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Chapter 6. Embolism formation and removal in grapevines: a phenomenon affecting hydraulics and transpiration upon water stress.

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Abstract

Drought signalling among grapevine organs has a dual component: a hydraulic signal controlled by xylem physiology coexists with chemical signals (involving hormones, especially abscisic acid, ABA), transported via xylem, phloem and parenchyma pathways. Under water deficits, with high levels of tension developing, gas-filled xylem vessels may become disrupted by breakage of water columns, producing the so-called cavitation (or embolism) that drastically reduces hydraulic conductance. In grapevine, petioles and roots have been shown to be more vulnerable to xylem cavitation than shoots. When grapevines are re-watered following a drought period, 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 plant organs occurs. To reintegrate vessel functionality, plants have developed different repair mechanisms, which involve active and energy-consuming processes in shoot conductive tissues, possibly involving the contribution of aquaporins. The role of ABA in xylem embolism repair during diurnal cycles is also apparent and discussed.

Introduction

Water moves throughout the plants via the xylem conduits, pulled by the suction generated at leaf level, when transpiring. This transport is therefore the effect of negative (sub-atmospheric) pressures, which can get values lower than water vapour pressure (Tyree and Sperry, 1989). In similar conditions, the water column inside the vessels is under tension in a thermodynamically metastable state, which can produce a rapid phase change of liquid water to vapour, with formation of microscopic gas bubbles, according to a process called cavitation. The increasing number and size of these bubbles can interrupt completely the xylem conduit arresting its function: this phase is called embolism formation (Sperry, 1995).

In grapevine this phenomenon starts at moderate drought stress conditions (Lovisolo and Tramontini, 2010), when stem water potential (Ψ_{stem}) is between –0.8 and –1.2 MPa, and in young plants it originates from the new small diameter vessels, expanding then radially to older, larger vessels (Brodersen et al., 2013b).

Periodical cycles of embolism formation and repair are known to occur in vascular plants without affecting substantially the overall conductance of the xylem, compensated by a redundancy of flow paths such as the high number of single interconnected conduits (Tötzke et al., 2013). In the vineyard, Choat et al. (2010) observed that, although Ψ_{stem} varies significantly along the season (- 0.3 – -1.8 MPa), stem percentage loss of hydraulic conductivity (PLC) never exceed the 30%. Besides the seasonal fluctuations, grapevine plants present diurnal cycles of embolisms formation and repair that in water stressed plants

produce picks of 70–90% of PLC in the afternoon and significantly lower values in irrigated plants (Zufferey et al., 2011).

Unfavourable environmental conditions (i.e. drought, freezing, and salinity) worsen the consequences of cavitation events by influencing, in the short period, leaf gas exchange and general plant water status, and potentially causing productivity losses, branches dieback or even the death of the entire plant, on a longer perspective. The majority of the studies conducted on causes and effects of embolism in grapevine are related to drought, owing to the typical growing conditions to which this crop is submitted and positive effect of a controlled water stress in the production of high quality wines. Moreover the physiological and mechanical processes generating cavitations strongly differ according to the type of stress, e.g. drought produces cavitations via the exogenous nucleation of gas bubbles while freezing for endogenous nucleation (Lo Gullo and Salleo, 1993). For all these reasons, the current chapter only considers the embolism related to water stress.

Organs affected

In vascular plants, according to the segmentation hypothesis proposed by Zimmermann (1983), the susceptibility to embolisms is reduced in proximity to the trunk, in order to protect as much as possible the vital parts of the plant. In grapevine this theory is confirmed, being the effects of cavitation more evident in leaves and roots than in stem (Schultz et al., 2003; Lovisolo et al., 2008a; Zufferey et al., 2011). However, roots are submitted to positive root pressures (Tyree and Sperry, 1989) which favour a generally faster recovery from the embolisms and allow the organ to operate at xylem tensions closer to cavitation limits than shoots (Tyree and Sperry, 1988). In the shoot, the embolism resistance increases acropetally (McElrone et al., 2004; Choat et al., 2005). Furthermore, at given Ψ_{stem}, xylem vessels with larger lumen and longer elements are more vulnerable to embolism than smaller ones, in spite of the higher conductive capacity (Lovisolo and Schubert, 1998; Choat et al., 2005). Mimicking a moderate water stress, also grapevine stem downward orientation reduces stem hydraulic conductivity and enhances embolism resistance, by inducing accumulation of auxin in the apex which in turn affects the density and the size of the xylem vessels (Schubert et al., 1999; Lovisolo and Schubert, 2000; Lovisolo et al., 2002b; Favero et al., 2010). The expected damage is in fact proportionate to the larger pit area and heavier conductivity losses (Cochard and Tyree, 1990; Lo Gullo and Salleo, 1993; Choat et al., 2008), but also positively related more to pore diameter of the pit-membrane than to vessel diameter (Tyree and Zimmermann, 2002). Also wood density provides mechanical resistance to the walls preventing microfractures in the xylem walls (Lens et al., 2013).

