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Mercurial inhibition of root hydraulic conductance in *Vitis* spp. rootstocks under water stress

Claudio Lovisolo [a,*], Sara Tramontini [a], Jaume Flexas [c], Andrea Schubert [a,b]

[a] *Dipartimento Colture Arboree, Università di Torino, Via Leonardo da Vinci 44, I-10095 Grugliasco, Italy*
[b] *IVV-CNR, Grugliasco (TO), Via Leonardo da Vinci 44, I-10095 Grugliasco, Italy*
[c] *Laboratori de Fisiologia Vegetal, Grup de Biologia de les Plantes en Condicions Mediterrànies, Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, E-07122 Palma de Mallorca, Balears, Spain*

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Abstract

This work aims to quantify the contribution to whole-root water transport of the fraction controlled by cellular metabolism in grape rootstocks upon water stress. We used mercuric chloride as inhibitor of cell metabolism on genotypes obtained from hybridization of *Vitis berlandieri* with either *Vitis rupestris* or *Vitis riparia*, and we found that the fraction of root water transport under metabolic control is higher in former, which are known to be more resistant to water stress. In addition, as these rootstocks showed lower vessel embolization during water stress, we suggested a possible role of cellular metabolism on the control of root embolism.

Keywords: Aquaporin; Cavitation; Drought; $HgCl_2$; Embolism; Grapevine; Hydraulic conductance; Mercury; *Vitis vinifera* L.; Water stress

1. Introduction

The grapevine has been used as model plant for our studies about hydraulics in plants under water stress (Lovisolo and Schubert, 1998) because it can endure severe water stress causing embolism in xylem vessels (Schultz and Matthews, 1988). At the shoot level *Vitis* spp. behave as lianas, having a high hydraulic conductivity of their main axis and little interferences with lateral branching (Lebon et al., 2004). Grape shoot hydraulic conductance depends on xylem development and on the extent of its embolization (Lovisolo et al., 2002), which occurs during transpiration when water availability becomes scarce. Embolisms can be repaired if plants are rehydrated, and we have shown that plant metabolism sensitive to mercuric chloride is required to repair shoot embolisms (Lovisolo and Schubert, 2006).

In roots, according to Steudle's model (Steudle, 2001), water moves overcoming a series of hydraulic resistances, which can be imputed to apoplastic, symplastic and transcellular pathways. Resistances of apoplastic routes can be predicted following physical lows. Cell-to-cell water pathways depend on water potential gradients driving cell-to-cell flows and on membrane water permeability, which in principle could vary among genotypes as well as in response to drought. To differentiate root cell-to-cell water transport from the overall root water transport, we used mercuric chloride as unspecific inhibitor of cell metabolism. Mercury inhibition has been in past routinely used as a tool to offset the contribution of aquaporins to water transport in plants, after Maggio and Joly (1995) performed first experiments in tomato whole-root systems. Since then, because of its efficiency in blocking many aquaporins, $HgCl_2$ has been used extensively at various concentrations (10^{-6} to 10^{-3} M) on several plants, as reviewed by Javot and Maurel (2002). However, today, it is assumed that not only water channels, but several other metabolic steps putatively affecting water transport, can be down regulated by $HgCl_2$ feedings to plant roots (Lovisolo and Schubert, 2006).

This work aims to estimate the contribution to whole-root water transport of the fraction controlled by cellular metabolism in grape rootstocks, i.e. the fraction related to cell-to-cell water pathways in root. Most of grape rootstocks are hybrids of *Vitis berlandieri*. To obtain different adaptation to drought they are either hybridizated with the xerophylic species *Vitis rupestris* or with the mesophylic species *Vitis riparia*. We grouped rootstocks

* Corresponding author.
E-mail address: claudio.lovisolo@unito.it (C. Lovisolo).

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of our experiment according to these two opposite climate adaptations, pointing to find functional justification of different rootstock responses to water scarcity studied for applied viticulture purposes (Carbonneau, 1985; Bavaresco and Lovisolo, 2000; Padgett-Johnson et al., 2003).

2. Materials and methods

2.1. Plant material and growth conditions

We used 2-year-old plants of seven different grapevine rootstocks, either derived from hybridization of *V. berlandieri* with the xerophylic species *V. rupestris* (140RU, 775P and 1103P), or from *V. berlandieri* with the mesophylic species *V. riparia* (SO4, 157.11, 420A, and K5BB). Three replicate plants per genotype were grown in 12 L containers filled with a substrate composed of sandy-loam soil/expanded clay/peat mixture (4:2:1 in volume), with a final pH of 7.3. Containers were placed in a greenhouse with no supplementary light or heating. Pots were fertilized once a month with 15 g of a complex (20-10-10) fertilizer. In January, plants were pruned to a single-bud spur. Average budbreak took place on 10 April. The single shoot of each plant was trained vertically upon a stick. Lateral shoots and clusters were removed immediately after formation. Plants were watered to container capacity (Ψ_{soil} about −0.01 MPa; Lovisolo and Schubert, 1998), each 3rd day until the beginning of the water stress, imposed by withholding irrigation to all plants during 10 days starting from May 31st (51 days after budbreak, DAB).

