Pearcy 1990; Flexas *et al.* 1998). Bertamini *et al.* (2007) showed that severe water stress does not affect the concentration of the D1 protein and reduces only slightly the electron transport capacities of different components of the electron transport chain. Also, decreases of V_{c,max,C_i} are sometimes due only to decreased g_m (Flexas *et al.* 2002a) and to errors in the calculation of C_i , as demonstrated by constant V_{c,max,C_c} . The errors in the calculation of C_i are due to the interference of heterogeneous stomatal closure, which has been shown to occur in grapevine leaves fed with ABA (Downton *et al.* 1988a) or subject to water stress (Downton *et al.* 1988b) or salinity (Downton *et al.* 1990). These errors become important (>10% error in the calculation of C_i) when average g_s drops below 30 mmol H₂O m⁻² s⁻¹ (Flexas *et al.* 2002a, 2009).

In many other conditions, however, Rubisco activity is truly impaired (Bota *et al.* 2004b), mostly due to decreases in its concentration (Bota *et al.* 2004b; Bertamini *et al.* 2006) and, to a lesser extent, to decreased activation state (Bota *et al.* 2004b). Moreover, in some cultivars there is a large decrease in the capacity for photoassimilate export out of the leaves (Bota *et al.* 2004a) resulting in accumulation of soluble sugars in leaves, which can induce feedback inhibition of photosynthesis. In some other cultivars and/or conditions, however, the decrease in the capacity for photoassimilate export is minor and sugars do not accumulate as a consequence of severe water stress (Quick *et al.* 1992).

Recovery of photosynthesis after water stress

The carbon balance of a plant during a period of water stress and recovery may depend as much on the velocity and degree of photosynthetic recovery as on the degree and velocity of photosynthesis decline during water depletion (Flexas *et al.* 2006). Relatively few studies have addressed the rate and limiting factors for the recovery of grapevine leaves after water stress. Still, there are some indications suggesting that previous water stress intensity is a crucial factor affecting both the velocity and the extent of recovery after re-watering. For instance, Tempranillo grapevines subjected to 'Stage 2' water stress (i.e. maximum stomatal conductance among 0.1–0.15 mol H₂O m⁻² s⁻¹) recovered completely overnight after re-watering, but 'Stage 3' plants of the same cultivar recovered only slowly during the next week, and did not reach the maximum photosynthesis rates reached before water stress (Flexas *et al.* 2009).

In addition to stress severity, differences associated with both cultivar and environmental conditions may affect recovery. For instance, in cultivars Airén and Chardonnay, g_s and A_N recovered completely but slowly (3–5 days to complete recovery) after a 'Stage 2' water stress. Plants of the rootstock Richter-110 recovered slowly (2 weeks for complete recovery) even after a 'Stage 1' water stress (Pou *et al.* 2008; Flexas *et al.* 2009). In contrast, several cultivars and rootstocks including Cabernet Sauvignon showed almost complete recovery 2 days after rewatering of 'Stage 3' water stressed plants (Guan *et al.* 2004), similar to 'Stage 3' *V. labruscana* (Liu *et al.* 1978). Furthermore, g_m , Rubisco and ETR recover quickly (1–3 days) after re-watering, although g_s remains lower for longer, becoming the most limiting factor for photosynthesis recovery (Flexas *et al.* 2009).

The reasons for sustained low g_s are unclear. Both in cases of rapid recovery of photosynthesis and those in which photosynthesis lasts few days after re-watering, leaf water relations recover fast after re-watering, being totally reversed the day after (Lovisolo *et al.* 2008a; Pou *et al.* 2008). In contrast, free *cis-trans* ABA also recovers control values quickly (1–3 days after re-watering), although hydrolyzable *cis-trans* ABA and phaseic acid remain high for longer (Liu *et al.* 1978; Pou *et al.* 2008). Hydraulic conductivity recovers quickly (1 day) in some experiments (Lovisolo and Schubert 2006) but slowly in others (Pou *et al.* 2008). Lovisolo *et al.* (2008a) proposed that ABA-induced g_s decrease allows embolism repair during the day after re-watering, but this mechanism may not work in cases where ABA is fully reversed in few days while conductivity and g_s are not (Pou *et al.* 2008). Clearly more studies are required to understand the dynamics and mechanisms involved in recovery of grapevine cultivars after water stress.

