Rootstock control of scion response to water stress in grapevine

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1. Introduction

The dehydration observed under drought conditions appears when an imbalance occurs between water extracted from the substrate and water lost by transpiration (Aroca et al., 2012). Therefore, in plants that are routinely grafted, such as grapevine (Vitis vinifera L.), rootstock effect on scion performance must be considered in the study of adaptability to stress conditions. Rootstocks provide tolerance to exogenous limiting factors, biotic (e.g., soil-borne pests) and abiotic (e.g., salinity, water or oxygen deficit), while influencing the ecophysiological behaviour of the scion and its berry quality (Bavaresco and Lovisolo, 2000; Padgett-Johnson et al., 2000; Soar et al., 2006; Tramontini et al., 2012; de Souza et al., 2009; Ibacache and Sierra, 2009; Rizk-Alla et al., 2011; Marguerit et al., 2012). Stomata are considered direct responsible of optimizing the balance between carbon gain and water loss of the plant (Rogiers et al., 2012), and the patterns of their response, in terms of timing and intensity, are genetically determined (Chaves et al., 2010). Significant efforts have been done in the selection of the optimal rootstock/scion combinations to satisfy specific grape growing needs (e.g., Koundouras et al., 2008; Hamdan and Basheer-Salimia, 2010; Komar et al., 2010). However, the effect of rootstock/scion interaction on plant adaptation to stress is still very much debated (Gambetta et al., 2012). Since drought conditions affect water transport from the soil through the plant into the atmosphere in a soil–plant–air continuum that is interconnected by a continuous film of water, measuring its influence on plant water transport requires considering both water extraction at the soil–root interface and water release at the leaf–air interface (Janott et al., 2011). Although the key role played by both rootstock and scion is acknowledged their relative contribution and differences obtained by specific combination of genotypes require some further analysis.

The aim of this study was therefore to clarify the relative role of rootstock and scion in the hydraulic response to limitations in water availability. In order to do so, we evaluated the differences observed between two grapevine rootstock genotypes (considered representative of two of the most widespread hybrids: Vitis berlandieri × Vitis rupestris, and V. berlandieri × Vitis riparia) and among four scion genotypes (cultivars), separately and in combination (i.e., on grafted plants). The relationship between stomatal conductance (gs) and leaf water potential (Ψleaf) was used to rank the scion genotypes for their ability to withstand water stress (Rogiers et al., 2012). Root hydraulic conductivity, in terms of short and long distance transport (over membranes and xylem, respectively) was considered in order to characterize the responses of the rootstocks. Aquaporins play a major role in transcellular water movement by facilitating the transport of water through cell membranes (Kaldenhoff et al.,...
2. Materials and methods

2.1. Plant material

For those experiments requiring the destruction of the root system, the investigation was conducted on 2-year-old plants of two different grapevine rootstocks, one derived from hybridization of V. berlandieri with the xerophytic species V. rupestris (140Ru), and the other from V. berlandieri with the mesophytic species V. riparia (SO4), with three replicates for genotype. The two genotypes were selected as characterizing the extremes in the results of Lisovolo et al. (2008). All the other experiments were conducted during two consecutive years (2011 and 2012) on 2-year-old (and 3-year-old the next year) plants of four cultivars (Cabernet Sauvignon, Grenache, Merlot, Syrah) grafted on the two above-mentioned rootstocks, with five replicates for each combination rootstock/cultivar. The four cultivars were selected taking into account their ecophysiological characterization obtained by previous authors: Grenache is near-isoosydrlic (Schultz, 2003; Santesteban et al., 2009), Merlot is anisoosydrlic (Williams and Baaza, 2007; Shellie and Glenn, 2008), Syrah is anisoosydrlic (Schultz, 2003; Rogiers et al., 2009; Santesteban et al., 2009), and Cabernet Sauvignon is classified as isosydyric (Chalmers, 2007) or anisoosydrlic (Williams and Baaza, 2007), depending on the authors. All plants were grown in a greenhouse with no supplementary light or heating, in 4L pots 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. Additional leaves of Cabernet Sauvignon, Grenache, Merlot, Syrah needed for the experiment on detached leaves were collected from the experimental vineyard located at the Faculty of Agriculture of the University of Turin (Grugliasco, Italy), from plants of the same clonal origin. To this aim, mature leaves (10th node onwards) were collected in the month of July. These leaves were used in order to study stomatal behaviour in absence of rootstock and shoot effects.

