OPTICAL VISUALIZATION OF EMBOLISM SPREAD IN DROUGHT-INDUCED LEAVES: REVEALING DIFFERENCES ACROSS THREE GRAPEVINE GENOTYPES

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Abstract:

Context and purpose of the study -Evaluation of xylem embolism is an important challenge in identifying drought tolerant genotypes within the context of climate change. Visualization methods such as the optical vulnerability technique (Brodribb et al. 2016) has been shown to be a reliable and accessible approach to observe the spread of embolism in dehydrating leaves (Hochberg et al. 2017; Lamarque et al. 2018). In this study we use the optical technique to examine the development of leaf embolism in three grapevine cultivars as a method to characterize their drought-tolerance strategy.

Material and methods -Potted plants of Grenache, Semillon and Syrah were grown outdoors in 2018 under well-watered conditions. Leaf embolism formation and spread was evaluated in four individuals per genotype by monitoring changes in light transmission through the xylem after the irrigation was cutted-off. For each plant, a mature leaf was placed on a scanner and imaged every 5 minutes until complete desiccation. Simultaneous measurements of stem water potential (Ψ_{stem}) were registered using psychrometers properly installed on the main stem. The accuracy of the psychrometers was evaluated by measuring the leaf water potential in adjacent leaves previously bagged with aluminum foil using a Scholander pressure bomb. The stack of images obtained were analyzed using the ImageJ software as described in Lamarque et al. (2018). The percentage of embolism ($\%_{emb}$) was calculated as the cumulative number of embolised pixels normalized to the total number of embolised pixels throughout the dehydration. Finally, the $\%_{emb}$ was represented as a function of Ψ_{stem} and different events were colored using a continuous scale respective to their time of appearance.

Results -Embolism formation and spread in the leaves were detected at different times for each cultivar since the beginning of drought. While Grenache showed the first embolism event at around 48 h of desiccation (-0.48 MPa), Semillon showed its first event after 72 h (-1.5 MPa). Syrah plants were placed in between the other two genotypes showing the first embolisms at -0.68 MPa. The vulnerability curves ($\%_{emb}$ vs Ψ_{stem}) constructed from the data obtained followed a sigmoidal function for all genotypes and showed a great variability between individuals. In spite of this, the time and water potentials at which the main embolisms occurred was significantly different between cultivars where Grenache showed an early cavitation (P_{50} at -1.43 MPa), followed by Syrah (P_{50} at -1.65 MPa) and Semillon (P_{50} at -2.08 Mpa). The optical technique tested in this study revealed genotype differences in the temporal appearance of leaf embolism suggesting a different strategy to tolerate dehydration.

Keywords: Embolism, drought, xylem cavitation, vessels, grapevine.

1. Introduction

Loss of plant functionality by cavitation is an important trait in identifying drought tolerant genotypes in changing environments. However, lack of information on the variation in vulnerability within species and/or individuals, largely due to hydraulic methodological limitations, remains a gap of knowledge in the propagation and spread of embolism.

Most traditional methods to evaluate the hydraulic function and vulnerability to cavitation may be prone to artifacts as they require excition of the plant organ (root, leaf, stem) thereby perturbing the vascular system before the measurement (Wheeler et al. 2013, Torres-Ruiz et al. 2014). Recent advances has been made using visual methods such as micro-computed tomography (microCT) and magnetic resosnance imaging (RMI) providing exciting insights about embolism location and formation in different plant organs (Brodersen et al. 2013, Choat et al. 2015, Charrier et al. 2016). However, these

approaches have a reduced field of application as they require expensive and limited available equipment as well as long acquisition times.
An alternative to these techniques, Brodribb et al. (2016) have recently introduced a new optical method where embolism formation and spread can be continuously monitored by visual light transmition in leaf veins as long as they dehydrate. This technique has been shown to be reliable and accessible, providing a new feasible method to characterize loss of function by cavitation during water stress (Hochberg et al. 2017).
The purpose of this study was to compare the formation and development of leaf embolism in three grapevine cultivars using the optical technique as a method to characterize their drought-tolerance strategy.