Cross-talk between water stress responses and berry growth and ripening processes

Following historical linkages between research on grapevine physiology and viticulture techniques, there is a wide and heterogeneous literature related to drought effects on grape berry development, as reviewed by Ollat *et al.* (2002) and Keller (2005). Knowledge of the phenomenon focuses on

(i) effects of an impaired plant metabolism (especially photosynthesis and transpiration) on the accumulation of sugars and secondary metabolites in berry, (ii) consequences at the berry level of both the chemically-mediated long distance signalling between root and shoot (essentially ABA and cytokinin) and the whole-plant hydraulic control via both the xylem and the phloem from root to berry and (iii) berry metabolism adaptations to severe osmotic stress in berry cells.

Water influx into fruits occurs through xylem and phloem and during ripening water flow via the xylem markedly decreases from the onset of *véraison* when the main source of water to berries becomes the phloem sap. Evidence for ceased xylem functionality in berries has been reported from studies of apoplastic dye perfusion through the pedicel (Greenspan *et al.* 1996; Rogiers *et al.* 2001; Bondada *et al.* 2005; Keller *et al.* 2006; Tilbrook and Tyerman 2009) even though some studies have been doubtful about the validity of the interpretation of dye uptake experiments (Tyerman *et al.* 2004). Nevertheless, the hydraulic isolation of the berry due to development of a xylem discontinuity in the pedicel or inside the berry has been considered a way to prevent the loss of solutes in berries (Sarry *et al.* 2004). Keller *et al.* (2006) have demonstrated that berry xylem functionality is retained during ripening; the detected decline in xylem water influx into ripening grape berries is due to the apoplastic phloem unloading coupled with solute accumulation in the berry apoplast. More recently, Tilbrook and Tyerman (2009) showed that, according to varietal differences, the activity of semi permeable membranes in mesocarp cells is the key controller of flow in to and out of the berry, dictated by xylem flow. As drought lowers leaf water potential by concentrating osmolites in leaf, drought effects drive water flows between leaf and berry.

Drought effects on accumulation of secondary metabolites in berry were thoroughly investigated since the 1980s. Several studies have measured the effects of water stress on polyphenols (Kennedy *et al.* 2002; Ojeda *et al.* 2002; Koundouras *et al.* 2006; Pedreira dos Santos *et al.* 2007; Poni *et al.* 2007) and flavors (Oliveira *et al.* 2003; Koundouras *et al.* 2006; Bindon *et al.* 2007; Pedreira dos Santos *et al.* 2007). Matthews and Anderson (1988) stated that in the anisohydric Cabernet Franc berry, skin polyphenol and anthocyanin concentrations increased as a consequence of berry volume reduction due to water stress, even though, also expressing data on the basis of surface area, an increase in polyphenol concentration was detected. Roby *et al.* (2004) found that the concentration of polyphenols such as anthocyanins and proanthocyanidins of berry skins increased after water stress conditions independently from the differences in berry size due to water availability. There was still uncertainty in whether the increases in skin anthocyanins and proanthocyanidins resulted from a differential growth of exocarp cells compared with pulp cells rather than direct effects on phenolic biosynthesis. Recent researches on anisohydric Cabernet Sauvignon (Castellarin *et al.* 2007a) showed that the expression of some enzymes of the phenylpropanoid biosynthetic pathway increased as a consequence of early and late water stress application, and also, that this increase was more effective on the biosynthesis of tri-hydroxylated anthocyanins. Early applied water stress was

also proved to accelerate the accumulation of sugar and the onset of ripening.

Consequences of chemically(ABA)-mediated signalling and the hydraulic control between root and shoot at the berry level

ABA production in drying roots and its translocation and accumulation in the shoot drive several physiological mechanisms in grapevine leaves (Lovisolo *et al.* 2002a, 2008a), as reviewed above. In grape berries, ABA is considered a promoter of ripening as its concentration increases at the beginning of *véraison* (Gagné *et al.* 2006; Wheeler *et al.* 2009). Exogenous ABA, used to promote ripening in berries (Peppi *et al.* 2006; Cantin *et al.* 2007; Peppi and Fidelibus 2008) induces the accumulation of the mRNA of *VvmybA1*, a regulatory gene of anthocyanin biosynthesis of grape, leading to an increase of anthocyanin accumulation in Cabernet Sauvignon berry skins (Jeong *et al.* 2004). ABA also activates invertases (Pan *et al.* 2005) and *VvHT1* (*V. vinifera* hexose transporter 1) (Çakir *et al.* 2003). The effect of ABA on UFGT (UDPglucose:flavonoid 3-*O*-glucosyltransferase), a key-enzyme of the flavonoid biosynthesis, has recently been proved, as well as its influence on the quality of color, the skins of ABA treated Crimson Seedless berries having lower lightness and hue than ABA-untreated control plants (Peppi *et al.* 2008).