The current-year growth portions are more prone to cavitation than older parts of the plant because primary xylem conduits, with thinner and weaker primary cell walls and a greater surface of exposed pit membrane than secondary conduits, tolerate lower air-seeding thresholds (Choat et al., 2005). In addition,

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"cavitation fatigue" has been observed on vessels which already embolized once, which oppose therefore a lower resistance to new cavitation events (Stiller and Sperry, 2002). Therefore, the accumulation of repeated stress and structural integrity of pit membranes in the primary xylem vessels increase the likelihood of water flow disruption (Brodersen et al., 2013b).

In leaves the situation is complicated by the fact that the xylem dysfunction due to stress is a combination of tension-induced wall collapse and xylem cavitation, being the first more readily recoverable than the second, and probably more distributed in the minor veins, while cavitation affects mainly the leaf midrib (Blackman et al., 2010). In grapevine petioles a conductivity loss of 50% was observed at Ψ_{stem} of -0.95 MPa and of more than 90% at -1.5MPa (Zufferey et al., 2011). In this organ, the level of damage is influenced by the stomata reactivity, in terms of speed and intensity, and by its control on transpiration (Domec and Johnson, 2012). Because of this role of barriers to embolism propagation, leaves can be assimilated to sort of hydraulic fuses (Zufferey et al., 2011). In this regard, abscisic acid (ABA) action is also relevant for avoiding cavitation, as it is the hormone devoted to induce stomatal closure in response to drought conditions (reviewed in grapevine by Lovisolo et al., 2010). Under either soil or atmospheric water stress, ABA is synthesized remotely in the roots or locally in the near tissues of the same leaf (Lovisolo et al., 2002a; Rogiers et al., 2012). Pantin et al. (2013) propose that this hormone triggers stomatal closure with a dual mechanism: biochemical and hydraulic. The former is widely acknowledged for its message targeted to the guard cells, while the latter is proposed by the same authors as an indirect effect of long-distance ABA signals. The suggested mechanism would involve the propagation of impairing water potential from the parenchyma around the xylem vessels, across the mesophyll to the stomata, as the consequence of a drop in water permeability at the level of the bundle sheath cells (Shatil-Cohen et al., 2011; Speirs et al., 2013), with a synergistic effect on both leaf water potential and transpiration rate (Dodd, 2013). A combination of hydraulic and hormonal signal in grapevine is thus the main mechanism to maintain adequate leaf water status and stomatal control of water loss (Rogiers et al., 2012; Domec and Johnson, 2012, Tramontini et al., 2013a; Figure 9.1.).

Spread and recovery

The embolism spread from gas-filled to adjacent water-filled vessels is limited by the small pore size of the pit membranes, which connect adjacent conduits (Choat et al., 2008; Brodersen et al., 2010). However, when the differential pressure reaches a critical point, the embolisms start spreading, most likely by air seeding (Brodersen et al., 2013b). According to the air-seeding hypothesis, the maximum (rather than the average) diameter of the pit membrane influences the occurrence of embolism spread (Choat et al., 2008). Narrow diameter elements called "vessel relays", which develop contemporarily to

the main xylem conduits as a product of the normal cambial activity, play a major role connecting large-diameter, adjacent vessels (Brodersen et al., 2013a).

The spread potential of embolisms is favoured by the extent of intervessels connectivity (Tyree and Zimmermann, 2002), the proximity of main xylem conduits (Brodersen et al., 2011) and the amount and orientation of vessel relays (Brodersen et al., 2013b). On the other hand, the ray parenchyma, by constituting separate modular units, allows neighbour xylem sectors to work independently, avoiding systemic embolisms spread (Brodersen et al., 2013b). The same living ray and paratracheal parenchyma is also involved in the xylem reversible gel occlusion and water refilling phase (Sun et al., 2008; Brodersen et al., 2010).