2.2. Leaf, root and soil water potentials

Leaf water potential ($\psi_{leaf}$) and root water potential ($\psi_{root}$) were estimated with a pressure chamber (Scholander et al., 1965; model used: Soil Moisture Equipment Corp., Santa Barbara, CA, USA). $\psi_{leaf}$ measurements were done between 10:00 and 12:00 AM on fully expanded leaves from primary stems, immediately after detaching the leaf from the stem, unless differently specified (e.g., experiment on detached leaves). $\psi_{root}$ was measured on the two most basal leaves early in the morning (around 8:00) after keeping them enclosed overnight in a reflective plastic envelope. Leaf bagging blocks transpiration and allows leaf water potential to reach equilibrium with root water potential. Soil water potential ($\psi_{sat}$) was calculated from the moisture potential curves previously obtained for that specific substrate (Lovisolo et al., 2008).

2.3. Root hydraulic conductance and putative transmembrane path

We measured hydraulic conductance, its transmembrane water movement component, and the intensity of xylem embolization. All measurements were based on a destructive method with a controlled tension–pressure apparatus (Lovisolo et al., 2002) and taken in sequence on the same plant material as described by Lovisolo et al. (2008). The root system was gently cleaned from soil under water and cut at the interface between root and shoot. After measurement of weight and volume, it was immersed into a tension–pressure chamber filled with tap water and its stalk was clamped with a rubber sleeve. An initial conductance was measured by applying a negative pressure of $-80$ kPa for 5 min through the sleeve: this tension simulates the suction forces that cause water transpirative transport in the potted roots. The same pressure was then imposed for 5 min after having treated the root with a solution of 0.05 mM HgCl$_2$ for 60 min (+15 min for stabilizing the system) in order to inhibit mercury-sensitive aquaporins. The difference between this conductance and the previous conductance defines the portion of conductance sustained by transmembrane water movement. The same pressure was again imposed for other 5 min after a flushing of $+100$ kPa for 5 min to the whole root, in order to free the system from embolisms. The difference between this value and the previous defines the conductance achievable by the system through the apoplastic pathway in absence of embolisms.

2.4. Stomatal conductance

In order to exclude the root effect and to concentrate on the characteristics induced by the grafted cultivar, this measurement was conducted on detached leaves, treated as self-contained functional units, as described by Brodribb (2009). Stomatal conductance ($g_s$) was measured on fully developed leaves with a portable Gas Exchange Fluorescence System (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). Measurements were conducted by clamping the leaves in the leaf chamber, where photosynthetic active radiation, (PAR: 1200 μmol m$^{-2}$ s$^{-1}$) and temperature (25°C) were kept constant. A zero-point for CO$_2$ was set at the beginning of each day of measurements. In the case of the experiment on attached leaves, after measuring $g_s$, the same leaf was used for measuring $\psi_{leaf}$. The collection of data was concentrated within two weeks (between 31 May and 15 June 2012) in order to minimize any potential seasonal fluctuation (Alsina et al., 2011). In the case of the experiment on detached leaves, seven leaves per replicate were excised under water and immediately placed in 50 mL tubes with their petiole immersed in deionised water. Leaves were kept in the laboratory under constant artificial light (400 μmol m$^{-2}$ s$^{-1}$ PAR) and temperature (24–27°C) and transpiration was continuously monitored on one of them. When the leaf reached a steady state in transpiration (usually after 20 min, with a coefficient of variation <3% for 3 min, Nardini and Salleo, 2005), all tubes were simultaneously emptied from water. Six measurements of $\psi_{leaf}$ were then taken with a 4-min interval on the remaining leaves. Later on, in analysis of the correlation between $g_s$ and $\psi_{leaf}$, it was decided not to...
consider the first two measurements of $g_s$ due to the observed ‘Iwanoff effect’ described by Dürring (1993), which produced an increase in $g_s$ immediately after removal of the leaf from water. Four replicate measurements were done for each cultivar.