ABA application has little or no effect on berry sugar content at harvest (Jeong *et al.* 2004; Peppi *et al.* 2006). The accumulation of sugars in berries requires the coordinated expression of sucrose transporters, invertases, and monosaccharide transporters. The expression of the glucose transporter homologue (*VvHT1*, *V. vinifera* hexose transporter 1), isolated from grape berries at *véraison*, is regulated by sugars and ABA (Vignault *et al.* 2005). Phloem influx into the berry is accompanied by a decrease in cell turgor (Thomas *et al.* 2006), which influences the expression of many genes at the onset of ripening (Deluc *et al.* 2006). Water stress, lowering cell turgor, particularly if applied *pre-véraison* (Thomas *et al.* 2006), may induce an increase in sugar influx and ABA, influencing several key steps of the phenylpropanoid biosynthetic pathway. In particular, the expression of genes F3H, F3'5'H, LDOX and DFR involved in the biosynthesis of anthocyanins, proanthocyanidins and flavonols, increases in water-deficit conditions (Castellarin *et al.* 2007a, 2007b). The increase in total anthocyanins results predominantly from an increase of the F3'5'H expression, responsible for the biosynthesis of tri-hydroxylated anthocyanins. During ripening, the cumulative expression of genes strictly associated to the anthocyanin accumulation UFGT and GST (the latter probably playing a role in the transport of anthocyanins into the vacuole (Ageorges *et al.* 2006)), is strongly upregulated under water stress conditions (Castellarin *et al.* 2007a).

The ripening process *per se* is not accelerated by water deficit in Cabernet Sauvignon or Merlot, since no changes in timing of berry growth and sugar accumulation occur after water deficit (Castellarin *et al.* 2007a). Furthermore, no earlier downregulation of the genes responsible for proanthocyanidin biosynthesis, which is expected when an early ripening occurs,

is detected when water deficit is applied (Castellarin *et al.* 2007b). The effects of early or late water stress on proanthocyanidin and flavonol concentration and on the expression of genes responsible for their synthesis are still unclear.

Myb transcription factors MybA and Myb5a are higher in water-stressed vines respect to control vines in Merlot, as well as MybC which is upregulated from *post-véraison* to harvest; MybB and MybD are not influenced by water availability (Castellarin *et al.* 2007b). In conditions where the accumulation of ABA is higher, the m-RNAs of *VvmybA1* are also higher (Yamane *et al.* 2006); these results suggest that the endogenous ABA level affects the expression of *VvmybA1* that controls the expression of the anthocyanin biosynthetic enzyme genes. In Merlot berry skins, the genes *NCED1* and *NCED2* are only transiently upregulated (Castellarin *et al.* 2007b) at the onset of *véraison* after water stress. Moreover, *ACPK1* and *rd22*, both correlated with the ABA metabolism, the former in grape berries (Yu *et al.* 2006), the latter in other species (Iwasaki *et al.* 1995), are not at all or only partially upregulated in the experiments reported by Castellarin *et al.* (2007a, 2007b).

The root and shoot ABA-mediated responses to water stress conditions, or, more generally, to abiotic stresses, are relevant to vine productivity and yield. As described earlier, water stress influences ABA accumulation at the root, shoot and leaf level, and also affects berry quality (Keller 2005). However, a connection between ABA and berry quality has not yet been clarified.

Cytokinin signalling downregulation in ripening berries may represent another point of cross-talk between water stress responses and developmentally-driven ripening processes. Cytokinin concentration sharply decreases at the onset of ripening (Alleweldt *et al.* 1975), and a zeatin *O*-glucosyltransferase (Zhang *et al.* 2008), as well as a gene encoding a cytokinin synthase (Carra *et al.* 2009), are downregulated in mature berries. Since the repression of cytokinin metabolism is a known response to water deficit in grapevine shoots (Stoll *et al.* 2000), the relative abundance of ABA and cytokinin may be regulated in the berries by water stress-like signals, and may be important for the coordination of the ripening process. However, direct evidence of drought-induced repression of cytokinin metabolism in berries is still lacking.