While part of the water flow is interrupted by embolism, the plant is still able to compensate the reduction in hydraulic conductance through an ion-mediated up-regulation of the remaining conduits (Trifilò et al., 2008). However, the same xylem anatomical redundancy, which in grapevine composes a complex interconnected network of tracheids and vessels, can represent a complication of the recovery phase, such as the water refilling of affected conduits (Brodersen et al., 2010), reinforcing the importance of the spatial organization of xylem vessels in the embolism spread and repair (Brodersen et al., 2013b). The simultaneous effect produced by vessels connections is one of the reasons for which characterization and quantification of the recovery time on a single isolated vessel results especially hard (Nardini et al., 2011a).

According to the in vivo observations performed on grapevine by Brodersen et al. (2010), the refilling phase occurs at Ψ_{stem} of -0.6 – -0.8 MPa with an overall average refilling time of about 2.3 h for a 20 μm diameter vessel and 17.3 h for a 150 μm diameter vessel. Due to the longer time required repairing embolisms in larger diameter vessels, these are expected to be the most likely subjected to refilling delays (Brodersen et al., 2010). Furthermore, the anatomic conformation of the vessel walls influences their surface wettability, bubbles removal and gas dissolution during the recovery phase (Kohonen and Helland, 2009; Zwieniecki and Holbrook, 2009; Brodersen et al., 2010). Also the geometric properties of pits and vessels, the developing contact angle and the gas concentration in the sap characterize the dynamics of gas bubbles elimination from the xylem conduits (Zwieniecki and Holbrook, 2000; Konrad and Roth-Nebelsick, 2003).

At a smaller scale, embolism refilling is under metabolic control imposed at cell level through the ionic effect on osmotic forces displacing water molecules (Nardini et al., 2011b) and through the membrane intrinsic proteins, i.e. aquaporins (AQPs), expression and activation (Kaldenhoff et al., 2008). The main mechanism producing the displacement of water molecules from contact cells and functional vessels into the embolized unit is in fact the creation of an osmotic potential inside the embolized vessel. This is obtained by the parenchyma cells secreting solutes into the vessel and, by this, favouring the passive movement of water into the embolized vessel, accelerated by the contribution of the AQPs (Hacke and

Sperry, 2003; Brodersen et al., 2010).From this perspective, the role of living parenchyma cells surrounding xylem channels, from where AQPs act, becomes even more crucial than that of anatomical barrier (Salleo et al., 2004).

The AQP-mediated water transport facilitates a rapid exchange of water across cell membranes (De Boer and Volkov, 2003) and its contribution to the plant recovery is quantitatively relevant. Lovisolo and Schubert (2006) observed that, on grapevine plants treated with mercurial reagents applied with the scope of excluding the plasmamembrane activity, the recovery of shoot conductivity after 24 h was of the 60% versus the 80% observed on untreated plants. The same treatment, imposed to roots of stressed plants, reduced by the 40% the root hydraulic conductivity with noticeable differences between rootstock genotypes (Lovisolo et al., 2008b; Tramontini et al., 2013b).

More the embolism creation is frequent, lower is the capability of the plant to recover (Lo Gullo and Salleo, 1993). After a recovery phase, not all the vessels are refilled with water: they represent a source for future embolism formation during the next Ψ_{stem} drop, contributing to the cavitation fatigue of the system (Hacke et al., 2001).

In leaves, where the xylem is largely composed by primary conduits, therefore scarcely resistant to cavitation, the ability to recovery from disruption is very high. Here Pou et al. (2012a) observed that the application of mercurial reagents produced a significant, although relatively small, inhibition of leaf conductivity of 30-40% only on well-watered vine plants, letting hypothesize a relative increase of the apoplastic contribution compared to symplastic path under stress conditions, as previously observed on herbaceous species.

The process of xylem refilling occurs even when the bulk of still functioning xylem is under tension (Salleo et al., 2004) according to a finely regulated process discussed in detail by Nardini et al. (2011a).

According to Nardini et al. (2011a), all the hypothetic mechanisms aimed at explaining water refilling imply the involvement of the vessel-associated cells (i.e., the living cells surrounding the xylem conduit). In this model, the native osmotic gradient driving the water movement along the vessel acts contemporarily to radial water flowing through membranes of the vessel-associated cells toward repairing xylem. The activation of several PIP1 and PIP2 AQP genes during embolism refilling has been reported from different organs of some woody species (Secchi and Zwieniecki, 2012), among which grapevine petioles (Perrone et al., 2012b). These water channels could favour the embolism removal by acting in two directions: axially, on the cell-to-cell water flow through the cortical parenchyma and/or radially on the water flow from parenchyma cells to xylem vessels (Perrone et al., 2012b). The regulation of AQPs activity during water stress seems to interact with ABA signals (Maurel et al., 2008), for which Kaldenhoff et al. (2008) suppose an indirect chemical control on the complementary AQP-mediated cell pathway during the recovery phase. This hypothesis has been recently reinforced by observations on

grapevine leaf tissues concerning the transcriptomic responses activated during recovery phase (Perrone et al., 2012b).