2.2. Water relations

At the end of the 10-days drought period soil water potential (Ψ_{soil}) was calculated according to soil moisture/water potential curves previously assessed for the pot substrate (Lovisolo and Schubert, 1998). Water potential of the root stalk (Ψ_{root}) was measured using a Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on the two most basal leaves, wrapped in the evening before measurement with a double layer (inside plastic and outside aluminum) bag and assumed to represent the water potential of the corresponding root stalk xylem (Liu et al., 1978).

Root hydraulic conductance was first calculated *in vivo* ($^{calc}L_h$) with the transpirative flux method (Martre et al., 2001) under steady-state conditions, based on the ratio between E_{plant} (determined gravimetrically) and the difference in water potential between soil and root stalk ($\Delta\Psi_{soil\text{-}root}$).

Root hydraulic conductance (L_h) was also measured through a controlled tension–pressure apparatus (Lovisolo et al., 2002), using the method of Lovisolo and Schubert (2006), modified as follows. Whole-root systems, gently freed from the pot soil underwater, were cut at the interface between root and shoot, weighed and immersed into a tension–pressure chamber filled with tap water; volume of whole-root systems was recorded; the root stalk, outside of the pressure chamber, was clamped with a rubber sleeve. A measurement of 'undisturbed' conductance was taken by applying an apical suction (−80 kPa) for 5 min through the sleeve to the cut root stalk apical end. This tension is thought to be close to physiological values and is expected to give measurements of root hydraulic conductance close to the *in vivo* measured values. Also the whole aerial cut portion (shoot more leaves) was weighed.

2.3. Inhibition of water flow with mercuric chloride and flushing of root xylem embolism

A solution of $HgCl_2$ (0.5 mM) was used to inhibit water transport controlled by cellular metabolism according to Lovisolo and Schubert (2006). Feeding of $HgCl_2$ was done in the tension–pressure apparatus, directly on the excised root system, after the L_h measurement. The 0.5 mM $HgCl_2$ solution was aspired into the root by applying a −80 kPa suction for 1 h, and the root then left immersed for 15 min without applying tension or pressure before taking another measurement of L_h, named $^{Hg}L_h$. Following this measurement, leaving the same root system in the chamber, a basal pressure (+100 kPa) was applied for 5 min to the $HgCl_2$ solution in the chamber in order to flush out xylem embolisms; thereafter the measurement at −80 kPa was repeated and named $^{flushed}L_h$. It is possible that only embolism present in secondary roots were flushed out, whereas in finest branched roots 5 min at +100 kPa could be not sufficient; however, in preliminary experiments we tested both higher pressure and/or longer time, but both strategies caused mechanical damage to the root.

2.4. Experimental design and statistical analysis

Three replicate plants per genotype were used. Experiments were laid down following a randomized design. Results were submitted to one-way ANOVA followed by a Duncan's test of differences between the means. In addition, standard errors of the means have been calculated.

3. Results

Genotypes used were commercial hybrids of *V. berlandieri*; three of them crossed with *V. rupestris* (BxRu), while the others crossed with *V. riparia* (BxRi). At the end of the water stress period, root and shoot were more developed in BxRu than in BxRi plants. Water uptake from the soil was higher in BxRu than in BxRi plants, resulting in lower soil water potential in BxRu hybrids. Water loss to the atmosphere was higher in BxRu, as shown by higher plant transpiration. Root stalk water potential was higher in BxRu than in BxRi plants, and this reflected on higher $^{calc}L_h$ (Table 1).

According to the *in vivo* measurements, on the excised root system L_h was higher in BxRu than in BxRi plants. After mercury inhibition, $^{Hg}L_h$ was about 40% lower than L_h in both BxRu and BxRi plants, while the absolute reduction of root hydraulic conductance was 49.7×10^{-9} m^3 MPa^{-1} s^{-1} in BxRu, and 18.8×10^{-9} m^3 MPa^{-1} s^{-1} in BxRi plants. After ejection of embolisms by pressure flushing, root hydraulic conductance ($^{flushed}L_h$) increased in BxRu plants to a value about 1.4 times higher than L_h, and about 2.6 times higher in BxRi plants (Fig. 1).