High sugar accumulation in ripening grapes generates a severe osmotic stress in berry cells and is accompanied by the expression of thaumatin-like proteins similar to osmotins (Tattersall *et al.* 1997; Salzman *et al.* 1998; Davies and Robinson 2000). Osmotins are induced by ABA, thus, it could be that ABA and high sugar concentration may interact to trigger in the berry a defence response, which may also be triggered by water stress. Supporting this idea, transcripts of genes associated with responses to pathogens were shown to be significantly upregulated in the skin of water-stressed plants compared with well watered controls (Grimplet *et al.* 2007). ABA and sugar signals converge in the induction of *VvMSA*, an ASR (ABA-, stress- and ripening-induced) protein that acts as a transcriptional activator of the hexose transporter *VvHT1* (Çakir *et al.* 2003). ASR proteins are also induced by drought and salt stress, further supporting the idea that processes characterising berry development and ripening overlap with water stress responses.

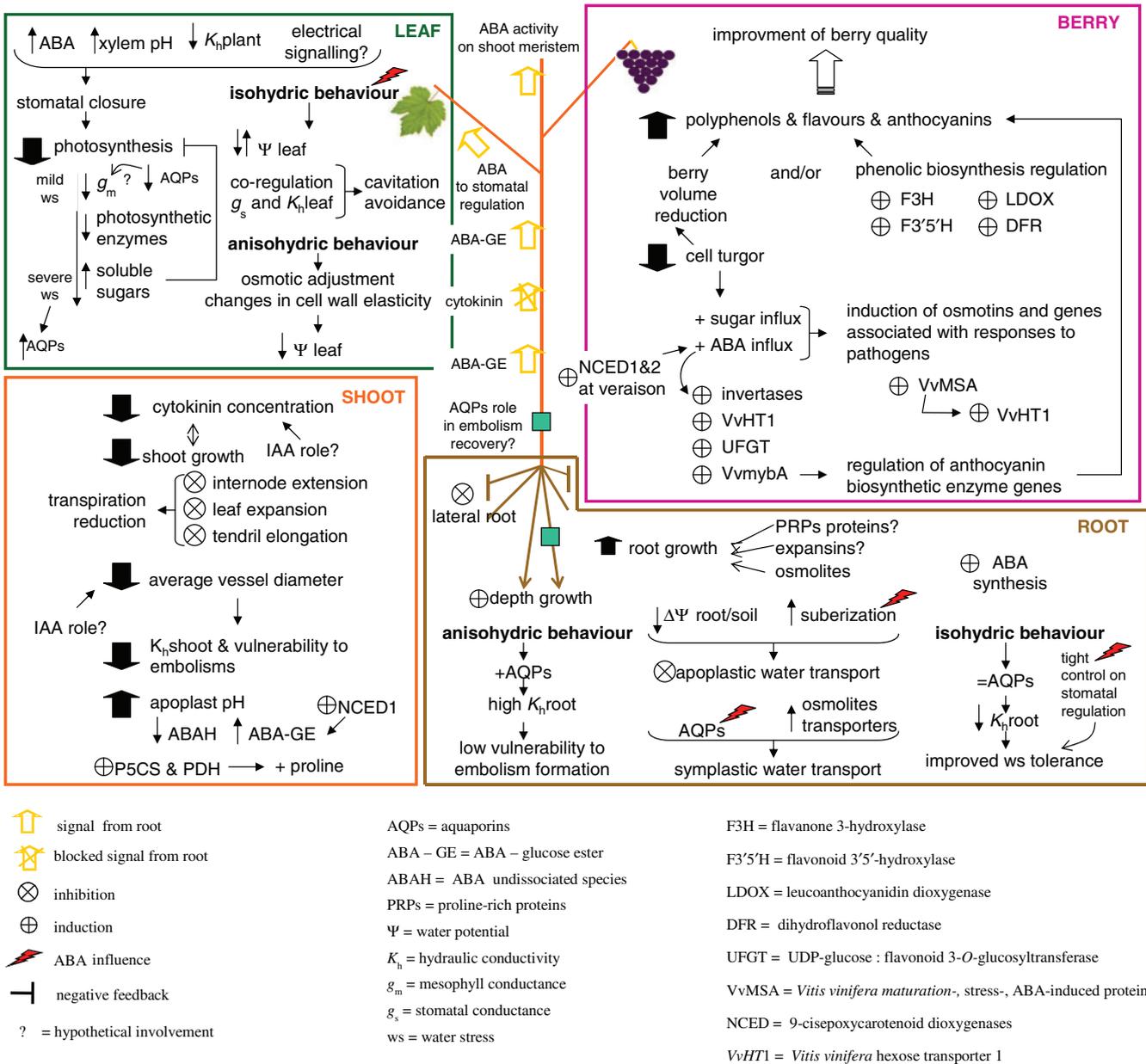


Fig. 3. Summary of water stress effects on grapevine root, shoot, leaf and berry. **LEAF:** Different signals contribute to stomatal closure, and decrease in photosynthesis. Downregulation of aquaporins (AQPs) may decrease mesophyll conductance to CO_2 . The accumulation of soluble sugars causes inhibition of photosynthesis. In isohydric varieties, leaf water potential is kept above a certain threshold by co-regulation of g_s and leaf hydraulic conductivity, avoiding cavitation. In anisohydric varieties, leaf water potential decreases along with osmotic adjustment and changes in cell wall elasticity. **BERRY:** Berry quality, and the biosynthesis and accumulation of polyphenols, is affected in both isohydric and anisohydric varieties. Phloem influx is accompanied by a decrease in cell turgor and berry volume. Water stress, especially when occurring pre-veraison, induces an increase in sugar influx and ABA, which can influence key steps of the phenylpropanoid biosynthetic pathway and may trigger a defense response upon water stress. **SHOOT:** Water stress inhibits internode extension, leaf expansion, elongation of tendrils, and therefore limits transpiration. Decreases in the average diameter of xylem vessels lowers xylem hydraulic conductivity and may prevent embolism, as smaller vessels are less susceptible to cavitation. AQPs may assist in embolism repair. The apoplast is alkalinized and the ABA pool is enriched in the low permeability conjugated form ABA-GE, a long-distance stress signal. Cytokinin concentrations can fall by 50% and account for growth inhibition. Auxins may regulate cytokinin biosynthesis and also xylem vessel size. **ROOT:** Roots