Vitis sp. is also subject to seasonal type of embolism recovery process: the spring vessels filling after winter cavitation which is, however, driven by positive (over-atmospheric) root pressure (Sperry et al., 1987).

Genotype effect

In ecological terms, the balance between conductive efficiency and embolism vulnerability observed on a plant is the result of the combined effect of genotype specificity and environmental conditions during growth. Some cultivars are less prone than others to cavitation due to the increasing elasticity of their cellular walls under water stress conditions (e.g Chardonnay in Alsina et al., 2007 and Robichaux et al., 1986). Also the contribution provided by the AQPs activity in different genotype is a character influencing the water transport capacity of the plant, therefore the avoidance of cavitation and efficient water refilling of vessels (Lovisolo et al., 2008b; Tramontini et al., 2013b; Table 9.1.). Cavitation events in leaves can be interpreted either as a signal for stomatal closure or as the effect of limited stomatal control (Zufferey and Smart, 2012). Furthermore, the ecophysiological characterization of grapevine cultivars in terms of iso and anisohydric behaviour could be interpreted as a differential vulnerability to embolism resulting from the variation in hydraulic conductance (Schultz, 2003). This aspect is debated as confirmed by certain results (Chouzouri and Schultz, 2005; Lovisolo et al., 2008a; Zufferey et al., 2011; Pou et al., 2012b) but not supported by others (Alsina et al., 2007) probably due to the different experimental set up (Schultz and Stoll, 2010). However, also the differential regulation of AQPs expression (Vandeleur et al., 2009), ABA concentration (Soar et al., 2006; Perrone et al., 2012a) and differential sensitivity of AQPs to ABA (Pantin et al., 2013) in response to drought conditions, interact with iso and anisohydric behaviour in grapevines, straightening the relationship between cavitation and the stomatal behaviour. As mentioned above, ABA is an active mediator of drought avoidance (i.e. stomatal closure) and tolerance (i.e. embolism recovery) mechanisms and is involved in the grape berry ripening phase as well (Castellarin et al., 2007; Giribaldi et al., 2010 and 2011; Table 9.1.). The combination of these aspects with the acknowledged importance of a controlled water stress in enhancing grape quality, confirm the need for a better understanding of ABA signal differential actions generated by different grapevine genotypes.

Conclusions

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According to Tyree and Zimmermann (2002) the xylem organization of a plant is the result of the combination of safe and efficient water distribution. All the steps involved in the embolism formation and repair are dynamic and complex, including passive hydraulic mechanisms, active energy-expensive processes, hormonal (particularly ABA) and hydraulic (AQP) signalling (Figure 9.1.). Through them, the plant imposes a conspicuous control over the flow and distribution of water in the xylem system, responding actively to the changing environmental conditions (Brodersen and McElrone, 2013).

Table 6. 1. Summary table on the main aspect involved in the embolism formation and repair. The authors propose a classification based on the separation between mechanisms devoted to avoid the embolism formation, therefore supporting plant resistance, and those involved in the recovery phase, therefore in plant tolerance. Symbols "+", "-" and "±" indicate positive, negative or two-direction relationship between the parameter and its effect towards resistance or recovery of the embolism.

	Embolism	
	Avoidance → Resistance	Tolerance → Recovery
Anatomy	+ woody density - cavitation fatigue + number of vessels - size of vessels (lenght and diameter) - maximum pore diameter and pit area	+ root pressure - gas concentration in the sap - number of vessels -vessels diameter geometric property of pits and vessels developing cavitation angle ± intervessel connectivity ray parenchyma and vessel relays
Hydraulic and metabolic control	± stomatal conductance, involving AQP activity and ABA signal for stomatal closure	osmotic forces, ± stomatal conductance, involving AQP activity and ABA signal promoting AQP synthesis/activation

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Figure 6.1. Scheme of the main mechanisms involved in the embolism formation and repair. The brown-coloured area marks the processes located at the root level as the green-coloured area marks those happening in the leaves. All the processes in the middle, concerning embolizing, functional and refilling vessels, parenchyma and phloeam, can be indiscriminately located at any point of the xylematic system.