2.5. Transmembrane water path in leaves

In order to compare the four cultivars for their transmembrane water movement on foliar tissues, the methodology proposed by Terashima and Ono (2002) was followed and adapted. Each detached leaf was divided in two parts along the median vein. The half without petiole was used as control and the half with petiole was left absorbing a solution of 50 mM of HgCl$_2$ for 60 min before the measurement. During this period, the leaf was exposed to light (1000 μmol m$^{-2}$ s$^{-1}$) to help uptake of the solution through the petiole by transpiration. From each half-leaf 20 foliar disks with a diameter of 1.2 cm were obtained with a circular cutter and weighted. The disks were floated on 40 mL of deionized water in a Petri dish with the adaxial surface upwards and were shaken gently for 15 min. They were then dried on absorbing paper for 5 min and weighted again. Twelve replicate leaves were used for each cultivar. The increase in turgidity of the leaf discs was calculated with the following equation:

$$\text{Average weight increase} = \left(\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}}\right) \times 100$$

The obtained value was treated as a measure of the hydraulic conductivity of the plasma membrane of the leaf disc, such as a reasonable approximation of the transmembrane component of water transport, independent from water movement via vasculature.

2.6. Statistical analysis

Data were expressed by means and corresponding standard errors. Results were submitted to one-way ANOVA using the statistical software package SPSS (version 20, SPSS Inc, Cary, NC, USA). Correlated variables were interpolated in figures by regression curves plotted by means of Microsoft Excel® software.

3. Results

3.1. Rootstock – soil and leaf water potentials

In order to characterize rootstock components of the control of stress effects, we analysed the relationship between $\Psi_{\text{soil}}$ and $\Psi_{\text{leaf}}$, during two consecutive years, in plants grafted on 140Ru or SO4. At the same values of $\Psi_{\text{leaf}}$, plants grafted on 140Ru showed more negative $\Psi_{\text{soil}}$ than plants grafted on SO4. This difference was increasingly evident with lower $\Psi_{\text{leaf}}$ and was not influenced by the scion cultivar (Fig. 1). Plant development, measured as leaf area, was not different between the two rootstocks (data not shown).

3.2. Rootstock – root hydraulic conductance and transmembrane water path

The diverse relationship between $\Psi_{\text{soil}}$ and $\Psi_{\text{leaf}}$, observed in the two rootstocks suggested differences in the hydraulic conductance of the root system. We analysed this parameter in excised roots of not grafted plants of 140Ru and SO4, at the same time evaluating the component of conductance controlled by xylem embolization. Root hydraulic conductance, measured at −80 kPa on the excised root before and after Hg treatment, was always higher in 140Ru than in SO4. However, Hg treatment reduced the conductance by 56% in 140Ru and 16% in SO4, a result not far from the range recently obtained on rootstocks by Gambetta et al. (2012) after H$_2$O$_2$ aquaporin inhibition. Pressure flushing induced an increase of conductance of 197% and 369% respectively (Fig. 2).
3.3. **Scion – stomatal conductance on attached leaves**

While assessing the pattern of $g_s/\Psi_{\text{leaf}}$ in detached scion cultivar leaves, we established in grafted plants whether the genotype-dependent patterns of $g_s$ adaptation to leaf water status would be affected by the rootstock. A situation consistent to all the four cultivars can be observed: at the same level of $\Psi_{\text{leaf}}$. In fact, plants grafted on 140Ru tend to have higher $g_s$ values than plants of the same cultivar grafted on SO4 (Fig. 3). This was observed at $\Psi_{\text{leaf}}$ levels higher than −1 MPa; only Grenache (Fig. 3b) expressed this condition for $\Psi_{\text{leaf}}$ values more negative than −1.1 MPa. The result of a two way ANOVA conducted on $g_s$ values for the two factors ‘cultivar’ and ‘rootstock’ confirmed that the difference in the mean values among the different rootstocks was greater than would have been expected by chance after allowing for effects of differences in rootstocks ($P=0.031$) and that the difference in the mean values