grow into deeper, wetter soil layers. PRP genes play a role in the initiation of new roots on grape stem cuttings, probably by altering the cell wall mechanical properties to enable root emergence. Increased suberization of the exodermis and/or endodermis increases the hydraulic resistance along the apoplastic pathway. Root symplastic water transport is improved by osmotic adjustment and by increased activity of AQPs. Anisohydric behavior: increased expression of AQPs avoids a major reduction of K_h and so avoids vulnerability to embolism. Isohydric behavior: no change in AQP transcript levels, which combined with increased suberization leads to a lower K_h , however tight control on stomatal regulation avoids excessively low xylem water potentials and therefore minimises the risk of cavitation.

Mature grape berries accumulate high amounts of free proline, a well known compatible osmolyte that accumulates in tissues of water stressed plants. Although accumulation of free proline is likely to exert a protective action on grape cellular processes, the molecular determinants of proline homeostasis in berries are largely unknown. In most plant species the reciprocal rates of proline biosynthesis and catabolism are controlled by transcriptional regulation of the rate limiting biosynthetic enzyme Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) and the catabolic enzyme proline dehydrogenase (PDH) (Verbruggen and Hermans 2008). In grape berries, however, the expression of *P5CS* and *PDH* remains relatively unchanged along ripening (Stines *et al.* 1999; Deluc *et al.* 2006), despite the rise in concentration of ABA, which is required for the expression of *P5CS* genes in *Arabidopsis* (Strizhov *et al.* 1997). By contrast, in water stressed grapevine, shoot and berry increase in free proline is accompanied by upregulation of both *P5CS* and *PDH* (Cramer *et al.* 2007; Deluc *et al.* 2009), as well by upregulation of the key ABA biosynthetic gene *NCED* and of several ABA-responsive genes. Stines *et al.* (1999) cloned a single *P5CS* gene from grapevine, and suggested that its protein product may be regulated post-translationally. However, the publication of the grapevine genome sequence draft evidenced that *P5CS* may be represented by a family of two or three genes in grapevine (Jaillón *et al.* 2007). Of two family members included in the *Arabidopsis* genome, *P5CS1* is induced by osmotic stress while *P5CS2* is developmentally regulated and is required for embryo vitality (Székely *et al.* 2008). Thus, a yet uncharacterised member of the *P5CS* family may be responsible for developmentally regulated proline accumulation in ripening grape berries. Alternatively, proline might be translocated to the berry through the phloem.