2. Material and methods

Plant materials - The experiment was carried out during 2018 season at the ISVV-INRA and the University of Bordeaux (France).One year-old seedlings of own rooted *Vitis vinifera* L. "Grenache", "Syrah" and "Semillon" were planted in 7 L pots containing 1 kg of gravel and 5.5 kg of commercial potting soil (70 % of horticultural substrate and 30 % sand). Plants were grown outside under well-watered conditions in a drip irrigated platform for two months approximately. The plants were irrigated with nutritive solution (NH4H2PO4 0.1 mmol.L^{-1}; NH4NO3 0.187 mmol.L^{-1}; KNO3 0.255 mmol.L^{-1}; MgSO4 0.025 mmol.L^{-1}; 0.002 mmol.L^{-1} Fe, oligo-element (B, Zn, Mn, Cu, Mo)) to avoid any deficiency during their development and the surface of the pots was covered with a plastic bag to minimize water losses by soil evaporation.

Plant measurements - Leaf embolism formation and propagation was evaluated in four individuals per cultivar by monitoring changes in light transmission through the xylem after the irrigation was cutted-off (Brodribb et al. 2016). The plants were placed in a room with controlled conditions at 26°C and 50% of HR. For each plant, the abaxial side of a mature leaf (still attached to the parent vine) was fixed on a scanner (Perfection V800 Photo, EPSON, Suna, Japan) using a transparent glass and adhesive tape and imaged every 5 min until complete desiccation. The imaged area consisted of half leaf including the midrib and the scanner magnification was set to give enough resolution of the midrib and at least eight major (second order) veins. Each leaf was automatically scanned every 5 min using a computer automation software (AutoIt 3) until the leaf was observed to turn from green to brown indicating cell death. After 5 days of desiccation and Ψ_{leaf} lower than -3.5 MPa the plants presented pronounced and severe leaf damage. Simultaneous measurements of stem water potential (Ψ_{stem}) were registered using psychrometers (ICT Internationale, Armidale, NSW, Australia) properly installed on the main stem. The accuracy of the psychrometers was evaluated by measuring the leaf water potential in adjacent leaves previously bagged with aluminum foil using a Scholander pressure bomb.

The stack of images obtained were analyzed using the ImageJ software as described in Lamarque et al. (2018). The percentage of embolism ($\%_{emb}$) was calculated as the cumulative number of embolised pixels normalized to the total number of embolised pixels throughout the dehydration. Finally, to visualize the dynamics of embolism spread through the leaf, spatio-temporal colour maps of cavitation formation were created for some of the samples by colouring the embolism area in each sequence using a colour scale of Ψ_{stem} over time.

Statistical analysis - Vulnerability curves that corresponded to the percentage of embolized pixels (cumulative embolisms) as a function of stem water potential (Ψ_{stem}) were fitted using R (R2.13.2, Foundation for Statistical Computing, Vienna, Austria) based on the following equation (Pammenter and Van der Willigen, 1998):

$$\mathrm{PLC} = \frac{10\ 0}{1+e^{(\,slp/25.(\Psi-\Psi_{50}))}} \quad (1)$$

where Ψ_{50} (Mpa) is the xylem pressure inducing 50% loss of hydraulic conductivity and slp (% MPa^{-1}) is the slope of the vulnerability curve at the inflexion point. The xylem pressure inducing the 12% (Ψ_{12}) and 88% (Ψ_{88}) loss of hydraulic conductivity were calculated as follow: Ψ_{12} = 50/S + Ψ_{50} and Ψ_{88}= -50/S + Ψ_{50}. One vulnerability curve was obtained per leaf per plant.

3. Results and discussion

Embolism formation and spread in the leaves were detected at different times for each cultivar since the beginning of drought. While Grenache showed the first embolism event at around 48 h of desiccation (-0.48 MPa), Semillon showed its first event after 72 h (-1.5 MPa). Syrah plants were placed in between the other two genotypes showing the first embolisms at -0.68 MPa.
The optical vulnerability curves (Figure 1) constructed from the data obtained followed the typical sigmoidal function for all genotypes as previously observed for grapevine and other species (Brodribb et al. 2016, Hochberg et al. 2017, Lamarque et al. 2018). The time and water potentials at which the main embolisms occurred was significantly different between cultivars where Grenache showed an early cavitation (Ψ_{50} at -1.43 MPa), followed by Syrah (Ψ_{50} at -1.65 MPa) and Semillon (Ψ_{50} at -2.08 MPa). These differences suggested that Semillon had a higher resistance to embolism formation than the other two cultivars even if they arrived to the same final condition at <-2.0 MPa. These differences where graphically observed by a progression colored map of the leaf embolism formation in different vein orders for each cultivar (Figure 2).