![Fig. 3. Relationship between stomatal conductance ($g_s$, mmol H₂O m⁻² s⁻¹) and leaf water potential ($\Psi_{\text{leaf}}$, MPa) on vine plants of Cabernet Sauvignon (a), Grenache (b), Merlot (c) and Syrah (d) grafted on two different rootstocks: 140Ru (filled squares) and SO4 (empty circles). The figures refer to data collected between 31 May and 15 June 2012. Each trendline label is bordered with the same style of the line to which it refers.](image)

3.4. **Scion – stomatal conductance on detached leaves**

The next step was to focus on the scion cultivar control of $g_s$, which was measured on progressively drying leaves (Fig. 4). For the cultivars Cabernet Sauvignon and Grenache, the dynamics of $g_s$
over time (40 min) almost overlapped, and showed the lowest $g_s$ values for the whole timeframe among all cultivars. Merlot was the least responding to the increasing water stress conditions. Syrah, on the contrary, initially had the highest $g_s$ values, but, after a steep decline, reached the same low levels of Cabernet Sauvignon and Grenache (Fig. 5a). From the $g_s/\Psi_{\text{leaf}}$ curves obtained on the same leaves, further information on the cultivar behaviour was obtained (Fig. 5b). As concerns $\Psi_{\text{leaf}}$, Grenache and Syrah represented the two extremes, the former with the less negative, and the latter with the most negative $\Psi_{\text{leaf}}$ values. The steepness of the interpolation curves was higher for Merlot and Cabernet Sauvignon, and in the latter case $g_s$ values dropped down by 60% between the 8th and the 20th min while $\Psi_{\text{leaf}}$ remained almost constant at $-2.0 \text{ MPa}$.

3.5. Scion cell-to-cell path

The previous observations concerning differences among cultivars at leaf level were integrated with information on the putative cell-to-cell water pathway (Fig. 6), as done for the rootstocks at the root level. Hg treatment significantly decreased leaf water uptake in all cultivars. Cabernet Sauvignon and Grenache were strongly affected by Hg treatment, losing respectively 30% and 67% of the uptake capacity of their foliar tissues. Merlot and Syrah also presented a reduction, albeit lower, of water uptake, of 28 and 20% respectively.

4. Discussion

Rootstocks are known to play a major role in grapevine tolerance to water stress by controlling and adjusting the water supply to shoot transpiration demand (Carbonneau, 1985; Soar et al., 2006; Alsina et al., 2011; Marguerit et al., 2012). This study aimed to characterize the influence of rootstocks genotypes in the adaptive response of scions to water limiting conditions.

What shown in Fig. 1 suggested that 140Ru depletes the water soil reserves faster than SO4. This could be explained by its very high vigour, in comparison with the moderate vigour of SO4 (Gambetta et al., 2012), requiring the extraction of greater amounts of water in order to compensate the water losses for canopy transpiration (Jones, 2012). 140Ru is also characterized by a high adaptation to water deficit, while for SO4 the classification varies considerably with the study: from high (e.g., Carbonneau, 1985; Cirami et al., 1994), to medium (e.g., Delas, 1992; Whiting, 2005), to low (e.g., Southey, 1992; Dry, 2007) and very low (Galet, 1998). The higher drought tolerance of 140Ru could not be completely explained with its capacity to explore larger and deeper soil volumes conferred by a more developed root surface, as the limited substrate volume (pot conditions) and water availability were the same for both genotypes. Valuable details were provided by the second experiment, on non-grafted rootstock plants (Fig. 2). The intrinsic higher stress resistance of 140Ru than SO4 was parallel to higher conductance of the excised roots (~80 kPa), to higher cell-to-cell water transport and to higher resistance to xylem cavitation processes, in agreement with observations made by Lovisolo et al. (2008).