Partial root-zone drying (PRD): an agronomical application of physiological theory

Knowledge about chemical and hydraulic root signals induced by soil water stress has stimulated new irrigation strategies. Partial root-zone drying (PRD) was developed to improve yield-to-irrigation ratios. PRD is designed to expose part of the root system to drying soil in order to produce the root drought signal, while the remaining roots in wet soil can maintain water supply and, therefore, leaf water potential (Dry and Loveys 1999). PRD enhances root hydraulic conductance in fruit trees; during PRD treatment, roots show higher uptake capacity than in whole root-zone irrigation treatment (Kang *et al.* 2002). Putative aquaporin stimulation by ABA produced by PRD may be involved. Prolonged exposure of roots to drying soil may cause anatomical changes as epidermis suberisation, collapse of cortex and loss of secondary roots. Alternate watering, after a period of soil drying, may improve this situation by inducing new secondary roots (Kang and Zhang 2004). In the field during a typical drying cycle (10–15 days), only roots near to soil surface feel dry soil whereas deeper roots extract water from wetter soil layers. Maybe this reduces the synthesis of drought chemical signals, like ABA, and, hence, probably reduces PRD effect. Also, the water redistribution process from wet to dry roots (the so-called 'hydraulic lift') in response to water potential gradients can contribute to decrease of ABA

biosynthesis (De la Hera *et al.* 2007). This phenomenon has been observed in several grapevines subjected to dry soil conditions or PRD treatment (Smart *et al.* 2005; Bauerle *et al.* 2008).

In order to improve PRD technology, further studies may be focused on intra-specific variation in the mechanisms of control of transpiration and their relative sensitivity to soil water deficit, i.e. isohydric and anisohydric behaviour of different grapevine cultivars (Schultz 2003a; Sadras 2009). Genotypically different responses to water stress, such as stomatal sensitivity to non-hydraulic signalling or the ability of the xylem to supply ABA (as described for Grenache and Chardonnay, see above) or rootstock vigour, could be very important factors in determining intensity of PRD response (Antolín *et al.* 2006; De la Hera *et al.* 2007). For example, de Souza *et al.* (2005a) suggest that PRD appears more successful with the more drought-responsive wine grape varieties.

Besides PRD, other techniques aiming at controlling the water balance in vineyards are being adopted in viticultural practice, as discussed by Keller (2005) and Chaves *et al.* (2007).

Conclusions

We have addressed grapevine responses to water stress by examining perturbations to physiological and molecular processes at the root, shoot, leaf and berry levels (Fig. 3).

Vitis genotypes have been described in relation to their isohydric or anisohydric response to water stress, linked to stomatal behaviour and non-stomatal effects. Stomatal regulation of grapevine is under ABA and hydraulic control; the latter linked to embolism formation and recovery in xylem tissues upstream the stomata. We have focused on ABA effects on stomata and their interrelationship with plant hydraulics from the root towards leaves and with photosynthetic assimilation. Using the daily maximum value of stomatal conductance as an indicator of water stress that allows comparison of plants with iso- and anisohydric behaviours, we have defined three stages of photosynthesis regulation in grapevines subjected to progressive water stress on the basis of the main causes of assimilation decline.

We have shown that in grapevine, xylem embolism occurs and repairs during diurnal cycles under ABA control, and that an almost full recovery of water potential is needed to promote repair mechanisms.

Aquaporins play a fundamental role in the control of plant water status. Different drought-defence strategies between iso- and anisohydric cultivars have been highlighted on the basis of drought-induced root ABA biosynthesis: an increase of suberisation of apoplastic barriers causes a reduction of water conductivity, but this is not compensated by an enhanced aquaporin-mediated cell-to-cell water transport.

Reverse genetics study on key genes of molecular pathways could provide a better understanding of drought tolerance mechanisms in grapevine. The *Vitis* genus is not usual for genetic transformation (Vivier and Pretorius 2002). However, progress has been recently made in grapevine transformation (Bouquet *et al.* 2006; Carmona *et al.* 2008; Burger *et al.* 2009). Today, a transgenic approach to grapevine improvement is more attractive than a classical breeding approach because a transfer of

individual traits as single genes with a minimum disruption of the original genome would leave the traditional characteristics of the cultivar intact (Bouquet *et al.* 2006).

All aspects reviewed in this paper, when taken together, show that grapevine fits well as a complex, modern model plant for molecular and physiological studies on both plant drought avoidance and tolerance. In particular, knowledge of drought effects on (i) differential development of root and shoot, (ii) long-distance water transport and hydraulic (involving xylem embolism) and hormone signalling, (iii) adaptations of the photosynthetic machinery and of stomatal behaviour and (iv) fruit ripening and technological quality for wine making, highlights the fundamental role that grapevine can play as a model crop. All these aspects are spread on different genotypes either used as rootstocks or as scions, which enlarge the genetic background facing peculiarities known in viticultural practice.

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