4. Conclusions

The optical technique used here resulted in a realible and simple method to visualize "in-vivo" embolism formation and propagation in grapevine leaves. In addition, the technique allowed us to revealed differences in the temporal dynamics of leaf cavitation in three grapevine cultivars under progressive drought.

5. Acknowledgments

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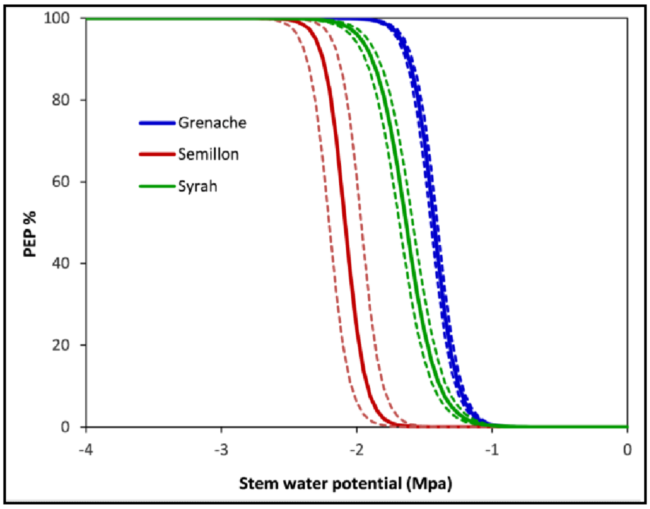

Figure 1. Optical vulnerability curves expressed as percentage of embolized pixels (PEP%) as a function of stem water potential in three grapevine cultivars (Grenache, Semillon and Grenache). Solid colored lines and short-dashed bands represent the mean observed embolism ± SE for each cultivar. The Pammenter model was first fitted per sample (n=4) per cultivar before calculating the mean Ψ_{50} and slope.

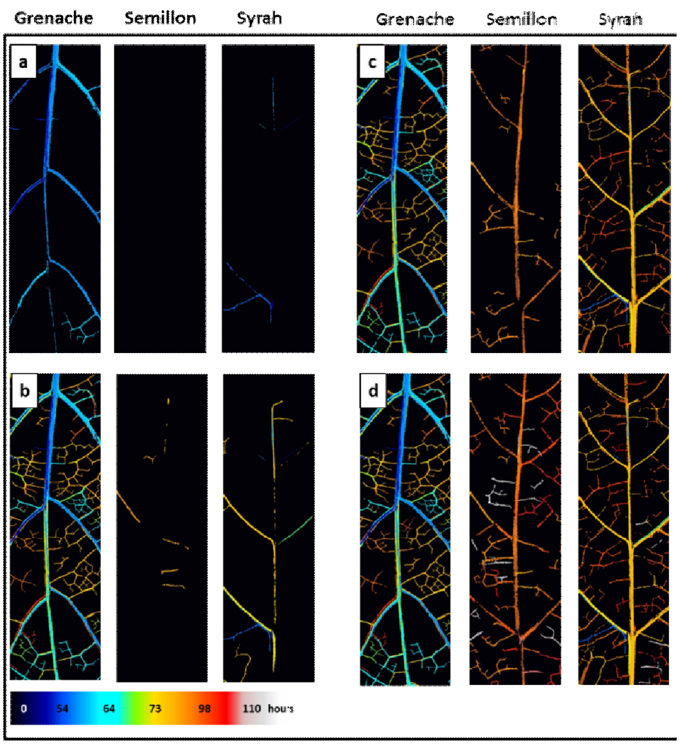

Figure 2. Representation of leaf embolism spread during the progress of dehydration in three grapevine cultivars (Grenache, Semillon and Syrah). Cavitations events are colored according to their time of occurrence and linked to the stem water potential at which they were recorded: a) -0.68 MPa; b) -1.63 MPa; c) -2.08 MPa d) <-2.8 MPa.