The higher putative aquaporin activity, or abundance, observed on 140Ru, could not only produce a more efficient recovery in xylem conduit re-filling after drought-induced embolisms (Lovisolo et al., 2008; Vandeleur et al., 2009) but also explain the tolerance of this genotype to higher tensions ($\Delta \Psi$) observed on grafted plants at any level of water availability (Fig. 1). However, the capacity of 140Ru to extract water from the substrate and to recover from cavitation events more easily than SO4 was not substantially reflected on the slightly higher $g_s$ at a given $\Psi_{\text{leaf}}$ observed on the four grafted cultivars (Fig. 3). This limited difference on the expected performances at scion level makes us to hypothesize the mediation by chemical signaling, and mainly ABA (Soar et al., 2006; Marguerit et al., 2012).
The component which conferred specific characteristics to the correlation between stomatal conductance and leaf water potential was the scion cultivar: the $g_s/\Psi_{leaf}$ curves measured in grafted plants (Fig. 4) have different slopes and shapes, corresponding to the ecophysiological behavior of each genotype. Slopes were cultivar-specific and not dependent on the rootstock. The curve of Cabernet Sauvignon, in spite of the most evident rootstock effect among the four cultivars (Fig. 3), maintained intermediate $g_s$ values on both rootstocks (Fig. 4). This intermediate ranking was consistent with the intermediate percentage loss in water uptake observed on foliar disks after Hg treatment, suggesting that stomatal conductance is linked with the transmembrane water movement capacity of the leaf, probably dependent on aquaporin activity (Pou et al., 2013). However, the $g_s/\Psi_{leaf}$ curve on excised leaves, where stomatal closure delayed the $\Psi_{leaf}$ drop below the threshold of $-2$ MPa (Fig. 5b), could not be explained within the context of the above-mentioned hydraulic forces and could imply the involvement of complex hormonal signalling in whole plants, so that Cabernet Sauvignon assumes isohydric or anisohydric behaviour, depending on the experimental conditions. Grenache, in spite of its $g_s/\Psi_{leaf}$ curves resemblance to Cabernet Sauvignon on whole plants, presented the tightest stomatal control on excised leaves (Fig. 5) and the highest effect of Hg-treatment among the four cultivars. This result is consistent with what was observed in Cabernet Sauvignon, with a more characterized isohydricity, probably supported by the predominance of a hydraulic control. Syrah, as expected, displayed a behavior opposite to Grenache, but only on excised leaves, with the highest values of $g_s$ and the most dramatic $\Psi_{leaf}$ drop (Fig. 5), and the lowest percentage loss in water uptake on Hg-treated leaves. However, on whole plants, therefore at less negative $\Psi_{leaf}$, the same cultivar expressed a behaviour closer to Grenache (near-isohydric) than to Merlot (anisohydric), supporting the results obtained under moderate water stress by Pou et al. (2012). Finally, Merlot showed the lowest variations of $g_s$ both in whole plants, and in excised leaves, confirming the general identifications as anisohydric cultivar. The scarce effect of the Hg treatment, similar as observed in Syrah, suggests that the low transmembrane water transport capacity negatively affected stomatal control, limiting transpiration in the former and favoring soil water depletion in the latter.

The observations done on whole plants and on excised leaves allowed to obtain a more complete picture of the cultivar effect on the hydraulic dynamics involved at plant and at leaf level. Further studies will be needed in order to integrate the current results with additional details on the component of variation due to hydraulic and hormonal signalling between the scion and rootstock (Marguerit et al., 2012). From the combination of the obtained results, we could interpret the rootstock effect as ‘quantitative’: the ability of roots to supply water relative to shoot transpiration demand displaced reciprocally the rootstock curves to higher/lower parallel positions. On the other hand, the scion effect was ‘qualitative’: the feedback loop between $g_s$ and $\Psi_{leaf}$ produced at leaf level modified the slope of the curves.

5. Conclusions

From the present study, it was concluded that rootstock and scion genotypes are able to confer to the plant traits of drought adaptability influencing the capacity of water extraction from the soil and sensitivity of the stomatal control. On both components (roots and canopy) the transcellular pathway seems to have strong relationships with strategies related to water translocation. Further research will be needed in order to identify the potential use of this information in studies of stress tolerance and adaptability of rootstocks and cultivars to specific ecological conditions.

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References


